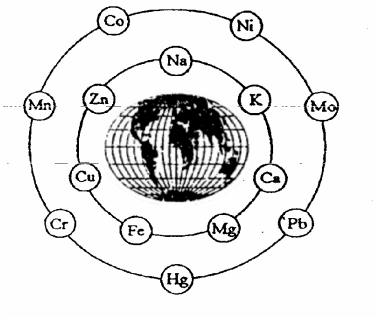
C O N T E N T S

Metal Elements in Environment, Medicine and Biology

Tome IV

ROUMANIAN ACADEMY
BRANCH TIMIŞOARA



GÂRBAN ZENO, DRĂGAN PETRU
EDITORS

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Tome IV

Proceedings of the 4th International Symposium on Metal Elements in Environment, Medicine and Biology, Timişoara, November 6-8, 2000

Publishing House "Eurobit" Timişoara
2000

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C O N T E N T S

FOREWORD

The series of International Symposia with the theme "Metal Elements in Environment, Medicine and Biology" (M.E.E.M.B.) organized under the auspices of Roumanian Academy – Branch Timişoara cover, by their large thematic area, some inter- and multidisciplinary domains. Their addresability is of interest for ecology, nutrition, technology and biotechnology, pharmacology, pathology, surgical plasties, toxicology etc. In this way, the approached topics are remarkable by theoretical and applicative importance.

Interdisciplinarity of studies dealing with metals regards lithosphere, hydrosphere, atmosphere as well as with their implications in the biosphere. In this context there are mentioned the researches on biometals with role in the vegetal and animal kingdom and in the human organism as bioconstituents and/or as biochemical effectors. There are also studied the metals with toxicogene potential and their undesirable effects on living matter.

Opening the series of International Symposia was agreed by the Roumanian Academy, personally by the vicepresident Acad. Nicolae Cajal, in 1993. Subsequently it was also sustained by vicepresident Acad. Alexandru Balaban and vicepresident Acad. Ionel Haiduc. Researches of this domain were also sustained by Acad. Prof. Pius Brânzeu MD, PhD in the frame of Branch Timişoara - Roumanian Academy.

The last symposia were honoured by Acad.Prof. Ionel Haiduc, PhD, with remarkable international contributions in the domain of organometallic compounds. Acad. Ionel Haiduc was the honorary president of the last two symposia of the series. At "M.E.E.M.B." 2000 being in the U.S.A., he contributed at this volume with a paper on scientometry.

Also, this scientific event, was sustained by the Comission of Biochemistry of the Roumanian Academy - president Prof. Mihai Şerban, corresponding member of Roumanian Academy. The series of International Symposia were organized at the beginning by the Roumanian Academy – Branch Timişoara with the contribution of University of Medicine and Pharmacy, University of Agricultural Sciences and Veterinary Medicine and later also by University Politehnica and West University Timişoara.

Fundamental and applicative investigations on metals, on their importance and their role in the environment, medicine and biology, present a top domain for the actual sciences to attend. To organize these series of scientific meetings started from the existence of a "Working group for the research of metals in environment, medicine and biology" founded in Timişoara around the years '79 and orientated predilectly to the study of the interactions metals-proteins (especially nucleic acids), embryopharmaco-toxicology (of cis-platinum and various metal ions) and to finding out the role of metals in the biogenesis of uroconcrements being of interest for biochemistry and biochemical pathology. The "Working group for the research of metals" functioned initially in the Institute of Public Health and Medical Research Timişoara-Laboratory of Embryology belonging to the Academy of Medical Sciences - Branch Timişoara and in the University Clinic of Urology from the University of Medicine and Pharmacy Timişoara.

Interdisciplinary research has been approached and extended, appearing departmental themes included in the schedule of scientific research of the Academy of Medical Sciences. Researchers from different institutes as well as the teaching staff showed a great interest for this domain.

Specialists from various universities and research institutes from our country and from abroad took part at this international symposia, all of them preoccupied by interdisciplinary studies on metal elements.

One can remark that each M.E.E.M.B. symposium contributed to the extent of international scientific cooperation, to diversifying and thoroughly study of inter- and multidisciplinary themes in the endless quest to make great progress in this field. Scientists are always looking for more and more powerful tools to search constantly growing databank of information.

All the mentioned aspects make us believe that, becoming well-known, the series of International Symposia with the theme "Metal Elements in Environment, Medicine and Biology" will focus the interest of more and more distinguished personalities and also young researchers on this domain. From this point of view we want to wish everyone success in the future.

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Acad. Prof. Toma Dordea, PhD, Eng.
President
Roumanian Academy-Branch Timişoara

PREFACE

The series of International Symposia “Metal Elements in Environment, Medicine and Biology” (M.E.E.M.B.) arrived at its 4th edition and continues to arise the interest of the roumanian and international scientific community because it joins the desiderations of fundamental and applicative research.

The editors express their gratitude to all prestigious personalities for their support during the series of International Symposia. In this context we thank to Acad. Prof. Nicolae Cajal MD, PhD, vice-president of Roumanian Academy. In 1993, for his helpful indications beginning on the first Symposium and the suggestion of continuity and to Acad. Prof. Pius Brânzeu MD, PhD, for continuous help in the development of this research domain.

Also, they are indebted to Acad. Prof. Alexandru Balaban PhD, Eng. and Acad. Prof. Ionel Haiduc PhD, who successively - as vicepresidents of the Roumanian Academy – encouraged and supported this manifestation.

We express the whole gratitude to Acad. Prof. Ionel Haiduc PhD – honorary president of the Symposium - recognized personality of the international scientific life with remarkable contributions in the domain of organometallic compounds studies - for the support given to this scientific meeting, for the approach to the topics of bioinorganic chemistry. In this volume of proceedings, among plenary lectures, was also included a paper referring to scientometry in biology, chemistry and medicine of Acad. Ionel Haiduc who could not take part at the session being in the USA as a visiting professor. Further on were introduced the papers of scientific communications and posters.

The symposia benefitted by the help of Acad. Prof. Toma Dordea PhD, Eng. – President of the Roumanian Academy - Branch Timişoara, Acad. Prof. Păun Ion Otiman PhD, Eng. - rector of University of Agricultural Sciences and Veterinary Medicine Timişoara, Prof. Ioan Carțiş PhD, Eng., member of the Academy of Technical Sciences - rector of University “Politehnica” Timişoara, personalities with solicitude and opening for cooperation ascertaining the location of symposium and pertinent suggestions in the scientific and organizing problems. The fruitful collaboration with the rectorate of University “Politehnica” assured the efficiency and the prestige of this Symposium. Also, we would like to express our gratitude for technical help to Prof. Gheorghe Ciuhandu PhD. Eng . - Mayor of Timişoara city.

For constant encouraging of the series of International Symposia “M.E.E.M.B.” we express our thanks also to Acad. Prof. Emanuel V. Sahini, PhD and to Acad. Prof. Gheorghe Zarnea PhD.

Further on there are reiterated some aspects mentioned in the prefaces of the previous editions of the Symposium.

A first aspect is represented by the inter- and multidisciplinary horizon of the moderne conceptions referring to the mechanisms of metals translocation which stay at the base of the so-called trophic chain soil-plants-animals-humans. In this context the problems have in view biochemical and pathobiochemical, physiological and pathophysiological as well as morphological and morphopathological aspects.

A second aspect is related also to the specific inter- and multidisciplinary character of the theme but in the acceptance of the applicative sphere with practical problems involving the habitual environment of humans (i.e. soil, water, air), macro- and micronutrients, chemotherapeutical products (for human and veterinary use),

pathological processes, materials utilized in prothesis and implantology, different residues with metal content discharged in the environment etc.

Research on metals permit to approach the problem of various metal compounds, represented by bioconstituents, biochemical effectors (activator/ inhibitors), as well as the metabolic processes involving metals. Such an approach make possible to investigate the chemical structure-biological activity relationship in the homeostatic context of the blood and tissular metallograms as well as of the pathological implications.

There is also a reverse side of this problem: situation in which the metallic compounds (in facto the metal ions) are of interest for using them as food supplements and as chemotherapeutical products. Such situations may appear in nutritional and metabolical diseases (dismineralosis), in cytostatic chemotherapy (e.g.: use of cis-platinum), in endocrinopathies (e.g.: use or restriction of metallic compounds as drugs and nutrients), in cardiovascular diseases (e.g.: use of magnesium containg compounds) etc. One can mention also iatropathies in the case of an excess of metal containing chemotherapeutical products.

In this preface we will reiterate the contribution of prestigious schools with international recognition in the study of metals – in time and space – as reference scientific forum for such researches. In this context we emphasize the contribution of the following schools: G. Eichhorn et al. (Bethesda-U.S.A.); J.E. Underwood (Nedland-Australia); A. Prasad (Michigan-U.S.A.); M.Anke et al. (Jena-Germany); L.G. Marzilli (Atlanta-U.S.A.); C.F. Mills (Aberdeen-United Kingdom); J. Durlach et al. (Paris-France); H. Sigel (Basel-Switzerland); J.Mc. Howell (Perth-Australia); V. Ferm (New Hampshire); P.J. Sadler (London-United Kingdom); M. Gielen (Brussels-Belgium); R. Smetana (Vienna-Austria); Joan Silverstone Valentine (Los Angeles-U.S.A.); G. Chazot (Lyon-France); Ph. Collery (France); J. Corbella (Spain); I. Pais (Budapest-Hungary; A.S. Kiss (Szeged-Hungary); M.J. Halpern (Lisbon-Portugalia); P.F. Zatta (Padova-Italy); M. Abdulla (Stockholm-Sweden); T. Theophanides (Athens – Greece); T. Ito (Tohoku-Japan);I. Haiduc (Cluj-Napoca-Roumania) a.o.

Finally, we wish to thank all the students from University “Politehnica” Timişoara, University of Agricultural Sciences and Veterinary Medicine Timişoara, University of Medicine and Pharmacy Timişoara and West University of Timişoara who helped us in different moments for a successful organization of the Symposium.

Timişoara, December 10, 2000

Gârban Zeno
Drăgan Petru

URANIUM IN THE FOOD CHAIN OF HUMANS IN CENTRAL EUROPE - RISKS AND PROBLEMS

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ABSTRACT

On an average, the uranium content of annual plant species decreased from the beginning of May to the middle of June by about 50%. The uranium uptake is faster than the substance formation. Assimilates dilute the uranium concentration in plants.

The granite weathering soil produced the uranium-richest vegetation. The lower strata of new red sandstone and muschel kalk contained about 25% less uranium and the diluvial sands 50% less uranium than granite. The differences are significant.

The uranium content of drinking and mineral water is also determined by the geological origin of the ground water reservoir. The water from diluvial sands often contains only 1 to 10% of the uranium amounts found in that from granite and muschelkalk.

Compared to control plants, wild and cultivated plants from the immediate vicinity of uranium slagheaps stored normal to eightfold uranium amounts. Leafy species accumulated much uranium whereas tubers, the thick parts of stalks and fruit stored less uranium.

The uranium content of 117 foodstuffs and beverages varied between 0.7 and 0.8 μg/kg dry matter in butter, honey and margarine and 105 μg in mixed mushrooms, 53 μg/kg dry matter in asparagus, 43 μg/kg dry matter in marjoram and 39 μg/kg in lettuce. As a rule, sugar-, starch- and fat-rich foodstuffs proved to be uranium-poor (fruit, seeds, flour, meat) whereas leafy vegetables, black tera, herbs and hen's'egg can be uranium-rich.

Food for babies and young children proved to be uranium-poor. However, its uranium content can increase to the 50fold of that of mother's or cow's milk due to its preparation with particularly recommended mineral water.

On an average, the uranium intake slightly increased from 2.2 to 2.7 μg/day between 1988 and 1996. It is considerably varied by regional (geological) effects. The test populations on the diluvial formations in Northern Germany only took in one third of uranium amounts, which are taken, in Thuringia. Women with mixed diets consume a significantly uranium-richer dry matter than men with the same diets. Women prefer uranium-richer group of foodstuffs (vegetables).

The uranium balances of people with mixed diets were balanced with 2.4 and 3.2% . Uranium is not accumulated in the body. The seeming absorption amounted to about 6% and thus, was extremely high.

The uranium content of drinking and mineral water needs public control. This is particularly necessary in areas with former uranium mining but it seems also important in other areas. The uranium content in the mineral water recommended for preparation of baby food should be analyzed in any case.

Key words: uranium in food chain of humans – Central Europe

INTRODUCTION

In the 16-km thick earth`s crust are assumed to occur 2.4 and 3.2 mg U/kg, resp. (Saager 1984; Hollemann and Wiberg 1985). Thus, uranium occurs more frequently than iodine, cadmium, mercury and selenium. It consists of the three natural radioactive isotopes 238 uranium (99.283%), 235 uranium (0.711%) and 234 uranium (0.005%). As a rule, granite contains much uranium (2.5 to 10 mg U/kg) (Rösler and Lange 1975; Vriend et al 1985, Kabata-Pendias and Pendias 1992). Slate and muschelkalk or dolomite can also be uranium-rich (3.0 - 4.1 and 2.2 - 2.5 mg U/kg) whereas sandstone (0.45 - 0.59 mg U/kg) and basalt (0.3 - 1 mg U/kg) store less uranium. As a rule, uranium forms organic, readily soluble and mobile complexes during the process of weathering. Oxides, carbonates and phosphates of uranium come into being under arid conditions. Microorganisms accumulate much uranium. Coal and peat can store much uranium.

Between 0.79 and 11 mg U/kg soil were found all over the world. The soil is often rich in uranium in the vicinity of power and phosphate stations (Kabata-Pendias and Pendias 1992). This is all the more true for the vicinity of uranium mines and uranium processing plants, which were concentrated in Saxony, Thuringia and the Bohemian Ore Mountains in Central Europe. E.g. the slagheaps of the former uranium processing plant of Crossen near Zwickau contain 130 mg U/kg. The sediments of brooks and rivers in uranium-exposed biospheres can store much higher uranium amounts (10 to 400 mg U/kg), which come from the uranium-rich water of uranium mines or uranium processing plants (Friedrich and Voland 1991).

The uranium transfer from soil and water via flora and fauna and the foodstuffs produced therefrom into the food chain of humans has hardly been investigated although the increased cancer incidence of the population with natural uranium exposure is well-known (Augustin and Zejda 1991). They may also result from the exposure to other radionuclides such as thorium, the daughter elements of uranium and several heavy metals (e.g. Cd, Pb). An increased occurrence of bronchial carcinomas in workers from uranium mining was also registered. They are, however, equally exposed to a multifactoral burden to arsenic, nickel, asbestos and the radiation of daughter elements of uranium and other elements (Mehlhorn 1982, Enderle and Friedrich 1995). The investigations aimed at following up the scientific basis of the uranium transfer in the food chain of humans, to register the "normal" uranium content in foodstuffs and beverages in Germany, to determine the uranium intake of people with mixed diets by means of the duplicate method, to draw up uranium balances, to detect the excretion of this element and to draw conclusions for human nutrition from the findings.

MATERIALS AND METHODS

The influence of the age of plants on the uranium content of the flora was investigated in lucerne, wheat and rape on 6 different, marked sites of the upper muschelkalk in Thuringia between May and June 14th, 1995.

The analysis of the influence of the geological origin of the soil on the uranium content in the vegetation was carried out with the ubiquitously spread plant species acre red clover in buds (Trifolium pratense var. sativum L.), meadow red clover in blossom (Trifolium pratense var. spontaneum L.), rye in blossom (Secale cereale L.) and wheat in shooting (Trticum sativum L.).

The samples were collected all over Germany with the help of phenological maps (anonymous 1953) and geological ordnance survey maps. The plants were generally collected when rye was in blossom. The geological origin of the sites was

checked with "reading stones". 10 samples of the 4 indicator plants were analyzed per geological origin.

The effect of a uranium exposure due to uranium mining and uranium processing was investigated in 14 species from the living area of Gauern and Wolfersdorf near Ronneburg in the immediate vicinity of the uranium slagheaps. The samples were collected in summer 1996 (table 1). After the collection, the samples were generally washed in bidistilled water and - in the case of foodstuffs - analyzed ready for consumption. In 1992, the 117 investigated foodstuffs were bought in the supermarkets of Berlin and the capitals of the Lands in the new German states and analyzed ready for consumption. The samples of drinking water were taken on 152 sites in the new German states. Furthermore, 77 samples of mineral waters were analyzed in threefold repetition in 1997.

Table 1 Species, origin and number of the investigated samples

Kind of analysis	Number of samples
age of plants (lucerne, wheat, rape)	24
geological origin (indicator plants)	440
influence of uranium mining (14 species)	34
Foodstuffs	702
drinking and mineral water	383
duplicate and balance studies, Jena 1988	98
duplicate and balance studies, Vetschau 1988	98
duplicate and balance studies, Wusterhausen 1988	98
duplicate and balance studies, Bad Liebenstein 1992	98
duplicate and balance studies, Bad Langensalza 1992	98
duplicate and balance studies, Chemnitz 1092	98
duplicate and balance studies, Freiberg 1992	98
duplicate and balance studies, Wusterhausen 1992	98
duplicate and balance studies, Greifswald 1992	98
duplicate and balance studies, Steudnitz 1996	98
duplicate and balance studies, Jena 1996	400
duplicate and balance studies, Ronneburg 1996	316
duplicate and balance studies, Mexico 1996	196
number of uranium determinations	**3815**

For the duplicate and balance studies of adults with mixed diets, 12 test populations from Germany and 2 from rural areas of Mexico were available. They consisted of at least 7 men and 7 women and collected all consumed foodstuffs and beverages as visually estimated dupli-cates on 7 successive days (Anke et al 1997). The balance studies were carried out with women and men from Jena and Ronneburg in 1996 (Anke et al. 1999) (see table 1).

Water was removed from the samples at 60°C up to air dry matter and afterwards 20 to 30 g were dried to weight constancy at 105°C. 500 to 1000 ml of beverages and samples of drinking water as well as 100 ml of the daily urine were dried and afterwards they were made matrix-free at 450°C. The ashes were dissolved in 25% HCl, diluted to 2.5% HCl, heated to 80°C in a water bath for 10 minutes and filtrated in glass retorts. In order to remove the carbonic acid from the mineral water, 100 nl HNO_3 suprapur were added to 100 ml water and afterwards it was treated in an ultrasound bath (10 minutes).

For the measurement of the different ashed samples, 1ml of the hydrochloric disintegration solution was filled up to 5 ml with bidistilled water and mixed with 1 ml

of the rhodium standard (200 g Rh/l). 238Uranium served as measuring isotope. The uranium analysis was carried out at the devices Elan 5000 and 6000 (Perkin Elmer), resp. The measuring conditions are described in detail by Seeber (1999). The detection limit of the uranium analysis was 23 ng/l (Elan 5000) and 15 ng/ (Elan 6000). The suitability of the sample disintegration was tested with refinding attempts in samples with a different matrix. The refinding rate was 94 to 109% (Seeber 1999). The accuracy of the analysis was checked with the help of several reference materials (NISTSR M 1575 Pine Needles; GBW 07602 Bush Twigs; CTA-OTL - 1 Tobacco Leaves; APS 1075 Trace Metals in Drinking Water). Deviations of <10% from the certified or informative uranium concentrations were registered.

Repeated measurements were regularly carried out during the whole measuring period in order to guarantee the precision of the measuring procedure. The findings varied within a range of 10% around the average value. The relative standard deviation of the results amounts to 5.3%.

The t-test, the one-factorial and the simple several-factorial variance analysis and the calculation of the least significant difference were used for the statistical evaluation of the results. The preparation of the data and the statistic calculations were carried out with the programmes Fox Pro (version 2.6 for windows, Microsoft Ltd) and SPSS/PC+ (version 6.01 for windows, SPSS inc.).

RESULTS AND DISCUSSIONS

The effect of the age of plants on the uranium content in the vegetation

Like in the case of other, but not all macro, trace and ultratrace elements (K, Na, P, Fe, Mn, Zn, Cu, I, Mo, Ni), the uranium content in lucerne, wheat and rape decreases significantly with increasing age (Anke et al 1994). The uranium intake of annual plant species is faster than the substance formation and was double the amount at the beginning of May than in the middle of June in the species presented in table 2. The assimilates apparently dilute the uranium content of the vegetation with increasing age.

Table 2. Effect of the age of plants on the uranium concentration in the vegetation in µg/kg dry matter

Species		Time of sample collection				Influence of age	
(n;n;n;n)		May 4th	May 17th	May 31st	June 14th	LSD[1)]	%[2)]
lucerne	$\bar{x}$	9.2	6.3	6.0	3.9	3.3	42
(6;6;6;6)	s	3.3	2.3	1.5	1.3		
wheat	$\bar{x}$	9.0	10.8	6.5	4.7	37	52
(6;6;6;6)	s	1.5	2.8	3.6	1.6		
rape	$\bar{x}$	6.5	4.3	3.8	3.9	2.4	60
(6;6;6;6)	s	1.4	1.8	1.8	0.8		

1) least significant difference 2) 4th May = 100%, 14th June = x%

Therefore, the time of harvesting takes a highly significant effect on the uranium content in the green fodder for the fauna and on vegetables and herbs. Grassses, herbs and leguminous plants follow this trend homogeneously. Therefore, the uranium exposure of game in uranium-contaminated areas is particularly intensive in early spring.

The influence of the geological origin of the living area on the uranium content of the vegetation

On the average of Germany, the weathering soils of the granite deliver the significantly uranium-richest plant forage and foods (table 3). Their uranium content was equated with 100 and the uranium concentration of the flora on the other sites was related to it. The lower strata of new red sandstone and muschelkalk follow as uranium suppliers with a significant distance to granite but the uranium content in the flora growing on them did not differ. Plants on the Triassic sediments new red sandstone and keuper, the gneiss and particularly the diluvial sands store significantly less uranium than those on the weathering soils of granite, the lower strata of new red sandstone and muschelkalk. The geologically induced effects on the uranium concentration in the flora are so important that they deserve nutritive attention. Uranium is apparently taken up by plants in accordance with the offer in the soil. The granites described as uranium-rich and their weathering soils produced the uranium-richest vegetation in these investigations as well. The low uranium sorption capacity of diluvial sands may also be the cause of the modest uranium content in the vegetation growing on them.

Table 3: Relative uranium content in the flora depending on the geological origin of the soil

Geological origin of the soil	Relative number
granite weathering soils	100
Weathering soils of the lower strata of new red sandstone	75
muschelkalk weathering soils	74
loess	72
phyllite weathering soils	67
slate weathering soils	64
boulder clay	64
weathering soils of new red sandstone	55
keuper weathering soils	52
gneiss weathering soils	50
diluvial sand	46
least significant difference	**9**

Uranium content in drinking and mineral waters

The uranium content in drinking water is equally affected by the geological origin and anthropogenic uranium emissions (Fiserine 1994). This is also true for the drinking water in Germany (table 4). It was astonishing that, on an average, the drinking water in the area of Ronneburg contained the same uranium concentrations as that in Thuringia, far away from the uranium mining area and without any connection to it.

Table 4: Uranium content in the drinking water in the new Lands of Germany in µ/l

Land of Germany	(n)	x	s	minimum	maximum
Thuringia	(55)	2.4	2.0	0.11	8.6
Saxony	(31)	1.1	1.2	0.09	4.5
Saxony-Anhalt	(19)	0.76	0.74	0.09	2.8
Brandenburg	(22)	0.31	0.26	0.08	1.0
Mecklenburg-Western Pomerania	(25)	0.28	0.24	0.05	0.94
New Lands of Germany	**(152)**	**1.3**	**1.6**	**0.05**	**8.6**

On the other hand, it was detected that drinking water from Thuringia contained nine times more uranium than that from Mecklenburg-Western Pomerania. The drinking water from Brandenburg also stored only one eighth of the uranium amount found in Thuringia. Thus, the diluvial formations of Northern Germany do not only produce uranium-poor foodstuffs but also uranium-poor drinking water. On an average, the drinking water from Saxony and Saxony-Anhalt delivered between twice to three times more uranium than that from Thuringia (table 4). The comparison of the extreme uranium values of the drinking water shows that the lowest uranium concentrations in the individual Lands only varied between 0.5 and 0.11 µg/l. The maximum values are regionally very different and vary between 0.94 and 8.6 µg/l. The uranium content in the drinking water all over the world varies to the same extent as in Germany (Seeber 1999). The uranium concentration in local drinking water can take considerable effect on the uranium intake via home-made (tea, coffee) and locally produced beverages. The uranium content of mineral water is subjected to an even much wider range than that of drinking water. It varies between 24.5 and 0.015 µg/l all over Germany. Examples are presented in table 5. The highest uranium concentration of 24.5 µg/l was found in the alwa bonalwa spring in the Black Forest. 6 of the 77 mineral waters contained less uranium than the detection limit with < 0.015 µg/l. It is striking that the mineral waters from Thuringia, Hesse, North Rhine Westphalia, Saxony and the southern

Table 5: Uranium content in German mineral waters in µg/l

Name	Origin	Land*	$\bar{x}$	s
alwa bonalwa-Quelle	Bad Peterstal-Griesbach	BW	24.5	0.5
Hessberger**	Heßberg	TH	11.2	0.6
Delta Graf Simion-Quelle	Steinheim-Vinsebeck	NW	9.1	0.6
Taufrisch Mineralbrunnen	Leissling	ST	8.8	0.1
Leisslinger Mineralbrunnen	Leissling	ST	8.8	0.6
Waldecker	Volkmarsen	HE	6.7	0.7
Rennsteig Sprudel **	Schmalkalden	TH	6.7	0.1
Schlossquelle Friedrichroda	Friedrichroda	TH	6.3	0.2
Bad Vilbeler Elisabethenquelle	Bad Vilbel	HE	6.3	0.5
Fortuna Quelle	Eichenzell-Lütter	HE	6.0	0.6
Justus Brunnen	Eichenzell-Lütter	HE	6.0	1.0
Thüringer Waldquell	Schmalkalden	TH	5.5	0.1
Margonwasser	Müglitztal-Burkhardswalde	SN	5.2	0.2
Engelbert Brunnen	Bochum	NW	0.017	0.019
Wenden Quelle	Dodow	MV	0.016	0.003
Bischofs-Quelle	Dodow	MV	<0.015	-
hella Mineralbrunnen **	Trappenkamp	SH	<0.015	-
Bad Brambacher	Bad Brambach	SN	<0.015	-
Leichte Brise	Goslar	NI	<0.015	-
Kaiser Friedrich Quelle	Offenbach	HE	<0.015	-
Franken Brunnen Silvana Quelle	Pechbrunn	BY	<0.015	-

*BB Brandenburg, BE Berlin, BW Baden-Württemberg, BY Bavaria, HE Hesse, MV Mecklenburg-Western Pomerania, NI Lower Saxony, NW North-Rhine Westphalia, RP Rhineland-Palatinate, SN Saxony, ST Saxony-Anhalt, SH Schleswig-Holstein, TH Thuringia
** suitable for the preparation of baby food

part of Saxony-Anhalt contain > 5 µg U/l, whereas the North-German mineral waters from Mecklenburg-Western Pomerania, Schleswig-Holstein, Lower Saxony, Brandenburg, Berlin, Bavaria and Rhineland-Palatinate are usually uranium-poor (Seeber et al 1987, Seeber 1998). More than two thirds of the mineral waters in Germany stored < 1 µg U/l in 1997 (Fig. 1) and thus proved to be particularly uranium-poor.The strikingly high uranium concentration of the alwa bonalwa spring (24.5 µg/l) from the Black Forest is due to the natural uranium deposits in this region. Frindik and Fischer (1984) also found extremely high U activities of 740 and 380 mBq/l (60 and 31 µg U/l) in two mineral waters from the Black Forest. The mineral waters from the Thuringian Forest also contain increased uranium amounts with 5.5 - 11.2 µg/l. The existence of smaller uranium deposits in the Thuringian Forest is known as well (Meinel and Mädler 1995).

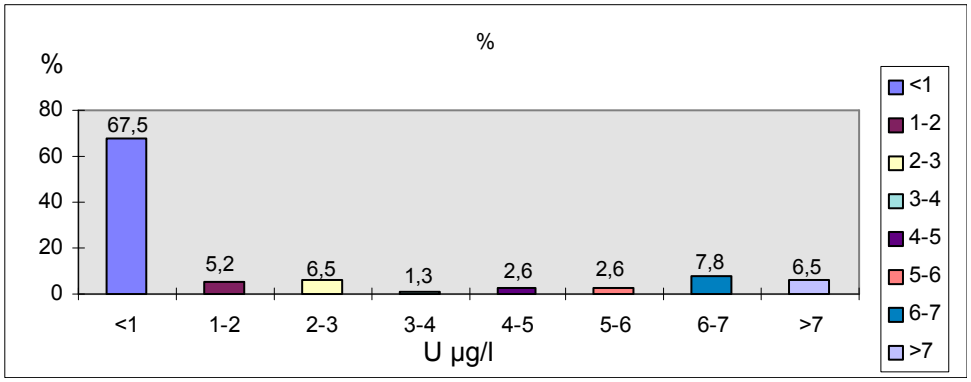

Fig. 1: Frequency distribution of the uranium content in mineral waters

Although there are large uranium deposits in Saxony, the uranium content in Saxon mineral waters is surprisingly low. Apart from *Margonwasser* (5.2 µg/l), the other mineral waters contained uranium concentrations of below 1 µg/l.. It should also be mentioned here that even some mineral waters which are suited for the preparation of baby food contain very high uranium amounts. This finding is not surprising since only the limit values for sodium, nitrate, nitrite and fluoride are necessary for the labelling of the waters.

The influence of anthropogenic uranium exposures on the uranium content of the flora

The effect of uranium mining and uranium processing on the uranium content of several wild and cultivated plants which serve as feeds for farm animals and as raw products for plant foods was systematically investigated in the immediate vicinity of slagheaps near Ronneburg. The analyzed species and parts of plants grew on agriculturally productive land or in housegardens directly beside the uranium slagheaps. The regions with uranium mining in the Ore Mountains are hardly used as productive land or gardens but by forestry or they lie fallow. Apart from water, they hardly deliver any uranium into the food chain of humans. Meadow red clover and white clover from uranium-exposed areas near slagheaps stored eight and fifteen times more uranium than control plants in Thuringia. Several raw

materials for foodstuffs such as rape seed and wheat grains accumulated three times and twice more uranium than the cultures from control areas.

Several vegetables and herbs also stored similar uranium amounts whereas tubers (peeled potatoes), thick parts of stalks (onions) and fruit (cucumbers, apples, tomatoes and onions) did not differ from those in control areas. Therefore, compared with drinking water, the uranium exposure due to the consumption of herbs, fruit and potatoes cultivated in house garden is very limited.

Table 6: The uranium content of several wild and cultivated plants, vegetables and fruit from a control and a uranium-mining area

Plant species (n;n)	**Control site**		**Uranium mining**		**Effect of site**	
	s	$\overline{x}$	$\overline{x}$	s	p	% [1)]
meadow red clover (19;11)	2.0	7.4	57	38	<0.001	770
white clover (25;11)	10	15	68	23	<0.001	453
rape seed (10;6)	0.10	0.71	2.2	0.8	<0.01	310
chives (23;7)	4	10	29	12	<0.001	290
French beans (12;6)	1.2	3.1	8.1	2.3	<0.01	261
lettuce (20;6)	18	34	73	14	<0.001	215
wheat grain (16;14)	0.13	0.58	1.2	0.8	<0.05	207
parsley (13;6)	8	28	54	21	<0.01	193
carrots (18;8)	1.5	4.4	8.2	3.7	<0.05	186
potatoes, peeled (26;14)	1.4	2.3	2.9	1.9	>0.05	126
cucumber (15;8)	2.6	7.0	8.5	4.5	>0.05	121
apple (25;9)	1.7	2.7	2.8	1.5	>0.05	104
tomato (9;9)	1.2	5.2	5.0	2.4	>0.05	96
onion (22;6)	3.1	5.2	4.3	3.1	>0.05	83

[1)]control site = 100%, Ronneburg = x%

The uranium content of several foodstuffs and beverages

The uranium content of the 117 investigated foodstuffs is presented in table 7 by means of characteristic foodstuffs. Mixed mushrooms accumulate by far the most uranium. Asparagus harvested very early contained much uranium with about 50 µg /kg.

The same comes true for leafy vegetables (lettuce) and parsley. Astonishingly, hen´s eggs also deliver relatively much uranium. It may get into the hen`s food chain via phosphates and/or calcium carbonate. Apart from livers and kidneys, animal foodstuffs are generally significantly uranium-poorer than several (leafy) vegetables and herbs. Meat, milk, cheese and particularly starch-, sugar- and fat-rich foodstuffs are extraordinarily uranium-poor. The uranium content of commercial foods for babies and children is also pleasantly low (table 8). However, the uranium intake of babies can be considerably increased by the use of uranium-rich mineral waters which are particularly recommended for the preparation of baby food. Thus, the uranium-richest mineral water suitable for the preparation of baby food contained 11.2 µg U/l. Baby food produced with it contains 9.5 µg U/l, i.e. the 50fold uranium content of cow`s or mother`s milk (Iyengar 1982). Experiments with animals showed that the gastro-intestinal uranium absorption of new-born babies is much higher than that of adults

(Sullivan 1980, Sullivan and Gorham 1982). This potential risk needs experimental clarification.

Table 7: The uranium content of several foodstuffs in µg/kg edible proportion (fresh matter, fm) and in µg/kg dry matter (dm)

Foodstuff	U	U	U µg/dm	
	dm, %	µg/kg fm	$\bar{x}$	s
mixed mushrooms	6.0	6.3	105	39
asparagus	4.6	2.4	53	32
lettuce	7.3	2.9	39	27
parsley	18	5.7	32	15
paprika, sweet	88	17	19	10
hen`s egg	25	4.1	16	11
cucumber	5.2	0.6	12	5.0
black tea	94	8.3	8.8	2.5
kidneys of cattle	24	2.1	8.8	5.5
carrots	7.0	0.6	8.0	4.3
trout fillet	29	1.5	5.1	3.4
herring fillet	35	1.5	4.2	1.5
coarse wholemeal bread	54	2.0	3.7	2.2
mutton	33	1.0	3.1	2.9
potatoes, peeled	18	0.6	3.0	0.6
Emmentaler	63	1.8	2.9	1.4
beef	27	0.6	2.4	1.3
apple	10	0.2	2.0	0.3
milk	12	0.2	1.9	1.6
coffee	96	1.6	1.7	0.4
wheat flour	86	1.5	1.7	1.0
white bread	64	1.1	1.7	0.5
pork	28	0.4	1.5	0.4
mustard seeds	91	1.1	1.2	0.1
oat flakes	88	1.1	1.2	0.3
vanilla pudding	86	1.0	1.1	0.3
semolina	18	0.2	1.1	0.2
bananas	87	0.9	1.0	0.6
sugar	100	1.0	1.0	0.2
butter	85	0.6	0.7	0.2

Table 8: The uranium content of commercial food for babies and children (powder) in µg/kg edible proportion (fm) and in µg/kg dry matter (dm), resp.

Foodstuff	U	U	U µg/dm	
	dm, %	µg/kg fm	$\bar{x}$	s
adapted milk nutrition	89	2.7	3.1	1.5
milk pudding for children	89	2.6	2.9	1.6
milk nutrition	83	1.7	2.1	0.2
partly adapted milk nutrition	86	1.1	1.3	0.5

The uranium content of traditional German beverages varies between 0.30 µg/l in beer and 1.3 µg/l in white wine (table 9). The uranium content of the water

used for the production of beer, lemonade and coke as well as for home-made tea or coffee is of decisive importance.

Table 9: The uranium content of several beverages in µg/l

Beverage	Uranium in µg/l	
	$\bar{x}$	s
white wine	1.27	1.05
coke	0.92	0.52
lemonade	0.89	0.51
red wine	0.68	0.35
vermouth	0.39	0.21
fruit juice	0.36	0.31
beer	0.30	0.28

The uranium intake of adult Germans and Mexicans with mixed diets

The mean uranium intake of 12 test populations from Germany and of 2 test populations from rural Mexican areas depending on time and sex (table 10) is significantly varied by the living area. Therefore, it only reflects the tendency of the increase from 1988/1992 to 1996.

Table 10: The uranium intake of adult Germans and Mexicans depending on time and sex (µg/day)

Period (n;n)		women		men			
		s	$\bar{x}$	$\bar{x}$	s	Fp	%
1988, G*	(147;147)	2.5	2.2	2.2	2.3		100
1992, G	(294;294)	1.0	1.5	1.7	1.9	> 0,05	113
1996, G	(168;168)	2.0	2.6	2.8	2.4		108
1996, M**	(98;98)	1.6	2.2	2.5	2.6	> 0,05	114
Fp	G	< 0.001				—	
	M	> 0.05					
%	G	118		127			
	M	85		89			

* Germany ** Mexico

On the other hand, it showed clearly that the uranium intake of Mexicans with mixed diets did not differ from that of Germans in 1996.

It was astonishing that there was no difference between the uranium intake of men and women although men had a mean 24% higher dry matter intake (Anke et al 1997). Women apparently prefer uranium-richer groups of foodstuffs than men. The uranium content of the consumed dry matter is a better indicator of the sex-specific uranium intake because it excludes the varying influence of the individual dry matter intake (table 11).

Table 11. The uranium concentration of the ration and beverage dry matter taken in by adult Germans and Mexicans with mixed diets depending on time and sex (µg/kg dry matter)

Period (n;n)		women _ x	women s	men _ x	men s	Fp	%
1988, G	(147;147)	9.0	7.7	5.8	6.5		75
1992, G	(294;294)	3.1	4.8	4.5	4.8	<0.001	94
1996, G	(168;168)	9.6	10	8.0	6.8		80
1996, M	(98;98)	4.2	7.0	7.2	6.6	> 0.05	103
Fp	G	< 0.001				-	
	M	< 0.01					
%	G	130		138			
	M	70		90			

German women with mixed diets actually took in a significantly uranium-richer dry matter whereas there were no sex-specific differences with regard to the consumed uranium concentration in Mexicans. On an average, the Mexicans with mixed diets took in a 10% to 30% uranium-richer dry matter than the Germans in 1996. Though time also affected the uranium concentration of the consumed dry matter, it is mainly varied by regional influences (table 12).

Table 12: The uranium content in the dry matter of the duplicate samples depending on the land

Land (n;n)		women _ x	women s	men _ x	men s	sex influence Fp	sex influence % [1)]
Thuringia		8.9	9.9	8.2	7.0		83
Saxony		1.8	4.2	4.2	2.6	< 0.001	100
Brandenburg		2.5	3.4	2.7	3.1		79
Regional influence	% [2)]	34		33		-	
	Fp	< 0.001		< 0.001			

[1)]women = 100%, men = x %, [2)]Thuringia = 100%, Brandenburg = x %

Independent of time, Thuringians eat a significantly uranium-richer dry matter than Saxons and particularly people from North Germany. The cause of this finding is almost certainly the significantly lower uranium content in the local drinking water of the diluvial areas in Germany. On an average, the uranium content in the dry matter taken in by the Thuringian test persons varies between 6.0 and 18 µg/kg in women and between 6.3 and 12 µg/kg in men.

The test persons from Brandenburg and Western-Pomerania only took in 4.9 and 2.2. µg U/kg dry matter (Seeber et al 1998).

In spite of the purchase of foods in supermarkets with a supraregional offer of goods, the living area affected the uranium intake highly significantly via drinking water. Due to the modest uranium content in the drinking water, the uranium offer in the areas with former uranium mining and uranium processing near Ronneburg corresponds with the intake typical of Thuringia. Local drinking water from this living area can contain considerable uranium amounts. Age and weight of the test persons

Table 13. Uranium content in the dry matter of the duplicate samples in µg/kg dry matter

Place and year (n;n)		women		men		sex influence	
		s	$\bar{x}$	$\bar{x}$	s	Fp	%
Jena	1988 (49;49)	10.0	17.6	11.9	6.9	< 0.05	67
Steudnitz	1996 (49;49)	10.6	10.9	9.3	5.0		85
Jena	1996 (70;70)	6.1	8.9	8.0	6.8		90
Ronneburg	1996 (49;49)	12.2	10.5	5.9	8.3		56
Bad Liebenstein	1992 (49;49)	5.0	6.2	7.2	7.5		116
Bad Langensalza	1992(49;49)	2.3	6.0	6.3	6.9		105
Chemnitz	1992 (49;49)	1.3	4.0	4.7	2.2		118
Freiberg	1992 (49;49)	2.2	4.5	3.6	2.9		80
Greifswald	1992 (49;49)	3.3	4.9	3.2	2.0		76
Vetschau	1988 (49;49)	2.2	2.3	3.3	5.8		143
Wusterhausen	1992 (49;49)	1.6	3.2	2.2	0.6		70
Wusterhausen	1988 (49;49)	1.7	3.1	2.2	1.3		71
influence of the place	%	18		18		-	
	Fp	$< 0{,}001$		$< 0{,}001$			

as well as the season affect the uranium intake of people with mixed diets to a limited, but significant extent. The uranium intake rises with increasing age and weight; more uranium is taken in in summer than in winter.

Uranium intake of adults with mixed diets

It was possible to draw up uranium balances for 2 test populations from Jena and the uranium-exposed area near Ronneburg (table 14). Both sexes from the 2 sites excreted 9% of the uranium renally and 91% faecally. The uranium balance was balanced with 2.4% in women and 3.2% in men. Uranium is apparently not accumulated in the body as it is the case in other elements (e.g. Cd). The negative uranium balance can be caused by respiratory air and tobacco smoke (Fisenne et al 1987; Igarashi et al 1989)

Table 14: Intake, excretion, seeming absorption and balance of uranium in adults with mixed diets

Parameter (n;119;119)		women		men			
		s	$\bar{x}$	$\bar{x}$	s	p	%[1)]
intake		1.51	2.91	2.85	1.67	>0.05	98
excretion	urin, µg/day	0.18	0.26	0.25	0.16	>0.05	96
	faeces µg/day	2.41	2.72	2.69	3.25	>0.05	99
	urin, %	8.7		8.5		-	
	faeces, %	91.3		91.5			
seeming absorption, %		6.5		5.6			
Balance	µg/day	-0.07		-0.09			
	%	-2.4		-3.2			

[1)] women = 100%, men = x%

Compared to findings in animal experiments, the seeming uranium absorption was astonishingly high with about 6% in both sexes. Investigations in humans do not exist. Much lower absorption rates were registered in animal experiments (Sullivan 1980a, Harrison and Strather 1981, Tracy et al 1992). They are certainly caused by considerable uranium offers in mg/kg body weight.

Discussion of the uranium transfer in the food chain of humans

The uranium transfer in the food chain of humans is significantly affected by the geological origin of the soils and the groundwater basin as well as the living area of the flora and the drinking water reservoir. In accordance with its occurrence in the soil, the uranium gets into the flora and is comprehensively stored in young plants. The uranium content/ kg dry matter is diluted by assimilation with increasing age. Apart from wild mushrooms, young and leafy plants (asparagus, lettuce, herbs) store particularly much uranium. As a rule, starch-, sugar- and fat-rich parts of plants (seeds, fruit) contain less uranium. Animal foodstuffs deliver less uranium into the food chain of humans than the flora. Hen`s eggs, kidneys and livers can accumulate relatively much uranium. In accordance with the uranium offer, mutton contains more uranium with 3.1 µg/kg dry matter than beef (2.4 µg/kg dry matter) and pork (1.5 µg/kg dry matter).

On an average, the uranium intake of German and Mexican people with mixed diets amounted to about 2 µg/day between 1988 and 1996. It is determined by regional (geological) influences which are mainly due to the uranium content in the drinking water. The test populations from Mecklenburg and Brandenburg on the diluvial formations consumed a dry matter which contained only one third of the uranium amount which is taken in in Thuringia. The uranium intake in Ronneburg, an area with abundant uranium occurrence in the environment, was in accordance with that in Thuringia without any anthropogenic uranium exposure. The uranium content in the drinking water offered there was low.

Most of the uranium (about 40%) is taken in by people with mixed diets via beverages, mainly soft drinks (Fig. 2). In both sexes, about 16% of the consumed uranium get into the daily ration of people with mixed diets via bread, cake and pastries, 16% and 14% via fruit, vegetables and potatoes, 8 and 12% via meat, sausage and fish and 12% via milk and cheese. Soft drinks are the main uranium suppliers of people with mixed diets and they are responsible for the regionally (geologically) different uranium intake. The uranium conten in the drinking water, particularly in the former areas with uranium mining and processing in Saxony, Bohemia and Thuringia (Ore Mountains, Vogtland) must be subjected to permanent checks which should also include other exposed granite areas. The increased risk also exists for babies who get commercial baby food which is prepared with uranium-rich water.

The highest uranium intake of a test person in Germany found in 1414 duplicates amounted to 17.9 µg/day or 250 ng/kg body weight/day. Proceeding from the nephrotoxicity of uranium, Wrenn et al (1985) recommend a limitation of the uranium intake of humans to 187 µg/day or 2.6 µg/kg body weight and day. This value is ten times higher than the highest uranium intake found in the duplicates. According to the present level of knowledge, uranium-induced health risks can be excluded in adults with mixed diets.

Throughout the world, there are no obligatory limit values for the uranium content in drinking and mineral water, whose uranium content varies extremely (Fisenne 1994). Drinking water may contain 20 µg U/l in Canada (anonymous 1978 and 1980) and 8 µg U/l in the USA (anonymous 1991). An increase to > 20 µg U/l is assumed to intensify the risk of cancer (anonymous 1991). The clarification of this problem seems necessary, also under the aspect of babies with commercial baby food, who might absorb more uranium than adults.

CONCLUSIONS

1. On an average, the uranium content of annual plant species decreased from the beginning of May to the middle of June by about 50%. The uranium uptake is faster than the substance formation. Assimilates dilute the uranium concentrations in plants.

2. The granite weathering soils produced the uranium-richest vegetation. The lower strata of new red sandstone and muschelkalk contained about 25% less uranium and the diluvial sands 50% less uranium than granite. The differences are significant.

3. The uranium content of drinking and mineral water is also determined by the geological origin of the ground water reservoir. The water from diluvial sands often contains only 1 to 10% of the uranium amounts found in that from granite and muschelkalk.

4. Compared to control plants, wild and cultivated plants from the immediate vicinity of uranium slagheaps stored normal to eightfold uranium amounts. Leafy species accumulated much uranium whereas tubers, the thick parts of stalks and fruit stored less uranium.

5. The uranium content of 117 foodstuffs and beverages varied between 0.7 and 0.8 µg/kg dry matter in butter, honey and margarine and 105 µg in mixed mushrooms, 53 µg/kg dry matter in asparagus, 43 µg/kg dry matter in marjoram and 39 µg/kg in lettuce. As a rule, sugar-, starch- and fat-rich foodstuffs proved to be uranium-poor (fruit, seeds, flour, meat) whereas leafy vegetables, black tea, herbs and hen`s eggs can be uranium-rich.

6. Food for babies and young children proved to be uranium-poor. However, its uranium content can increase to the 50fold of that of mother`s or cow`s milk due to its preparation with particularly recommended mineral water.

7. On an average, the uranium intake slightly increased from 2.2 to 2.7 µg/day between 1988 and 1996. It is considerably varied by regional (geological) effects. The test populations on the diluvial formations in Northern Germany only took in one third of the uranium amounts which are taken in in Thuringia. Women with mixed diets consume a significantly uranium-richer dry matter than men with the same diets. Women prefer uranium-richer groups of foodstuffs (vegetables).

8. The uranium balances of people with mixed diets were balanced with 2.4 and 3.2%. Uranium is not accumulated in the body. The seeming absorption amounted to about 6% and thus, was extremely high.

9. The uranium content of drinking and mineral water needs public control. This is particularly necessary in areas with former uranium mining but it seems also important in other areas. The uranium content in the mineral water recommended for the preparation of baby food should be analyzed in any case.

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TRACE ELEMENTS IN VEGETATION OF DANUBE FLOODPLAIN: DEFICIENCY OR EXCESS ?

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ABSTRACT

It is documented that the Danube floodplain functions as a buffer of the longitudinal fluxes of sediments and associated trace metals. Whether the carrying capacity of the floodplain ecological systems with respect to metals retention is likely to be exceeded or not, it might be indicated by the comparison of concentration in plants with known levels of excess / toxicity. In this paper we make this comparison, and also the comparison with known levels of deficiency, when applicable. Some elements (Ag, As, Ba, Cd, Co, Pb, Sb, Se) were in the normal range, whereas other elements showed in some cases deficiency levels (Cu, and occasionally Mn, Zn), or excess / toxicity levels (Cu, Cr, Fe, Hg, Mn, Ni, V, Zn, Zr). These cases were dependent on species, ecosystem type, position of landscape on the upstream – downstream gradient and location near point sources of pollution. Probability of real deficiency/toxicity effects is discussed and future research directions are proposed.

Key words: vegetation of Danube floodplain – trace elements

INTRODUCTION

Contrary to the high number of studies concerning toxic and essential elements interactions at individual biological level (e.g. Goyer 1997 for review), studies attempting to investigate causal connections between elements at ecological system level are rare (Palmborg et al 1998, Breitberg et al 1999). Two types of mechanisms contribute to these connections: mechanisms involving abiotic (physico-chemical) transformations, and mechanisms involving effects on the biological compartments (Vadineanu, 1998). Effects on biological compartments occur directly either through deficiency (in the case of essential elements) or by excess / toxicity (in the case of toxic elements). Nutrition science tackles the problems of requirements and deficiency of elements at individual level (e.g. Anke et al 1999), and do not consider the effects at higher biological levels and at ecosystem level, not to speak about landscape level. However, premises for approaches at these levels exist, because of the intensive long term studies of certain landscapes (e.g. Neagoe 1999a,b Iordache, 1999). Toxicology and ecotoxicology should tackle, by definition, the problems linked to excess / toxicity at all biological and ecological levels (Ramade, 1992). Cairns (1993) suggested landscape ecotoxicology as a future research field, and Fahrig and Freemark (1995) made an attempt to develop a conceptual model of landscape scale effects of toxic events on species. According to Vadineanu et al (2000b), the investigation of the toxic and deficiency effects on biological compartments which either sustain biological fluxes between ecosystems, or are involved in mechanisms controlling the intensity of abiotic fluxes between ecosystems, is of interest for landscape ecotoxicology and nutrition science because the fluxes connecting the ecological systems underpin the landscape functioning.

The capacity of floodplains and deltas to retain macro-nutrients and metals is generally accepted (Puckett et al 1993). In particular, it is documented that Danube floodplain and delta function as buffer of the longitudinal fluxes of nitrogen,

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phosphorous, sediments (Cristofor et al 1993, Vadineanu et al 1997) and associated metals (Iordache et al 1997a, 2000). Detailed structural studies of the Danube floodplain vegetation have been made in the last decade (Cristofor et al, 1997, Gheorghe, Topa and Vadineanu, 2000). Further characterization of its functional role demonstrated that plant communities are strongly involved in macro-nutrients and metals retention in the Danube floodplain (Cristofor et al, 1998a,b, Iordache et al 1998, Topa, Gheorghe and Vadineanu, 2000). This confirmed results concerning other regional wetlands (for instances, Nebesnyy et al, 1993, estimated that 22966 t of heavy metals are retained in the wetlands of NV Black Sea by reed stands - core Danube Delta not included). Based on the above statements, we may expect, on the one hand, that interactions of elements cycling at plants level takes place, and, on the other hand, that these interactions are relevant for landscape ecotoxicology and nutrition science, i.e. that they may modify the retention capacity of the ecological systems. Beside its relevance at landscape level, an eventual excess of trace elements in plants is also important at ecosystem level, by affecting the consumers populations, and by potentially increasing the trace elements content in animals consummed by humans above acceptable limits.

Aspects concerning heavy metals distribution in the Danube floodplain vegetation have been reported by Pantelica et al, 1998, Iordache, Onete and Vadineanu, 1999, Iordache et al 1999, but an overall analyses of the concentrations with regard to deficiency and excess was not made by now. Here we present preliminary results concerning the hypotheses that heavy metals occur in deficiency or excess concentrations in the vegetation of the Danube floodplain, and propose future research directions.

MATERIALS AND METHODS

The method for assessing the occurrence of deficiency/toxicity consisted in the following steps:

- identification of hyperacumulators plants in our species list by comparison to the literature (Raskin, 1994, Salt, Smith and Raskin, 1998)
- comparison of trace element concentrations in plants with minimum and maximum deficiency or toxicity levels as indicated in literature (table 1)
- comparison of the trace element concentrations in soil with toxicity levels for plants as indicated in the literature (table 1)
- indirect assessment of metals bioavailability in the studied ecosystems through its control parameters (table 2, Iordache et al 1997b, Cazacu and Adamescu, unpublished data)

When a metal concentration in a plant species in an ecosystem is higher than the maximum toxicity level, the species is not an hyperacumulator, the concentration in the ecosystem soil is higher than the toxicity levels for plants, and the values of the bioavailability control parameters indicate potentially high bioavailability of metals in soil, we can consider that is most likely to have toxicity effects.

Three data sets were used in the analyses: data concerning Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ni and Zr distribution in dominant plant species of 14 ecosystems of the Small Island of Braila landscape (km 175-220 on the Danube) (Iordache et al 1998). Three transversal transects (called G, H, and I) consisted in 4 (G), 6 (H), and 4 (I) stations, started from the Danube shore and ended in the internal part of the landscape. Aboveground parts of plants (only leaves in the case of the shrub *Rubus caesius*) were sampled in July-August 1996 in replicates from 9 individuals, dried at 60^{o}C, homogenized in agate mill, and used for preparing a single composite sample.

Fe, Mn, Zn, Cu, Cr, Ni, and Zr were determined by X-ray fluorescence (XRF). Values obtained on control samples by flame atomic absorption spectrometry (F-AAS, after dry mineralization at 450^{o}C) were not different than those obtained by XRF. XRF analyses on plants were also checked by instrumental neutron activation analyses (INAA, Pantelica et al, 1998) and no significant differences were obtained. Cd and Pb were analyzed by F-AAS. A characterization of the studied ecosystems is presented in table 2, including metals distribution in soil/sediment. Methods for the determination of the bioavailability control parameters (table 2) are presented elsewhere (Iordache et al, 1997b, Cristofor et al, 1997). We mention here only that soil metals were determined by XRF, the available method at the analyses time, and that values obtained by F-AAS on control soil samples were smaller than those obtained by XRF.

Table 1. Elements concentrations in plants and soil (ppm) used in the data interpretation.

	1	2	3	4	5	6	7	8	9	10	11
Ag						5-10					
As		2.1	0.02			5-20	25-50			2	15-50
Ba						500					
Co	0.7-8.3		2.4	81		15-50			6.7		25-50
Cr	0.5-16		7	69		5-30	4-15	63	12.6		75-100
Cd						5-30			9.6		3-8
Cu	2-196	7	0.036-22	26	2-5	20-100		5-10	50.6		60-125
Fe	100-17300					>1000					
Hg			0.02	0.5		1-3			23		0.3-5
Mn	36-24220				10-30	400-1000	100		2310	1500	1500-3000
Ni			7	40		10-100			25.8	35	100
Pb			16	47		30-300	10				100-400
Sb						150					5-10
Se						5-30					5-10
V						5-10				150	50-150
Zn	8-7023	36.2	10.7-69	192	10-20	100-400			1269	110	70-400
Zr						15					

Legend: **1** = Backround values for vegetation (range, Denny et al. 1995), **2** = Herbaceous vegetation in pristine site.(average, Pascoe et al , 1996), **3** = Aquatic macrophytes in pristine site (average, range, Manny et al, 1991, Pascoe et al , 1996), **4** = macrophytes in polluted site (average, Manny et al, 1991) **5** = Deficiency level in various herbaceous species (Kabata-Pendias and Pendias, 1992), **6** = Excessive or toxic level in various herbaceous species (Kabata-Pendias, and Pendias, 1992), **7** = Toxic level for vegetation (average, range, Crowder et al,1989), **8** = Toxic levels for aquatic macrophytes (average, range, Guilizzoni, 1991), **9** = Levels at which invertebrates distinguish contaminated aquatic macrophytes (average, Stewart et al, 1992), **10** = Acceptable levels in soil for production of healthy food (average, Kabata-Pendias and Pendias, 1992), **11** = Levels in soil considered toxic for herbaceous vegetation (range, Kabata-Pendias and Pendias, 1992).

1. data concerning Ag, As, Ba, Co, Hg, Sb, Se and V distribution in several plant species from H and I transect (samples from 1996) obtained by INAA.
2. data concerning Fe, Mn, Zn, Cu, Cr, Cd and Pb in 1999 in ubiquitous species in 10 floodplain landscapes of the Lower Danube River System located between km 100 and km 790, and including the Small Island of Braila, as described in

Table 2 General characterization of the studied ecosystems in the Small Island of Braila (annual average and range of parameters values, November 1995 to December 1996). Grey areas indicate values higher than levels considered toxic for herbaceous vegetation, as presented in table 1.

Parameter / Ecosystem code	H1	H2	H3	H4	H5	H6*	I1	I2	I3	I4	G1	G2	G3	G4
Ecosystem type	1	2	3	4	4	6	1	2	5	2	1	2	3	7
Direct anthropogenic impact	0	1	0	0	0	0	0	1	0	1	1	2	2	3
Water level (cm)	132	-208	26	63	82	110	48	-106	78	-221	30	-233	-1	-50
Min water level	-100	-400	-150	-150	-80	15	-150	-300	-150	-400	-200	-390	-145	-120
Max water level	420	80	210	260	320	290	298	163	343	48	305	65	190	-10
Water speed when flooded	1	3	3	3	3	3	2	3	2	1	2	3	3	-
Water pH (average)	7.96	8.35	7.26	7.42	7.36	7.86	8.00	7.89	7.83	NA	7.92	NA	8.17	7.44
Min water pH	7.25	8.25	6.25	6.03	6.10	7.00	7.31	7.62	6.76	NA	7.34	NA	7.21	6.90
Max water pH	8.44	8.44	8.37	8.47	8.32	8.55	8.58	8.21	8.90	NA	8.32	NA	9.37	8.12
Water O_2 (mg/l, top 50 cm)	NA	NA	NA	NA	NA	7.27	NA	NA	NA	NA	NA	NA	NA	NA
Min water O_2	NA	NA	NA	NA	NA	0.30	NA	NA	NA	NA	NA	NA	NA	NA
Max water O_2	NA	NA	NA	NA	NA	11.5	NA	NA	NA	NA	NA	NA	NA	NA
Soil type	1	2	2	4	4	4	1	3	3	3	1	3	3	3
Soil temperature (^oC, average)	13	12.3	12.3	12.4	12.3	15.5	13.5	12.9	13.8	12.3	12.7	12.7	13.2	12.4
Min soil temperature	0	0	0.8	1	0	8	0	0	0	0	0	0	0	0
Max soil temperature	26.4	26.5	23.7	22.4	23.3	21	24	22.3	27.3	22.4	22.2	24.8	23.8	22.6
Soil humidity (%, average)	28.8	30.2	44.6	43.2	46	47.9	27.5	29.5	36.2	29.1	25.3	18.4	27.5	27
Min soil humidity	23.1	14.3	32.6	38.4	36.4	32.4	8.1	5.9	23	14.9	13.4	2.9	5.6	17.1
Max soil humidity	37.9	41.1	52.4	51.2	50.9	58.5	39.8	41.6	46.5	41.1	30.6	28.3	35.5	32.4
Soil pH (average)	7.43	7.51	7.19	7.19	7.21	7.27	7.35	7.59	7.33	7.6	7.37	7.81	7.64	7.51
Min soil pH	7.02	6.64	6.53	6.45	6.65	6.99	6.29	6.03	5.98	6.22	5.95	6.99	6.84	6.02
Max soil pH	8.02	8.24	7.97	7.78	7.78	7.61	8.11	8.9	7.95	8.1	8.2	8.35	8.61	8.26
Soil Eh (top 5 cm, mV, average)	126	360	108	54	-38	NA	308	269	213	409	194	289	138	210
Cr in soil (ppm d.w., average)	227	167	159	164	154	152	189	156	160	157	144	139	129	129
Cu in soil (ppm d.w., average)	81	156	99	84	63	67	76	147	84	81	84	42	48	48
Fe in soil (% d.w., average)	2.86	4.20	5.11	4.83	4.98	4.75	2.84	3.82	3.60	3.88	3.38	3.32	3.94	3.94
Mn in soil (ppm d.w., average)	832	1213	861	994	843	938	798	1168	1037	1218	1009	896	1383	1383
Ni in soil (ppm d.w., average)	129	94	103	99	103	101	111	104	86	88	86	79	75	75
Pb in soil (ppm d.w., average)	107	83	104	65	58	71	36	49	49	67	38	33	53	53
Zn in soil (ppm d.w., average)	126	268	276	229	203	199	116	197	191	251	144	101	105	105
Zr in soil (ppm d.w., average)	401	300	208	231	196	182	306	288	295	232	347	351	295	295

Legend: ***Ecosystem type:*** 1 = shore, 2 = natural levee, 3 = internal zone of intermediary altitude, 4 = depression, 5 = oxbow lake, 6 = shallow lake, 7 = internal zone isolated by artificial levee, ***Anthropogenic impact:*** 0 = none, 1 = low, 2 = high, 3 = very high, ***Water speed when flooded:*** 1 = high, 2 = low, 3 = low or stagnant***, Soil type:*** 1 = sandy and silty strips alternating, 2 = sand and silt in equal parts (>90%), 2 = silt (>70%) 3 = clay (>90%), * = Studied in April – October 1997, **NA** = not available

Vadineanu et al 2000a, Vadineanu, Cristofor and Iordache, 2000. Sampling was done from natural / planted forests, grasslands (located on levees and in depressions), marshes, and shallow lakes. Composite samples were prepared similarly to the first data set. Fe, Mn, Zn, and Cu determination was performed by

F-AAS. Cr, Cu, Pb, and Cd were determined by graphite furnace AAS. Certified concentrations of a reference material were determined within ±10%.

The major limit of the data sets, with respect to our hypotheses, is that only composite samples were analyzed. We could not assess the statistical significance of the differences observed between measured concentrations and toxicity/deficiency levels (as required, Forbes and Forbes, 1994). Another problem consisted in the available literature information about deficiency/excess thresholds. We considered the extensive review performed by Kabata-Pendias and Pendias (1992), relevant for terrestrial vegetation, the review made by Guilizoni (1991), relevant for aquatic macrophytes, and other articles as mentioned in table 1. We found a lack of specific information concerning emergent macrophytes from marshes and flooded depressions. For many metals, there is a lack of information in the case of aquatic macrophytes, too. It was mentioned in the text when metals concentration in emergent or aquatic macrophytes was compared to deficiency/excess values not specific to this plant groups. Because of these limits, the results from below should be seen as preliminary, useful for the design of a research program having as objective to test the hypotheses.

RESULTS AND DISCUSSIONS

None of the plant species analyzed was identified as hyperacumulator. All metals concentrations presented below are given in ppm on a dry weight base.

Ag, As, Ba, Cd, Co, Pb, Sb, Se

Ag ranged from 0 (below the detection limit) to 0.328, As from 0.110 to 1.040, Ba from 19 to 93, Cd from 0.01 to 1.98 (tables 3, 4), Co from 0.26 to 12.49, Pb from 0.01 to 10.52 (tables 3, 4), Sb from 0 to 0.22, and Se from 0 to 0.38. None of these metals have reached excess/toxicity levels specific to herbaceous vegetation (deficiency levels not applicable or not available, levels specific to macrophytes not available).

Hg, Ni, V, Zr

Hg ranged from 0.086 to 2.15. Excess concentrations (> 1) occurred in the case of *Rubus caesius* and *Salix* sp. (leaves) from the Fundu Mare island (one of the islands of the Small Island of Braila landscape). V ranged from 0.28 to 5.40, with excess values (> 5) in the case of *Stachys palustris* from Fundu Mare island levees. The excess values were not higher than the maximum of the toxicity range. Concentrations of these metals in soil of the studied ecosystems were not available.

Ni concentrations ranged between 2 and 63, with > 90% of the values higher than the minimal excess level for herbaceous plants (10), but no values higher than the maximal reported excess value of 100. Ni in soil exceeded in several cases (table 1) toxicity levels for plants. However, we should mention that the toxicity level in soil refer to concentrations estimated by AAS, whereas our concentrations were estimated by XRF and were proved to be systematically higher than estimation by AAS. These remark holds for all metal concentrations in soil. Deficiency levels for herbaceous vegetation and levels for macrophytes were not available.

Most of the Zr concentrations in plants were above the toxic level (15). The range was 11-150, with 20 % of the values higher than 100. Highest values occurred in *Bidens* sp., *Elymus repens, Equisetum palustre*, *Polygonum hydropiper*, *Rubus caesius*, *Sparganium erectum,* and *Xanthium strumarium*. The only reference available for comparison, beside that of Kabata-Pendias and Pendias (1992) reporting the excess value for herbaceous plants, was Kovacs et al (1984), who reported concentration of Zr in submerged macrophytes ranging from 0.22 to 26.78.

Cu, Cr, Fe, Mn, Zn

These were the metals with the most complex situation. Their concentrations, and the values of excess or deficiency are presented in tables 3 and 4. The biggest number of excess values occurred in the case of Mn, followed by Fe, Cr, Cu, and Zn.

Mn concentrations in herbaceous vegetation and shrubs of shores, levees and depressions. were frequently higher than the minimal excess values reported in the literature, 100. The highest Mn concentration occurred in *Stachys palustris* (347), but it was much smaller than the upper limit of the toxicity range, 1000 (that means that there are herbaceous species reported to show no toxicity symptoms at concentrations as high as 1000, which might be our case, too). One case of deficiency level occurred (25.85 in *Xanthium strumarium*). As the maximum reported value for deficiency is 30, this case can be considered as accidental. Mn concentrations in soil are not higher than the level in soil considered as toxic for plants. However, Mn content of plants is not only an effect of plant characteristics and soil total concentration, but also of the pool of available Mn, which is highly controlled by soil properties. Highly alkaline soils (at about pH 8, heavily limed soils) can produce Mn toxicity by excess of phytoavailable fraction (Kabata-Pendias and Pendias, 1992). Inspection of table 2 shows that pHs higher than 8 frequently occur in the studied sites. Low redox potential can also occur during and after floods and heavy rainfalls (Iordache et al, 1997b).

Mn concentrations in emergent, floating and submerged macrophytes were even higher, up to 5214 in *Salvinia natans*. Most of them were higher than toxicity levels reported for herbaceous vegetation. However, it is known that plants adapted to waterlogged conditions are commonly more tolerant to Fe and Mn, which are mobile at low redox conditions in soil and water column. It is worth noticing that our values are much higher than those reported for Danube Delta (concentrations of 37.2-44.6. Mn in aquatic macrophytes, Naffea al Azzawi, 1987), but in accordance with values for macrophytes of the Danube system, (147-2970, Guilizoni, 1991). The water chestnut, *Trapa natans*, is reported to be an accumulator plant for Mn (up to 1% d.w. in leaves) and Fe (Gulizzoni, 1991). Our data for this species shows lower values of Mn. This may be due to differences in metal accumulation between different populations of identical species, as has also been recorded by other authors (e.g. Coughtrey and Martin, 1978). Fully submerged plants were reported to contain 2 to 3 times more Mn than emergent plants (Yalynskaya and Lopotun, 1994). This is confirmed by our data. Rootless *Ceratophyllum* takes up elements through leaves and can strongly concentrate Mn at low redox conditions (Guilizzoni, 1991). We found Mn concentration up to 4423 in this *Ceratophyllum demersum*. The situation of those species which have a well-developed root-rhizome system and totally submerged foliage, such as *Myriophyllum* and *Potamogeton* is much more complex (Guilizzoni, 1991). Physiological experiments on macrophyte growth have identified sediments as the major source of nutrients and trace metals for most rooted aquatic plants (Greger and Kautsky, 1991). To our knowledge, there are not available data concerning the toxicity of sediment Mn for macrophytes, and thus the sediments toxicity can not be assessed in this way. Toxicity levels of Mn in macrophytes were missing too. The studied landscapes (table 4) did not differ from the point of view of Mn concentrations in plants, based on the available data.

The situation of Fe is similar to that of Mn. Fe concentrations in herbaceous vegetation and shrubs of shores, levees and depressions were in many cases higher than the excess value reported in the literature, 1000. Highest values were measured in *Rubus caesius* leaves (4700). Plant injury due to Fe toxicity is most likely to occur

in strongly acid soils, and flooded soils (Kabata Pendias and Pendias, 1992). There are no acid soils in the Danube floodplain (table 2), but floods are regular in those areas not diked. Very high concentrations are present in most of the macrophytes (up to 1.05 % d.w in the case of *Ceratophylum demersum*), but statements about excess/toxicity cannot be made due to lack of information about excess/toxicity levels.

Cr concentrations in herbaceous vegetation and shrubs of shores, levees and depressions were only in several cases higher than the minimal excess values, 4. All concentrations were smaller than the maximal excess value, 30. The highest measured value was 12.6 and occurred in *Echinochloa crus-galli*. All concentrations in soil were higher than the maximum toxicity level reported for plants, 100. Plants from two landscapes presented concentrations at excess level in all herbaceous plant species investigated (table 4): the landscape located at km 606 (at the confluence of Danube with Olt river), and the landscape located at km 687 (at the confluence of Danube with Jiu river). Cr concentrations in emergent macrophytes were comparable with those in herbaceous vegetation (information about toxicity levels in this case was missing) and with values in reed of Danube Delta (Keller, Lajtha and Cristofor, 1998) Highest Cr concentrations from all plant species were measured in floating and submerged macrophytes (up to 45.81 for *Ceratophyllum demersum*). These values were smaller than toxicity level, 63, reported to reduce growth rate of macrophytes (Guilizoni, 1991), but higher than 12.6, the concentration at which snails, amphipods, and a microcrustacean could distinguish between contaminated and noncontaminated *Potamogeton foliosus* exported from a riparian wetland to a river, and preferred noncontaminated vegetation (Stewart et al, 1992).

Zn exceeded the excess/toxicity levels in herbaceous vegetation in a few cases (maximum concentration 180 in *Bidens cernua*). One case of concentration at deficiency level occurred (8.41 in *Xanthium strumarium*). All concentrations in soil were in the range of toxicity level for plants, 70-400. Concentrations in sediment of marshes and shallow lakes were not higher than 1250, reported to strongly reduce biomass production of *Potamogeton* sp. (Greger and Kautsky, 1991). Zn concentrations in macrophytes were in some cases higher than those in herbaceous vegetation, up to 355 in *Typha latifolia*. Information on deficiency/excess values in macrophytes was not available

Cu concentrations in herbaceous vegetation had values at excess level in some landscapes (especially in the landscape located at the confluence of Danube with Olt river) Values at deficiency levels were found in several plant species sampled in the Small Island of Braila (all Cu concentrations in *Bidens* sp., *Equisetum palustre*, most concentrations in *Elymus repens* and *Xanthium strumarium*). This might be important, as these species are highly dominant in some of the studied ecosystems (table 3). However, Cu concentrations in soil were in the range reported as toxic for plants (60-125), with the maximum value even higher (156). The deficiency in plants, if real, might be explained by the fact that Fe reduces Cu absorption from soil solution by plants (Kabata-Pendias and Pendias, 1992) We obtained high Fe concentrations in plants, related to redox conditions for high Fe availability in soil. Cu concentrations in emergent macrophytes were comparable to those in herbaceous vegetation, and in many cases lower than the deficiency levels for herbaceous species. Cu concentrations in floating and submerged macrophytes were also comparable with those in herbaceous vegetation, and with other data from Danube system (1.5-3.92, Guilizoni, 1991). However, because the toxicity level for aquatic macrophytes is much lower (5-10) than for herbaceous vegetation (20-100) many concentrations in macrophytes had values at toxicity level (up to 21.75 in *Salvinia natans*).

Table 3 Metals distribution in plant species of ecosystems from Small Island of Braila (km 175 – 220, average values, ppm d.w., sampling in 1996).

Species	Ec.	R. A.	Fe	Mn	Zn	Cu	Cr	Cd	Pb
Herbaceous vegetation and shrubs on shores, levees and depressions									
Aster tripolium L. (1753)	G3	0.64	2603	181	73	**2.2**	1.2	ND	0.14
Bidens tripartita L. (1753), *B. cernua* L. (1753)	H1	9.94	2920	150	120	**1.7**	3.5	0.04	1.40
	H2	25.53	4000	150	180	**2.7**	1.1	0.06	2.80
	H3	0.78	4500	200	180	**3.5**	1.2	0.22	0.50
	I1	0.22	1285	120	76	**2.7**	2.3	ND	0.10
	I2	54.50	2703	186	121	**1.6**	1.5	ND	2.13
	I3	4.11	950	280	120	**2.8**	1.2	ND	1.25
	I4	66.57	1880	151	72	**3.2**	1.8	ND	1.63
Cynodon dactylon (L.) Pers. (1805)	G3	20.68	1125	125	37	**4.4**	4.3	ND	0.10
Echinochloa crus-galli (L.) P.Beauv. (1812)	H1	26.33	762	100	48	**3.2**	12.6	0.15	2.19
	I1	1.14	968	117	80	**4.1**	7.0	ND	0.07
	I3	24.15	532	290	38	**3.5**	2.7	ND	0.07
Eleocharis palustris (L.) Roem. & Schult. (1817)	G3	3.78	481	135	29	9.7	6.7	ND	0.07
Elymus repens (L.) Gould (1947)	G1	14.70	374	37	56	12.8	5.7	ND	0.42
	G2	40.86	2824	195	32	**3.5**	2.4	ND	0.35
	G3	52.12	3521	86	80	6.2	2.8	ND	1.40
	G4	21.00	1589	62	38	**2.0**	3.2	ND	1.20
	H2	21.76	2000	120	80	**2.9**	2.4	0.21	1.22
	I2	1.81	1279	180	85	**2.4**	1.7	ND	0.26
Equisetum palustre L. (1753)	G1	4.68	1015	217	72	**2.4**	1.3	ND	1.48
	I2	3.01	985	180	63	**2.7**	1.8	ND	0.25
	I3	1.04	1554	180	63	**4.2**	2.3	ND	0.25
Juncus gerardi Loisel. (1809)	G3	1.89	652	64	67	**4.0**	4.7	ND	0.10
Lycopus europeus L. (1753)	I4	5.41	154	130	90	**3.5**	2.5	ND	0.05
Lythrum salicaria L. (1753)	H3	4.77	863	180	48	**4.4**	3.3	0.16	0.75
	H4	0.45	524	123	94	**2.1**	5.0	0.98	0.12
	I2	5.85	549	150	52	5.8	3.1	ND	0.15
Poa pratensis L. (1753)	G4	2.17	236	68	65	6.3	2.6	ND	0.04
Polygonum hydropiper L. (1753)	I1	36.08	1524	170	60	16.4	1.6	ND	0.13
	I4	3.44	3000	140	72	6.6	2.0	ND	2.00
Rorippa sylvestris (L.) Besser (1822)	G1	2.01	1347	198	46	7.0	1.2	ND	0.02
Rubus caesius L. (1753)	H2	1.23	4500	100	40	9.1	3.2	0.06	5.01
	H3	6.90	2000	80	50	7.2	2.4	0.06	1.63
	I2	2.28	2642	150	56	5.8	1.2	ND	0.55
	I4	5.89	4700	120	68	8.8	2.5	ND	0.03
Stachys palustris L. (1753)	H2	0.24	894	111	50	5.2	5.0	0.35	0.10
	H3	34.17	4251	347	75	6.4	1.0	0.09	1.63
Xanthium strumarium L. (1753)	G1	52.83	250	40	48	**2.6**	1.5	ND	3.90
	G2	41.77	250	40	48	**2.6**	1.5	ND	3.90
	H1	25.30	215	50	70	5.2	2.2	0.11	2.61
	H2	45.82	157	26	43	14.0	2.0	0.07	0.46
	I1	43.07	153	110	44	**3.2**	1.7	ND	0.01
	I2	21.09	162	147	42	**2.9**	1.4	ND	0.30
	I3	61.19	375	223	49	**4.2**	1.9	ND	0.10
Emergent macrophytes in marshes and flooded depressions									
Phragmites australis (Cav.) Trin. Ex Steud. (1841)	G4	65.25	*1913	*2924	23	5.1	3.56	ND	1.43
Oenanthe aquatica (L.) Poir. (1798)	H3	8.20	*1487	*900	45	5.2	*5.40	0.08	3.50
	H4	1.44	984	*782	49	**1.9	*10.30	1.98	0.08
Scirpus lacustris L. (1753)	H4	2.09	*1547	*580	65	6.1	1.98	1.37	0.01
	H5	18.79	958	*654	50	**2.6	*4.25	0.14	0.90
Sparganium erectum L. (1753)	H3	36.79	*1654	*1354	17	**4.2	1.24	0.05	5.20
	H4	37.14	*1000	*1900	40	**3.4	3.54	0.13	6.12
	H5	24.36	*2229	*652	18	**3.3	2.68	0.25	2.80
Typha latifolia L. (1753)	H4	17.12	874	*857	68	6.1	1.90	0.08	2.25
	H5	29.94	952	*1042	45	**3.4	3.80	0.13	1.50

Floating and submerged macrophytes in marshes and shallow lakes									
Ceratophyllum demersum L. (1753)	H4	32.08	*1369	87	68	4.5	8.65	1.14	5.20
	H5	21.82	*1582	*102	73	6.0	7.52	0.12	6.34
Species	St.	R. A.	Fe	Mn	Zn	Cu	Cr	Cd	Pb
Ceratophyllum demersum L. (1753)	H6	41.91	950	*125	80	5.2	6.89	1.17	2.10
Nuphar lutea (L.) Sibth. & Sm. (1809)	H6	30.19	*2165	*253	89	6.5	3.69	0.10	2.91
Nymphaea alba L. (1753)	H4	0.39	*1285	*164	*125	4.7	3.25	1.05	1.35
	H5	0.78	*1498	*298	*106	3.5	2.87	1.03	0.25
Nymphoides peltata (S.G. Gmelin) O. Kuntze (1891)	H4	0.27	967	*146	27	9.7	3.65	0.10	2.20
	H5	0.56	*1368	*167	89	5.8	2.15	0.13	0.09
Potamogeton pectinatus L. (1753)	H6	4.43	*1658	*117	75	12.5	2.35	1.12	4.50
Salvinia natans (L.) All. (1785)	H4	9.90	*1547	90	48	11.3	18.40	0.12	4.30
	H5	3.48	*2158	*120	75	10.5	16.50	0.35	2.90
Trapa natans L. (1753) floating leaves	H6	6.88	*2150	*210	*120	6.5	4.20	0.17	0.90

Legend: Grey areas = values higher than excess levels as presented in table 1, Values in white = values lower than deficiency levels as presented in table. R. A. = relative biomass abundance, Ec. = ecosystem, ND = not determined, *, ** = *excess and **deficiency by comparison with levels specific to herbaceous species.

Table 4 Metals distribution in plant species of Danube floodplain ecosystems (average, ppm d.w., sampling in 1999).

Species	Km	Fe	Mn	Zn	Cu	Cr	Cd	Pb
Herbaceous vegetation and shrubs on levees and depressions								
Lythrum salicaria L. (1753)	175	553.12	132.65	84.85	**4.45**	3.21	0.16	0.33
Rubus caesius L. (1753)		230.45	86.27	37.83	9.39	3.41	0.06	4.59
Xanthium strumarium L. (1753)		156.58	**25.85**	42.69	14.04	1.98	0.07	0.46
Lythrum salicaria L. (1753)	606	617.75	78.38	22.69	21.88	5.20	0.18	0.75
Rubus caesius L. (1753)		327.57	46.82	59.25	20.81	5.61	0.12	5.25
Xanthium strumarium L. (1753)		433.21	48.06	71.07	23.03	7.18	0.11	1.92
Lythrum salicaria L. (1753)	621	224.75	47.68	22.42	5.34	3.14	0.15	1.31
Rubus caesius L. (1753)		323.01	76.75	29.08	13.44	4.68	0.08	7.01
Xanthium strumarium L. (1753)		333.79	152.83	**8.41**	13.08	2.98	0.10	0.01
Rubus caesius L. (1753)	687	706.70	96.95	65.60	10.55	6.24	0.01	5.11
Xanthium strumarium L. (1753)		1379.55	101.12	65.02	17.01	10.23	0.12	8.93
Rubus caesius L. (1753)	700	298.17	123.50	45.59	13.08	3.18	0.35	0.05
Xanthium strumarium L. (1753)		22.29	88.60	39.36	19.65	10.19	0.27	0.07
Lythrum salicaria L. (1753)	747	407.28	39.79	171.72	11.58	3.17	0.15	0.34
Rubus caesius L. (1753)		236.70	106.80	41.39	8.99	1.89	0.15	0.04
Xanthium strumarium L. (1753)		300.73	76.63	31.66	13.53	3.86	0.05	7.89
Rubus caesius L. (1753)	790	290.96	44.17	55.23	14.59	3.56	0.25	1.52
Xanthium strumarium L. (1753)		264.38	44.95	51.52	20.70	5.03	0.63	0.09

Emergent macrophytes in marshes and flooded depressions

Sparganium erectum L. (1753)	100	*1698.30	*113.71	31.73	10.18	3.62	0.25	0.05
Typha latifolia L. (1753)		384.07	79.03	*355.1	**3.50	2.23	0.13	1.30
Scirpus lacustris L. (1753)		*3896.22	50.47	**2.06	**2.64	1.13	0.14	0.22
Phragmites australis (Cav.) Trin. Ex Steud. (1841)		459.14	46.63	**7.12	**1.45	1.97	0.06	1.13
Sparganium erectum L. (1753)	175	*4514.20	*557.66	77.47	7.44	1.59	0.05	9.80
Typha latifolia L. (1753)		833.20	60.20	13.89	5.52	1.76	0.08	0.02
Sparganium erectum L. (1753)	614	673.08	*101.77	45.07	11.41	1.66	0.22	0.07
Typha latifolia L. (1753)	621	889.76	*419.09	14.91	**3.77	1.81	0.19	0.02
Sparganium erectum L. (1753)	747	*1069.73	52.85	36.16	10.65	*5.72	0.22	0.07
Phragmites australis (Cav.) Trin. Ex Steud. (1841)		53.95	47.56	14.44	**3.71	*7.16	0.15	0.03

Floating and submerged macrophytes in marshes and shallow lakes

Trapa natans L. (1753) floating leaves	100	602.21	*116.48	15.71	4.30	3.27	0.20	0.03
Trapa natans L. (1753) submerged leaves		*7107.63	*339.34	68.52	13.85	10.41	0.14	10.52
Ceratophyllum demersum L. (1753)		*10528.9	*341.37	52.00	11.05	45.81	1.14	4.30
Nymphoides peltata (S.G. Gmelin) O. Kuntze (1891)		967.03	*145.59	26.59	9.72	3.65	0.10	2.20
Salvinia natans (L.) All. (1785)		*3433.86	*466.77	93.20	11.87	7.14	0.43	6.68
Ceratophyllum demersum L. (1753)	175	*1049.48	*4423.1	*191.7	7.67	9.34	0.12	1.99
Nymphoides peltata (S.G. Gmelin) O. Kuntze (1891)		*1433.16	*482.78	56.31	6.63	2.89	0.13	0.03
Salvinia natans (L.) All. (1785)		*3374.68	*5214.8	*229.7	17.66	30.93	0.35	5.86
Salvinia natans (L.) All. (1785)	666	*6856.52	*650.36	*124.2	15.22	5.84	0.07	9.09
Trapa natans L. (1753) floating leaves	747	792.83	*120.14	41.62	8.44	9.86	0.24	2.43
Trapa natans L. (1753) submerged leaves		*8823.44	*3747.4	*111.7	19.32	8.86	1.01	7.88
Ceratophyllum demersum L. (1753)		*1508.57	*1595.2	61.85	5.78	3.75	0.54	4.01
Nuphar lutea (L.) Sibth. & Sm. (1809)		355.25	74.38	17.93	3.18	2.32	0.17	0.05
Salvinia natans (L.) All. (1785)		*2105.24	*1913.9	*127.3	21.75	8.88	0.60	0.19
Nymphoides peltata (S.G. Gmelin) O. Kuntze (1891)	786	*3428.93	94.60	63.72	18.96	2.15	0.35	2.00

Legend: Grey areas, values in white and * as in table 3, Km = the distance from the Danube mouths to the investigated landscapes

We will complement the above analyses with several comments, in order to point out some limitations of this kind of approach:

- Toxic levels of metals in plant tissues due to high levels in soil/sediment may increase the uptake of other toxic elements, even if they are at normal concentrations in the sediment, and thus also the metals effect on the plants (Greger and Kautsky, 1991). More knowledge about interaction between heavy metals is needed.

- The effects of toxic substances on vegetation can be assessed by changes in the community structure, physiological activity, and ultrastractural components of macrophytes. To date, ecotoxicologists have emphasized the effects of the toxicant at the species level. Field studies that have taken into account the variations in plant communities or populations as a result of pollution are rare. Thus, most of the excess/toxicity levels are based on effects at species level.
- By generalizing the information provided by Falkner and Falkner (2000) with regard to the algae *Scenedesmus* sp., we may expect that gradual increase of a contaminant concentration at a time scale comparable to the life cycle of the organisms allows survival at concentrations much higher than if the organisms were exposed immediately to high concentrations. Such a gradual increase is most characteristic in large systems like Danube floodplain (Vadineanu, 2000a), where most of the contaminants input occur in a diffuse form, either by flood or by atmospheric deposition. Consequently, we may expect tolerance of plant species to concentrations higher than those considered toxic based on acute toxicity experiments at species level.
- Even if toxicity excess at species or community level are not attained, effects relevant at landscape scale might occur by affecting through bioaccumulation other consumers compartments involved in fluxes between ecosystems, or by export of contaminated vegetal biomass to other ecosystem (especially the main river channel). The trophodynamic consequences of sustained inputs of energy-rich organic matter when such inputs contain toxicants that may bioaccumulate is unkown.

The main conclusions are:

- There are many limitations for testing the considered hypotheses. Some of them are due to data quality, some of them to gaps in the knowledge base.
- Toxicity in plants due to low soil redox potential and presence of alkaline salt layers is most probably to occur in the levee and depressions ecosystems. This may be the case of Fe and Mn. Related to Fe excess, Cu deficiency seems to occur in the same type of ecosystems.
- Some landscapes located at the confluence of polluted rivers with the Danube may present excess of Cu and Cr in herbaceous vegetation.
- Cu in submerged and floating macrophytes is at excess/toxicity levels.

As future research directions, we propose:

- the characterization of the distribution of metals found at deficiency or excess values in herbaceous vegetation i) in plants by analyses of replicates, and ii) in soil at chemical speciation level. Arguments for this direction are provided also by Iordache et al 2000, and Vadineanu et al 2000b.
- assessment of the aquatic macrophyte role in the mobilization of metals from sediments and their export to Danube river. Arguments for this direction are given also in Iordache et al 2000.
- characterization of metals concentrations in sheep, cattle and pigs grown in the floodplain

Without improved knowledge on this aspects, decisions based on the assessment of the ecological effects of contamination in the Danube floodplain would have a high degree of risk.

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THE DETERMINATION OF PLATINUM BY RADIOCHEMICAL NEUTRON ACTIVATION ANALYSIS IN NEURAL TISSUES FROM RATS, MONKEYS AND PATIENTS TREATED WITH CISPLATIN

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ABSTRACT

Cisplatin is one of the most used antineoplastic drugs, essential for the treatment of germ cell tumours. Its use in medical treatment of cancer patients often causes chronic peripheral neuropathy in these patients. The distribution of cisplatin in neural tissues is therefore of great interest. Rats and monkeys were used as animal models for the study of sensory changes in different neural tissues, like spinal cord (ventral and dorsal part), dorsal root ganglia and sural nerve. The study was combined with quantitative measurements of the content of platinum in the neural tissues of the animals mentioned and in the neural tissues of human patients. For the determination of platinum in the tissues neutron activation analysis has been used. The platinum results indicate that platinum becomes accumulated in the dorsal root ganglia and in the sural nerve.

Key words: platinum, neural tissues, chemotherapy, cisplatin

INTRODUCTION

Cisplatin – cis – diamminedichloroplatinum – has been known for more than 25 years as one of the most used antineoplastic drugs. Cisplatin-based combination chemotherapy cures more than 90 % of testicular cancer patients (Daugaard et al., 1983; Daugaard et al., 1990).

The cytostatic treatment with cisplatin combination chemotherapy may cause serious side effects in particular neuro-, nephro- and ototoxicity. Patients treated with cisplatin, often develop a chronic peripheral neuropathy, which mainly affects distal sensory nerves but also central neural tissue may be involved. The mechanism causing cisplatin neuropathy is unknown (Daugaard et al., 1987; Gastaut and Pellisier, 1985; Roelofs et al., 1984; Krarup-Hansen et al., 1993). Animal neural tissue has been tested to reproduce electrophysiologically and histopathologically features of cisplatin in a model that simulates the pathological changes in humans (Krarup-Hansen et al., 1999; Krarup-Hansen, 1996) .

The understanding of neurotoxic effects, which includes peripheral neuropathy and a number of more central neurotoxic phenomena, necessitates knowledge of the distribution of platinum within the different kinds of neural tissues. Therefore, the purpose of this investigation was to identify the major sites of platinum accumulation within neural tissues after treatment with cisplatin. This problem can only be solved satisfactory by the use of a sensitive and selective analytical method, like the radio-

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chemical neutron activation analysis (RNAA). ICP-MS and XRDF-techniques can principally be used for the same purpose, but they may often be hampered by spectral interferences, which are difficult to remove without loss of sensitivity (Pyrzynska, 1998). Using RNAA the aim of this study was to quantify the content of platinum in neural tissues from rats, monkeys and patients treated with cisplatin. Preliminary results have earlier been published (Rietz et al., 1994; Heydorn et al., 1998).

MATERIALS AND METHODS

Rats

Wistar adult rats, weight about 200 g, were treated with cisplatin by an intraperitonal injection with Platinol R at a dosage of 2 mg/kg rat weekly for 7 and 12 weeks, corresponding to a total dose of cisplatin of 14 mg/kg and 24 mg/kg, respectively.

Monkeys

Macaca-monkeys, weight about 8 kg, were treated with infusional cisplatin chemotherapy 3 weekly with a dose of 4-6 mg/ kg to total cumulated dose of 60 mg/kg. Pre- and post hydration was given to diminish renal toxicity.

Patients

The patient's were treated with combination chemotherapy including infusional cisplatin to cumulative total doses in the range of 153-457 mg cisplatin/m^2 body surface area (Daugaard et al., 1983). Pre- and post hydration was given in adequate amounts to diminish renal toxicity.

The material included tissues of female and male patients, with different kinds of malignant neoplasias including ovarian, cervical and testicular and other types of cancers.

Preparation of tissue

After dissection of the relevant tissue, slices of fresh tissue were cut and placed in preweighed ½ dram polyethylene vial; the final weight was determined and the vials were frozen at -80 ^{o}C before performing neutron activation analysis. The term "fresh sample weight" refers to the weight of the material immediately after dissection of the relevant tissue and cutting slices of the tissue. All calculations of Pt-concentrations are based on this weight.

The RNAA method

The analytical method, described earlier (Rietz et al., 1994; Heydorn et al., 1998) , permits the determination of Au and Pt in biological materials and is based on neutron activation analysis with radiochemical separation of gold. The ^{199}Au daughter of ^{199}Pt is a favourable indicator for the determination of platinum in biological materials, were radiochemical separation is to be carried out.

$$^{198}\mathrm{Pt}\ (\mathrm{n},\gamma)\ ^{199}\mathrm{Pt} \xrightarrow{\beta^-} {}^{199}\mathrm{Au}$$

For the determination of platinum a high sensitivity is needed, and this requires both a long irradiation time and a high neutron flux density, However, the radiation decomposition of biological materials limits the duration of the irradiation. The limit of detection for platinum is determined by the formation of ^{198}Au, from which the ^{199}Au indicator of Pt cannot be separated. Therefore, an irradiation time of 6 hours at approximately 4×10^{17} n/m^2s in the rotating facility of the Danish DR3 reactor was chosen as a compromise. Data for platinum and gold indicator nuclides are given in Table 1.

After irradiation, complete decomposition of the irradiated material is achieved by digestion with a mixture of hydrochloric, nitric and perchloric acids under strict temperature control in a modified Bethge digestion apparatus. Before starting the digestion procedure an inactive Au-carrier solution, with approximately 5 mg of Au, is added to the sample.

Table 1. Data for platinum and gold indicator nuclides

Atomic weight	Platinum A_r = 195.09			Gold A_r = 196.97	
Indicator nuclide	^{191}Pt	^{197}Pt	^{199}Pt	^{199}Au	^{198}Au
Mother nuclide	^{190}Pt	^{196}Pt	^{198}Pt	^{199}Pt	^{197}Au
Isotopic abundance	0.01 %	25.3 %	7.20 %	-	100 %
Cross section	150 b	0.74 b	3.58 b	-	98.65 b
γ-ray energy, keV	538.91	191.36	542.96	158.38	411.80
γ intensity	13.7 %	3.7 %	14.8 %	36.9 %	95.5 %
Half-life	2.96 d	18.3 h	30.8 min	3.14 d	2.70 d
k_o-factor				1.03E-3	1.000
Counting efficiency	1.80 %	4.23 %	1.75 %	4.75 %	2.30 %
Resolution, keV				0.98	1.20

The separation of gold, including the ^{199}Au indicator of Pt, from other radioactive species in the irradiated sample is achieved by electrolysis of a 1 M hydrochloric acid solution.

The electrolytic deposition of gold on a niobium cathode ascertains highest radiochemical purity with a complete elimination of the 159.4 keV γ-ray interference from ^{47}Sc at a potential of 800 m V between a graphite anode and the niobium cathode, performed during 5 hours. The only significant interference comes from the second order reaction of the naturally occurring gold in the sample matrix (double neutron capture).

$$^{197}\mathrm{Au}\,(\mathrm{n},\gamma)\ ^{198}\mathrm{Au}\,(\mathrm{n},\gamma)\ ^{199}\mathrm{Au}$$

Correction for this interference is based on the counting of a gold comparator, normally performed during 24 hours. The precision of the Pt determination for the tissue sample is hereby not significantly affected.

The yield of the separation was determined by re-irradiation of the separated sample together with a reference containing the same amount of gold as was added to the sample.

Conditions of method

Irradiation

The samples were irradiated for 6 hours at a flux density of 4×10^{17} neutrons $m^{-2}s^{-1}$.

Standards

Pt: 6.0-6.5 μg Pt/ml 2 M HNO_3

Au: 15.3-16.4 ng Au/ml 1 M HNO_3

Digestion

The digestion of the material was performed about 2 days after the end of irradiation during 3 hours at temperatures, rising from 75° C to 285° C in a modified Bethge digestion apparatus, containing the irradiated sample and a mixture of conc. HCl, HNO_3 and $HClO_4$ + 5 mg of Au-carrier.

In earlier publications, e.g. 10, a mixture, containing hydrochloric and nitric acid, obtained from the digestion medium by distillation, was collected in a trap. After

cooling, this mixture was returned into the flask. The retaining nitric acid in the digestion mixture prolongs the time of electrolysis as the following step of the procedure. This was indicated by the time needed to collect the amount of gold carrier added including the amount of the indicator nuclide ^{199}Au on the electrode.

Therefore, contrary to the earlier publications, e.g. 10 – we discarded the distilled acid mixture immediately after the trap was filled and replaced the discarded amount of concentrated hydrochloric acid. This was performed until the end of digestion, which was indicated by the yellow colour of the solution in the Erlenmeyerflask and a colourless acid distillate. This technique ensures that all nitric acid is removed from the digestion medium at the end of the digestion process, which is important to the formation of a chloro-compound of Pt, H_2PtCl_6. This compound (complex) shortens the duration of the following electrolysis from previously 5 hours to 2-3 hours. Furthermore, the Pt-chloro-compound ensures that the chemical yield of the whole analytical separation procedure seldom will be below 80 %.

Electrolysis

The electrolytic separation is performed during 2-3 hours at 800 mV using a rotating Nb-cathode and a graphite anode. The gold deposit on the cathode is dissolved in 1 cm^3 of aqua regia, transferred to a halfdram polyvial and sealed.

Counting

The counting is performed in a γ-X-detector with resolution of 1.78 keV at 1333 keV, rel. efficiency 35 %.

Counting-time:	Samples	24	hours
	Au-standards	24	hours
	Pt-standards	1	hour
	Gain	0.2	keV/channel
	8192 channels		

Radiochemical yield

The radiochemical yield of the whole separation procedure is determined by re-irradiation of the sample for 30 s in the pneumatic tube of DR3 with a fluence rate of $2\text{-}3\times10^{17}$ n/m^2s. A solution of 5 mg Au/ml of 1 M HCl, used as comparator, was irradiated simultaneously with the sample.

RESULTS AND DISCUSSIONS

The results are presented in the tables 2-4. A view at the platinum results, obtained for the different tissues, shows, that a relation between the cumulated doses administered and the platinum concentrations was not found. Furthermore, it can be seen that the content of gold in the samples is very variable, but the corrections for the gold content are insignificant. The corrections must be taken into account, because the gold content of the sample interferes the platinum determination. To our knowledge no biological reference material has hitherto been certified for platinum.

The uncertainties given in tables 2-4 represent one standard deviation, and comprising counting statistics and flux variations between the sample and the comparators. Counting contributes most to the uncertainties of the results (< 5 %). Other sources of variation (flux variations between samples and comparators, weighing, transfer operations) are small in comparison.

Radiochemical Neutron Activation Analysis (RNAA) was chosen as the analytical method for the determination of the platinum content of all neural tissues. The reasons for this choice are quite obvious:

RNAA is an attractive method due to the absence of any reagent blank. Matrix effects (e.g. interference from Ca or/and from Au, naturally occurring in the sample matrix) can be either eliminated or calculated very precisely. The analytical results are corrected for these interferences. However, with neural tissues as biological material these interferences are negligible (Rietz et al., 1994).

Table 2: Platinum and gold found in samples of neural tissues from 4 rats

Material	Sample size (mg§)	Cumulative dose (mg cisplatin /kg rat)	Apparent Pt (ng ± SD)	Observed Au (ng ± SD)	Pt-correction (ng ± SD)	Chemical Yield (% ± SD)	Actual Pt (ng ± SD mg/kg ± SD)
Rat no. 1-4 Spinal cord ventral part*	29	14	3.4 ± 0.2	0.0391± 0.0001	0.777± 0.006	93±3	2.8±0.3 0.100±0.003
	33	24	8.7 ± 0.3	0.3095± 0.0005	4.429± 0.041	95±3	4.5±0.3 0.140±0.004
	79	24	9.3 ± 0.3	0.507± 0.001	2.849± 0.026	83±3	7.8±0.4 0.100±0.003
	45	24	2.5 ± 0.2	0.0369± 0.0004	0.177± 0.003	89±3	2.7±0.2 0.060±0.002
Rat no. 2-4 Spinal cord dorsal part*	33	24	5.9 ± 0.2	0.1112± 0.0003	0.804± 0.005	96±3	5.3±0.3 0.160±0.005
	32	24	5.1 ± 0.1	0.1030± 0.0006	0.499± 0.006	81±3	5.7±0.3 0.180±0.005
	45	24	9.7 ± 0.3	1.406± 0.002	6.355± 0.086	95±3	3.6±0.3 0.080±0.002
Rat no. 1-4 Dorsal root ganglia*	12	14	16.8 ± 0.2	0.0557± 0.0004	0.227± 0.003	95±3	17.4±0.6 1.45±0.04
	42	24	20.2 ± 0.3	0.0537± 0.0004	0.318± 0.006	88±3	22.5±0.9 0.54±0.02
	25	24	19.9 ± 0.2	0.0601± 0.0002	0.644± 0.005	82±3	23.5±0.9 0.940±0.030
	33	24	8.9 ± 0.2	0.322± 0.001	1.667± 0.017	64±2	11.3±0.5 0.34±0.01

- segment level ThXII-L3

§ fresh sample weight

Furthermore, good sensitivity, precision and reasonable detection limits are characteristics of the RNAA method. The method used in this investigation has a detection limit of about 1 ng/g (1 ppb), which is sufficient for the determination of platinum in neural tissues. Adsorption voltammetry combined with detection by the catalytic hydrogen ware (Hoppstock et al., 1989) can achieve limits of detection at the 0.1 ng/g level (0.1 ppb), however, the performance of this technique requires a very high analytical standard in order to recognize and minimize possible interferences due to surfactants and residues from decomposition acids. As mentioned before our method does not have these serious limitations. The results of our RNAA-method seem to be within a reasonable range compared to the results, described by

Gregg et al. (Gregg et al., 1992). They determined the Pt-content of neural tissues, using electrothermal atomic absorption spectrophotometry (ET-AAS) with a detection limit of 20 ng Pt/g.

Table 3: Pt in neural tissues of monkeys

Material ***Monkey no. 1 and 5***	**Sample size (mg§)**	**Cum. dose (mg cis-platin /kg)**	**Apparent Pt (ng ± SD)**	**Observed Au (ng ± SD)**	**Pt-correc-tion (ng ± SD)**	**Chem. Yield (% ± SD)**	**Actual Pt (ng ± SD mg/kg ± SD)**
Spinal cord ventral part*	84	60	10.261 ± 0.436	0.0443 ± 0.0008	0.162 ± 0.005	94.6 ± 3.3	10.675 ± 0.461 0.127 ± 0.005
	52	60	4.427 ± 0.363	0.0186 ± 0.0005	0.055 ± 0.002	84.0 ± 3.0	5.202 ± 0.432 0.101 ± 0.008
Spinal cord dorsal part*	81	60	9.433 ± 0.422	0.0015 ± 0.0004	0.062 ± 0.003	99.5 ± 3.3	9.418 ± 0.424 0.117 ± 0.005
	50	60	4.715 ± 0.213	0.0333 ± 0.0003	0.118 ± 0.002	76.3 ± 2.7	6.025 ± 0.279 0.122 ± 0.006
*Dorsal root ganglia**	43	60	123.733 ± 0.711	0.0871 ± 0.0008	0.373 ± 0.008	88.3 ± 3.3	140.840 ± 0.805 3.30 ± 0.02
	23	60	35.717 ± 0.394	0.1316 ± 0.0006	0.521 ± 0.010	96.03 ± 3.40	36.65 ± 0.41 1.63 ± 0.02
Sural nerve	36	60	71.538 ± 0.658	0.0203 ± 0.0006	0.095 ± 0.003	92.3 ± 3.3	77.403 ± 0.713 2.17 ± 0.02
	16	60	60.462 ± 0.393	0.1329 ± 0.0006	0.497 ± 0.009	78.2 ± 2.8	76.682 ± 0.503 4.68 ± 0.03

* segment level ThXII-L3
§ fresh sample weight

Table 4: Platinum content of human neural tissues

Pa-tient no.	Material	Sample size (mg§)	Cum. dose (mg cis-platin /kg)	Apparent Pt (ng ± SD)	Observed Au (ng ± SD)	Pt-correction (ng ± SD)	Chemical Yield (% ± SD)	Actual Pt (ng ± SD mg/kg ± SD)
1	Spinal cord*	288	153	7.910 ± 0.240	0.457 ± 0.001	2.308 ± 0.048	81.70 ± -0.42	6.857 ± 0.300 0.024 ± 0.001
2		617	457	125.442 ± 0.602	0.487 ± 0.001	1.871 ± 0.032	96.70 ± 0.42	127.788 ± 4.33 0.207 ± 0.007
3		58	200	11.6 ± 0.4	0.0114 ± 0.0004	0.052 ± 0.002	79.5 ± 0.3	14.58 ± 0.56 0.25 ± 0.01
4		23	400	1.2 ± 0.4	0.0132 ± 0.0005	0.068 ± 0.003	94.6 ± 0.4	49.61 ± 18.26 0.05 ± 0.02
1	Dorsal root ganglia*	21	153	6.620 ± 0.168	0.0677 ± 0.0-004	0.342 ± 0.007	87.44 ± 0.42	7.180 ± 0.287 0.347 ± 0.014
2		163	457	188.193 ± 0.696	0.516 ± 0.001	1.982 ± 0.034	82.00 ± -0.42	227.087 ± 8.12 1.397 ± 0.050
3		18	200	36.3 ± 0.4	0.0099 ± 0.0004	0.044 ± 0.003	90.7 ± 0.3	39.95 ± 1.43 2.24 ± 0.08
4		22	400	30.8 ± 0.7	0.0714 ± 0.0008	0.451 ± 0.019	85.6 ± 0.3	35.47 ± 1.34 1.59 ± 0.06
1	Sural nerve	54	153	30.648 ± 0.340	0.0633 ± 0.0004	0.438 ± 0.008	88.31 ± 0.42	34.186 ± 1.18 0.635 ± 0.022
2		86	457	44.268 ± 0.372	0.0551 ± 0.0004	0.345 ± 0.006	79.43 ± 0.42	55.298 ± 1.89 0.643 ± 0.022
3		57	200	106.9 ± 0.7	0.0177 ± 0.0007	0.082 ± 0.005	85.9 ± 0.3	124.34 ± 3.98 2.18 ± 0.07

* segment level ThXII-L3
§ fresh sample weight

CONCLUSIONS

The radiochemical neutron activation analysis method described here permits the determination of platinum in biological materials with good precision (SD <5 %) and reasonable sensitivity (detection limit: 1 ng Pt/g material). The analytical technique used in this investigation enables to obtain quantitative information about the distribution of platinum among the different kinds of animal and patient tissues.

Table 5. Order of neural platinum concentrations in different species pre-treated with cisplatin

	Rat	Monkey		Patient
Increasing order ↓	The spinal cord ventral part The spinal cord dorsal part	The spinal cord		The spinal cord
	The dorsal root ganglia	The dorsal root ganglia	The sural nerve	The dorsal root ganglia
	No exam			The sural nerve

Furthermore, it has hitherto not been possible to reproduce the histopathological features of cisplatin neuropathy in an animal model that simulates the pathological changes in humans [6, 8]. Determination of the platin concentration in different rat tissues revealed that platin had become accumulated in the dorsal root ganglia and in the dorsal part of the spinal cord. All effects on peripheral nerves observed in animals (mice, rats) were reversible, whereas cisplatin neuropathy in man is usually non reversible. The investigations, using monkeys as an animal model, are not finished yet.

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CLINICAL ASPECTS AND BIOCHEMICAL PATHOLOGY IN OXALIC UROLITHIASIS - PECULIARITIES OF METALLIC COMPOSITION

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ABSTRACT

Studies of clinical interest based on data of biochemical pathology and physiopathology reveal that simple or mixed oxalic urolithiasis is predominantly localised at the level of the excretory system.

The biogenesis process of cristallyzation nuclei is consequtive to metabolic disorders but also to urodynamic disfunctions which lead to the formation of microuroconcrements named "starters" or "primers". In a second step of this heterogenous nucleation, takes place the formation of lithiasis which is clinical decelable. This process is due to the existence of an excedent of oxalic ions (often together with other compounds – the case of mixte lithiasis) and of the presence of diverse metallic ions such as: Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, etc.

Studies on surgically removed or spontaneously eliminated uroconcrements have been done with the goal to decelate the types of lithiasis, i.e. oxalic lithiasis (simple lithiasis); oxalic-phosphatic lithiasis, oxalic-uric lithiasis, oxalic-cholesterol lithiasis a.o. (mixed lithiasis), using infrared spectroscopy (IRS). In order to determine the content of metals and to establish the metallogram of uroconcrements, atomic absorption spectroscopy (AAS) was used.

In this way, by investigations made with IRS and AAS, we could conclude on the pecularities of biochemical pathology in the oxalic lithiasis, and the obtained data are medical guidelines in the clinic of urology.

Key words: oxalic urolithiasis (simple and mixed), metallograms

INTRODUCTION

In the biochemical pathology of urolithiasis the study of the problems concerned with etiology, pathogenesis and composition implies a complex inter- and multidisciplinary approach. Within the framework of paraclinical investigations aimed at an urgent elucidation of the aspects dealt with, there is a requirement for current examinations such as radiology, clinical chemistry and microbiology.

The thorough study of lithiasis, with the aim of obtaining some accurate data of clinical usefullness, involves investigations founded on the modern applications of biochemistry, biophysics, electronics and computers. This way, the morpho-functional information regarding renal excretion, urodynamics, uroconcrement formation etc., are extended to and completed with compositional data. This information may stand for an underlying bases for prophylactic, therapeutic and metaphylactic measures to be undertaken.

The main nutrients of concern in biochemical pathology of calcium oxalate crystal formation are ascorbic acid, amino acids, calcium, magnesium, phosphorus,

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vitamine D, vitamine B_6, natrium and water (Gârban et al., 1981; Matouschek and Huber, 1981; Rushton and Spector, 1982; Coe and Parks, 1988;.Tiselius et al., 1995)

Ascorbic acid and amino acids are metabolised to form oxalic acid. The increase in oxalic acid causes hyperoxaluria. Hypercalciuria is caused by excessive intestinal absorption of calcium, impaired renal concentration of calcium and excessive skeletal mobilization of calcium. Calcium and oxalic acid bind together and crystallize. Magnesium competes with calcium in the urine to bind with oxalate. Thereafter, oxalate can be excreted in the urine and will not be able to bind calcium and form crystals.

It is important not to restrict the level of phosphorus in the diet. Once phosphorus is limited, parathyroid hormone is activated and it begins to remove calcium from the bones. Calcium acceeds into the blood stream and eventually in the urine.Sodium is a nutrient of concern because as it moves through the renal tubules, calcium follows it and ends up in the urine, providing and environment favourable for crystal formation.

There are 3 theories with regard to the physiological process of how calcium oxalate stone formation occurs. They consist of the: a) precipitation-crystallization theory; b) matrix-nucleation theory; c) crystallization-inhibition theory.

a) The precipitation-crystallization theory implies that the bladder's urine is suprasaturated with stone-forming crystalloids, calcium, oxalate and urea which results in calcium oxalate urolithiasis. The theory is supported by hyperparathyroidism, excess vitamine D in the diet, decreased calcitonin secretion by the thyroid gland, defective tubular reabsorption of calcium and pseudohyperparathyroidism.
b) The matrix-nucleation theory implies that there is organic material present that is the nucleus for crystalloid material forming. This nucleation encourages the continued growth of the crystalloid material resulting in stone formation. This could be due to increased renal excretion with glomelular filtration, decreased tubular reabsorption or increased tubular secretion. To prevent calcium and oxalate from combining to form crystals and then growing to form uroconcrements, magnesium oxide is included in the diet.
c) The crystallization-inhibition theory implies that there is a reduction of crystalloid inhibitors, which results in stone formation.

The formation of calcium oxalate stones can lead to secondary infections with urease – producing bacteria, leading to a more alkaline urine. Calcium oxalates stones are unaffected by changes in pH from 4.5-8.0. The bacteria cause inflammatory responses, hematuria, pyuria and proteinuria.

MATERIALS AND METHODS

Investigations were made on some surgically removed calculi (SRC) as well as on some spontaneously eliminated calculi (SEC) - in the University Clinic of Urology, Timişoara. Out of 173 calculi of simple and mixed various urolthiasis, 52 calculi were included in our study. These represented (simple or mixed) oxalic lithiasis.

The research on the qualitative composition of calculi was performed by means of an accurate and expedient method – IR spectroscopy. The applicability of this method to paraclinical investigations has been noted by Beischer (1955); its usage for large scale investigations has been used after the 70's by Bellanato et al., 1973; Hesse et al., 1974; Dragan et al. 1981, and by others. The determination of metal concentration in calculi was made by means of AAS – a physical method of high precision conceived by Walsh (1955), extended to various studies by Pinta (1971) and to biochemical pathology (Gârban et al., 1987, 1990).

In a preliminary stage of our research, in order fo find out the type of urolithiasis, we proceeded to recording standard IR spectra of the chemically pure compounds present in calculi: organic (oxalic acid, uric acid, xanthine, cystine, cholesterol) and inorganic (phosphates, carbonates) compounds. The standard IR

spectra show the transmittance (T%) as a function of wavelength (λ) or of wave number (ν^{-1}), i.e.

$$T\ \% = f(\lambda) \quad \text{or } T\ \% = \Phi(\nu^{-1})$$

On the basis of standard IR spectra there have been defined the values of wave numbers (ν^{-1}) corresponding to vibration types characteristic of each molecule. After having started with these, we successively proceeded to: a) defining the data base characteristics; b) adopting the identification algorithm for the unknown compound (with special reference to the investigated calculi); c) elaboration of the program and its implementation on a computerized minisystem; d) establishing the composition and defining the urolithiasis nature. Methodological details have been provided in previous works (Drăgan et al., 1994).

In the next step – after having recorded the IR spectra of calculi – we applied the computerized procedure for establishing the composition.

For the recording of IR spectra of the standard compounds and of the calculi we made use of a Spekord 75 type apparatus. The samples were KBr – compressed and the spectral range was λ = 2.5 - 25 nm and ν^{-1} = 4.000 – 500 cm^{-1}.

Further on, our research dealt with the determination of metal concentrations in calculi. On this purpose by means of AAS, the following metals were determined: Na, K, Ca, Mg, Zn, Fe, Cu, Pb, Mn. The spectro-chemical process underlying this outstanding analytical method is determined by the number of atoms absorbed in time ($dN_{b\rightarrow h}$) by the compound (particularly by the calculi). Therefore

$$dN_{b\rightarrow h} = B_{bh} \cdot N_b \cdot g_v \cdot dt$$

where: B_{bh} - the absorption probability;
N_b - the number of atoms in the lower transitional state;
g_v – spectral volume density;
dt – duration (time derivative)

In the analytical practice the characteristic radiation for the element dealt with, is emitted by the lamp with craterized cathod (i.e. with the corresponding parameters for each metal: frequency - ν and wavelength - λ).

Investigations were performed by means of a PYE UNICAM series Sp 1900 apparatus in the spectral range of λ = 189-855 nm. Details are presented in a previous paper (Gârban et al., 1983)

The obtained analytical data were classified according to lithiasis types; averages (X) and standard deviations (SD) have been calculated for each metal (expressed as μg/g calculi).

RESULTS AND DISCUSSIONS

In oxalic urolithiasis the main involved compound is oxalic acid (HOOC-COOH) a metabolic end product in the human organism (Smith et al., 1981; Sivagnanam and Khan, 1998; Drăgan et al., 1994). This is a relatively strong dicarboxylic acid with pK's ranging between pK_{a1} = 1.46 and pK_{a2} = 4.40. The importance of oxalic acid and oxalates, especially calcium oxalate (CaOx), in human pathology is due to their low solubility. For example, the solubility of CaOx is 0.67 mg/100 ml in water at pH = 7.0 and 13^{O}C (Leskovar, 1978).

The predominant localization of oxalic lithiasis is at the level of the renal excretory system, yet there also have been described prostatic, bile, intestinal and even pancreatic localizations. In literature is mentioned the fact that in localizations other than the excretory system traiectory, there usually occur mixed calculi: with phosphates in prostatic lithiasis, with cholesterol in bile lithiasis and with carbonates in intestinal lithiasis.

Naturally, in urine as a metastable solution – the process is amplified by the presence of various organic and inorganic metabolites, that favourise co-precipitation processes.

The oxalates existing in the organism originate from both endogenous and exogenous sources. The quantity of the exogenous part is dependent on feeding and intestinal absorption. Endogenesis is assured by way of tissue and microbiological biosynthesis. A synoptic display of the biochemical pathways is provided by Fig. 1

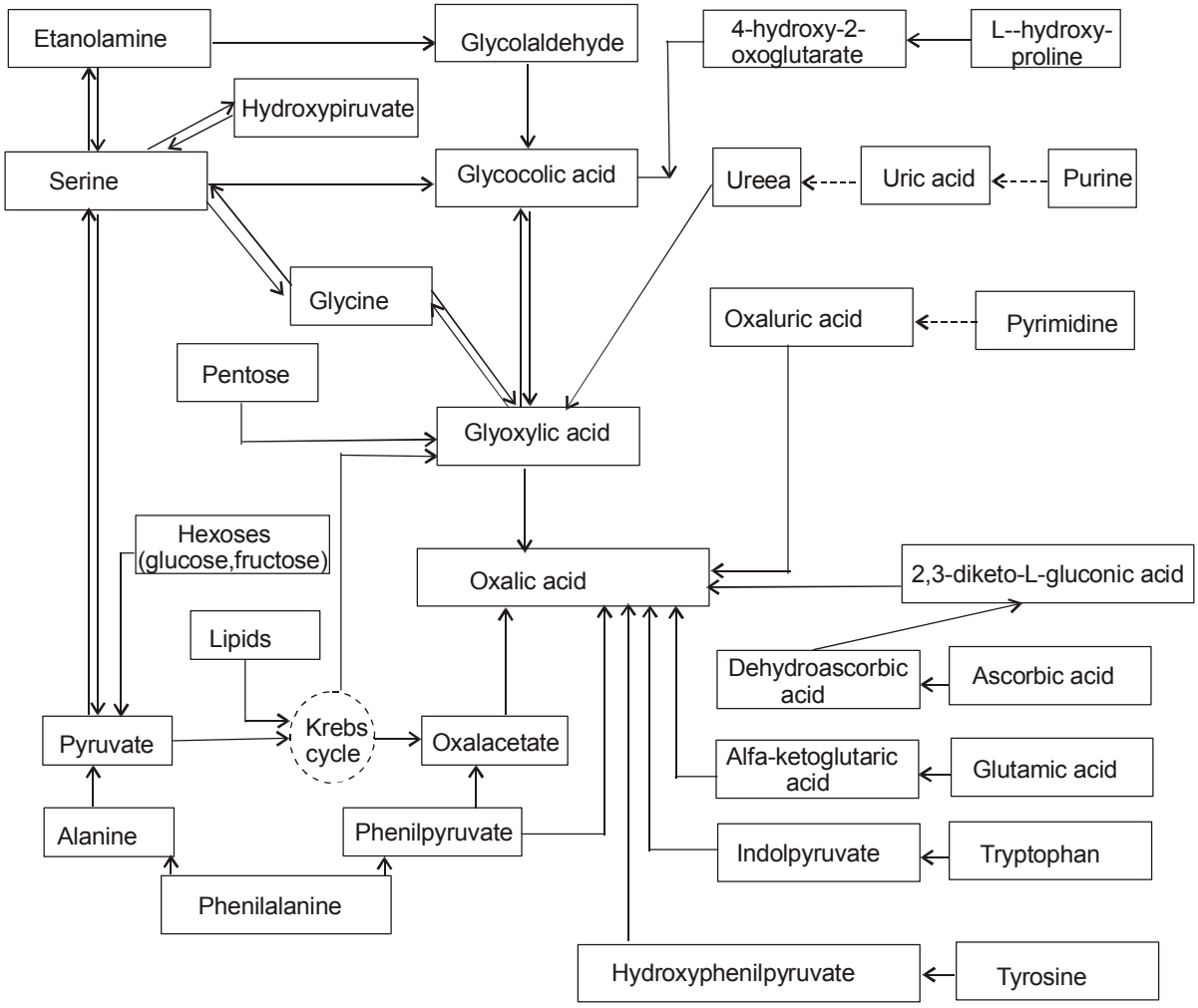

Fig. 1. Biochemical pathways of oxalic acid biosynthesis – general presentation

Calcium combines with oxalates in the intestines. This reduces calciums' ability to be absorbed. Sometimes oxalate or calcium oxalate stones form because there is not enough calcium in the intestines.

Generally, there are accepted three phases for stone formation: α) initiation of uroconcrements formation as “starters” or “primers”; β) growth of the crystalloid material forming a urolithiasis; γ) evolution – process that can be characterized by the evolution of uroconcrements or its limitation.

Oxalic acid and its salts of exogenous origin represent a relatively small percentage. According to Leskovar (1978) the quantity of exogenous oxalic acid is only of 10-20%. Among the exogenous sources, vegetable foods are richer in oxalates than those of animal origin. Foods obtained by way of fermentative processes (e.g. cheese) have increased amounts of oxalates. Oxalemia is a function of the absorption of oxalate anion at the intestine level. This process is conditioned by the presence of Ca^{2+}, Mg^{2+}, Fe^{2+}, PO_4^{3-} and by the morpho-functional status of the intestinal mucous membrane (lesions, transit, pH etc.). The intestinal absorption of Ox^{2-} and Ca^{2+} depends on the reduction in the food-derived calcium share, that favours the intestinal absorption of oxalate and viceversa. In various digestive diseases – such as Crohn’s disease, ulcerous colitis, resection or bypass of the ileum, chronic pancreatitis and steatouria, the unabsorbed fatty acids combine with the intestinal Ca^{2+} preventing thereby the formation of insoluble $Ca(COO)_2$ and allowing for an increased $C_2O_4^{2-}$ absorption through the small bowel and colon (Smith et al., 1981; Coe and Parks, 1988).

An important aspect regarding the perspective of oxalic uroconcrements biogenesis can be offered by the dishomeostasis of hydro-electrolytic metabolism. Data refering to the normal status of metal concentrations in blood (Table 1) and in urine (Table 2) offer a view over homeostasic values. Their pertubation due to physiologic and/or physiopathologic causes constitutes a predictive sign – generally - of the risk of lithiasis occurrence.

Table 1. Concentration of main metals present in blood

Metal	Chemical simbol	UM	Average	Range
Aluminium	Al	µg / 100 mL	17.00	
Calcium	Ca	mEq / L	5.00	4.50-5.50
Cobalt	Co	µg / 100 mL	0.03	
Copper	Cu	µg / 100 mL	114.00	81.00-147.00
Iron	Fe	mg / 100 mL	115.00	50.00-170.00
Magnezium	Mg	mEq / L	1.85	1.50-2.50
Manganese	Mn	µg / 100 mL	0.142	
Mercury	Hg	µg / 100 mL	0.30	
Nickel	Ni	µg / 100 mL	3.00	
Lead	Pb	µg / 100 mL	4.60	
Potasium	K	Eq / L	4.40	3.50-5.50
Sodium	Na	mEq / L	140.00	133.00-144.00
Tin	Sn	µg / 100 mL	3.30	
Zinc	Zn	µg / 100 mL	1.00	0.70-1.50

In the case of oxalic urolithiasis (simple and mixed) other causes also intervene.

Endogenous sources display a high variability. There are known various pathways of oxatite formation. Thus, in the organisms oxalic acid is produced by way

of tissue biosynthesis (within the frames of the intermediate metabolisms), also existing however, a microbiological biosynthesis performed in the digestive tube.

Table 2. Concentration of main metals present in urine

Metal	Chemical simbol	UM	Average	Range
Aluminium	Al	mg / 24 ore	$1.10 \cdot 10^{-3}$	$0.70 \cdot 10^{-3} – 1.60 \cdot 10^{-3}$
Calcium	Ca		3.30	0.60-8.30
Cobalt	Co		$0.70 \cdot 10^{-4}$	$0.05 \cdot 10^{-3} – 0.70 \cdot 10^{-3}$
Copper	Cu		$0.50 \cdot 10^{-3}$	$0.30 \cdot 10^{-3} – 0.70 \cdot 10^{-3}$
Iron	Fe		$0.70 \cdot 10^{-2}$	
Magnezium	Mg		1.35	0.42 - 2.40
Manganese	Mn			$0.10 \cdot 10^{-3} – 1.40 \cdot 1.40 \cdot 10^{-3}$
Mercury	Hg			$0.70 \cdot 10^{-3}$ - $1.10 \cdot 10^{-3}$
Nickel	Ni			$2.00 \cdot 10^{-3} – 1.10 \cdot 10^{-3}$
Lead	Pb		$0.40 \cdot 10^{-3}$	$0.16 \cdot 10^{-3}$-$1.10 \cdot 10^{-3}$
Potasium	K		34.00	16.00-56.00
Sodium	Na		60.00	25.00-94.00
Tin	Sn			$0.13 \cdot 10^{-3}$ - $0.25 \cdot 10^{-3}$
Zinc	Zn		$1.80 \cdot 10^{-2}$	$1.10 \cdot 10^{-2}$ - $3.30 \cdot 10^{-2}$

The tissue biosynthesis is accomplished within the intermediate metabolic framework, independent of the oxalates provided by foods. Oxalic acid may result from protides, sugars and lipids, during the metabolic processes at the level of organ and tissue.

Biosynthesis originating from protides primarily concerns amino acids. Thus, by way of oxidative deamination, glycocolic acid is transformed to glyoxylic acid, alanine is transformed to pyruvate, and than within Krebs cycle, to oxalacetate which, turns into oxalic acid. Glutamic acid – so important by its quantity (about 40%) present in the organism – may evolve through secondary catabolic pathways (in cases of excessive presence or in some enzymic deficiences) to ketoglutarate and then to oxalic acid.

The aromatic amino acids, phenylalanine and tyrosine, evolve towards phenylpyruvate and hydroxyphenylpyruvate, respectively, which lead to oxalic acid. From among the heterocyclic amino acids tryptophan and hydroxyproline also constitute oxalic acid precursors.

It is a noteworthy fact, that purines and pyrimidines resulting during the biological turn-over of nucleoproteins may also evolve to oxalates. Uric acid originating from purines may lead to ureea, glycoxilic acid and, finally, to oxalic acid. This accounts for the fact that there were found subjects presenting successively oxalic and uric lithiasis or mixed calculi with oxalates and uric acid salts. Generally speaking, this observation is important in considering the fact that excessive uric acid excretion may cause the setting in of crystallization nuclei for oxalic calculi.

Sugars also have a share in the oxalate biosynthesis. In this process take part pentoses and hexoses (glucose, fructose, etc.). Sugar – based oxalogenesis is performed within the specific processes of the intermediate metabolism.

There is a limited oxalic acid synthesis from lipids. Oxalic acid results from the biological turn-over of glycerol and, to a lower extend, of fatty acids whose metabolites enter the Krebs cycle, wherefrom they may evolve to oxalates (Matouschek and Huber, 1981; Leskovar, 1978).

Ascorbic acid – either from foods and / or therapeutic origin – also stands for an oxalic acid source. Calcipherols influence – by calcium retention – the formation of calculi.

It should particularly by mentioned, that on the intermediate metabolic pathways appear two important precursors, glycine and glyoxylic acid. This main sequence of the metabolic pathways for the production of oxalic acid is displayed by formulae in fig. 2, mentioning there the reversible or irreversible character of the transformations.

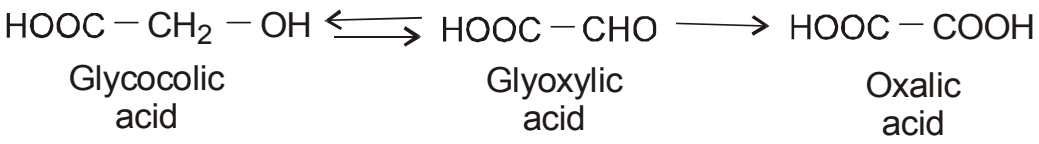

Fig. 2 Precursors for oxalic acid

The conversion of glyoxylic acid to oxalic acid is accomplished with the participation of three enzymes: lactate dehydrogenase, glycolic acid oxidase and xanthine oxidase. Lactate dehydrogenase (LDH), despite its substrate specificity, also intervene in the reversible transformation of glyoxylic acid to glycine. Moreover, LDH catalyzes the dismutation reaction of glyoxylic acid into oxalic acid and glycine. Glycolic acid oxidase catalyzes the oxidation of glycine to glyoxylic acid, yet also the irreversible transformation reaction of glyoxylic acid to oxalic acid. Xanthine oxidase, a non-specific agent for oxoalogenesis, also intervenes in the oxidation of glyoxylic acid to oxalic acid (Leskovar, 1978; Thomas et al., 1995).

Except for the tissue biosynthesis, reffered to above, the endogenous share of oxalate is also accomplished by the way of the conversion performed by microorganisms present in the digestive tube. The bacterial and fungus flora contributes to the biosynthesis of oxalic acid and of some of its precursors. As an outcome of the intestinal absorption, there is observed an increase of oxalemia and oxaluria.

In the case of hyperoxaluria, there is a significant increase of plasmatic oxalate level, pool size and turn-over rate, which lead to lithogenic processes. In the presence of other organic and inorganic compounds there appear co-precipitation processes that lead to uroconcrements. Metals intervene to a large extent in their evolution and in the development of calculous formations. Thus, while the sodium salt is readily soluble, the calcium salt – prevailing in oxalic urolithiasis – is scarcely soluble. In our research involving IR spectroscopy, we recorded the IR spectra of the chemically pure compounds. Thus, we recorded the spectrum for oxalic acid (fig. 3) the underlying compound of oxalic lithogenesis. We procceded similarly, with the other compounds too, present in the composition of calculi.

The IR spectra for SRC and SEC – recorded in a subsequent second stage – served to the determination of the qualitative chemical composition by computer.

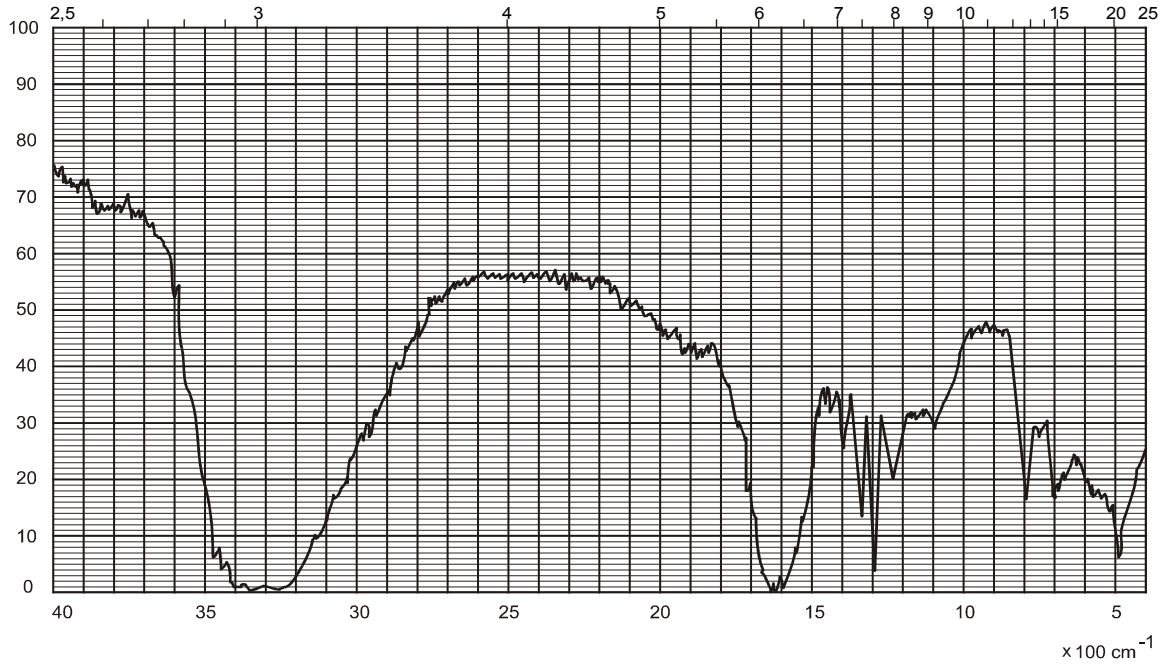

Fig. 3. Infrared spectrum of oxalic acid (standard spectrum)

By applying the aforegoing procedures to the undertaken cases, we detected oxalic calculi (free of other organic compounds or inorganic anions) and mixed calculi in whose composition also were phosphates or cholesterol, beside oxalates. Fig. 4 presents the IR spectra recorded for a surgically removed calculus with oxalate.

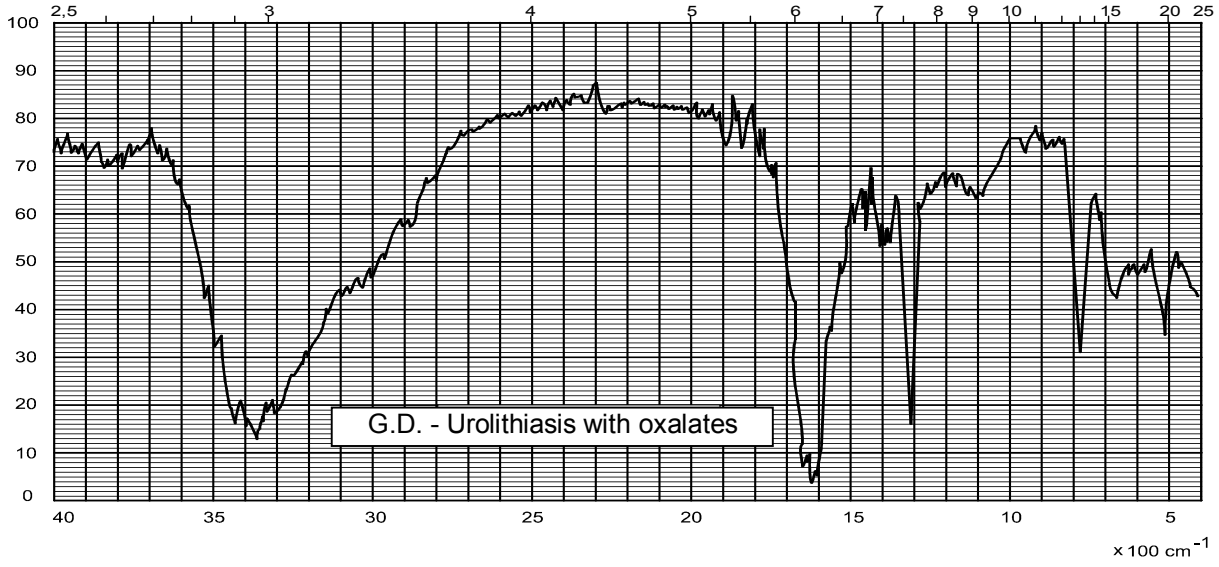

Fig. 4. Infrared spectra of some surgically removed calculi:
case G.D. – calculi with oxalates

Similarly, in Fig. 5 is shown the IR spectra of a calculus with oxalates and phosphates.

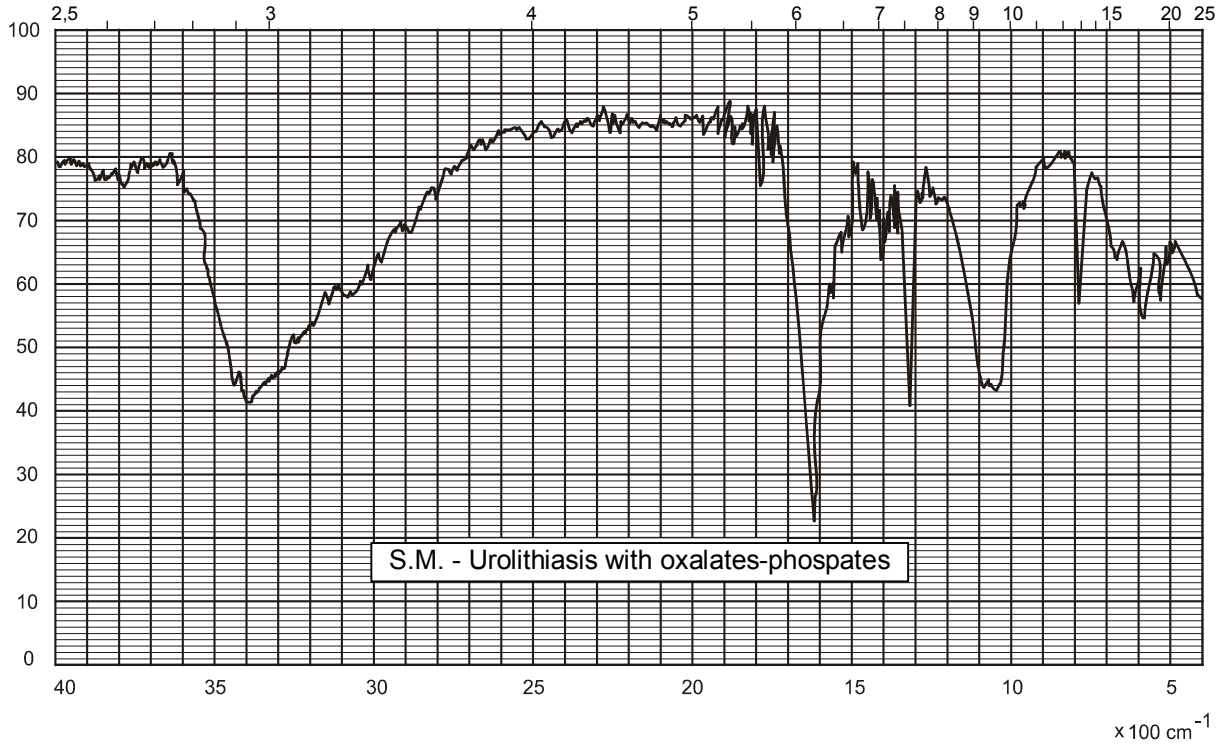

Fig. 5. Infrared spectra of some surgically removed calculi:
case S.M. – calculi with oxalates and phosphates

The procedure based on IR spectroscopy allows defining the composition of calculi with accuracy and expediency (Bellanato et al., 1973; Hesse et al., 1974; Drăgan et al., 1983; Gârban et al., 1983). The importance of defining the composition consists in the fact that it allows the establishment of etiology and of therapeutic implications.

The ingredients of oxalic nature are, prevailingly, mono- and di-hydrated calcium oxalate and (more rarely) di-hydrated iron oxalate Hodkinson, 1977; Drăgan et al., 1986). Often, these appear with their mineralogical denominations: Whewellit, $Ca(COO)_2 \cdot H_2O$; Whedellit $Ca(COO)_2 \cdot 2H_2O$; Humboldtin $Fe(COO)_2 \cdot 2H_2O$.

Into the mixed calculi enter numerous other compounds. Among these, phosphates display a high degree of compositional diversity, owing to the present metal ions (Sutor, 1969; Drăgan et al., 1998; Gârban et al., 1998).

Using the computer program, the investigated cases were introduced in groups as a function of urolithiasis types. We found simple urolithiasis with oxalates and mixed (binary) urolithiasis wherein the predominant compound was oxalate while the secondary ones were phosphates or cholesterol. Table 3 presents the synoptic situation of the cases undertaken for study.

Generally, it is a known fact that in urolithiasis there prevail those cases wherein there are present oxalates-phosphates, being followed by cases with phosphates, oxalates, uric acid, etc. (Khan et al., 1992; Drăgan et al., 1993).

By extending the investigations over the main metals present in the composition of calculi one gains new information regarding their participation at the process of co-precipitation. These are involved in lithogenesis by way of initiating heterogenous nucleation and, later on, by deposits on the matrix of the uroconcrement under formation.

Table 3. Synopsis of the investigated cases – types of urolithiasis

Urolithiasis type	Calculi composition	Investigated cases with oxalic urolithiasis								
		No. of cases			Overall, out of which					
					Men			Women		
		Total	SRC	SEC	Total	SRC	SEC	Total	SRC	SEC
Simple	Oxalates	25	23	2	11	10	1	14	13	1
Mixed	Oxalates-phosphates	13	23	-	8	8	-	5	5	-
	Oxalates urates	9	5	1	4	4		5	4	1
	Oxalates-cholesterol	5	8	-	-	-	-	5	5	-

Note: SEC - spontaneously eliminated calculi; SRC - surgically removed calculi

It is known that the metals present in the organism change their concentration as a function of age. Thus, the increase of concentration (by accumulation), may determine competitive interactions in the organism, thereby modifying the normal metabolic processes and leading, in certain cases, to disease states. It is likely that such mechanism may be implied also in some processes of urolithiasis having a congenital component (e.g.: xanthinic and cystinic lithiasis, also encountered sometimes in childhood).

Beyond the metal concentration changes in the organism, conditioned by age, there also may occur variations due to biorhythms (Berg, 1982; Gârban et al., 1998). Thus, in urolithogenesis the chronobiochemical modifications of the hydroelectrolytic metabolism do have a peculiar importance. The circadian and cirannual variations of metal concentration in the renal excretion are involved in co-precipitation that proceeds heterogenous nucleation and uroconcrement formation.

In our studies, metal concentrations, determined by means of AAS, have been expressed as μg / g calculi in order to confer a unitary way of the exposure. In such situations, owing to the wide variations of concentration, it is sometimes preferable to change units from one element to the other (e.g.: mg, μg, ppm, etc.). Data dealing with the studied cases are entered in Tabel 4.

Beyond the decelated metals mentioned in our research on calculi, literature data reveal in the blood serum: Al, Ba, Cd, Cs, Cr, Li; Mo, Ni, Sr etc. (Altman and Dittmer, 1968) and in urine: Al, Cd, Cs, Cr, Li, Mo, Ni Sr etc. (Altman and Dittmer, 1968)

The occurrence of the metals in uroconcrements is an outcome of their presence in urine (regarded as a metastable solution) wherein there are commenced the co-precipitation processes. Berg et al. (1990) found that in the urine of the patients with calculosis there is an increase of Ca and Mg concentration, while the concentrations of Na and K display minor variations as compared to persons from a control group.

Table 4. Metallograms of simple and mixed urolitiasis

Metals		**Urolithiasis type** (metals concentration in µg/g calculi)			
		Oxalates (n=25) X ± SD	Oxalates-Phosphates (n=13) X ± SD	Oxalates-Urates (n=9) X ± SD	Oxalates-Cholesterol (n=5) X ± SD
Alkaline	Na	2473.19±804.26	4732.73±1013.84	1714.70±604.51	958.73±442.39
	K	831.79±183.57	991.15±346.41	472.17±103.74	576.91±269.13
Alkaline-earth	Ca	236611.11±27156.09	183321.23±34753.12	203719.00±73491.07	91783.47±23415.33
	Mg	1629.31±513.11	7134.50±1901.83	409.27±131.72	303.16±99.44
Trace elements	Zn	341.78±105.42	563.61±157.08	159.16±70.41	51.77±11.14
	Fe	426.83±217.79	233.56±77.01	243.52±94.72	45.39±19.87
	Cu	18.93±6.14	17.77±4.81	21.01±9.16	23.51±13.07
	Pb	72.37±30.86	223.16±94.73	69.73±28.12	71.69±23.08
	Mn	34.19±16.07	18.23±6.03	16.83±8.46	9.17±3.87

The homeostasis of hydroelectrolitic metabolism – prevailingly regarding metals may be disturbed as a function of nutrition (water, foods), ambiental (geographic area, professional environment) and metabolic factors. Under certain circumstances, in the urine there may occur increased quantities of electrolytes as well as of organic metabolites. As a result of metabolic decompensation, urine changes its pH, osmolarity, density and, evidently, composition. Thus appear the premises for a heterogenous nucleation, with the participation of the mineral and organic components (Schultz et al., 1989; Thomas et al., 1995).

Around the central nucleus (crystalloidal or colloidal) is formed the fibrillar network of mucoproteins that is charged with crystals (Gârban et al., 1981). The system formed this way, will evolve to uroconcrements of varying shape, size, composition, localisation and clinical evolution.

In the etiopathogeny of urolithiasis metals play an important role. They may intervene either indirectly as effectors (inhibitors-activators) of metabolic processes, or directly as substituents engaged in competing interactions owing to the difference in the solubility products of oxalic phosphatic salts etc. Therefore, in a larger context, there is made use of the notions of lithogenic and litholytic substances (Berg, 1990; Grases et al., 1994). In this respect, it is shown that from among these metals Zn^{2+} has a dissolving effect for Ca^{2+}; Al^{3+} favours lithogenesis by way of being antidissolving, while Fe^{3+} also favours lithogenesis by blocking pyrophosphates (Matouschek and Huber, 1981).

The establishment of the calculi composition – involving organic and inorganic compounds – is of interest for the setting up of a conservative treatment, in view of the known fact that there exists a tendency for relapse if surgery has been performed during the fully evolutive stage of the disease. The correction and restoration of the hydro-elctrolytic equilibrium is an essential requirement in both cases of either surgically removed or spontaneously eliminated calculi.

In this regard it is noted that there is a need for metabolic recompensation, aimed at avoiding undesirable secondary effects. In this respect there is followed up The optimization of the dietary prescriptions and, prevailingly in the case of oxalic urolithiasis, the setting up of an oxalic-deprived diet.

A knowledge of the mechanisms of oxalogenesis and of the composition of uroconcrements by means of IR spectroscopy, computer and AAS investigations provides one with accurate data that are useful for clinical guidelines in the therapeutics and metaphylaxia of urolithiasis.

CONCLUSIONS

1. The application of IR spectroscopy and the computer processing of the results allows for an expedient investigation of the qualitative composition of calculi and for a definition of the oxalic urolithiasis types (simple and mixed)
2. Determination, by means of AAS, of the concentration of alkaline and alkaline-earth metals permitted revealing the fact that in the urolithiasis series, the alkaline metals decrease according to:

Oxalates-phosphates > oxalates > oxalates-cholesterol

and the alkaline/earth decrease according to:

Oxalates > Oxalates-phosphates > oxalates-cholesterol

3. The investigated trace metal elements are found in much varying (as a matter of order of magnitude) concentrations, from one urolithiasis to another. Thus we found out that Fe, Pb and Cu prevailed in oxalic lithiasis, while Zn and Fe in lithiasis with oxalates-phosphates.
4. The analytical data concerning the etiopathogeny of oxalic urolithiasis provide important information to medical guidelines in the urology clinic.

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SCIENTOMETRIC COMMENTS ON BIOLOGY, CHEMISTRY AND MEDICINE

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1. THE VISIBILITY AND IERARCHY OF SCIENCE JOURNALS

The scientific production of countries, institutions and individuals is closely monitored by the American Institute of Scientific Information, Philadelphia, USA (ISI) with the aid of *Science Citation Index* and derived instruments (e.g. *Journal Citation Reports*, etc.).

The statistical analysis of citations in the scientific literature produced a ierarchy of science journals according to the so-called *impact factor.* The impact factor is defined as the ratio between the number of citations received by a journal in a given year and the number of papers published in that journal in the two previous years. The impact factor became a measure of the quality of a journal (better journals are cited most frequently) and is frequently used in evaluations of institutions or individual scientists. This tendency generated numerous controversies about the fairness of the procedure when it comes to evaluations of individuals, but it can be taken as a good measure of the quality of research performed in a given institution or country.

The impact factor should be used with caution because the citation habits of scientists in various disciplines and subject areas are not the same. Thus, in biology and chemistry the scientific publications tend to have more extensive lists of cited references than the papers in mathematical journals. As a result, the number of citations accumulated by chemical and biological papers in a certain period is considerably greater than mathematical papers, and this will influence the impact factors of the respective journals. This is illustrated in Table 1, which gives for comparison the average discipline impact factors (year 1998) for subject areas in chemistry, biology and mathematics. The conclusion is that the impact factor should be only used within a discipline and not for comparisons between different disciplines.

"Metal Elements in Environment, Medicine and Biology"- Gârban Z., Drăgan P. (Eds.)
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Table 1. Average discipline impact factors for year 1998

Biology	1.65	Chem	1.31	Math	0.39
Bio-biochem	2.59	Chem-anal	1.31	Math-appl	0.53
Bio-biophys	2.06	Chem-appl	0.78	Math-cyb	0.61
Bio-biotech	1.20	Chem-inorg	1.64	Math-info	0.50
Bio-genet	2.34	Chem-org	1.70	Math-stat	0.64
Bio-immun	2.51	Chem-phys	1.81		
Bio-med	1.32	Chem-polym	0.90		
Bio-micro	1.68				

In addition to the subject area of the journal, the impact factor is also influenced by other features, such as the type of journal and articles (letters, full papers, reviews), the number of authors per paper, the extent of the interest for a given subject area, the size of the journal (number of papers published in one year), etc. Thus, review articles tend to accumulate a larger number of citations and consequently journals and serial publications containing such papers will have very high impact factors. Also, journals like *Nature* and *Science*, which publish cutting- edge research results, have unusually high impact factors, as illustrated in Table 2.

Table 2 Top journals and periodicals with very high impact factors (1998)

Nat. Genet.	40.36	Nature	28.83
Annu. Rev. Biochem.	39.00	Science	24.39
Cell	38.69	Chem. Rev.	20.23

There are small variations of the impact factors from one year to another, as illustrated in Table 3 for a selected list of chemical journals (years 1998 and 2000). The order of magnitude is, generally, maintained and the variations should not cause concern.

Table 3. Impact factors of selected chemistry journals for years 1998 and 2000

Specification	**1998**	**2000**
Chem. Rev.	20.23	20.04
Acc. Chem. Res.	12.89	13.26
Angew. Chem. Int. Ed.	8.03	8.55
J. Am. Chem. Soc.	5.73	6.02
Chem. Commun.	3.41	3.69
J. Org. Chem.	3.50	3.69
J. Phys. Chem. B	3.39	3.86
Organometallics	3.47	3.17
New J. Chem.	1.80	3.01
J. Organomet. Chem.	1.61	1.63
Coll. Czech. Chem.	0.55	0.96
Pol. J. Chem.	0.51	0.57
Rev. Roum. Chim.	0.19	0.26
Hung. J. Ind. Chem.	0.25	0.20
Rev. Chim. Bucharest	0.10	0.19

The 1988 impact factors for a series of biochemistry, biology and medical journals are listed in Table 4. It can be seen hat these journals exhibit large variations and the visibility of a scientific publication in these subject areas will be greatly influenced by the journal in which it appears.

Table 4 Impact factors (1998) of selected biochemical and biomedical journals

J. Cell Biol.	12.79	J. Cell Biochem.	2.78
Am. J. Human Genet.	10.87	Biochem. Pharmacol.	2.72
Adv. Immunol.	10.71	Arch. Biochem. Biophys.	2.50
Bioessays	7.58	Eur. J. Cell Biol.	2.49
Adv. Microbiol. Physiol.	7.20	Biochim. Biophys. Acta	2.48
J. Biol. Chem.	7.20	Environ. Toxicol. Chem.	2.34
J. Mol. Biol.	5.80	Hum. Immunol.	2.17
Adv. Virus Res.	5.75	Adv. Drug Delivery	2.15
Eur. J. Immunol.	5.44	Cell Tissue Res.	2.08
Crit. Rev. Toxicol.	4.97	Anal. Biochem.	1.99
Biochemistry (USA)	4.63	Anticancer Drug Res.	1.89
Biophys. J.	4.52	Bioorgan. Med. Chem.	1.78
Genetics	4.45	Chem. Immunol.	1.55
Cancer Gen. Ther.	4.35	Biophys. Chem.	1.52
Biol. Rev.	3.99	J. Agr. Food Chem.	1.43
Biochem. J.	3.86	Arch. Environ. Health	1.41
Environ. Sci. Technol.	3.51	Toxicology	1.38
Clin. Chem.	3.42	Chem. Biol. Interact.	1.20
Eur. J. Biochem.	3.25	Eur. J. Med. Chem.	1.12
Int. Immunol.	3.19	Biometals	1.06
Exp. Cell Res.	3.05	Biol. Trace Elem. Res.	0.85
Bioscience	2.98	Fundam. Clin. Pharmacol.	0.75

From the above presentation follow some practical *conclusions*:

a) The (young) scientists should be aware of the ierarchy of scientific journals (determined by the impact factor) when deciding where to publish some new results;

b) Papers published in obscure or local journals with little visibility, not characterized by an impact factor, will have a very small chance to be noticed and read by the scientific community; publication in such journals should be avoided as much as possible when significant research results are communicated.

c) Presentation of works at conferences (as preliminary results or even as comprehensive lectures) should be followed by publication in scientific journals, since these have a greater visibility than the volumes of abstracts or even proceedings.

d) Not all “foreign” journals are of the same value. Preference should be given to the *international* journals characterized by an impact factor.

2. THE PUBLICATION PROFILE OF SOME COUNTRIES

It is obvious that no country can afford to develop to the same extent all science disciplines and subject areas. Some areas are privileged, enjoy certain priority while others are more or less neglected and as a result the share of chemistry, biology and medicine in the scientific production of various countries varies greatly. A recent American report prduced by the National Science Foundation “*Science and Engineering Indicators-2002”* provides interesting information about this aspect. Thus, Table 5 shows the percentage of scientific publications in various subject areas for a number of Central and East European countries in the year 1999. In all of these chemistry and physics are dominant, but the share of medical and biological papers varies greatly from country to country.

Table 5 The share of various disciplines (percentage) in the scientific production of Central and East European Countries (in 1999)

	BG	**CZ**	**HU**	**PL**	**RO**	**RUS**	**MD**
Clinical medicine	12.7	11.8	21.5	12.0	3.0	3.9	4.3
Bio-medical research	14.3	14.9	16.2	8.6	3.3	10.6	2.0
Biology	5.5	8.2	5.6	5.4	2.0	5.0	4.3
Chemistry	26.5	26.4	27.5	29.7	36.8	25.0	23.7
Physics	20.5	21.0	16.0	30.0	34.4	38.5	58.1
Earth and space	5.2	4.0	3.4	3.6	1.7	5.8	1.1
Engineering and technology	10.6	6.2	5.0	6.5	11.9	7.3	4.8
Mathematics	3.2	2.1	2.8	3.2	6.6	1.2	1.1
Psychology	0.3	1.7	0.4	0.4	0.3	0.7	0.5
Social sciences	1.0	3.7	1.0	0.5	0.1	1.7	0.0
Health	0.0	0.1	0.1	0.0	0.1	0.0	0.0

In some countries (e.g. Romania) only a few excellence centers contribute scientific publications (in international journals!) in biology and medicine related subject areas, and a great disproportion between various discplines is noted. This contrasts greatly with highly advanced countries, where the share of medical and biological publications is much higher (Table 6).

Table 6 The share of various disciplines (percentage) in the scientific production of some advanced countries (in 1999)

	USA	CND	JPN	UK	GER	FR	
Clinical medicine		32.2	29.8	30.0	34.0	29.6	27.7
Bio-medical research	17.0	15.6	14.5	14.4	14.9	15.4	
Biology	6.1	11.3	5.9	6.8	5.5	5.4	
Chemistry		7.6	8.5	16.0	9.3	14.7	14.0
Physics	10.4	7.3	21.2	11.0	18.4	18.9	
Earth and space		6.1	7.3	2.5	5.6	4.8	6.4
Engineering and technology	5.8	7.2	7.9	6.0	5.8	6.0	
Mathematics		1.8	1.9	1.0	1.5	2.1	4.0
Psychology		3.4	3.9	0.4	2.7	1.6	0.9
Social sciences		4.2	4.1	0.4	4.6	1.4	1.4
Health		1.5	1.5	0.1	1.7	0.2	0.1

The differences in the publication profiles of various countries reflect the national priorities and areas of expertise in the respective disciplines.

The data presented above suggest a practical *conclusion*: At least some Central and East European countries should pay more attention to biomedical research by supporting financially and otherwise the existing centers of excellence, developing their human resources and research infrastructure and stimulating the contribution to the international scientific literature, in order to bridge the gap which separates them from highly developed countries.

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STUDY OF THE COPPER ACCUMULATION CAPACITY OF MEADOW VEGETATION AND COPPER SUPPLY FOR ANIMALS DURING THE PASTURE PERIOD IN DIFFERENT MOUNTAINS AREA (CENTRAL AND SOUTH BULGARIA)

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ABSTRACT

The study traced out dynamics on the transfer of essential trace element Cu during the grazing period (May-September) by using from 135 plants samples (Rhodope mountain) and 65 alpine plants samples from the Balkan mountain.

With an advance of the vegetation phase a significant decrease of the Cu content of the pastures at the altitude of 1200 and 1500 m was estimated. Significant differences were found in the accumulating capacity of the flora ($p< 0.05$) as a result from the different vegetation phase. At the same altitude syenite formation (Rhodope 1400-1500 m) transferred in the beginning of pasture period significant more Cu into grass (11.81±0.52 mg/kg DM) in comparison to granite weathering soils (7.0-7.9 mg/kg DM) in the other region (Beklemeto-Balkan mountain). The geological structure in North Bulgaria restrict the accumulation of Cu into pasture grass at the altitude over 1450 m. In the beginning of pasture period a normal amount of Cu were found in 60% of the investigated area. With vegetation progress Cu concentration of all alpine pastures (Balkan mountain) decreased significantly to 6.21 mg/kg DM. The needs of Cu were 22% less than the requirements of the sheep (8.0 mg/kg DM). The same results in the Rhodope region has been estimated. Only in July and August a marginal copper deficiency has been observed (1200 m a.s.l.). In September the new meadow vegetation contains about 40-50% much copper as the requirement for ruminants (13.6 mg/kg).

The dynamic changes in the Cu content of grass during the pasture period didn't demand a correction on the Cu offer to growing and lactating ruminants. An additional Cu-supplementation under Se-Zn-deficient condition (antagonistic effect Cu-Zn, Se-Cu) may lead to secondary Cu-toxicity.

Key words: copper accumulation, mountain plants.

INTRODUCTION

Copper as trace element ranks in 25th position in the table of chemical element availability in the earth's crust (Kabata-Pendias and Pendias, 1992; McDowell, 1993). The total amount of copper in the soils on the territory of Bulgaria ranges between 2 and 14 mg/kg. The humus layer in the soil surface horizon of mountain meadow soil and forest mountain soil contains insufficient Cu reserve, namely 5 mg/kg (Donchev et al., 1962; Alexiev and Krasteva, 1970; Stanchev et al., 1982). The great mobility of Cu ions within the soil and the low pH permit an extremely high Cu transfer rate to plants (Machalett and Bergmann, 1993; Lambert et

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al.,1993). A significant decline in the Cu level was found in the tested plant community during the April-June period (Anke et al., 1994; Mertz, 1993; Szentmihalyi et al., 1986) with the lowest values recorded in the grasses and the slightest changes found in the forage legumes. The Cu supply to sheep reared in the mountainous areas basically depends on the growth stage of each plant species.

The studies of Angelow (1995) and Kafedjiev et al. (1998) in the mountain permanent pastures of Sredna Gora (Panagyurishte) and the Balkan Mountains (Troyan) during the April – September period reveal a decline of Cu in plants to 4.5-5.5 mg Cu/kg DM which is indicative of Cu deficiency. These results partially coincide with the data reported by Alexiev and Stoyanov (1984) and Todorov and Dardjonov (1997) regarding the meadow vegetation in different regions of Bulgaria. The reasons for these differences should be sought for in the different geological origin of soils, the grassland botanical composition, the plant growth stage at the sample collection, and the antropogenic pollution of each region (Anke et al., 1999; Grün et al., 1996).

The aim of the study was to examine the changes in the level of the Cu trace element in mountain regions of various geological structures and altitude over the period May-September. The results are compared to the optimal Cu levels needed by small ruminants grazed in the regions.

MATERIALS AND METHODS

The biological material used consisted of 200 plant samples originating from the pasture areas in the region of the Central Rhodopes and the Central Balkan Mountains. The samples were collected in the course of two successive years from May through September on a total of 30 standard plots sized 4 m^2 and 2 m^2 and located at different altitude. Drying of the samples was performed at 65^0 C and burning to ash at 450^0 C in a muffle oven. The ash residue was dissolved in 20% HCl and transferred quantitatively from a 25 ml flask to a 50 ml one. The Cu concentration was measured on an AAS-3 (Carl Zeiss, Jena) and calculated in mg/kg DM. The data were statistically analyzed using a software package Statistic-Excel 95.

RESULTS AND DISCUSSIONS

The soil geological origin plays an important role in the Cu transfer to pasture grass. The highest transfer rates along the nutrient chain “soil-plant” were effected by slate, gneiss and phyllite. The results from the pasture mapping in the area Kainadina (1200 m a.s.l., Central Rhodopes) are presented in Fig. 1.

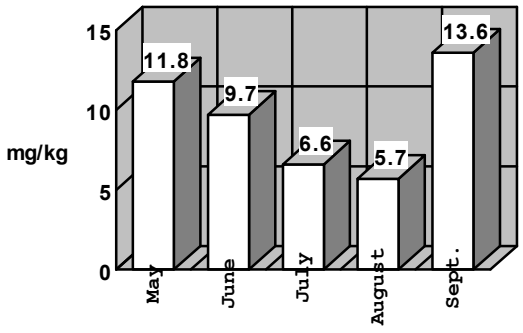

Fig. 1. Copper content of meadow grass in Central Rhodope during the deferent seasons (1200 m)

During the sheep’s grazing period the Cu content of meadow grass was found to vary within a wide range (13.6 – 5.7 mg/ kg DM). As maturity increased the Cu concentration dropped significantly by 18% in June, 44% in July and

52% in August. The elevated Cu level in the vegetation was due first of all to the prevalence of gneiss structures and the enhanced accumulation capacity of the dominant grasses such as Agrostis capillaris, Festuca fallax and the legume species Trifolium repens, Trifolium pratense. Similar findings are reported by Cheshmedjiev (1980) namely 7.18–12.07 mg Cu/kg DM in the mentioned species. Cu values exceeding 8 mg/kg DM point to upset balance between available Cu and the Cu needed by the animal organism. In May the Cu excess amounted to 48% and then declined to 21% (June) and in the two months to follow reached typical values of marginal and chronic deficiency (6.6 and 5.7 mg/kg DM, correspondingly).

At the end of the examined period (September) the newly grown plants managed to accumulate substantial amount of Cu that is similar to the values found in the analysis of samples at the beginning of the period. The sheep were supplied a surplus of Cu reaching 70%. The unbalanced Cu uptake ranging from Cu excess to Cu deficiency is particularly unfavourable especially in the first two months and the last one of the grazing period. This necessitates strict control of all the copper antagonists such as Zn, Se, Ca in the grass vegetation as they are directly responsible for suppressing the Cu transfer in the animal organism. On average, during the trial period the sheep grazed on pastures up to 1200 m a.s.l. had an uptake of 9.48 mg Cu/kg DM which is by 18% higher than is normally needed by growing or lactating sheep.

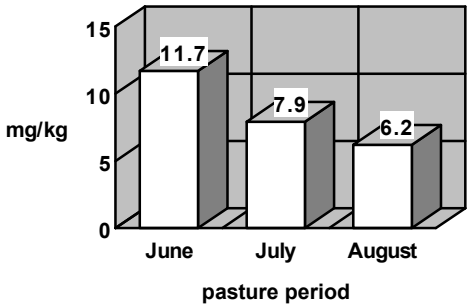

Fig. 2. Copper content of meadow grass in Central Rhodope during the deferent seasons (1200 m)

During the spring-summer period sheep reared and grazing at altitudes higher than 1400 m were exposed to different levels of available Cu (Fig. 2).

On examining the Cu transfer to pasture vegetation on the rough grazings in the Novak region (Central Rhodopes) a proportional decline of the trace element content was found reaching 33% in July and 48% in August. At the end of the grazing period Cu is reduced to deficiency levels of 6.16 mg/kg DM that guarantees 77% supply to the sheep diet.

All the differences among the individual subperiods were significant at $P < 0.01$ and $P < 0.001$. In the studies of Cheshmedjiev and Djarova (1984) on the Rila mountain pastures, particularly on the prevailing species of Nardus stricta considerably lower content of Cu was found (4 mg/kg DM) compared to the results from the grass samples of mountain pastures in the Central Rhodopes. On analyzing the mean data for the Cu content of sheep pastures at two different altitudes the following regular phenomena were found (Table 1).

Table 1. The copper content of sheep pastures at two different altitudes (mg/kg CB)

Pastures	May	June	July	August
	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$	$\bar{x} \pm S_{\bar{x}}$
upland (1200 m) (n = 15, 15, 15, 15)	11,81 ± 0,52	9,70 ± 0,43	6,57 ± 0,25	5,66 ± 0,26
alpine (1400 m) (n = 0, 15, 15, 15)	-	11,75 ± 0,61	7,94 ± 0,43	6,16 ± 0,24
p	-	< 0.05	< 0.01	> 0.05
%	-	121	121	109

Compared to alpine pastures (higher than 1400 m a.s.l.) the lower content of the trace element found in the mountain pastures (up to 1200 m a.s.l.) from samples collected at the same phenological date was due to the different growth stage of the grassland communities in June through August. The difference of 21% found in the available Cu was maximal in June / July and then declined down to 9% in August.

On the basis of data obtained in the same growth stage of the grassland communities, namely 11.81 mg Cu/kg DM at 1200 m a.s.l. versus 11.75 mg Cu/kg DM at 1400 m a.s.l., it appears that in this case the geological structure and the botanical composition of the pastures examined play a secondary role for the Cu level in meadow vegetation. No significant differences were found in the Cu accumulation capacity of plants on gneiss and syenite geological structures. Similar results were recorded from the grass samples on mountain and alpine pastures at different altitudes in the region of Troyan in the beginning of the grazing period (Fig. 3).

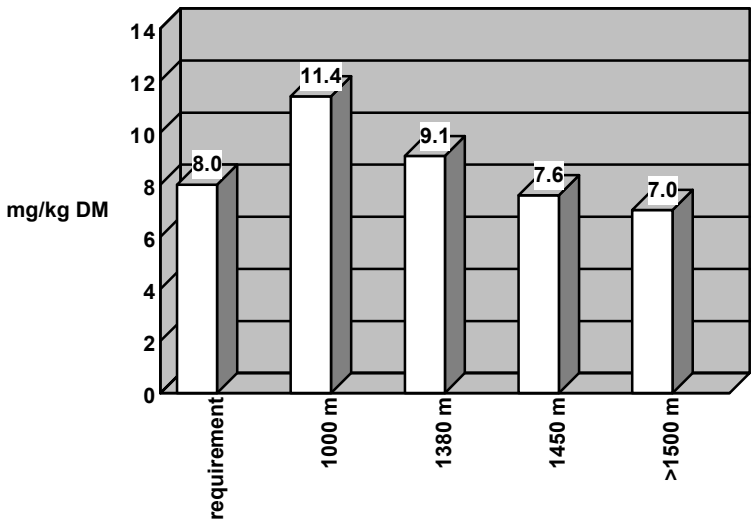

Fig. 3. Copper content of meadow grass at different altitudes (mg/kg CB)

Notably, in the early grazing period the Cu content (11.4 mg/kg DM) available in the meadow grass of pastures at 990 up to 1000 m a.s.l. exceeded the needs of the sheep by 42.5%. Up to 1400 m a.s.l. the Cu content receded to normal limits and proceeded to fall down to its lowest average value of 7.03 mg/kg DM between 1500 m and 1600 m a.s.l. The gradual decline in the Cu level should be attributed to the changing botanical composition of the pastures at different altitudes.

The homogenous composition of alpine pastures with prevalence of Nardus stricta and the lower soil pH in the mountain pastures up to 1000 m a.s.l. predetermines the extremely low Cu transfer in the plants and thus the animal Cu supply is at risk. In instances of proportional utilization of mountain and alpine grazings the average Cu supply of ruminants in early June reaches 8.34 ± 1.95 mg Cu/kg forage. The problem of Cu supply of ruminants reared above 1450 m a.s.l. is intensified as grass maturity increases. In late September the mean Cu content of meadow grass is about 6.21 ± 1.35 mg Cu/ kg DM. Such quantity can guarantee only 78% of the sheep needs of copper. Therefore, copper supplementation of animal diet rations is recommended on condition that Zn and Se are strictly controlled in the forage fed. In the presence of Zn deficiency the Cu supplement would intensify the antagonistic effect between Cu and Zn. On the other hand, under conditions of Se deficiency the peroxidation processes in the organism are enhanced, the cell membrane permeability is upset which might lead to increased transfer of Cu to the liver and kidneys.

Having in mind the integrated nature of the problem of sufficient copper supply to the animal organism during the grazing period, additional tests of the animals are needed using selected organs and secretions. That will shed more light on the biological assimilation potential of the trace element in the organism under conditions of either Cu-excess (beginning of grazing) of Cu-deficiency (end of grazing).

CONCLUSIONS

The study on the copper supply to ruminants in the grazing period revealed its irregular pattern. In the start of the period the Cu content of grass exceeded up to 50% the needs of sheep and goats. This effect is more evident in the Rhodopes region. As maturity of meadow grass increased, significant decline in the Cu content was found reaching its minimum in August (5.66-6.16 mg/kg DM) regardless of the altitude. These levels are within the marginal and chronic deficiency range and provide for 71 to 77% of the organism needs. In September the Cu content of meadow grass on alpine pastures (above 1400 m a.s.l.) in the Rhodopes was recorded as twice higher than the results from the Central Balkan Mountains area (13.6 versus 6.21 mg/kg DM, accordingly). The naturally upset balance of trace element content affects the fauna in both regions which on its behalf necessitates additional control of the mineral and biochemical status of livestock at the end of the grazing period.

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METAL ELEMENTS TRANSFER IN THE SOIL-PLANT SYSTEM AS A RESULT OF TOWN MUD DISTRIBUTION ON AGRICULTURAL LAND

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ABSTRACT

The mud resulted in the cleaning process of worn out city waters (from the sewerage system) of Timisoara contain a high concentration of organic matter (30 %) and also metal elements (Cu, Zn, Cr, Pb, Mn, Ni, Cd) with high polluting potential. Spread on agricultural land, the mud becomes a good fertilizer for the maize crop, yielding about 5.2 – 20.7 q/ha (9.8 – 39.9 %). The chemical analyses praise the direct conection between the presence of metal elements in mud and their transfer from soil to plant and crop.

Key words*:* soil, town mud distribution, metal elements transfer, crop.

INTRODUCTION

The agriculture needs organic matter for soil fertility conservation, on the one hand and environmental protection, on the other hand, searches for solutions to relieve industry, agriculture and settlements of the growing amounts of wastes involved in environmental pollution. The distribution of organic wastes, other than those of agricultural source, on agricultural land in order to turn into account the present fertilizing elements, involves the risk of soil polluting with metal elements and pathogenic agents and further their transfer to plants, animals and man. The countries of the E.U. use 30 % and England 40 % of town mud in vegetal yield. (Chassin, 1992). Researches made in our country between 1980-1990 (Lixandru, 1985; Ionescu- Sisesti, 1986) confirm the fertilizing value of town mud but also the accumulation danger of metal elements, above the admitted values in soil. Till now, the researching results from abroad and our country can't be easily generalized because of the differences of each settlement, from the viewpoint of economic activities generating the worn out waters of the town sewerage network. On the other hand, the characteristic features of soils establish a distinct buffer capacity for the presence of toxic elements in mud. (Borza, 1997; Rauta C. and Carstea St., 1984)

MATERIALS AND METHODS

The testing of the mud fertilizing value and it's polluting potential has been made between 1995 -1997, within the framework of a stationary experiment including growing doses of mud (10,25,50,75 and 100 t/ha) comparative with two witness alternatives: untreated soil and chemical fertilized soil.

The mud proceeds from the drying beds after its crossing through the methanetank and it was once applied at the beginning of the experiment. The chemical fertilizers have been applied every year.

The field experiment has taken place at the didactic research station Timisoara on a poor glazed cambic chernozem soil having a middle fruitfulness (humus content 3,26%, a middle nitrogen and potassium content and a poorly

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content of phosphorum). The quality of the applied mud and the alterings induced in soil and crops have been established by laboratory analyses using the official methods of the romanian agrochemistry laboratories.

RESULTS AND DISCUSSIONS

The analyses performed for town mud before its application in the experimental field, show the following specific features: moisture= 49.3%; pH= 5.8; organic matter=36.3%, total nitrogen = 0.97%; P=0.58%; K=1.42%; Ca=0.35%; Mg=0.30%, Cu=509 ppm; Zn=619 ppm; Pb=261 ppm; Mn=214 ppm; Cr=982 ppm; Co=25 ppm; Cd=23 ppm.

The bacteriological and parasites analyses show the absence of the environmental contamination danger by mud parasites.

The yield results during three years prove a good fertilizing effect of the town mud materialized in production spores comprised between 9.8-39.3%. The maximum spore has been registered at the application of 100 t mud/ ha, comparable with the fertilizing effect of N_{120}, P_{80} chemical fertilizers. (Table 1)

The symbol N120 signifies the dose of nitrogen fertilisiers and P80 represents the dose of phosphorusfrom saline fertilisation products.

In Table 1 noted DL represents the limit differences (Săulescu and Săulescu, 1967). For example DL5% represents the appearance probabillity of the errors to limit differences.

Table 1. The effect of town mud application on the maize crop

Treatment	Years			Average kg / ha	Production increase		
	1995	1996	1997		kg/ha	%	Significance
Untreated	6375	6476	3000	5283	-	100	
10 t mud / ha	7273	6853	3283	5803	520	109,8	
25 t mud / ha	7770	8583	3533	6628	1345	125,4	**
50 t mudl / ha	8153	8946	3900	7000	1717	132,5	***
75 t mud / ha	8330	9105	4100	7178	1895	135,8	***
100 t mud / ha	8500	9263	4320	7361	2078	139,3	***
N_{120} P_{80}	8603	8750	4683	7345	2062	139,0	***
DL 5% = 837	DL 1% = 1174		DL 0,1% = 1658				

Note: asterisks mean: * 5% > p >1%; ** 1% > p > 0.1%; *** 0.1% > pa

The remaining effect of town mud during the three experimental years is positive and the greatest benefit have been achieved in the first experimental yea. The benefits are maintaining high during the second and third years of the experiment (Table 2).

Table 2. The remaining effect of town mud application on the maize crop for grains

Treatment	Crop increase (kg/ha)/year			Crop increase (%)/year		
	1995	1996	1997	1995	1996	1997
Mt – untreated	-	-	-	100	100	100
10 t mud / ha	898	377	283	114	106	109
25 t mud / ha	1395	2107	533	122	133	117
50 t mud/ ha	1778	2470	896	127	138	129
75 t mud / ha	1955	2629	1100	130	141	136
100 t mud / ha	2125	2787	1320	133	143	144
N_{120} P_{80}	2228	2274	1583	135	135	156

The chemical analyse of the maize plant shows an increase of the nitrogen content (2.4 to 3.2%) and that of trace elements (Cu, Zn, Pb, Ni, Mn, Cr) on the account of these elements in town mud.(Table 3)

Table 3. The variation of chemical composition in maize plants fertilized with town mud

Treatment	%			ppm					
	Nt	P	K	Cu	Zn	Pb	Ni	Mn	Cr
Mt-untreated	1.78	0.18	1.22	5.6	20.8	11.5	14.0	64.5	7.6
10 t mud/ha	1.90	0.17	1.15	6.5	21.0	11.0	14.2	68.5	7.9
25 t mud/ha	2.10	0.17	1.15	6.0	20.6	12.4	15.5	70.6	8.2
50 t mud/ha	2.20	0.19	1.19	6.1	23.2	12.2	14.7	62.7	8.3
75 t mud/ha	2.10	0.18	1.14	6.5	23.3	11.6	15.7	64.9	8.1
100 t mud/ha	2.10	0.18	1.16	6.1	24.1	13.0	15.2	69.5	9.2
$N_{120}P_{80}$	1.90	0.18	1.21	6.4	22.5	11.0	13.9	70.3	7.2

The town mud had a lowering effect on the soil reaction (pH = 6.45-6.20) in the variant using the maximum dose of town mud (Table 4). The soil acidification has a temporary character because the value of basic saturation is the same as for the untreated soil. The organic matter in town mud had positive influence on the growing of humus content (2.7/3.2%) and phosphorus content (34.3-48.5%)(Table 4).

The trace elements (Cu, Zn, Pb, Co, Ni, Cr, Cd) from the town mud can be find again in concentrates shapes in soil depending on the applied doses (Table 4)

Table 4.The variation of some chemical features of soil fertilized with town mud

Treatment	pH	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)	Cu (ppm)	Zn (ppm)	Pb (ppm)	Cr (ppm)
Mt-ntreated	6,45	2,7	34	530	18	48	47	95
10 t mud/ha	6,30	2,7	37	510	20	52	47	101
25 t mud/ha	6,25	2,7	37	505	20	51	50	103
50 t mud/ha	6,20	3,2	44	520	30	75	56	115
75 t mud/ha	6,20	3,1	46	520	27	85	56	117
100 t mud/ha	6,20	3,1	48	520	30	81	62	130
$N_{120}P_{80}$	6,20	2,6	34	530	18	49	45	98

The highest increases have been established for Cu, Zn, Pb, Cr. It becomes obviously the direct relation between the trace elements content in mud, their accumulation in soil and the transfer in plants which points out the possible contamination danger of soil and crops as a result of mud application on the agricultural grounds.

CONCLUSIONS

The filtering wastewater town mud contains a high content of organic matter and nutritive elements but also trace elements with high polluting potential through their concentration in soil and transfer to plants.

Because of the effervescence process of mud in methanetanks and the long storage period the parasite contamination danger is absent. The mud application on agricultural grounds has raised the maize yield and the remaining effect is positive. In plants the applied mud has grown the nitrogen content and also that of trace

elements. Under the influence of mud application the soil pH has diminished, the humus and phosphorus content has increased and also that of the metal elements with toxic potential (Cu, Zn, Pb, Cr).

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HEAVY METALS INVOLVEMENT IN CELL DIVISION RUNNING

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ABSTRACT

High concentrations of heavy metals and aluminium are toxic for plants, because they affect a number of physiological and biochemical processes such as mineral nutrition, photosynthesis, respiration, growth, development and yield of plants (Kiss et al., 1997). In our experiments, in order to determine the tolerance of 12 Secale cereale genotypes, different concentrations of Pb^{2+}, Cd^{2+} and Al^{3+} were used. Since all the genotypes had a reduced tolerance and regeneration capacity of the roots, cytological analysis were necessary to determine the heavy metals involvment in cell division running. The roots, grown in heavy metal solutions, were fixed and analysed using the Feulgen method. Compared to control (nutritive solution) all of the metals repressed the cell division.

Key words: *Secale cereale,* heavy metals, aluminium, mitosis

INTRODUCTION

Besides the genetic conditions, environment plays an essential role on the plant development. Soil is one of the more important elements of it (Pamfil, 1999). In the Western part of Romania, soil have some specific features: high acidity and high concentration of heavy metals. The main source of acidity and heavy metals in this area is a natural one, the substrate on which the soil was originally formed (Ianos and Goian, 1995). Besides, acidity and heavy metals concentration rise constantly due to the human activities (industrial activities, mineral fertilizers, waste mud, industrial and household waste).

In our experiments, in order to determine the tolerance of 12 *Secale cereale* genotypes to heavy metals and high acidity soils, different concentration of cadmium, lead and aluminium were used. Cadmium and lead were selected since they are two of the most plentiful heavy metals in Banat (1.5ppm Cd^{2+}, 50ppm Pb^{2+}), and aluminium is the main negativ factor in natural high acidity soils (100 ppm Al^{3+}) (Lăcătuşu et al., 1992).

MATERIALS AND METHODS

Biological material: 12 genotypes of *Secale cereale L.*, provided by the Gene Bank Suceava were used.

Tolerance test (Gustafson, 1996)

The seeds were surface sterilized with sodium hipochlorite 1% and germinated in dark for 48 h at 23^0C, on moist paper.

The seedlings were placed on plastic mesh floating on 3.5 l of an aerated nutritive solution (0,40 mM CaCl2, 0,65 mM KNO_3, 0,25 mM $MgCl_2$ x $6H_2O$, 0,01 mM $(NH_4)_2SO_4$, 0,04 mM NH_4NO_3), in plastic tanks. The tanks and the seedlings were placed in a germinator, maintained at 25^0C, with a continue lighting.

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After 48 h, the plantlets were transferred to nutritive solution suplemented with different concentrations of metals. The concentrations of the metals in natural conditions and a common concentrations (200 ppm) for all of them were used (Table 1).

Table 1. Experimental variant

Variant	Metal	Metal concentration (ppm)
V_1	Control (nutritive solution)	0
V_2	Lead	50
V_3		200
V_4	Cadmium	1.5
V_5		200
V_5	Aluminim	100
V_6		200

After 24 h, they were transferred again to nutritive solution and maintaned for 48 h. Plants grown exclusivelly in nutritive solution were used as a control. At each experimental step, the root length was determined.

The roots growth after the stress cessation was the test to determine the tolerance and the regeneration capacity of the different genotypes.

Cytological analysis

The roots from the interest plants were fixed and colured with Schiff reactiv and aceto - carmin. From each slide 25 fields were analised.

Mitotic index and the indexes of all of the mitotic stages were determined. The chromosomal aberations were observed. Data were statistically processed (Butnaru şi Moisuc, 1979).

RESULTS AND DISCUSSIONS

The low regeneration capacity after the stress cessation, pointed out a negative influence of the heavy metals and aluminium on the roots growth. In order to determine the influence of metals on cell division running, citological analysis of the roots were performed.

Cadmium, even in low concentration (1.5 ppm) influenced the cell phenotype and the chromosomal apparatus activity. The meristematic cells wall became thicker and the cytoplasmic matrix were detached from lamina. In some cells degraded chromatine and citomixis was visible. The mitotic index was reduced compared to control (d = -2.96±0.59) (Table 2). On the methaphasic chromosomes euchromatine and heterochromatine zones were very distinct.

The cadmium high concentration (200ppm) produced the same anomalies as the low concentration. Besides, nuclei with an amorphous structure, lateral disposed and sticked to lamina were observed; a pronounced plasmolysis was also visible. In comparison to control, the mitotic index decreased (d = -5.16±0.47). In interphase the chromatine of some cells was streaked because of the different coil degree, pointing out a differentiation in the cell cycle.

Contrary effects in the different stages of mitosis were observed: uncoiled in prophase, when coiling usually take place and, the genetic material was strongly condensed after cell division.

Table 2. The influence of heavy metals (Cd^{2+} and Pb^{2+}) and aluminium (Al^{3+}) on the mitotic index

Specification	Mitotic index (I_M)	Diference d±sd	%
Control	6,92±0,00	-	100
Cd^{2+} 1,5ppm	3,96±0,59	-2,96±0,59	57,22
Cd^{2+} 200ppm	1,76±0,47	-5,16±0,47	25,43
Pb^{2+} 50ppm	1,05±0,56	-5,87±0,56	15,17
Pb^{2+} 200ppm	0,47±0,09	-6,45±0,09	6,79
Al^{3+} 100ppm	3,21±0,79	-3,71±0,79	46,38
Al^{3+} 200ppm	2,40±1,01	-4,52±1,01	34,68

In the presence of lead in low concentration (50 ppm) the number of the mitotical cells was smaller compared to the control (d = -5,87±0,56). In the interphasic nuclei the heterochromatine was strongly condensed. In prometaphase the heterochromatic zones were thicker.

The concentration of 200 ppm lead determined an amorphous (9.65%) and picnotic aspect of the nuclei. In the most cases, zones of degraded chromatine were observed. The mitotic index was strongly repressed (d = -6,45±0,09).

Aluminium in concentration of 100ppm determined the detaching of cytoplasmic matrix from lamina. In the euchromatic and heterochromatic zones a lot of gaps had appeared and the chromosomes were broken up. The mitotic index decreased compared to control (d = -3.71±0.79). In the presence of high concentration of aluminium large nucleoli and many fragmentated chromosomes were observed. The mitotic index was lower compared to control (d = -4,52±1,01).

In our experiments the action of the different metals on the mitosis stages were observed. The lead blocked the cells in metaphase, and the cell division was interrupted. Cadmium and aluminium had a similar but lower effect. The metaphasic index was also higher compared to control, but was lower in comparison to lead.

Amorphous nuclei appeared especially in the presence of lead and high concentration of cadmium (Table 3).

Table 3. The influence of heavy metals (Cd^{2+} şi Pb^{2+}) and aluminium (Al^{3+}) on the different stages of mitosis

	Prophasic index (I_P)	Metaphasic index (I_M)	Anaphasic index (I_A)	Telophasic index (I_T)	Amorphus nuclei %
Control	22.0±0.0	25.0±0.0	27.8±0.0	25.0±0.0	-
Cd 1.5ppm	26,3±3,1	32.0±4.3	16.5±2.8	25.2±3.3	-
Cd 200ppm	10.3±6.2	64.3±8.0	11.4±4.7	3.9±3.2	0.9±0.8
Pb 50ppm	-	91.7±6.8	-	8.3±6.8	2.7±0.4
Pb 200ppm	36.1±14.9	63.9±14.9	-	-	9.7±1.7
Al 100ppm	31.3±5.5	46.02±5.5	10.04±3.7	12.7±2.7	-
Al 200ppm	34.9±6.8	50.3±2.5	11.0±3.1	3.9±2.7	-

CONCLUSIONS

The heavy metals (cadmium and lead) and aluminium inhibited the cell division. It turned out that the mitotic index decreased because of the specific aberations (i.e. citomixis) which prolonged the mitosis stages.

Cadmium in high concentration (200 ppm) and the lead had the most harmful effect on the cell division running.

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BEHAVIOUR OF METAL-PORPHYRIN ENZYMES: INTERACTION OF HORSERADISH PEROXIDASE WITH POLYPHOSPHATE

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ABSTRACT

In a previous paper, we have shown that the blood catalase can be separated from hemoglobin in the erythrocyte hemolysate, using the precipiting effect of the sodium polyphosphate, and avoiding solvent denaturation of hemoglobin or ammonium sulfate fractionation procedures. In this study, we have determined the solubility curves of the horseradish peroxidase-polyphosphate complexes, depending of the ionic strength and pH values of the reaction media. The obtained solubility curves of the enzyme-polyphosphate complexes at determined ionic strength as function of the pH values are individual characteristics for each enzyme, as well as molecular weights, optimal pH, optimal temperatures a.o. These enable a very fast way for the separation and purification of the enzyme from various natural sources, with a good rate of recovery and a high enough degree of purity.

Key words: Horseradish peroxidase, organometalic complexes

INTRODUCTION

The horseradish peroxidase is a hemensyme with many praotical utilityes as well as imunology, ocological dianosis or chemical and clinicallaboratory. In imunology, the peroxidase is usad for preparing peroxidasc marked antibodics and for antigan identification or quantitation. In oncological diagnosis, the peroxidasic activities are determined, owing their known depression or even failing from limohoeyesc and reticulocytes occurring in cases of sarcoma or mieloma diseases. In clinical laboratory, the peroxidase beside the glucose oxidase are used are used for the enzymatic – colorimetric test (GOD-PAP) of blood glucose.

The peroxidase preparation using usual methods (Theorell in Colowick and Kaplan, 1955) is very laborious, implying many purification procedures. In a previous paper, we hawe shown that the blood catalase can be separated from hemoglobin in erythrocites hemolysates, using the precipiting effect of the sodium polyphosphate, and avoiding solvant denaturation of hemoglobin or ammonium sulfate fraction (Contrea et. al., 1997).

In this study, we have determined the solubility curvers of the horseradish peroxidase-polyphosphata complexes at varioous values of ionic strength, as function of the pH value of the reaction media.

MATERIALS AND METHODS

The horseradish samples were colected from the 5'th Farm of the Didactic Experimental station of the University of Agricultural Sciences and Veterinary

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Medicine in Timişoara. The horseradish routs after cleaning from the soil residues and washing in runnung water ware cut in pieces and extracted in a juice extractor of Mallinatype, and the resulted juice were mixod with monosodiun-dihidrogen phosphate solutions, with the concentration of 0.05; 0.1; 0.15 and 0.2 M. respectively.

The determination of the solubility curves were carryed out according the Nitschmann's and. Col. Procedure for the plasma proteines fractionation (1959).

For the constant maintaining of the ionic strength of the analysed after addition of the sodium polyphosphate solution as precipiting agent of the enzyme, we have substitued the Elving's and col. (1956) buffer system of constant ionic strength used in our previous researches with an conductometric and acidimetric adjustment of the conductivity and pH, at the corresponding values of the metioned molarities, using NaCl solutions of the sama molarityes for the calibration of the instrument. The resulted conductivities were: 7; 14; 20 and 26 ms respectively.

The determination of the pH values were carryed out with the pH-100 IAMC Otopeni pH-meter equipped with an combined glass-ClAg electrode manufactured by the "Raluca Ripan" Chemistry Institute of Cluj – Napoca, and the conductivityes with Radelkis OK-102 1 conductivity meter. The pH range studied were tetween 3 and 7, with 0.2 pH units intervals.

After 30 min. stirring at room temperature and then left stanting indistubed at refrigerator overnight, the extracts were centrifugeg for 30 min. at 3000 r.p.m. in a refrigerated centrifuge Janetzki K-70. The supernatants were neutralised with NaOH 0.1 M to pH=7 and determined the peroxidase activity. The residue from each centrifuge tube were dissolved with phosphate buffer 0.1 M, with pH= 7 and as well were determined the peroxidase activity.

The peroxidase activity were determined according Luck (Bergamayer, 1965) with p-phenilendiamine method, modified as follows:in a spectrophotometer cell with 10mm length are added 1.0 cm^3 sample; 0.5 cm^3 phosphate buffer 0.1 M pH=7; 0.1 cm^3 Hydrogen peroxide 0.03 M and 0.1 cm^3 p-phenilendiamine 1%. On put the cover, mix vigorously and messure the absorbancy at 485 nm witnin the first two min. at 15 sec intervals, at a Specol spectrocolorimetre.

RESULTS AND DISCUSSIONS

The solubility curves of the horseradish peroxidase in presence of sodium polyphosphate at various conductivity values of solution as function of pH is shown in fig. 1.

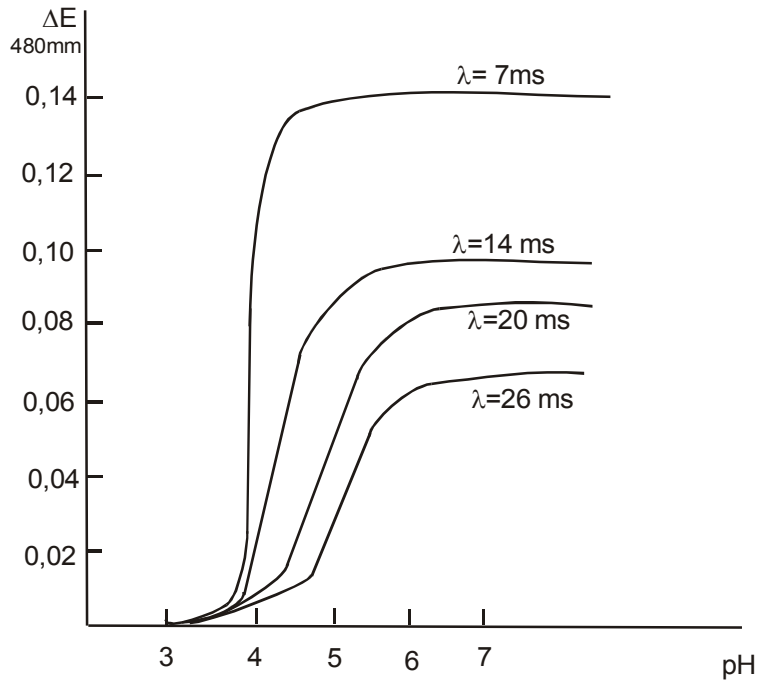

Fig 1. The peroxidasic activities curves in the supernatats of peroxidase-polyphosphate complexes at determined ionic strenght values, expressed as solution conductivities beteen 7 and 26 mS, as function of pH values of the resction media.

It can be observed that in the described experimental conditions we obtain a curves family congruents to the pH values of 3.6-3.8, at which almost the peroxidase activities are lost in the supernatant solutions. Therefore at this pH values, the peroxidase is precipited under form of poly phosphate complexes, regardless of the ionic sirength used for the extraction of the peroxidase in the presence of the polyphospate. It is not recommanded to work at ph values below 3.6, because the hoss of the peroxidase activity at more acidic media.

Based on above experimental data, we have worked a rapid method for the preparation a concentrate solution of horseradish peroxidase useful for determining the glucose acoording the enzymatic colorimetric test (GOD-PAP) based on the Trinder reaction (Trinder, 1969).

We give below a technical procedure for preparing this solution.

400 g horseradish roots are scrapped and extracted for two hours with 2000 cm^3 NaH_2PO_4 0.2 solution, under mechanically stirring. After standing in refrigerator overnight, the suspension is passed inte centrifuge cups and centrifuge for 30 min. To the supernatant is added 200 cm^3 sodium polyphosphate solution 12%, and the pH become 4.4. Next HCl N solution is added dropvise to the pH 3.8, and left standing overnight in refrigerator. Next day the solution is centrifuged again. Discard the supernatant, and the peroxidase-polyphosphate complex is disolved in 50 cm^3 phosphate buffer with pH=7. The total amount of the peroxidase activities resulted is near 3000 POD I.U.

The obtained peroxidase solution is of satisfactory purity for the enzymetic – colometric glucose determination.

CONCLUSIONS

1. The interaction of horseradish peroxidase with sodium polyphosphate were invastigated at four ionic strength values betwcen 0.05 and 0.2 and pH range between 3 and 7. The obtained solubility curves shows that at pH of 3.8 the peroxidase is precipited under form of peroxidase-poliphosphate complexes irraspective of ionic strength of the solution, and can be separated from other chemical constituents of the horseradish extract.

2. A technical procedure for the preparation of a concentrate solution with high peroxidase activity were worked out, useful for the enzymatic – colorimetric determination of glucose by the Trinder's glucoseoxidase-peroxidase method in the clinical laboratoris.

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ELEMENT COMPOSITION OF DIFFERENT VARIETIES OF RICE

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ABSTRACT

Element composition (calcium, magnesium, zinc and iron) was determined in different varieties of cultivated and wide type rice (Oryza sativa L.) by atomic spectroscopic methods. It was established, there are higher magnesium, zinc and iron contents in the cultivated than in the wild varieties of rice according to the other cereal varieties. The distribution of the macro- and microelements depends on the species and the variety, too. The husk of the rice contains more magnesium amount than its inner, starchy part. The increase of the calcium content is higher than the increase of the magnesium in the husk, therefore the mole ratio of calcium to magnesium is 0.7 in the husk and only 0.08 in the inner, starchy part of the rice. This means, there is more calcium-fitate than magnesium-fitate in the husk. The inner husk (bran) is also immensely rich in microelement (zinc and iron). The white rice contains the lowest amount of the microelement. The husked (cargo) rice has higher micro element content than the white rice, because the highest macro element content is in the surface of the husked rice (in the bran). The bran is the best part of the rice considering the alimentation with the highest macro and microelement contents.

Keywords: calcium, magnesium, zinc and iron contents in *Oryza sativa L*. and its parts, mole ratio of calcium to magnesium.

INTRODUCTION

The rice (*Oryza sativa L.*) is a main food source for the Oriental people, but it is important in the European people's meal, too. Nowadays not only the cultivated rice but also the wild variety (Indian rice) is grown.

The country of origin of the Indian rice is in North America (USA, Canada, region of the Great Lakes). The Indian rice is the primal food of the Amerindian. The Indian rice has been cultivated in Europe since 1989, on the region of Kisújszállás in Hungary, and its considerable part is exported for Western Europe. The Indian rice has black husk and nut-taste.

The growing of the cultivated rice was in the Eger district and on the environs of Tiszafüred in Hungary already in the Turkish occupation days. After many years, the rice cultivation again begins in the Banat during the reign of earl Karácsonyi in the XIX century (Vadányi, 1941).

The rice cultivation takes place almost with flood in the temperate-zone. The water covering may be permanent or periodical, but somewhere the rice cultivation carries out without flood, too (Simon-Ipsits, 1992 and Bíróné et al, 1998).

According to our previous investigations with different cereals (Kiss et al., 1998 and 1998-a) it was established, that the magnesium and zinc contents in the wild varieties of plants are higher than those in the cultivated varieties. Likewise it was shown by previous investigations, that the distribution of the element contents is not equal in different parts of wheat, rye, soybean, potato, pea and so on (Csikkel-

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Szolnoki and Kiss, 1995-a, 1995-b, 1996, 1996-a and 1996-b). Therefore we made our investigations in order to see, whether this mentioned fact is supported or not by the rice grains (Csikkel-Szolnoki and Kiss, 1999).

MATERIALS AND METHODS

The cultivated rice samples (Sandora and Ringola varieties) were sent from DATE, Agricultural Water and Environment Management Department (Szarvas) and we got the Indian rice from Indian Rice Kft. (Kisújszállás). Thanks for them.

Not only the total seeds (paddy, without husking), but also the husked rice (cargo), white (fretted) rice and chaff (surface husk) and the bran (inner husk) were analysed in different varieties of rice.

Digestion

The rice samples were dried at 105°C for 3 hours. Then approximately 0.2 g of sample was digested with 5cm^3 of concentrated nitric acid in Berghof B type microwave apparatus (Csikkel-Szolnoki, 1995).

Table 1. Parameters for digestion:

Warming:	Digestion:	Cooling:
power supplied 80% (850 W)	power supplied 80%	power supplied 0%
temperature 140 ˚C	temperature 190 °C	temperature 100 ˚C
time 2 min 30 sec	time 7 min 30 sec	time 10 min
P-range 5 ˚C	P-range 20 °C	P-range 20 ˚C

Instrumentation

The elemental analyses were performed by means of atomic spectroscopy. Jobin Yvon 24 sequential ICP-atomic emission spectrometer was used (Winge, R.K. et al, 1985, Csikkel-Szolnoki and Kiss, 1994 and 1997).

Experimental parameters for ICP-AES measurement:

Frequency of Rf generator: 40.68 MHz
Power supply of Rf generator: 0.8 kW
Flow rate of plasma gas (argon): 12 dm^3/min
Flow rate of aerosol carrier gas: 0.37 dm^3/min
Flow rate of sheath gas: 02 dm^3/min
Flow rate of nebulization (Babington): 1.4 cm^3/min
Wavelengths: Ca 393.366 nm, Mg 279.553 nm
Zn 213.856 nm, Fe 238. 204 nm

Every measurement was made in triplicate.

RESULTS AND DISCUSSIONS

Results of the analyses (calcium, magnesium, zinc and iron) were collected in the Table 2.

It was established, that higher magnesium contents were measured in the total seed of the wild rice (about +15%) than in the cultivated varieties. But in the cases of the husked (cargo) rice the magnesium contents were higher in the cultured varieties than in the wild variety (about +25%).

Both the total seeds and the husked varieties have about half amounts of calcium in the Indian wild rice than in the cultivated ones. For these reasons the mole ratio of the calcium to the magnesium is lower in the wild rice than in the cultivated varieties both in the total seeds and in the husked (cargo) rice.

Table 2. Element composition of the rice grains depending on the varieties mg/kg

Name of sample	Ca	Mg	Ca/Mg mole ratio	Zn	Fe
Total seed (paddy)					
Indian	129±6,5	628±25.1	0.12	38.9±2.1	52.0±3.3
Sandora	224±10.1	544±16.2	0.25	23.8±1.5	46.9±2.5
Ringola	372±11.9	563±22.5	0.40	15.1±1.3	21.4±0.8
Husked rice (cargo)					
Indian	67.2±4.7	507±11.2	0.08	49.8±3.1	46.8±2.6
Sandora	188±10.5	625±20.0	0.18	32.1±2.0	38.3±2.4
Ringola	218±11.6	649±26.6	0.20	21.8±1.2	20.8±1.1
White rice (fretted)					
Sandora	179±11.1	533±18.1	0.20	27.1±1.8	31.4±1.9
Ringola	192±11.5	422±15.2	0.28	20.0±1.0	19.4±1.0
Chaff (surface husk)					
Indian	610±19.5	776±29.0	0.48	33.3±1.8	73.0±2.6
Sandora	732±36.6	754±31.1	0.59	17.3±0.9	56.4±1.9
Ringola	1040±21.8	1160±26.7	0.54	17.5±0.7	41.5±1.5
Bran (inner husk)					
Sandora	448±15.9	473±17.0	0.58	41.2±1.6	49.9±2.6
Ringola	455±16.4	402±14.5	0.69	33.2±1.8	32.3±1.3

Indian rice has higher concentration from the microelements (zinc and iron) than the cultivated varieties.

There is the same tendency in the cases of different plant species: wheat (Kiss et al, 1998, barley, oat (Kiss et al, 1998/a) and rice (Csikkel-Szolnoki and Kiss, 1999). Namely the wild varieties of cereals have higher macro (calcium, magnesium and so on) and micro (zinc, iron and so on) element content than the cultivated ones.

The above mentioned fact is valid not only in the cases of the total seeds but in the white rice (fretted), in the chaff (surface husk) and in the bran (inner husk).

There is generally higher magnesium content in the husk (skin, periderm) of the plant seeds than in the inner, oleaginous, starchy part or containing protein (Table 3). The inner husk (bran) is immensely rich in microelements (zinc and iron), too.

The chaff made by husking contains the most amount of the magnesium than the bran (inner husk) and the fretted white rice.

Calcium and magnesium contents are the same in the bran and in the chaff of different rice varieties in mg/kg, therefore the mole ratio of calcium to magnesium is 0.5-0.6. These values are lower in the inner, starchy part of the rice (husked and white rice, 0.08-0.20). This result has diatetical importance.

Calcium and magnesium are found as calcium- and magnesium-fitate in the husk. The increase of the mole ratio of calcium to magnesium shows in the husk, that the fitate is mainly in the calcium bound.

Table 3. Magnesium contents in different parts of seeds and their ratio mg/kg

Plants	Skin	Inner part	Ratio
Wheat (Triticum aestivum)	1840	448	4.11
Maize (Zea mays)	913	480	1.90
Pea (Pisum sativum)	1870	845	2.21
Bean (Phaseolus vulgaris)	3450	1060	3.27
Horse bean (Vicia faba)	1810	950	1.91
Soybean (Glycine soybean)	1590	1360	1.17
Sunflower (Helianthus annuus)	2170	1900	1.14
Rice (Oryza sativa)	890	600	1.48

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BIOGENESIS OF UROCONCRETIONS: BIOCHEMICAL PECULIARITIES OF IONIC AND NON-IONIC STRUCTURES IN HETEROGENOUS NUCLEATION

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ABSTRACT

The lithogenetic process is characterized, in a first stage, by the formation of the so-called "starters" or "primers", resulted by precipitative mechanisms, involving the presence of a single anion, or by coprecipitation involving the presence of two or even three anions. In the case of coprecipitative processes, revealing a heterogenous mechanism, take part: alkaline monovalent metals (Na, K); alkaline-earth divalent metals (Ca, Mg); metal trace elements (Zn, Cu, Fe, Mn etc.)

In the process of uroconcrement biogenesis can compete compounds with ion and/or non-ion structure. Among the compounds with ion structure are evidently included all metal ions, e.g. Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} etc. whose presence, in the medical clinic, is searched by specific methods for the clinical chemistry.

Special peculiarities reveal the anionic compounds which can have organic nature, e.g. oxalates, cystine (and often together with other amino acids) or inorganic nature, e.g. phosphates (mono-, di- or tribasic), carbonates (mono- or dibasic), which are not so frequent met in the specific lithiasis for humans.

It can also be remarked that in the specific biogenetic processes of uroconcretions, organic non-ion compounds of purinic nature (uric acid, xanthine, 2,8-dihydroxyadenine) or of sterolic nature (cholesterol) can participate; the last one has a reduced incidence in urinary lithiasis.

The paper shows structural aspects of different ion or non-ion compounds which compete in the process of heterogenous nucleation.

Key words: metal ions, urolithogenesis, heterogenous nucleation

INTRODUCTION

Metals are important components of the human organism. They are not produced or distroyed in the body. Being present in our environment, i.e. food, water, air, soil they are introduced in the organism mainly by food and water. Usually the concentration of some essential metals in food dcreases as consequence of various processsing techniques and may cause a low intake in humans. In certain conditions some metals may have increased concentration in food, drinking water, air. In a balanced system, the amounts taken in excess are excreted in the urine (Underwood, 1981; Anonymous, 1997; Daranyi et al., 1998).

In the acceptance of the concepts of pathobiochemistry the approach of urolithogenesis is made taking into account "heterogenous nuclei" which contain organic and inorganic metabolites and are defined, in the pathology of urolithaisis, as "starters" or "primers". Once initiated, the nucleative process lead to the microcrystals and, in some conditions, to uroconcretions formation. The biogenesis of

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uroconcretions implies also the presence of a “matrix” with concentric fibrilar aspect containing neutral mucoproteins or mucopolysaccharides (glucosaminoglycans).

Investigating the crystallization in metastable solutions Leskovar and Hartung (1978) revealed that the process were preceded by “starters” formation which accelerate the precipitation. The authors exemplified the induction of crystallization by calcium oxalate considered as “starter” in uric acid (at pH 5.0 - 6.0) or phosphate (at pH 6.5-6.8) precipitation. In such case the starter of lithogenesis is not only a crystallization nucleus but also a product of coprecipitation.

1. PECULIARITIES OF IONIC AND NON-IONIC COMPOUNDS IN THE BIOGENESIS OF UROCONCRETIONS

In the last years more and more authors are interested in the determination of the alkaline, alkaline-earth and even of the trace elements concentration in uroconcretIons in order to elucidate their implication in the heterogeneous nucleation mechanisms and to give a real guideline in the metaphylaxy and prophylaxy of urolithiasis in the urological clinic (Joost and Tessadri, 1987; Dragan et al., 1993; Olcott et al., 1997).

The evaluation of the actions of metals and their role in urolithogenesis is possible by direct way - in vivo experiments on laboratory animals or by indirect way - in vitro investigations.

In vivo the metallic compounds are administered in the feedstuff or in the tap water of the animals in a well known concentration. But the estimation of the homeostatic aspects in ad libitum consumption of metals is rather difficult and offer no accurate data form scientific point of view.

In case of in vitro investigations the peculiarities of crystallization are studied in solutions with specific components of the uroconcretions. There are followed the effects of pH, ionic strenght, optimum concentration to avoid precipitation or coprecipitation with the apppearance of crystallization nuclei.

An other category of research are performed on uroconcretions, on patients with urolithaisis or risk of lithiasic recurrency. In such cases the indirect evaluation of metals role is possible,i.e. the establishment of the urolithiasis type and the metallic composition of uroconcretions. These researches allow us to estimate the types of the organic anionic, organic non-ionic and inorganic anionic compounds in uroconcretions.

Urolithiasis – the most frequent diseases of the urinary tract (about 12–40% among kidney diseases) – affects about 3% of the active people. In the last decades its frequency increased both in adults and children, its formation being a multifaceted process (Joost and Tessadri, 1987; Coe and Parks, 1988).

Problems concerning with the biogenesis of lithiasic concrements constitutesa a special domain when taking into account the location of lithiasis in the organism, e.g.: sialolithiasis, rhinolithiasis, urolithiasis, flebolithiasis a.o. as well as the type of lithiasis.

The human urine, considered as a metastable solution, contains beside nitrogenous compounds, protein, polysaccharides, organicx acidsalso anions and ionorganic cations (Na, K, Ca, Mg, Zn, Cu, Mn, Fe). All these ions participate in the lithogenic processes. Their concentration in uroconcretions is different, depending on the type of urolithiasis. The variations are due to the solubility differences of the compounds, the pH and the molality of the medium as well as to the morphofunctional status of the kidney and urinary tract (Hammarsten, 1929; Berg et al., 1990; Garban et al., 1987).

2. STRUCTURES OF THE MAIN PARTICIPATING COMPOUNDS IN UROLITHOGENETIC PROCESS

2.1. Organic anionic compounds

This class of compounds includes the ions generated by the carboxylic groups present in the composition of oxalic acid, cystine which can made electrovalent bindings with metallic ions M^{n+}.

Concerning the ionic structures, specific to organic anions, presented in fig.1 one must mention the fact that these structures preced the formation of “starters” (primers) which later on will generate the mycrocrystals and uroconcretions.

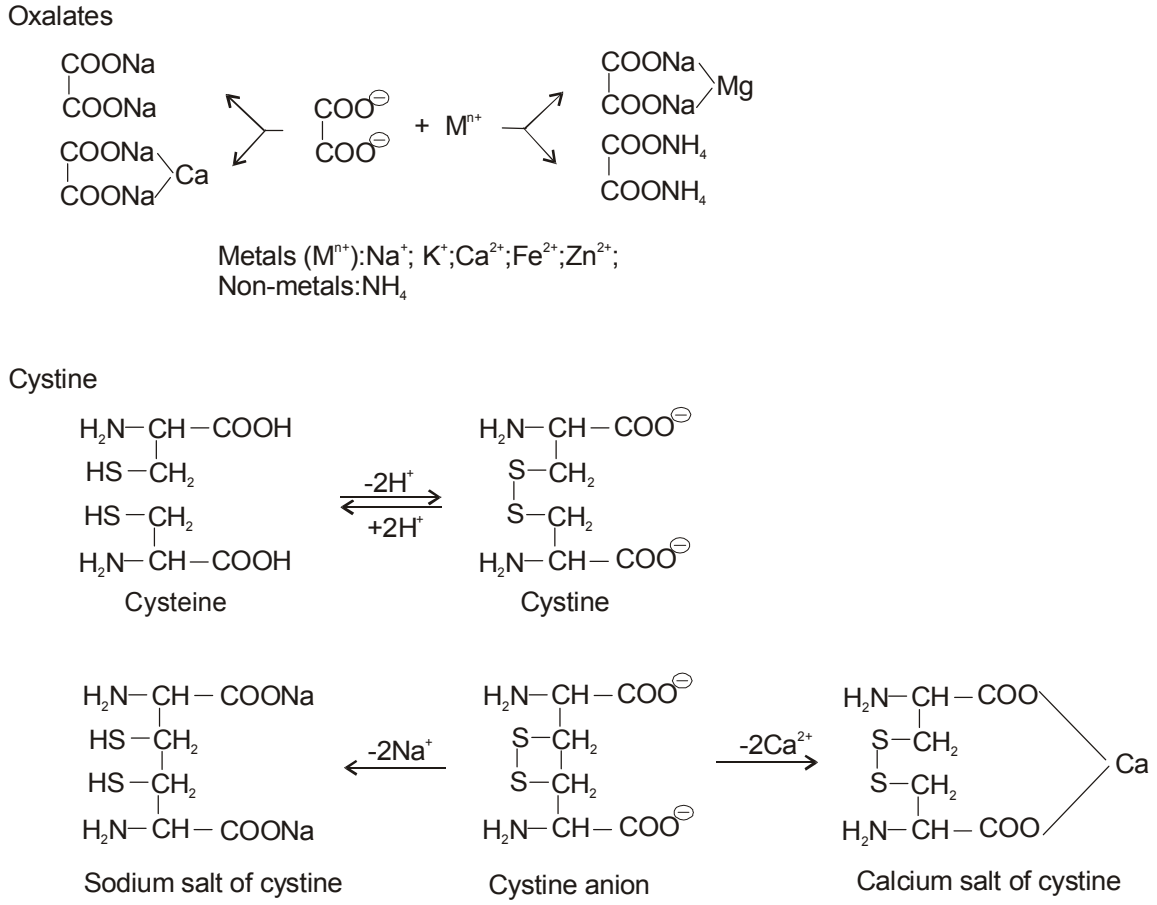

Fig.1. Organic anionic compounds of urolithogenetic process

The oxalic acid resulted from metabolic processes appears as a bidentated oxalic anion to which monovalente and frequently divalente metals can bind.

In cystinic lithiasis the binding of metals is made like in oxalic ones, i.e. electrovalently. Cystine has in its composition carboxylic groups which by ionization form a bidentated ion. And this one can fix mono- and divalente metallic ions.

2.2. Organic non-ionic compounds

In this class of compounds there are discussed those substances which in the presence of an –OH type polar group may generate structures able to polarize the medium and, as alcoolates, might constitute the precursors of complexes formation with metals. Among these one can mention uric acid and its derivatives as well as cholesterol (fig.2).

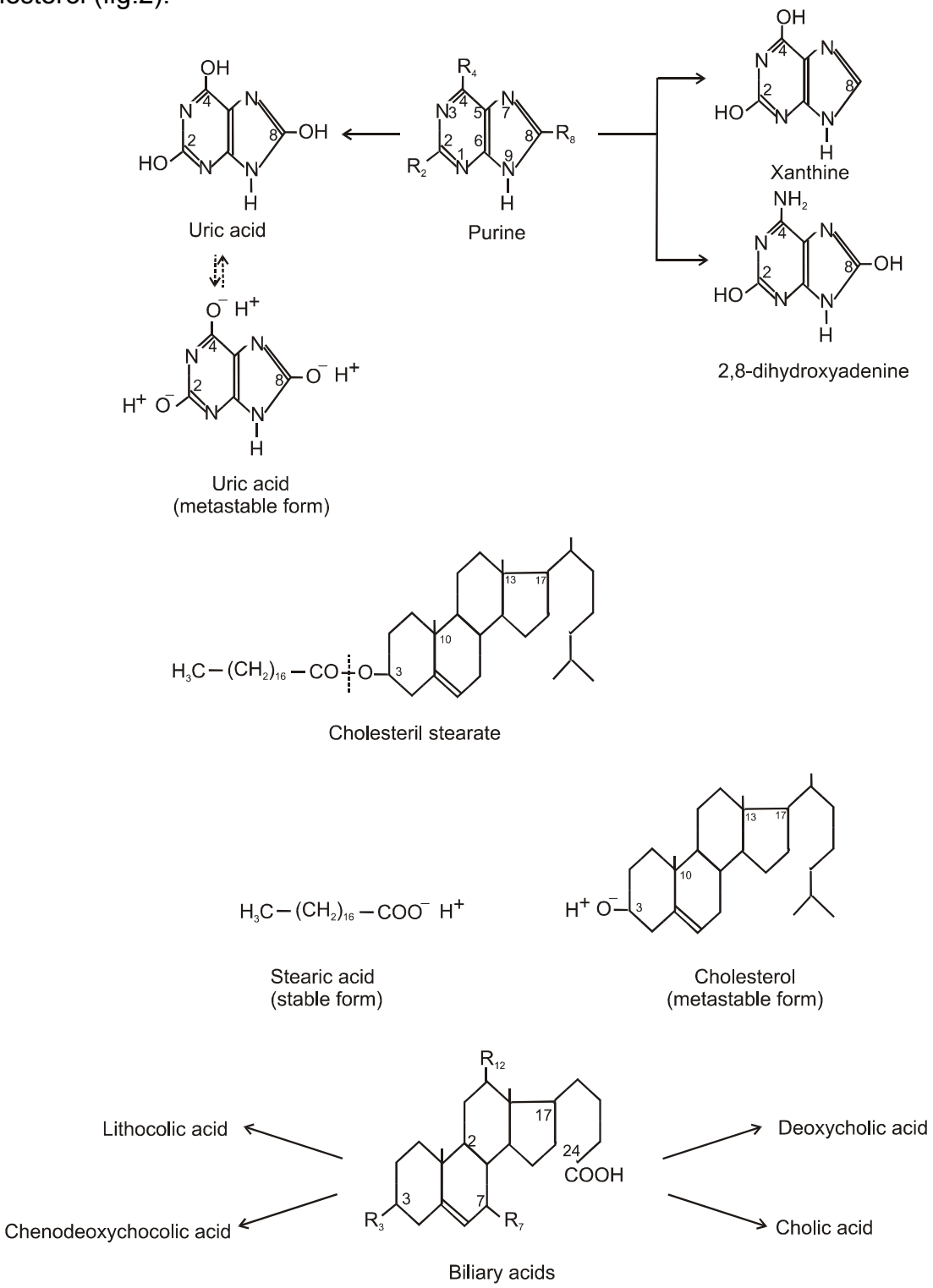

Fig.2. Organic non-ionic compounds of urolithogenetic process

Such complexes were studied in case of adenine and guanine. In case of adenine the metal ions bind to N_7 and to the N aminic of C_6 and in case of guanine to N_7 and O of C_6. To the metallic cations there are concomitantly complexed two water molecules. A peculiar case is represented by guanine to which M^{2+} cations can be complexed between two guanine bases. The bindings occur at the level of the same atoms, obviously without water molecules.

To the formation of the bioinorganic complexes of purine - M^{2+} type the bindings are chelatic ones between the purine (electron donor) and M^{2+} ions (electron acceptor) – fig.3. There are presented the complexes of divalentr metal ions with various nucleobases and with uric acid.

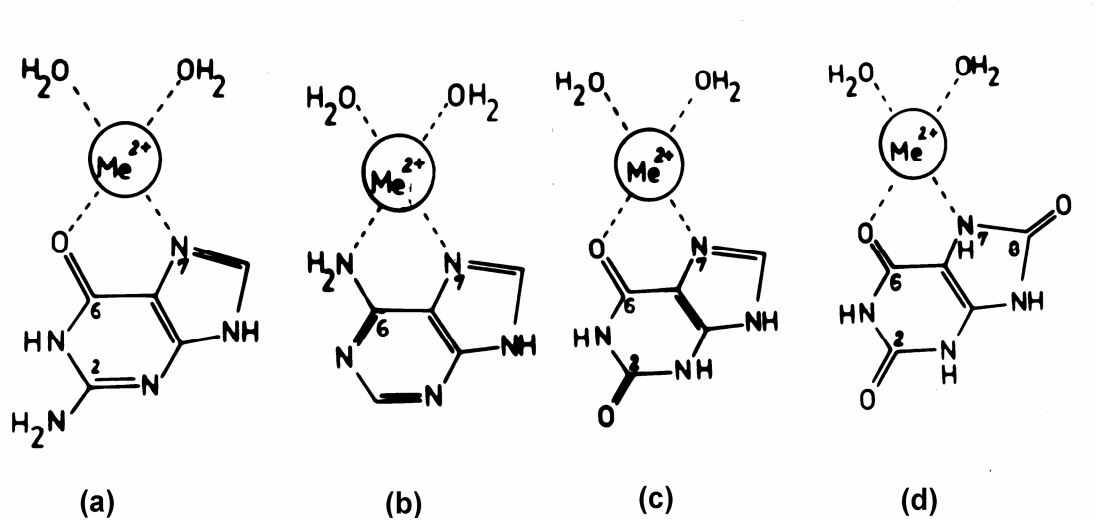

Fig.3. Purine - M^{2+} type aquated complexes formation

The selective affinity of divalent metallic cations towards bases, according to Zimmer (1971), shows a decrease of their binding capacity in the series:

$$Ca^{2+},\ Mg^{2+} < Co^{2+},\ Ni^{2+} < Mn^{2+} < Cu^{2+}$$

attesting the fact that trace metals (with distinctive electron on d orbital) have a more accentuated complexating effects.

Mono-, di- and trihydroxylated purines (hypoxanthine, xanthine) may generate also complexes like those described in case of adenine and guanine, and all these hydroxy purines are eliminated by kidneys.

These complexes, once appeared in the urine, constitute the starters and determine the perpetuation of precipitative and coprecipitative processes on the calculi matrix. Thus the system evolutes from primary urolithiasic nucleus to calculous concretions.

Generally, the concretions with sterolic composition are specific for billiary lithiasis. In such lithiasis alongside hydroxylated derivatives (like cholesterol) carboxylated derivatives (cholic acid type) are present, too. Cholic acids result from cholanic acid, i.e. lithocolic acid (3-hydroxycholane); chenodeoxycolane acid (3,7-di-hydroxycolane); deoxycholic acid (3,12-dihydroxycolane) – fig.2.

2.3. Inorganic anionic compounds

Are referred to phosphate and carbonate present in urolithiasis. In medical practice phosphatic urolithiasis is frequently found while carbonate appears rarely in mixed urolithisis playing a minor role.

In fig.4 the binding mode of various alkaline and alkaline-earth metals to phosphates and carbonates is presented.

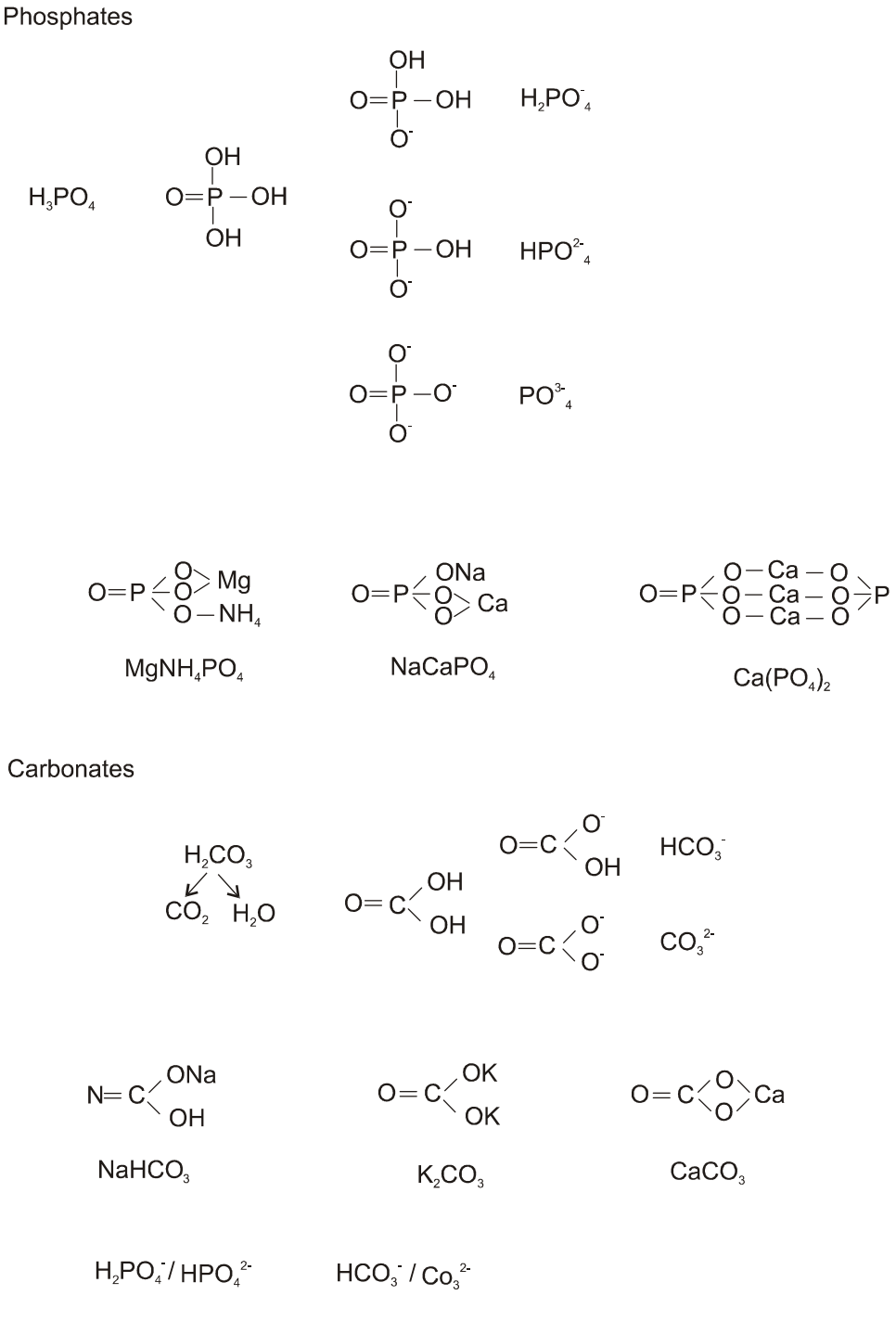

Fig.4. Binding mode of various metals to phosphates and carbonates

The binding modes of metallic cations to the inorganic anions present in uroconcretions explicate the starters (primers) formation in the precipitative and coprecipitative processes occurred in situ and the developing of concretions in time.

3. SHORT OVERWIEV CONCERNING HETEROGENOUS NUCLEATION

Urolithogenesis is a process characterized initialy by the appearance of the so-called “starters” or “primers” resulted by precipitative mechanisms, involving the presence of a single specific anion (e.g. oxalate) or coprecipitative ones involving - ino tempore - two or more anions (e.g. oxalate, phosphate, a.o.).

To anionic (organic and inorganic) compounds may bond: metallic ions, such as Na^+, K^+, $Ca^{2+,}$ Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Pb^{2+} a.o.; non-metallic ions (NH_4^+); non-ionic compounds. The binding of metallic and NH_4^+ cations is an ionic one in case of anionic compounds from uroconcretions (e.g.: oxalic acid, phosphoric acid a.o). In case of purine derivatives (e.g. uric acid, xanthine a.o.) the metals bind as aquated complexes (Garban et al., 1983; Dragan et al., 1981).

Precipitation stay at the basis of simple why coprecipitation at the basis of mixed (binary and ternary) uroconcretions formation. In the uroconcrement biogenesis can compete compounds with ion and/or non-ion structure. Beside these usually anionic structures there are ionic structures generated by some conditions: the pH , ionic strenght, osmolality a.o. – situations in which such forms may generate hydroxylated derivatives of purines (uric acid, xanthine, 2,8 –DHA).

Kidneys play a major role in maintaining balance with respect to water, mineral electrolytes, hydrogen ions and various organic compounds in the organism. The mineral electrolytes could penetrate the human organisms by absorption from respiratory tract, skin and intestine in variable amounts. They are distributed by blood in the liver, kidney and other organs, a part of them is used, an other is accumulated and the remainder is excreted in the urine (Rhoades and Pflanzer, 1992).

It is well known that the concentration of metals in the organsims is due to homeostasis mechanisms and may be influenced by environmental factors, age, ccumulations a.o. They can act indirectly as effectors (activators – inhibitors) of metabolic processes or directly as bioconstituents. The excess of metals can lead to competitive interactions in the biological systems modifying the normal metabolic processes and sometimes inducing diseases (lithiasis, endocrinopathies, enzymopathies, hemopathies a.o.)

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THE INFLUENCE OF COMPLEX COMPOUNDS WITH COBALT UPON ENZYMATIC ACTIVITY OF SOME MICROMICET SPECIES

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ABSTRACT

Microscopic fungi of Aspergillus, Trichoderma, Rhizopus, Penicilium and other species are producents of a whole number of hydrolytic enzymes (Pektinas, Cellulolas, Amilas, Proteas), that are widely adopted in different branches of national economy.

As objects of investigations three species of micromicets have been uses: Aspergillus flavus C-90 (producent of Cellulosus and Xylanas), Rhizopus arrhizus (producent – Pectinas), Aspergillus niger 33 (producent of Amilas).

As complex compounds under investigation have been used $Co(PC)_2 \cdot 4H_20$, $Co(PC)_3 \cdot H_20$, $Co(benzoate)_2$, $Co(ac \cdot ac)_3$, $[Co(DH)_2(Thio)_2]_3F[SiF_6] \cdot 1,5H_2O$, $[Co(DH)_2(Thio)_2]_2[SiF_6] \cdot 3H_2O$.

It was been established that complex compounds with Co(II) and Co(III) can exert both stimulating and inhibiting effect upon biosynthessi of hydrolytic enzymes of micromicets. The mechanism of effect in every particular case is evidently different and is being determined by: pecularities extra-cellular enzymic systems of strains-producents; composition of coordinating compound (combination); concentration of complex compound, added into the medium.

For the strains under investigation complex compounds with cobalt which can be applied as stimulators of biosynthesis of hydrolas have been discovered: for Aspergillus flavus; $Co(ac.ac)_3$, *stimulating biosynthes is of xylanas and* β*-glucozidase.*

Appart from these, $Co(benzoat)_2$, *stimulating biosynthesis xylanas: for Rhizopus arrhizus all the investigated compounds with cobalt can be used as stimulators of pectinas biosynthesis; for Aspergillus niger 33 as stimulators of biosynthesis amilas can be used Co(III) dioxymates with fluorine.*

Keywords: cobalt complex compounds – enzymatic activity

INTRODUCTION

Microscopic fungi of *Aspergillus, Trichoderma, Rhizopus, Penicilium* and other species are producents of a whole number of hydrolytic enzymes (Pektinas, Cellulolas, Amilas, Proteas), that are widely adopted in different branches of national economy. The use of these allows to intensify the processes of production, to improve the quality of a purposeful product, to apply resource preserving technologies (Galici, 1987; Graciova, 1987; Lobanov et al. 1989).

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One of scientific problems to be solved is to find the possibility of increasing their biosynthesis. Microorganisms, when being in constant contact with the environment, have adapted themselves to react quickly and exactly on the changes and to reconstruct their enzymic systems. High adaptability of microorganisms, to the changing external factors is the prerequisite for setting the above-mentioned problem. High adaptability of microorganisms made us have as an object investigation the increase of biosynthetic abilities of some species of micromicet-producents of hydrolytic enzymes.

To achieve the aim it is stipulated to use coordinating compounds. The possibility to use them as environmental factors, under which to activization (stirring up) the biosynthesis of hydrolytic enzymic systems can occur, is determined by the presence of metal atoms in their molecule (Rudic , 1993). Metals play a great part in enzymology. Many ions of metals are able to stirr up enzymic systems – Mg^{2+}, Ca^{2+}, Zn^{2+}, Cd^{2+}, Cr^{2+}, Cu^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Al^{3+}, etc.

The question of how the atoms of metals participate in the action of enzymes is very difficult. Depending on concentration, one and the same ion can either oppress or activate the enzyme.

In this paper the influence of various concentrations of complex compounds with Co(II) upon enzymic activity of some micromicets of strains *Aspergillus* and *Rhizopus* has been studied.

MATERIALS AND METHODS

As objects of investigations three species of micromicets have been used: *Aspergillus flavus C-90* (producent of Cellulosus and Xylanas); *Rhizopus arrhizus* (producent – Pectinas); *Aspergillus niger 33* (producent of Amilas)

Cultivation of Producents was carried out in shaker (180–200 turns per minute), under the temperature of 28–30^{o}C, on mediums of composition that has been chosen before.

Medium for cultivating *Aspergillus flavus C 90*:
vine – 10,0 g., beetrood marc – 10,0 g., $MgSO_4 \cdot 7H_2O$ - 0,5 g., KCl – 0,5 g., $FeSO_4$ – traces, $NaNO_3$ – 3,0 g., sugar beet marc – 10,0 g., water – 1,0 litre, pH 4,5 – 5,0.

Medium for cultivating *Rhizopus arrhizus*:
Beetroot pectin – 5,0 g., corn meal – 15,0 g., glucose – 10 g., $(NH_4)_2SO_4$ – 1,0 g., $MgSO_4$ – 0,7 g., $ZnSO_4$ – 0,025 g., water – 1,0 litre, pH 4,5 – 4,7.

Medium for cultivating *Aspergillus niger 33*: starch – 3,0 g., haricot bean flour – 9,0 g., wheat bran – 18,0 g., $MgSO_4$ – 0,5 g., KH_2PO_4 – 2,0 g., KCl – 0,5 g., water – 1,0 litre, pH 5,0.

In cultivating liquids of the producents under investigation which have been obtained without adding complex compounds (control) and with adding different concentrations (1, 5, 10 mg/l) of complex compounds, different types of enzymic activity have been determined.

This permitted to watch the changes of biosynthesis of hydrolytic enzymes.
Pectolitic activity has been determined by interferometric was (Graciova et al., 1982), according to the action on beetroot pectin.

Amilolytic activity has been determined according to the action on soluble starch.

Three types of cellular activity have been determined according to the action on Na-carboxymetilcellulose, filter paper and paranitrophenyl β–D–glucopyranozide. Xylanaz activity has been determined according to the action of oats on xylan (Graciova et al., 1982).

As complex compounds under investigation have been used $Co(PC)_2 \cdot 4H_20$, $Co(PC)_3 \cdot H_20$, $Co(benzoate)_2$, $Co(ac.ac)_3$, $[Co(DH)_2(Thio)_2]_3F[SiF_6] \cdot 1,5H_2O$, $[Co(DH)_2(Thio)_2]_2[SiF_6] \cdot 3H_2O$.

RESULTS AND DISCUSSIONS

Our investigations are devoted to the studying of the influence upon biosynthesis of hydrolytic enzymes of micromicets from the group of complex compounds. The common feature for these is the presence of atom Co(II) and Co(III) in the capacity of complexformer in the centre of their molecules.

The results obtained are presented in the tables 1–3.

The Investigations have showen that the degree of biosynthesis of enzymes under the influence of complex compounds depends both on the type of the producent, and on the used concentration of substance added into the medium. The effect of three different concentrations of coodinating compounds (1, 5, 10 mg in 1 litre of medium) upon biosynthesis of extra-cellular enzymes of three species of micromicetes has been studied.

Aspergillus flavus – producent cellulas and xylanas, *Rhizopus arrhizus* – producent of Pectinas, *Aspergillus niger 33* – producent of amylas.

Table 1. Change of enzymic activity of micromicet *Aspergillus flavus* under the influence of complex compounds that contain as complexformer atoms of cobalt

Complex compounds	Concentration (mg/l)	Xylanas activity	CMC-Na activity	FP	β-glicosidanas activity
$Co(PC)_2 \cdot 2H_2O$	1	+13,7	-3,34	-21,6	+3,89
	5	+2,0	+6,66	-17,69	-5,20
	10	+2,8	-3,33	-29,23	-25,97
$Co(PC)_3 \cdot H_2O$	1	+6,7	-7,78	-6,93	-6,97
	5	+13,7	+8,88	+21,54	+14,28
	10	+13,7	+7,77	-26,15	-6,49
$Co(benzoat)_2$	1	+24,6	-6,67	-4,62	+5,19
	5	+22,1	+6,66	+28,46	+1,29
	10	+18,5	+2,22	-4,62	0,00
$Co(ac.ac)_3$	1	+25,0	-12,23	-3,85	+14,28
	5	+2,0	-6,67	-16,16	+10,33
	10	+8,7	-6,67	-27,63	+16,68

One can see in the table that the effect of complex compounds on catalytic activity of enzymic systems of different species is manifested in different ways. It has been established that complex compounds with the atom Co(II) and Co(III) in the

capacity of complex former can, in some cases, stimulate biosynthesis of enzymes, in other ones, – they inhibit it.

It has been cleared up before that *Aspergillus flavus* produces extra-cellular hydrolytic complex possessing xylanaz, Na-Carboxymetilcellulose, β-glucosedase and cellobiohydrolytic (FB) activity.

The analysis of the results obtained (table 1) shows that the substances under investigation exert stimulating effect upon xylanase and β-glucozidase activity of *Aspergillus flavus*.

The most pronounces stimulating effect exert low concentrations (1 mg per 1 litre) of two substances with complex former Co: $Co(benzoat)_2$ and $Co(ac.ac)_3$.
While using these the increase xylase activity constitutes 24,6% and 25,0% correspondingly.

β-glucozidans of *Aspergillus flavus* react positively to carrying in complex substance $Co(ac.ac)_3$ – by increasing activity in 14,28 – 16,88%.

It has been established that the addition into the medium of cultivation of complex compounds with Co(II) and Co(III) do not exert any evident stimulating effect upon other types of activities of *Aspergillus flavus*, with the exception of $Co(PC)_3 \cdot H_2o$ and $Co(benzoat)_2$ substances, in the concentration of 5 mg, under which activity increase of cellobiohydrolase in 21,54% and 28,46% correspondingly is being observed.

Micromicet *Rhizopus arrhizus* (table 2) that synthesizes pectolytic enzymic complex is also sensible to the adding into the medum of low concentrations of complex compounds with cobalt. The most pronounces stimulating effect is being observed under the influence of picolinats Co(II) and Co(III) and dioxyminas Co(III) with fluorine. On adding $Co(PC)_3 \cdot H_2O$ into the medium of cultivation the increase of biosynthesis of pectinas makes up 67,22% under the concentration of 1 mg per 1 litre and 107,14% under the concentration of 5 mg per 1 litre.

Table 2. Change of pectinolytic activity of micromicets *Rhizopus arrhizus* under the influence of complex compounds containing as complexformer atoms of cobalt (%)

Complex compounds	Concentration		
	1	5	10
$Co(PC)_2 \cdot 4H_2O$	+47,37	-3,69	+45,21
$Co(PC)_3 \cdot H_2O$	+67,22	+107,14	-5,25
$Co(benzoat)_2$	+26,05	+15,32	+9,58
$Co(ac.ac)_3$	+12,27	+1,15	-13,49
$[Co(DH)_2(Thio)_2]_3F[SiF_6] \cdot 1,5H_2O$	+44,57	+97,15	+115,35
$[Co(DH)_2(Thio)_2]_2[SiF_6] \cdot 3H_2O$	+38,98	+87,46	+135,50

When using the complex substance $Co(PC)_2 \cdot 4H_2O$, pectolytic activity raises 47,87% (concentration 1 mg per 1 litre) and 45,21% (concentration 10 mg per 1 litre

Table 3. Change of amilolytic activity of micromicet *Aspergillus niger 33* under the influence of complex compounds containing as complexformer atoms of cobalt (%)

Complex compounds	Concentration		
	1	**5**	**10**
$Co(PC)_2 \cdot 4H_2O$	-12,97	-9,43	-9,43
$Co(PC)_3 \cdot H_2O$	-22,40	-70,74	-74,28
$Co(benzoat)_2$	-25,94	-9,43	-3,54
$Co(ac.ac)_3$	-79,0	-12,97	-3,25
$[Co(DH)_2(Thio)_2]_3F[SiF_6] \cdot 1,5H_2O$	+15,81	+20,33	+18,75
$[Co(DH)_2(Thio)_2]_2[SiF_6] \cdot 3H_2O$	+16,10	+19,64	+26,27

The highest indices of enzymic activity of pectinas have been recorded when having added dioxymines Co(III) with fluorine into to medium. The indices made up from 38,98 – 44,57% under the concentration of 1 mg per litre uip to 115,35–135,50% under the concentration of compexes – 10 mg/l.

The first three substances exert inhibiting effect upon the amilolytic activity of *Aspergillus niger 33*, i.e. the substance in this case appear to be inhibitors (the degree of inhibiting is up to 79,00%) (table 3), stimulating effect was only excerted by the combination of cobalt (III) with fluorine (up to 26,27%).

The most optimum are concentrations of 5 and 10 mg/l.

CONCLUSIONS

It was been established that complex compounds with Co (II) and Co (III) can exert both stimulating and inhibiting effect upon biosynthessi of hydrolytic enzymes of micromicets. The mechanism of effect in every particular case is evidently different and is being determined by:

– pecularities extracellular enzymic systems of strains-producents.

– composition of coordinating compound (combination).

– concentration of complex compound, added into the medium.

For the strains under investigation complex compounds with cobalt which can be appliedas stimulators of biosynthesis of hydrolas have been discovered.

– for *Aspergillus flavus* – $Co(ac.ac)_3$, stimulating biosynthes is of xylanas and β-glucozidas.

Appart from these, $Co(benzoat)_2$, stimulating biosynthesis xylanas.

– for *Rhizopus arrhizus* all the investigated compounds with cobalt can be used as stimulators of pectinas biosynthesis.

– for *Aspergillus niger 33* as stimulators of biosynthesis amilas can be used Co(III) dioxymates with fluorine.

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THE POSSIBILITY FOR APPLICATION OF THE METHOD FOR DETERMINATION OF IODINE BY KINETIC MEASUREMENT OF THE IODIDE CATALYTIC ACTIVITY

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ABSTRACT

Iodine can be found in small quantities in an organism, but it has great role in health protection of people and animals. Shortage of iodine causes reduced production of thyroid hormones, that bring disturbance of almost all metabolic processes in organism. Its shortage in nourishment of people and animals can be compensated by adding iodine to table salt that is usually mixed in animal feed. Regulations on food quality for people and animals define the smallest amount of iodine that have to be present in table salt, i.e. in animal mix and premix. In order to follow the quantity of iodine in premixes, as well in very complex analytical matrix, we tested the method of its detection by measuring catalytic iodide activity which catalyzes redox reaction between cerium(IV) and arsenic(III). The results of applied "recovery" test show that the method for determining iodine is applicable even in complex samples as premixes. The method has high accuracy (chemical income 97.8%), it is precise (variation coefficient 3.3%), and can be performed in relatively short period with a small amount of samples (detection limit is lower than 5 ng/ml).

Key words: kynetic measurement, iodine catalytic activity

INTRODUCTION

Iodine can be found in very small quantities in an organism, but it has great role in health protection of people and animals. Very well known is its role in the functioning of thyroid gland. Iodine presents an integral part of thyroid hormones T4 and T3 so it is very important for their synthesis as a microelement. Thyroid hormon regulates numerous metabolic processes in organism and they are important for mental development and body growth. Shortage of thyroid hormones causes disturbances in growth, development, reproduction, behavior and metabolic adaptation of an organism (Sinadinovic and Han, 1995). Iodine is introduced in body through food and water in the form of iodide. Its shortage in nourishment of people and animals can be compensated by adding iodine to table salt. Table salt is usually mixed in animal feed. Regulations on food quality for people and animals define the smallest amount of iodine that have to be present in table salt, i.e. in animal mix and premix (Trajkovic et al., 1983.).

In order to follow the quantity of iodine in premixes, as well in very complex analytical matrix, we tested the method of its detection by measuring catalytic iodine activity which catalyzes redox reaction between cerium (IV) and arsenic (III).

MATERIALS AND METHODS

The method for determining iodine by applying catalytic activity of iodide was applied in the samples of premixes (declared iodide value was 7 mg/kg), whereby from the same sample a total of 10 small and homogenous probes were performed at

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the quantity of 100 mg. In each sample additionally was added 1500 ngI^- which in final dilution had the value of 15 ng/ml. Samples prepared in this way were slightly heated with the presence of KOH, and than burned at the temperature of 600^oC for 2-3 hours. The samples were burned until they became white powder and than diluted in lukewarm water and diluted until appropriate volume.

The method of iodine determining with kinetic measuring by catalytic activity of iodine is based on the known redox reaction: 2Ce(IV) + As (III) $\rightarrow$ 2Ce (III) + As(V). Reduction of yellow Ce(IV) ion is reaction of first order and its speed depends on concetnration Ce(IV) ione and I^- ion.

The speed of changing of light yellow color of Ce(IV) ion in the presence of As(III) ion and I^- ion was measured on spectophotometry type MA 9524 with an additional termic printer. In a special work mode "cynetics", during the measuring the instrument constantly varied with the values of extinction. The printer wrote the values on a thermos paper in equal intervals of 2 seconds. In such a way a continual following of reactions in 1 minute, linear curve and its measuring is graphically determined.

The content of iodine in the measured samples was calculated in the following way:

$$\frac{tg\alpha_x - tg\alpha_o}{tg\alpha_s - tg\alpha_o} \times F = \text{ng of iodine per measured sample,}$$

whereby: x = unknown sample
o = blind probe
s = standard (burned)
F= proportion factor

RESULTS AND DISCUSSIONS

Table 1. Quantity of iodine in the probe where calculation was based on inclines of the recorded curves for the samples of premixes without standard addition of iodide.

Specification	Number of probe (n)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Inclining of the curve (tg of angle)	0.753	0.701	0.747	0.810	0.784	0.754	0.732	0.772	0.792	0.764
Quant.of iod. inprobe (ng/0,1g), $C_{0,n}$	793	787	739	854	826	794	771	813	835	805
$\Delta C=C_{0,n}-C_{0,av}$	-9	-15	-63	52	24	-8	-31	11	33	3
$(\Delta C)^2$	81	225	3969	2704	576	64	961	121	1089	9
$C_{0,av}$= 802±33 ng/0.1g; $\sum (\Delta C)^2$ / n-1= 1088.8; σ= ± $1088.8^{1/2}$ = ±33										

Applying Chauvent's criteria ($|\Delta C_n|\ \sigma^{-1} \leq q$), recommended by International bureau for measures and weights (Table 3), for n=10 follows that the maximum value of the coefficient is q=1.96. On the bases of this, it can be concluded that all the members of this series of measuring (Table 1 and Table 2) are correct and may be accepted.

Table 2. Quantity of iodine in the probe where calculation was based on inclines of the recorded curves for the samples of premixes with standard addition of iodide (1500 ngI^-).

Specification	Number of probe (n)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Incl. of the curve (tg of angle)	2.13	2.31	2.20	2.24	2.29	2.30	2.25	2.31	2.28	2.27
Quant. of iodine in probe (ng/0,1g), $C_{0+st,n}$	2240	2310	2200	2240	2290	2300	2250	2310	2280	2270
$\Delta C=C_{0+st,n}-C_{0+st,av}$	-29	41	-69	-29	21	31	-19	41	11	1
$(\Delta C)^2$	841	1681	4761	841	441	961	261	1681	121	1
$C_{0+st,av}$ =2269±36 ng/0.1g; $\sum (\Delta C)^2$ / n-1= 1287.8; $\sigma = \pm 1287^{1/2} = \pm 36$										

Table 3. Values of Chauvennet's factor depending on the number of measuring (Djuric and Petrovic, 1976).

Chauvennet's factor, q	1.15	1.54	1.73	1.86	1.96	2.13	2.24	2.40	2.58	2.81
Number of measuring, n	2	4	6	8	10	15	20	30	50	100

Calculated χ^2 function (Pirson's criteria) for the values given in the tables 1 and 2, were compared to the tabular values. For the number of degree of freedom, f=n-1=9 and the level of importance where β=0.05, it can concluded that the calculated values of χ^2 for both series of measuring are lower from the tabular values $\chi^2_{measured}<\chi^2_{tabular}$ (Djuric and Petrovic, 1976.).This means that the experimental data for the series of measuring the premix samples without the standard addition of iodide and the series of measuring the samples with the standard addition of iodide is subjected to normal statistical distribution.

When estimating the importance of systematic errors in investigation, we compared the received results (Table 3.) with the standard value as a referential material (additional standard quantity of iodine, C_{st}=1500 ng).

Table 4. The difference between the quantity of iodide in the premix samples with the standard addition and the premix samples without standard addition of iodine.

	Number of probe									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
$C_{0+st,n}-C_{0,n}$	1447	1523	1461	1386	1464	1506	1479	1497	1445	1465

From Table 4 it follows that the measured average value of standard addition of iodine represents $C_{st,av}$=1467.3±49.

The importance of systematic investigation errors was done by comparing the given average value of the standard addition of iodine (1467.3 ± 49 ng) with the additional referential value (1500 ng). Since the calculated t-value (2.11) is lower than the tabular value of the Student's coefficient ($t_{critical}$=2.26), for the number choice degree, f=n-1=9 and the level of importance β=0.05, we can conclude that applying of the mentioned method in determining iodine does not provoke systematic error.

Table 5. Values for reliability depending on factor K (standard deviation) (Duric and Petrovic, 1976.)

Reliability (%)	0	38.3	50.0	68.3	90.9	95.4	99.7
Factor K	0	0.50	0.68	1.00	1.65	2.00	3.00

Since that the reliability of the method is in function of standard deviation, based on Table 5, it follows that the applied method of detection of iodine measured by catalytic activity of iodide for K=2 has reliability at 95.4% since all the members in both measuring series (see Table 1 and Table 2) satisfy the interval from $C_{sr} \pm 2\sigma$. This means that for the series of measuring the premix samples without usual addition of iodide, the standard method error presents 8.3%. For the series of measuring the premix samples with the standard addition of iodide, the standard method error presents only 3.2%. Both series of measuring are highly homogenous in the results.

Table 6. Statistical survey of quality method.

Aditional quantity of standard iodine delution [ng]	**Average valueof measured iodine quantity [ng]**	**Chemical income [%]**	**Variation coeficient [%]**	**Deviation coeficient [%]**	**Standard error [%]**
1500	1467± 49	97.8	3.3	2.2	6.7

Using so called "recovery" test (Table 6.) chemical income of the method was determined, which was 97.8% with the variation coefficient of only 3.3%. The coefficient of deviation of the measured iodine content from the added standard represent 2.2%. This points to the conclusion that the method has satisfactory preciseness and accuracy.

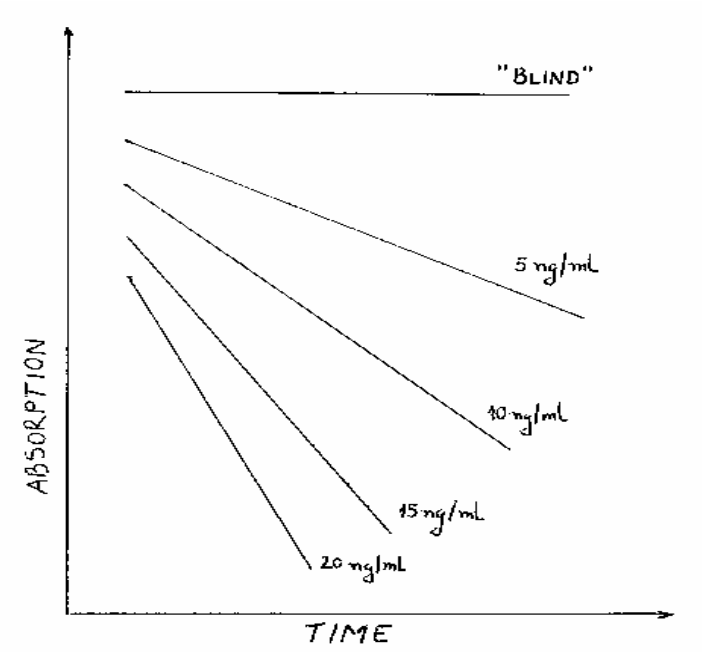

Fig. 1. The change of absorption in the function of time for standard dilutions.

From Figure 1 it is noticeable that the method is enough sensitive, since small changes in iodide concentration (standard dilution of 0, 5, 10 15, 20 ngI^-/ml) cause relatively great absorption changes in the function of time, i.e. important changes in the inclination of the recorded curves.

CONCLUSIONS

The preciseness of the method was checked by multiple determining of iodine on the same analytical sample in two fields of concentration. For the field of lower iodine concentration, a variation coefficient (VC) was 4.1%. For the field of higher concentration, the received VC was 2.2%. The preciseness of the method was determined by a recovery-test, which was in the span from 92.4-101.5%. Deviation of the measured iodine quantity from the standard added quantity was only 2.2%. Sine for the performing of the analyses relatively short time and small quantity of the samples is needed (detection border is lower than 5 ng/ml), the method of measuring catalytic activity of iodide may be applied in determining iodine in the samples form the environment with a complex analytical matrix.

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PREVENTION OF SELENIUM DEFICIENCY IN HUMANS BY SELENIUM RICH SOYBEAN

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ABSTRACT

Selenium (Se) offers a promising future in such widely diverse areas as cancer reduction, virology, immunology, reproduction and overall health for humans and animals. Therefore, there is much interest in increasing the dietary intake of Se. The amounts of Se in food are variable. In many geographic zones prevails Se deficiency. Recommending consumption of food rich in Se is therefore difficult. Metabolism and health benefits of Se depend on its chemical form that in food differ. Major form of Se that occurs naturally in foods is the L-isomer of Se-methionine (Se-met). Higher animals and humans cannot synthesize Se-met that is only active form of Se that can be incorporated in body proteins. Cereals and forage crops convert Se mainly into Se-met (> 80%) and incorporate it into protein in place of methionine (Met) because $tRNA^{Met}$ does not discriminate between Met and Se-met. For enriching soybean with Se was used foliar application of Se at certain stages of crops development because our earlier research showed that this way is more effective than application through soil. Concentrations of Se in soybean seeds produced in Middle Banat, using 10 - 130 g Se/ha rate varied from 0.175 - 5,43 mg Se/kg. Food obtained from Se reach soybean, particularly tofu and germs were especially rich in Se. Due to ability of soybean to absorb far more Se than grains and high nutritional quality of tested soy- food we concluded that it could be effective for nutritional Se deficiency prevention.

Key words: selenium, soybean, foliar enrichment, soy-foods, Se-methionine

INTRODUCTION

Food chain is the major pathway through which all nutrients that humans and animals need for life enter in the body. Deficiency of nutrients in the body and relatively high exposure to frequently toxic compound is widespread, particularly throughout the developing world. Numerous national and international studies point out that in developing countries high exposure to toxic elements (Pb, Cd, Hg) (Abdulla and al 1998) and organic contaminants (PCBs, PAHs) (Hietaniemy, 1996) are very common, as well as that intake of number of essential elements (Se, Zn, Cu, Ca, Mg) (Parr et al. 1991), antioxidative vitamins, natural antioxidants and potential anticancerogenic compounds as are isoflavonoids and lignins are low. Such a high intake of toxic compounds, in combination with a low intake of essential nutrients has a negative influence upon the health status of the humans and animals groups. Their susceptibility to a variety of diseases is increased.

Today a number of scientists all over the world connect imbalances in body functions with the limited adaptation of the affected organisms to inadequate dietary supply with nutrient and great differences in regard to priority of regulation. They think that probable causes of diseases that occurring from unexplained reasons lies in inadequate dietary supply with nutrient. To improve the quality of life of bout healthy and

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sick individual they use a nutritional approach, promoting use of functional food that contain needed quantity of all necessary nutrients, and natural bioactive products with curative properties - which safety and efficiency has been proven. Such food and bioactive products can regulate the immune, redox and elemental states in humans and animals, as well as optimising adaptation to various stressors by minimizing their harmful effects. Therefore they are applicable for combat against diseases of unknown and known pathologies, to slow down aging, and overall to enhance better health to humans and animals. On the other side, use of functional food and bioactive products may avoid, as much as possible, use of synthetic chemical compounds which the body identifies as foreign agents, and that often have undesired side effects that aggravate existing pathological condition.

Nutritional significance of selenium

Se is an essential element for humans and animals. It is incorporated into vital enzymes and proteins that protect cells and cell membranes against undesirable reactions. Good Se nutrition is of key importance for cellular defence as well as efficient energy metabolism. Se responsive disease has been observed in animals and humans. Interest for Se as micronutrient that may prevent diverse diseases in humans and animals is high. We don't have all the answers yet.

Researches conducted in the areas with the highest levels of Se founded that people in this regions had the lowest levels of particularly epithelial cancers. Opposite, studies conducted on people in regions with low dietary intakes of Se founded increased risk of cardiovascular diseases and cancer. Long-term study conducted on healthy residents and animals from the region with low levels of natural selenium indicates that increased Se intake greatly reduced the risk of many diseases. Short-term study showed that men fed a selenium-rich diet actually reported improvements in general mood, were able to think more clearly, and felt generally happier than at the start of the study. Our researches showed that Se-rich wheat consumption increasing Se and glutathione peroxidase (GSHPx) activity in humans and decreasing concentrations of thiobarbituric acid reactive substances (TBARS), lipid parameters (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides) and glucose in blood (Djujic at al. 2000).

The physiological Se requirements are 70 μg for adult man and 55 μg for women, respectively. Higher intake levels i.e. 200-300 μg is necessary for the protection against carcinogenic stress factors, in pregnancy and during lactation, whereas therapeutic doses are even higher. Bearing in mind that dietary Se intake depend on regional Se availability and the types of food consumed to provide a sufficiently wide margin of safety, the reference dose (RfD) for Se from all nutritional sources has been set at 350 μg/day for 70 kg adult American, corresponding to 5 μg/(kg body daily) or 5 times the recommended dietary allowance.

Occurrence of selenium in food and its metabolism

The amounts of Se in food are variable. It is required knowledge on existing Se levels in soil and plants to recommend diet that assure needed Se levels to maintain its balance in humans and animals, as well as to recommend adequate measures for assuring balanced Se nutrition. It can be forbidden that the chemical Se form in food differ to and that Se metabolism and health benefits depends on this.

Se is naturally found in foods high in protein, such as fish, meat, poultry, cereals and other grains. It can also be found in vegetables like mushrooms and asparagus. Brazil nuts are very high in selenium.

Normal form of Se in crops is Se-met and in vegetables Se-methylseleno-cysteine (SeMC). Ideally, Se should be supplemented in the form in which it occurs in foods. Se-met as the mayor natural food-form of Se is appropriate supplemental form of Se for humans and animals. SeMC, the mayor Se-compound in Se enriched onions, broccoli and garlic has potential antitumor properties. Therefore its potentially important application may be the use as chemoprotector against toxic side effects of drag such as cytostatic agents (Andreadou et al. 1996).

Higher animals and humans cannot synthesize Se-met that is the only active form of Se that can be incorporated in body proteins (Schrauzer G.N., 1998) and thus has beneficial physiological effects not shared by other Se compounds. Organs with a high affinity for Se-met are the skeletal muscles, the pancreas, the brain and the cells of the immune system. Average whole body half life of ingested Se-met, that can be metabolized directly to reactive forms of Se or stored, was 252 days or 150 days more in comparison with selenite and indicate that Se-met was utilized and reutilized excessively (Patterson et al. 1989; Swenson et al. 1991). Se-met supplemented animals maintain higher activity of Se-enzymes during depletion than selenite supplemented animals. Also, in nursing mothers, Se-met prevent the decline of plasma Se and glutathione peroxidase (GSHPx) activity, as well as the decline of Se in milk (McGuire et al., 1993; Alaejos M.S. and Romero C.D., 1995). Se-met metabolism is dependent on vitamin B-6 status, because B-6 – dependent enzymes are involved in the metabolic activation of Se-met (Soda et al., 1999), whereas tissue deposition of Se-met and its utilization for GSHPx synthesis depend on Met status (Luo X et al., 1987).

Cereals and forage crops convert Se mainly into Se-met and incorporate it into protein in place of methionine (Met) because $tRNA^{Met}$ does not discriminate between Met and Se-met. Se-met that is not required for growth by plants is produced along with Met in quantities depending on the amount of available Se (Schrauzer, 2000). In corn, wheat and soybean grown on Se-rich soil Se-met content is over 80% of total Se. Selenocysteine (Se-cys), methyl-Se-cys and γ-glutamyl-Se-metyl-cys remained at relatively low levels and were not significantly incorporated into plant protein (Yang et al., 1997).

Nutritional importance of soybean

The soybean is leguminous plant, characterized by the presence of nodes (little swellings) that contain bacteria *Rhizobium*, capable of fixing atmospheric nitrogen. Soya foods in China are over 4000 years part of diet. Western countries producing soybean for animal feeding from 19 century and since 1960's Soya foods became widely consumed by humans all over the world. Today soybean is an internationally important agricultural plant.

Soybean is one of the highest natural sources of high quality proteins, the highest source of dietary fibres, important source of oils and several compounds that demonstrated anti-cancerogenic activity as are isoflavonoids, protease inhibitors and phytic acid.

Epidemiological studies suggest that a high intake of soy foods may reduce the risk of certain types of cancer, including breast, prostate, endometrial and colorectal cancer. The low incidence of cancers, menopausal syndromes, reduced levels of serum cholesterol in Japan and China has been partially attributed to the high consumption of soybeans.

In soybean both storage proteins and lipid bodies are contained in the cotyledon (usable meat of the bean). The complex carbohydrate is also contained in the cell walls

of the cotyledon. The seed coat (outer layer of cells) makes up about 8% of the bean's total weight and is especially rich in crude fiber (35%). The row soybean is, depending on the variety, composed of approximately 18% oil, 4,9-9,5% of total sugar, about 5,3% crude fiber, 14% moisture and ash, and 38% protein. Of the total sugar content about 60% is sucrose, 10% is raffinose and 30% is stachyose. Raffinose and stachyose cause flatulence in humans and reduced feed efficiency in livestock (Hammond et al., 1993).

Eight essential amino acids are found in soybeans. They are necessary for human nutrition and are not produced naturally in the body. The storage proteins characterize heterogeneity in molecular weight and charge. About 90% of soybeans proteins are globulins (soluble in salts). Soybean albumins (in water soluble proteins) do not contains prolamines or glutelins.

Soybean globulins are composed of 4 fractions. The small globulins (2S fraction) represent 6-8% of total proteins and include two tripsin inhibitors (Kunitz's inhibitor - 21,7 kD and Bowman-Birk inhibitor (7,9 kD). This fraction also contains glycoproteins called lectins. A glycoprotein conglycinin (7S fraction) represent about one-third of total soybean protein (190kD). This fraction consists of three glycoprotein subunits, that are devoid of cistein and contain about 5% carbohydrate as mannose and glucosamine. Protein called glycinin (11S fraction) represents about 40% of soybean protein (350 kD). This storage protein contains six basic and six acidic subunits that show a complex pattern of assotiation-dissotiation involving disulfide bounds and hydrophobic bounding of the quaternary structure. Dissociation of subunits may be affected by appropriate conditions of ionic strength, pH, or solvent (Wolfe, 1972). The solubility of food-related functional properties of soybean proteins can be improved by limited hydrolysis with proteolitic enzymes, such s tripsin. Excessive hydrolysis of soybean proteins is normally undesirable because it gives rise to bitter-tasting peptides.

The nutritional quality of soybean protein is high, because it contains all the essential amino acids at levels greater than the FAO/WHO/UNU (1985) reference levels, and a digestibility comparable to those of egg-white or milk proteins, lower than animal proteins.

Moderate heat treatment is particularly beneficial for soybean proteins, because antinutritional factors as are trypsin and chymotrypsin inhibitors, or lectins are termolabile. Thus trypsin and chymotrypsin may impair efficient digestion of proteins, and thus reduce biological availability. Furthermore inactivation and compleation of trypsin and chymothrypsin by these inhibitors induce overproduction and secretion of these enzymes by the pancreas, which can lead to pancreatic hyperthropy and pancreatic adenoma. Soybean contains also lectins, glycoproteins that cause agglutination of erythrocytes. Lectins exhibit a high bending affinity for carbohydrates. In humans they impair protein digestion causing intestinal malabsorption of other nutrients. The latter consequences that result from binding of lectins to membrane glycoproteins of intestinal mucosa cells, are altering their morphology and transport properties. Both, protease inhibitors and lectins are termolabile. These antinutritional factors do not pose problems in home cooked soybean when heating conditions are adequate to inactivate them.

Soybean lipids contains significant amount (90%) of nutritionally important polyunsaturated fatty acids as are oleic ($C_{18:1}$), linolenic ($C_{18:2}$) and linoleinic ($C_{18:3}$) acids, as well as hay quantity of phospholipids (lectines and cefalines). Its content is usually 2-4%, but may be up to 8% of total lipids. Reduction of quantity of lipids in liver and blood vessels depends on their availability in the body.

Soybean is reach in vitamins. It contains carotene, vitamins E, K, B_1, B_2, B_6, nicotinamide, phantothenic acid, biotin and folic acid, whereas only soy germ contains vitamin C.

In comparison with wheat soybean contain more K, Mg, Ca, Cu, Fe, Zn, Fe and Se. Quantity of mineral elements on soybean depends on its availability from soil, sort, applied agricultural measures and climatic conditions. Investigations showed that soybean contain more Se than cereals, as well as that its quantity can be 100 times higher if adequate foliar application is applied.

Isoflavones are well-known phytonutrients of soybean that contain relatively high concentration of isoflavones (Walz, 1931). Soy foods made from whole soybeans or isolated and purified soy proteins contain relatively high concentration of isoflavones, primarily in the form of various β-glycoside conjugate (Coward, 1993) and low levels of aglicones. Amounts of genistein, daidzein, glucitein in their sugar-conjugated derivatives in soybean are 1-3 mg/g. Glucitein constitutes 5-10% of total isoflavones in most soybean foods and about 40% of total isoflavones in soy germ. Nutritional interest on isoflavonoids and lignans from soy foods as biologically active compounds that may have health benefits in hormone-dependent disease reduction, protect against chronic disorders including heart disease and bone loss, is today high. Much is known about the general metabolism of isoflavones in animals and humans. Interest in the health-related effects of soy isoflavones has surged in recent years. A daily isoflavone intake of 50 mg or higher has generally been used in clinical studies (Cassidy, 1994). The effect of varying dietary intakes of phytoestrogens is largely unknown.

Soybeans contain up to 2% saponins, important modulators in the promotion stage of cancerogenesis. Saponins are beside phytoestrogens and allergens thermo-stabile substances.

Nutritive value of 1 kg soybean1 is comparable with nutritive value of 2 kg of baby beef, 60 eggs, 8l of milk or 1,5 kg cheese (Maric and Plazinic, 1995).

Selenium enrichment of crops

One of approaches in treating marginal deficiencies of trace elements in humans and animals is in safe enrichment of food plants with deficient element. Techniques commonly applied for enriching food plants with mineral elements are soil applications, and foliar sprays**.** The production of Se-rich plants by mentioned techniques is the most safest and effective way to overcome natural deficiency of Se in humans and animals that eating them consume Se in natural form.

Experiences with Se fertilizations of a variety of crops in two Se deficient countries New Zealand and in Finland are provided encouraging results (Oldfield J.E., 1998). In Finland this is related to government concerns for human health and well-being, whereas in New Zealand government action was stimulated by the beneficial effects of organic Se on livestock productivity, an important item in the national economy. Our experience in this field is that foliar application of Se at certain stages of crops development is more effective than through soil. Applied procedure and combination for foliar supplementation of plants with Se (Djujic et al. 1988) have, in comparison with supplementation by means of root a series of advantages. By its application in agricultural praxis we now producing Se-rich crops as are wheat and soybean that contains enough Se for human and animal nutrition (Djujic and Milovac, 1998).

Bearing in mind the significance and beneficial effects of nutritional and super nutritional levels of Se for human and animal health, facts that Se deficiency prevails in the many geographical zones and that optimal way for increasing its consumption is to increase

Se content in certain foods, we have selected to rise its natural content in widely used crops. First crops that we obtained by applying own procedure for foliar application was Se rich-wheat. Its use in diet increased daily Se intake on the average by 18 μg in volunteers from Serbia. Here we present results of foliar Se enrichment obtained on soybean, second widely distributed and used plants by humans and animals. Aim was to produce soybean with desirable natural Se quantity for humans and animals and to find the optimal procedures for preparing functional nutritionally high valuable Se-rich foods that can assure desired intake of Se and other for human health important nutrients.

MATERIALS AND METHODS

In order to choose the proper foliar treatment for Se-rich soybean production a series of experiments was conducted in Middle Banat fields planted with soybean. The average Se content in top-soils selected for experiments was below 200 μg/kg. Fields planted with soybean were sprayed with compositions for foliar application that in solution contained 10, 15, 30, 50, 120 or 130g Se in selenite form that were used to spray 1 ha of the fields planted with soybean. Foliar procedure was same as in previous tests (Djujic and Milovac, 1998).

The effects of the Se application were determined in post-harvested soybean samples that, after air-drying were used for Se content and other relevant elements determination.

All samples were wet ashed. Methods used for Se, Ca, Mg, Fe, Mn, Zn, Cu, Cr, Mo, Ni, Al, As, Pb, Cd and Hg determination were atomic absorption spectrometry and inductively coupled plasma emission spectrometry. For analytical quality control was used NBS SRM-1567 reference material. In samples were beside Se determined concentrations of, protein, lipids, carbohydrate, water and ash (AOAC, 1990).

In soybean samples that contained needed quantity of Se (soybean treated with 10 and 15 g Se/ha) were determined essential amino acids, lipids, as well as fallowing vitamins: carotene, vitamins E, K, B_1, B_2, B_6, nicotinamide and folic acid (AOAC, 1990)

The rich-Se whole Soya seeds were utilized for production nutritionally highly valuable Se-rich Soya foods. With help of great enthusiasts doctors Draginja and Dusan Mladenovic (Mladenovic, 2000) that have great knowledge and experience in preparation foods from whole Soya seed were made:

- Soya milk (obtained from soybeans that are soaked, crushed and heated with water),
- Soya yogurt (fermented Soya milk),
- Tofu (coagulated Soya milk),
- Whey (liquid that stay after tofu straining),
- Okara (crushed and heated with water rest of soybeans after soy milk straining that contain non-extracted proteins and other nutrients)
- Germs with sprouts
- Gallets (cakes made from okara and integral wheat flour)
- Soya balls (sweets made with okara and wheat-meal)

In mentioned Se-rich Soya foods were determined Se content and composition, as well as calculated content of other important nutrients.

RESULTS AND DISCUSSIONS

Foliar application experiments

Results of the Se analysis in soybean seeds obtained after foliar application different amounts of Se in selenite form are presented in Table 1. As it can be seen from the results with use our procedure for foliar application 10 - 15 g Se/ha is enough for

obtaining Se rich soybeans, that in average contains 10 to 20 times higher content of Se than control. With higher Se doses obtained soybean seeds contained much more Se, even about 4000 times more than control.

Table 1. Content of Se, Zn, Cu and Mg in soybean seeds foliary treated with different amounts of Se

Rate of Se	Se, (mg/kg)	Zn, (mg/kg)	Cu, (mg/kg)	Mg, (g/kg)
Control	0,020	17,50	0,51	1,95
10 g Se/ha as Na_2SeO_3	0,175	23,56	0,73	2,45
15 g Se/ha as Na_2SeO_3	0,385	23,08	0,65	2,50
30 g Se/ha as Na_2SeO_3	0,926	19,52	0,64	2,40
50 g Se/ha as Na_2SeO_3	1,810	18,54	0,62	2,20
120 g Se/ha as Na_2SeO_3	4,285	17,50	0,65	1,90
130 g Se/ha as Na_2SeO_3	5,430	16,48	0,53	1,60

It is interesting that soybean seeds that contained optimal Se content (0,175 - 0,385 mg/kg) contain more Zn, Cu and Mg than control sample, as well as that with exceeding Se content in Soya seeds decreasing content of Zn, Cu and Mg to levels that are comparable with that in control (Table 1). Analyses did not show that applied Se treatment affect content of protein, lipid or carbohydrates, as well as Na, K, Ca, Mn or Fe content significantly.

Mineral elements in Se-rich soybean

On the basis of obtained results we decided to apply composition for foliar spraying that contain 12 g Se/ha on domestic sort of soybean "vojvodjanka" and obtained soybean seeds with 0,280 mg Se/kg. In Table 2 are presented data for Se and other relevant minerals content in Se-rich soybean seeds and Se-enriched what grain that we produced, as well as data on daily intake of minerals by our population (Djujic et al., 2000) and their recommended values (NRC, 1989; WHO, 1987 and 1989).

Table 2. Minerals content in Se-rich soybean seeds, Se-rich wheat grain and daily diet in Serbia

Mineral	Se-rich soybean seeds, (mg/kg)	Se rich wheat grains, (mg/kg)	Dietary intake, (mg/day)	RDA[1] or PTWI[2], (mg/day)
Ca	2650	530	620	1000 - 1200
Mg	2580	1400	342	310 - 420
Fe	58	78	13.7	10 - 15
Mn	17,2	34	4.9	2,0 - 5,0
Zn	23	25	12.7	12 - 15
Cu	0,75	3,2	2.07	2,0 - 3,0
Se	0,280	0,122	0,032	0,070 - 0,200
Cr	0,134	0,032	0,068	0,050 - 0,200
Mo	0,510	0,37	0,112	0,75 - 0,250
Ni	1,6	0,12	0,314	-
Al	6	4	8	3 - 17
As	0,018	0,004	0,014	0,003 - 0,160
Pb	0,037	0,023	0,130	0,455
Cd	0,048	0,011	0,023	0,065
Hg	0,012	0,003	0,006	0,045

[1](NRC, 1989); [2](WHO, 1987 and 1989)

On the basis of presented data Se-rich soybean seeds is not only rich in Se in natural form. It contains significant amounts of many essential elements and is particularly rich source of Mg. On the other side content of Al, elements due to which use of soybean and Soya dairy alternative products has been questioned its, is in

some cases even lower than in caws milk, and falls within acceptable limits given by WHO. Analysis of toxic elements in soybean shows that its quantity in soybean is also below the PTWI.

Essential amino acids in Se-rich soybean

Bearing in mind that one of limiting factors for Se binding is quantity of Met, as well as that soybean is today widely used source of proteins (substitution for meat and other animal sources of proteins) and that our idea was to produce, not only Se-rich soy foods, but functional food that assure all needed nutrient for organism in balanced ratio in Se-rich soybean seeds as well as in Se-rich wheat grain were determined essential amino acids contents (Table 3). In Table 3 are beside our data given data for essential amino acids pattern for main animal sources of food proteins, barley and maize and Recommended essential amino acids pattern for food proteins.

Obtained values for essential amino acids in Se-rich soybean seed showed that nutritional quality of soybean proteins is high. In comparison with animal proteins soybean contain less Met and cysteine as well as less phenilalanine and tyrosine. In comparison with cereals soybean proteins contain less Met and cysteine, than cereals and more lysine. These data indicates that the nutritional quality of soybean proteins can be improved by mixing it with another protein that is rich in Met, for example mixing soybean with cereals as is wheat and barley. On such way can be obtained the ideal nutritional quality of a proteins or protein mixture. They can contain all of the essential amino acids in proportion that produce optimum rates of growth and/or optimum rate optimum maintenance capability and beside this needed quantity of Se in natural form.

Table 3. Essential amino acids from Se-rich soybean seeds, Se rich wheat grain and other sources of food proteins (mg/g protein)

Amino acid concentration, mg/g protein	Soybean	Wheat	Egg[1]	Cow's milk[1]	Beef[1]	Barley[1]	Maize[1]	Recommended pattern[2], (mg/g)protein
Histidine	24	23	22	27	34	20	27	26
Isoleucine	63	39	54	47	48	35	34	46
Leucine	78	72	86	95	81	67	127	93
Lysine	64	29	70	78	89	32	25	66
Met+Cys	32	39	57	33	40	37	41	42
Phe+Tyr	42	75	93	102	80	79	85	72
Threonine	41	30	47	44	46	29	32	43
Tryptophane	13	11	17	14	12	11	6	17
Valine	53	42	66	64	50	46	45	55
Total	410	360	512	504	480	356	422	434
Protein, %	38	14	12	3,5	18	10	9	-

[1](Fennema, 1996); [2]The essential amino acids requirements of preschool children (age 2-5) generally recommended as a safe level for all age groups were given.

Lipids in Se-rich soybean

Data obtained for fatty acids content in Se-rich soybean seeds (Table 4) confirmed that soybean contain significant amount of nutritionally important polyunsaturated fatty acids. Soybean as well as wheat contains significant amount of

the linoleic ($C_{18:2}$) and linoleic ($C_{18:3}$) acids, the parent fatty acids of the n-6 and n-3 series respectively.

Table 4. Principal fatty acids of Se-rich soybean seeds, Se-rich wheat grain and main vegetable oils and fats, % (after [1]Fennema, 1996).

% of total lipids	Soybean	Wheat	Corn[1]	Sunflower[1]	Olive[1]	Milk[1]	Beef depot[1]
$<C_{12:0}$	-	-	-	-	-	12	-
$C_{12:0}$	-	-	-	-	-	3	-
$C_{14:0}$	-	3	-	-	-	11	4
$C_{16:0}$	11	16	6	6	15	36	41
$C_{18:0}$	3	8	2	4	-	15	17
$C_{18:1}$	23	29	44	31	75	21	20
$C_{18:2}$	74	59	48	57	10	1	4
$C_{18:3}$	4	5	-	-	-	-	1

Due to their importance for production the various metabolite s with important biological function, a nutritional strategy to provide optimum amounts and ratio of the n-6 and n-3 fatty acids series can be easily realised if Se-rich soybean and/or Se-rich wheat will be main components of functional nutritionally high valuable foods.

Vitamins in Se rich soybean

In Table 5 is given content of some vitamins in Se-rich soybean seeds and Se-rich wheat. On the basis of obtained data Se-rich soybean seeds are rich in vitamin E, K, nicotinamide, as well as in vitamins B_1, B_2 and B_6, although content of vitamins B_1, B_2 and B_6 is much higher in Se rich wheat grain. Bearing in mind that retention of vitamin E after boiling is 100%, and vitamins B_1, B_2 and B_6 from 45 - 95% it appear that production of nutritionally highly valuable Se-rich Soya foods may assure enough vitamin E and vitamins B_1, B_2 and B_6 for our needs.

Table 5. Vitamins in Se-rich soybean seeds and Se rich wheat grain

Vitamins	Soybean	Wheat	RDI[1], mg
Carotene, mg/kg	3,8	-	4,8 - 6,0
Vitamin E, mg/kg	15,0	0,136	0,008 - 0,010
Vitamin K, mg/kg	1,9	-	0,065 - 0,080
Vitamin B_1, mg/kg	9,9	124	0,0011 - 0,0015
Vitamin B_2, mg /kg	5,2	42,0	0,0013 - 0,0017
Vitamin B_6, mg/kg	11,9	67,6	0,0016 - 0,0020
Nicotinamide, mg/kg	25,1	-	0,015 - 0,019
Folic acid, mg/kg	2,3	-	0,18 - 0,20

[1]RDI Reference daily Intakes (NRC, 1989)

Nutritional characteristics of Se-rich Soya foods

The Se-rich soybean seeds were used for production Soya foods from whole seed. Soya dairy alternative as are milk, yogurt, tofu, whey were produced, as well as okara and germs with sprouts. In combination with Se rich integral flour were prepared Gallets and with wheat meal Soya balls with wheat meal. In products obtained from Soya seeds with 0,280 mg Se/kg and wheat with 0,122 mg Se/kg (Table 6) contents of Se were from 0,022 mg/l (in whey) to 0,360 mg/kg (in tofu).

Table 6. Se content in Soya foods produced from Se-rich soybean whole seed

Soya food	Se mg/kg (l^1)	Proteins, %	Lipids, %	Ash %
Soya milk	0,114[1]	7.12	6.20	0,63
Soya yogurt	0,120[1]	7.30	6.45	0,67
Tofu	0,360	19.15	2.84	0.71
Whey	0,022[1]	3.17	11.44	0.73
Okara	0,197	4.16	12.53	0.47
Germ with sprouts	0,319	36,45	20.78	0.68
Gallets	0,151	18,44	40.78	3.77
Soya balls	0,108	14,24	8,65	2,12

Tofu (Soya cheese) obtained from whole seed of Se rich soybean contained the highest level of natural Se-met (≥ 95% of total Se), At the same time it is rich source of proteins (19,15%) in which essential amino acids are in optimal proportion.

Soya milk and Soya yogurt prepared from whole seed of Se rich soybean contained the similar level of Se as Se-rich wheat (about 0,120 mg/l). Its dominant form is, as in Tofu, Se-met. Beside significant amount of highly valuable proteins (about 6%) in which essential amino acids are in optimal proportion they contains about 6% of lipids rich in polyunsaturated fatty acids.

Whey obtained from whole seed of Se rich soybean through was not rich in Se but contain high level of nutritionally important polyunsaturated fatty acids.

Ocara obtained from whole seed of Se rich soybean is on the second place looking Se content (0,197 mg/kg). About 80% of Se in Okara is in Se-met form. It is rich in lipids that contains nutritionally important fatty acids and also contain significant amount of phospholipids. Okara is the richest source of crude fiber and isoflavones.

Germs produced from Se rich soybean have ha the highest nutritional value regarding natural Se content (0,319 mg/kg), as well as content of other nutrients. It is the richest source of highly valuable proteins, crude fiber, lipids, minerals and other phytonutrients, as well as the form of soybean that beside mentioned vitamins contain vitamin C. Our investigations showed that it is important to impact proper procedure in its production. Only in this case oxidative changes tat in germ may be high can be prevented and obtained highly valuable nutritive product for human use.

Gallets is salted cake. Main components in gallets are Okara obtained from whole seed of Se rich soybean and integral flour obtained from whole grain of Se-rich wheat. Content of Se in gallets is high (0,151 mg/kg), as well as other mineral, protein and lipids. Optimal proportions of essential amino acids and polyunsaturated fatty acids in gallets provide its high nutritive value.

Soya balls are sweets. Main components in Soya balls are Okara obtained from whole seed of Se rich soybean and wheat-meal obtained from whole grain of Se rich wheat. Quantity of Se in them is about 0,100 mg/kg. In comparison with gallets they contains less lipids, but due to applied cooking in their preparations, not backing, as is case with gallets, contains more vitamin E.

On the bases of reported data all products obtained from whole seed of Se-rich soybean have high nutritional value. They may assure needed dietary intake of natural Se, highly valuable proteins, polyunsaturated fatty acids, most minerals and vitamins, as well as phytonutrients. In combination with cereals as are wheat, barley, ray, maize or rice can be optimized ratio of essential amino acids and lipids in soy foods and obtained highly valuable foods.

CONCLUSIONS

On the base of obtained results we concluded that:

- Foliar application procedure in which is applied composition with10-20 g Se/ha in selenite form assure obtaining Se-rich soybean which seeds can contain from 0,150 - 0,400 mg/kg Se in natural form in which content of Se-met is minimum 90%.
- Soya foods obtained from whole seed of Se-rich soybean (Soya milk, Soya yogurt, Tofu, Okara, Germs) or in combination with Se rich wheat grains (Gallets, Soya balls) must be expertly done, because value of some nutrients can be significantly diminished.
- Soya foods obtained from whole seed of Se-rich soybean alone or in combination with Se-rich wheat grains is functional nutritionally high valuable food that may assure desired intake of Se, highly valuables protein and unsaturated fatty acids, crude fiber, as well as many essential minerals, vitamins and phytonutrients necessary for promoting the human health and quality of life.
- To provide the full potentials of Se-rich soybean as a natural and all-purpose food supplement in the protection and preservation of human and animal health it is necessary to conduct waste investigation by clinicians.

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MAGNESIUM OROTATE IN THE TREATMENT OF VENTRICULAR ARRHYTHMIAS AFTER BYPASS SURGERY

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ABSTRACT

Ventricular arrhythmias are a relatively frequent complaint in cardiac patients after bypass surgery. The purpose of this study was to determine the effects of magnesium orotate in the treatment of these arrhythmias.

Methods: 23 coronary patients 1 month after bypass surgery were included in a 3 months study after non-invasive cardiovascular evaluation that included Holter monitoring and exercise testing. Usual medication was kept in all patients (betablockers, ACE inhibitors, aspirin) and magnesium orotate was given to 10 patients in a regimen of 500 mg bid.

The results showed a significant improvement of recurrence of ventricular premature beats both at rest and during exercise in the magnesium group, where also a better exercise tolerance and improvement of functional capacity were noted.

We conclude that magnesium orotate may become a useful adjunct to preventive treatment of ventricular arrhythmias after bypass surgery, probably due to its membrane- stabilizing effect.

Key words: ventriculat arrhythmias, magnesium orotate, bypass surgery

INTRODUCTION

Magnesium. Magnesium is on place four of all the cations in the body and it is present in more than 300 enzymatic systems, its presence being crucial for ATP metabolism. The role of magnesium is of enzyme activator in regulation of cellular energy metabolism, vascular tone and cell membrane ion transport.

Lack of magnesium was shown to determine a decrease in the concentration of intracellular potassium and an increase in calcium levels (Reinhardt, 1991).

Intravenous magnesium sulfate has been used therapeutically in critical situations such as torsade de pointes and ventricular arrythmias caused by digitalis (Yusuf et al, 1993; Ford, 1999) and proven to be safe and effective at the onset of myocardial infarction, leading to a 24% reduction in mortality (Yusuf et al, 1993).

Although all these reports have documented the effectiveness of magnesium in correcting lethal arrythmias, the rank of magnesium administration has not been well established in standard algorithms for arrythmia therapy.

Few controlled studies exist regarding the therapeutic uses of oral magnesium supplementation in chronic cardiovascular disease, although in the US daily allowances are recommended to prevent the risk of dying from heart disease (Ford, 1999).

Orotates. Orotic acid is a naturally occuring substance and a key intermediate in the biosynthesis of pyrimidines. Previous investigations suggest that orotate can protect recently infarcted hearts against a further ischemic stress and may be beneficial in certain types of cardiomyopathy.

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In the seventies many Russian and Bulgarian clinical investigators reported beneficial effects of potassium orotate in the treatment of angina, myocardial infarction and chronic heart failure. These investigators observed improved contractility, as assessed by the ventricular ejection period on nuclear angiography, lower incidence of complications and lower mortality rates in the patients on potassium orotate treatment compared with patients on cardiac glycosides, oxygen and anticoagulants (Lukomski et al 1967; Zharov, 1972; Ignatev et al, 1969).

In the Western world, Hans Nieper was the first to use orotates clinically prior to 1980 (Nieper, 1974). His argument was that being neutrally charged, they pass easily through cell membranes and ferry mineral atoms into cells, producing higher intracellular concentrations. Nieper combined potassium and magnesium orotates to treat cardiovascular disease.

The first International Symposium on Orotic Acid and Magnesium Orotate was held in november 1991 in Rudesheim, Germany (Williams, 1992). According to the studies of Williams (Williams et al, 1992) and Munsch (Munsch et al, 1992) performed on a rat model, the mechanism for the cardioprotective effect of orotic acid is consistent with the increase of the activity of all enzymes of the de novo pyrimidine pathway. Orotic acid stimulates the synthesis of pyrimidine bases in the heart, kidney and liver, by increasing the activities of uridine kinase and uridine phosphorylase, neither requiring phosphoribosyl-pyrophosphate (PRPP). These bases are transported by “salvage pathway” mechanisms from the kidney and liver to the heart for pyrimidine base and nucleoside synthesis, thus sparing the ischemic myocardium PRPP for the more needed ATP synthesis. In the failing heart, this mechanism seems to be crucial for maintaining the energy charge of the “high energy” adenylate compounds at an elevated level in the cytoplasm. This optimization of the phosphorylation state of the adenine nucleotides secures the energy metabolism of the stressed myocardium by “metabolic supplementation”.

The conclusions of the Hamburg symposium on magnesium orotate held in 1998 were more reluctant and stated that a number of studies indicate that orotic acid and its magnesium salt have a modest beneficial effect on the myocardium under conditions of stress and that further clinical testing is indicated to determine if the effects described could be of significant clinical benefit in the treatment of heart disease (Rosenfeldt, 1998).

MATERIALS AND METHODS

Aim of study. In patients with chronic heart failure, even on chronic cardiac glycoside, beta-blockers and associated antiarrythmic regimens, a worsening of their clinical condition is frequently due to supraventricular or ventricular arrythmias. In many cases these arrythmias prove to be life-threatening and are difficult to manage. Precipitating factors for arrythmia include associated potassium deficiency secondary to chronic diuretic therapy regimens, digitalis toxicity and ischemia.

Magnesium therapy has proven to be beneficial in counteracting all phases and processes of ischemic heart disease, from low-risk arrhythmias to sudden death.

Since orotate was proven to be efficient as transsarcoplasmatic carrier for Mg and also contributes to “metabolic supplementation” of the stresses myocardium, magnesium orotate seems to be the ideal oral form of administration of magnesium, in order to obtain maximal efficiency with minimal side effects. Reluctancy in the use of oral administration of other magnesium salts (oxide, carbonate) is linked to the laxative properties of these salts in higher doses, an effect completely absent in orotate.

The aim of the present study was to test the efficiency of magnesium orotate administered orally in ventricular arrythmias occuring in patients with heart failure.

Study Design The study was carried out on 23 selected patients (4F, 19M), mean age 56,98+/-11,29, with NYHA class heart failure II and III recruited over a period of 6 months in the Clinic of Cardiac Prevention and Rehabilitation of the Cardiology Institute Timisoara, Romania. Inclusion criteria were the presence of symptomatic ventricular arrythmias cls III-V Lown on standard ECGs and during 24 h Holter monitoring. Cardioactive medication was kept in all cases and included digoxin in 9 cases (39%), ACEI in 13 cases (56%), metoprolol in 18 cases (78%), amiodarone in 8 cases (35%), furosemide in 15 cases (63%) and spironolactone in 6 cases (26%).

All patients received magnesium orotate (Magnerot®) 500 bid for 14 - 28 days. Holter monitoring was repeated after a 20-day interval and results were compared to entry in each case.

RESULTS AND DISCUSSION

According to the required criteria at inclusion – presence of symptomatic ventricular arrhythmias, occurrence under cardioactive medication – the patients were classified as Lown IIIa – 8 patients, IIIb (bigeminy) – 3 patients, IV a (ventricular couplets) - 4 patients, IV b (unsustained ventricular tachycardia) – 6 patients, one with sustained ventricular tachycardia developed ventricular fibrilation and was successfully resuscitated, V (R/T phenomenon) – 2 patients.

The small number of patients in our study did not reach statistical significance as a specific group of arrhythmia occuring after cardiosurgery, therefore we present our results up to the date of the publication of this study in table 1, which analyses the type of arrhythmia, the associated cardioactive medication and the response to magnesium orotate. A larger study complete with statistical analysis will follow.

The results in the table show that ventricular arrhythmias occuring after cardiosurgery were considerably less severe after administration of magnesium orotate in most of the cases – 19 (82%), 3 cases did not respond and in 2 cases of extreme severity (Lown V) ventricular fibrillation occurred, successfully resuscitated in one case and fatal in the other (positions 20 and 21 in the table).

Magnesium orotate was very well tolerated in all cases, with no apparent side effects.

Table 1: Response to MAGNESIUM OROTATE 500 mg bid in 23 patients with ventricular arrhythmias after cardiosurgery

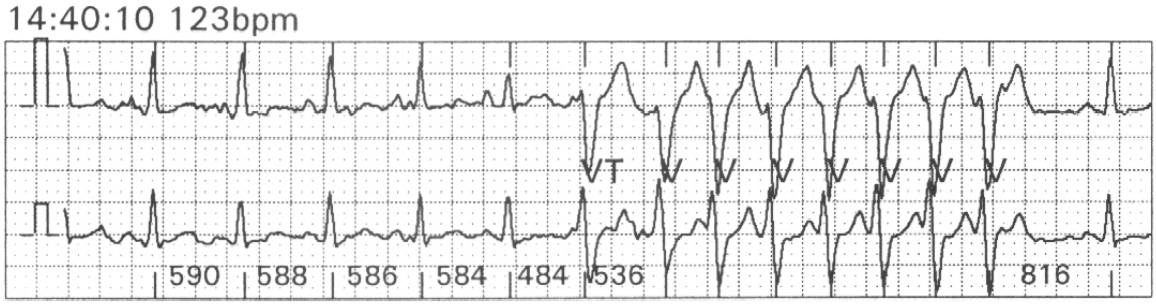
Nr.	Pt	Sex	Age	Medication	VA LOWN	Mg	VA LOWN res
1.	MT	M	75	ACEI, BB, DIG	IVa	500bid/4w	II/I
2.	CM	F	62	ACEI, BB	IVb	500bid/4w	I
3.	AA	M	71	ACEI, AMIO, DIG	IVb	500bid/2w	II
4.	BI	M	64	AMIO, BB	IIIa	500bid/4w	IIIa
5.	BM	M	72	ACEI, BB, DIG	IIIa	500bid/2w	I
6.	DN	M	70	AMIO,BB	IIIb	500bid/4w	II
7.	GD	M	46	AMIO,BB	IVb	500bid/2w	I
8.	PC	M	54	ACEI,BB	IIIa	500bid/2w	II
9.	MI	M	62	ACEI, AMIO, DIG	IIIa	500bid/2w	I
10.	MT	M	42	ACEI, AMIO,DIG	IVb	500bid/4w	IIIa
11.	HI	F	53	BB	IIIa	500bid/2w	IIIa

12.	AJ	M	49	BB	IIIb	500bid/2w	II
13.	HC	M	56	ACEI, AMIO	IVa	500bid/2w	II
14.	PP	F	52	BB	IIIb	500bid/2w	II
15.	CD	M	48	DIG,BB	IVb	500bid/4w	IIIa
16.	FS	M	73	ACEI, BB, DIG	IVb	500bid/2w	IIIb
17.	JC	M	68	BB	IIIb	500bid/2w	IIIb
18.	ZA	M	62	ACEI,DIG	IVa	500bid/2w	IIIb
19.	SE	F	49	BB	IIIa	500bid/2w	II
20.	GG	M	62	ACEI,AMIO,BB,DIG	V/VFIB res	500bid/4w	II
21.	PV	M	54	ACEI,BB	V	500bid/2w	VFIB, nonres
22.	GZ	M	61	BB	IIIa	500bid/2w	I
23.	DG	M	44	ACEI,BB	IVa	500bid/2w	II

Legend: ACEI= ACE inhibitor, BB=betablocker, AMIO=amiodarone, dig=digoxin
VFIB= ventricular fibrillation, CABG = coronary artery bypass graft
VES = ventricular extrasystoles, SVES = supraventricular extrasystoles
VA = ventricular arrhythmia

The favourable effect of magnesium orotate on ventricular arrhythmias is presented in the following case reports:

Fig 1a: GD (pos.7 in table 1), 46 yrs, male, 1 month after double CABG, on antiarrhythmic treatment with amiodarone 200mg/d + metoprolol 50 mg bid, presented symptomatic unsustained ventricular tachycardia (Lown IVb) and ventricular couplets (Lown IVa) on the 24h Holter monitoring.



Fg. 1a. Unsustained ventricular tachycardia, ventricular couplets

Fig 1b: The same patient after 14 days of treatment with magnesium orotate 500mg bid. Holter monitoring after 20 days. Rare VES, Lown I and SVES.

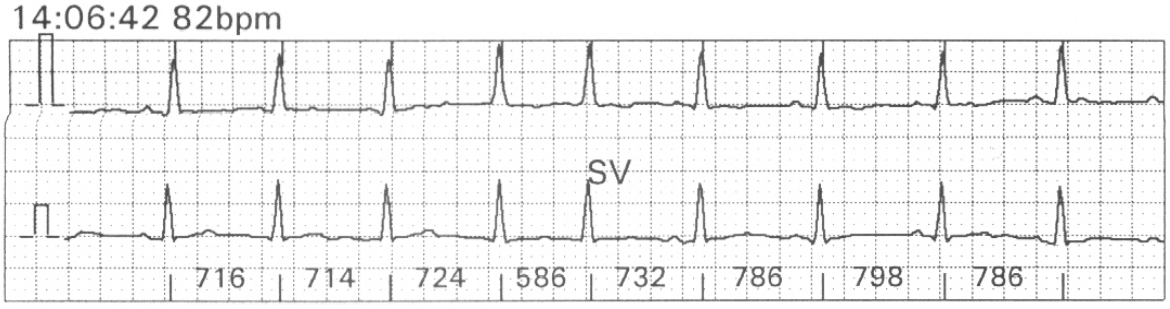


Fig. 1b. Sinus rhythm

Fig 2a: AA (pos. 3 in table 1), 71 yrs, male, 6 weeks after 3xCABG, on chronic treatment with enalapril 10mg bid, amiodarone 200/d, digoxin 0,25 mg/d, presented symptomatic unsustained ventricular tachycardia (Lown IVb) on the 24h Holter monitoring.

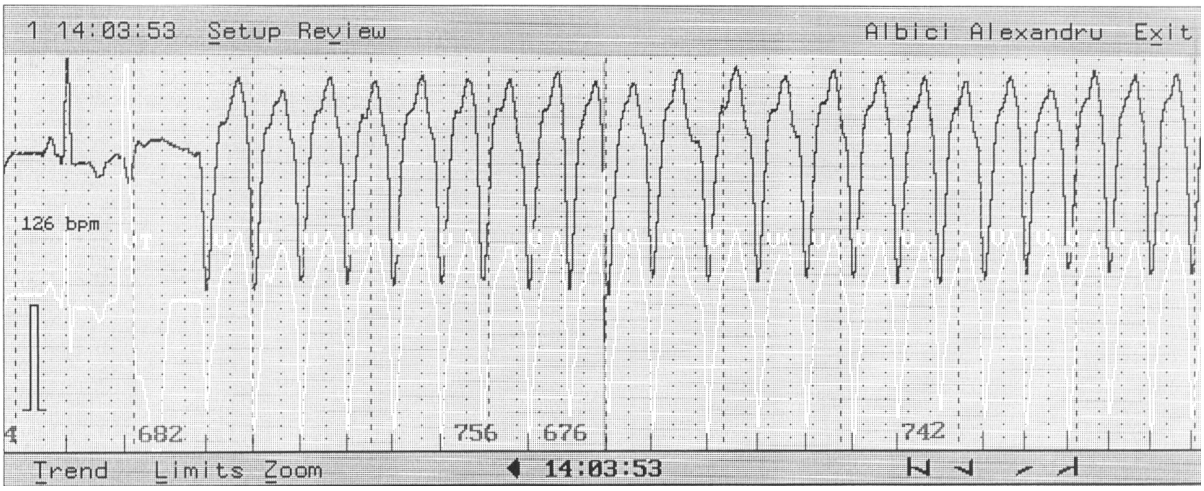


Fig. 2a. Unsustained ventricular tachycardia

Fig 2b: The same patient after 14 days of treatment with magnesium orotate 500mg bid. Holter monitoring after 20 days. Rare VES, Lown II. Example without VES.

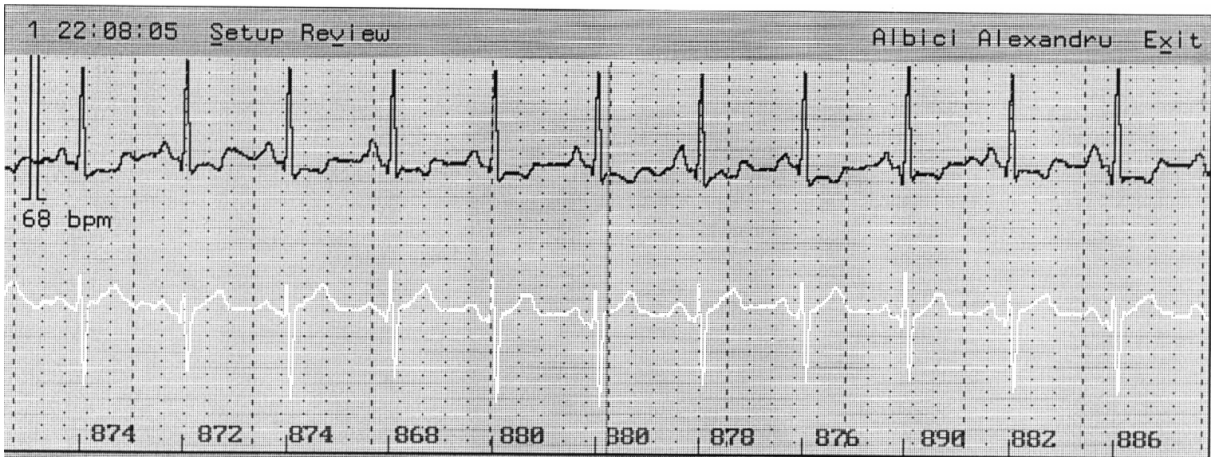


Fig. 2b. Sinus rhythm after treatment with Magnesium Orotate 500 mg bid for 10 days

Fig 3a: MT (pos. 1 in table 1), 75 yrs, male, 4 weeks after 3x CABG, on chronic treatment with enalapril 10mg bid, metoprolol 50 mgbid, digoxin 0,25/d, presented atrial fibrillation and ventricular couplets (Lown IVa) on the 24h Holter monitoring.

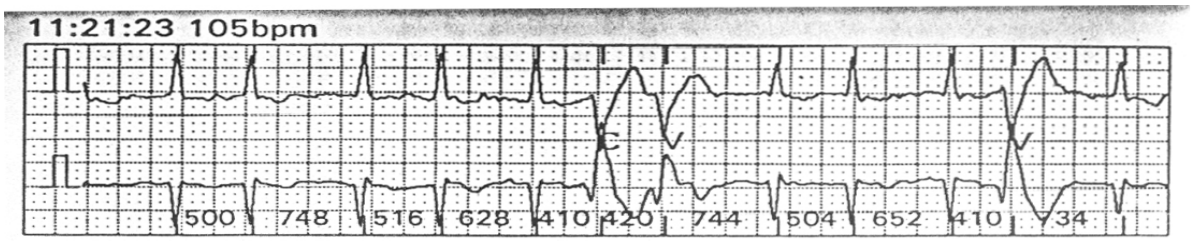


Fig. 3a. Atrial fibrillation. Ventricular couplets

Fig 3b: The same patient after 20 days of treatment with magnesium orotate 500mg bid. Holter monitoring after 20 days. Atrial fibrillation. Rare VES, Lown II/I

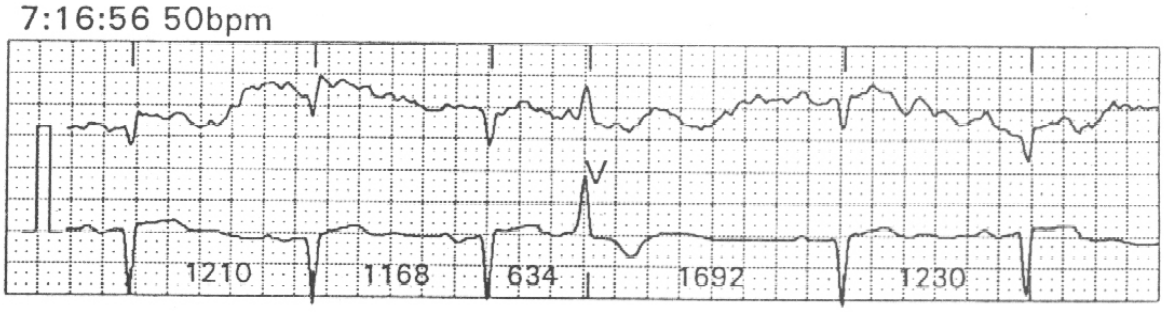


Fig. 3b. Atrial fibrillation. Rare VES

Oral supplementation Although most of the studies regarding the benefit of magnesium therapy were conducted with intravenous supplementation of magnesium sulphate, clinical evidence is growing that oral supplementations may be as efficient as intravenous use in chronic patients. Most clinicians seem reluctant to administer higher than daily recommended doses because of the undesirable laxative side effect of Mg oxide and carbonate. However, this is not the case of Mg orotate, a very well tolerated preparation, which offers the advantage of orotic acid supplementation with all the benefits derived from the key function it holds in regulation of energetic metabolism. The purpose of the present study was to test the efficiency of magnesium orotate administered orally in ventricular arrythmias occuring in patients after cardiosurgery.

Favourable effects of oral magnesium orotate on exercise tolerance and left ventricular function have been reported by Geiss et al in a pilot study on 14 CAD patients, active participants in an ambulatory rehabilitation program. Magnesium orotate decreased significantly LVESV and increased significantly EF and exercise duration (Geiss et al, 1998).

Shechter et al performed a randomised, placebo-controlled trial on 50 CAD patients to test the efficacy of oral magnesium supplementation in reducing endothelial dysfunction and improving exercise tolerance. They measured tissue magnesium levels in scraps of sublingual endothelial cells, found to correlate well with levels found in heart tissue. 72% of patients had lower than normal Mg tissue levels, and were randomised to receive either placebo or 365 mg of Mg oxide or carbonate daily. After 6 months, endothelial function and exercise duration were significantly better in the magnesium group, compared to the placebo group. It was also highly significant that none of the patients in the magnesium group experienced any arrhythmia during exercise. The authors conclude that magnesium may protect the heart against the detrimental effects of calcium overload and improve intracellular ATP production and glucose use (Schechter et al, 2000).

Serum levels Many investigators agree that normal serum levels of magnesium could still be associated with low tissue stores responsible for clinical effects. In our study, 12 patients had low Mg levels, 6 had normal levels and 5 had levels higher than 2,1 mmol, while calcium levels were normal in 19 cases and low in 4 cases.

Concomitant magnesium deficiency in K-depleted patients was reported to range from 38% to 42% (Whang et al, 1992). Uncorrected magnesium deficiency

impairs repletion of cellular potassium, a condition reffered to as refractory potassium depletion. According to Whang et al, refractory K repletion as a consequence of Mg deficiency may be operative in patients with congestive heart failure, digitalis toxicity, cisplatin therapy and in patients receiving potent loop diuretics. Therefore they recommend that serum Mg be assessed routinely and hypokaliemic patients be treated with both Mg as well as K to avoid the problem of refractory K depletion due to coexisting Mg deficiency (Whang et al, 1992).

In the ischemic myocardium, cellular calcium overloading is a major factor in the pathogenesis of arrhythmias and cell death. Magnesium ion is accepted as a natural calcium antagonist (Ziskoven, 1989). Mg deficiency reverses the optimal intracellular Ca:Mg ratio, the excess calcium becoming toxic to the cell.In response to high calcium levels in the ischemic myocardium, calmodulin, a calcium-sensing protein, binds to the tail end of the sodium channel protein causing a malfunction of these channels and consequently irregular cardiac activity (Balser, 1999).

Gottlieb et al have studied the prognostic significance of an abnormal serum magnesium concentration in 199 patients with heart failure. The serum magnesium concentration was less than 1.6 mEq/l in 19% of patients, within normal range in 67% of patients and greater than 2.1 mEq/l in 14% of patients. Patients with low serum magnesium concentration had more frequent ventricular premature complexes and episodes of ventricular tachycardia than did patients with a normal serum concentration. Patients with hypermagnesemia had more severe symptoms, worse renal function, but fewer ventricular arrythmias. The authors recommend routine measurement of serum magnesium concentration in patients with chronic heart failure (Gottlieb et al, 1990)

Sueta et al have found that the risk of developing potentially fatal ventricular arrythmias was reduced by more than half in patients with ischemic heart failure who received large intravenous doses of magnesium, 0.3 mEq/kg injection followed by continuous infusion of 0.08 mEq/kg/h over 24 h . They conclude that more studies are needed for establishing to what extent oral supplements of magnesium can be as efficient as intravenous doses (Sueta et al, 1994).

Investigators in Baltimore, Maryland report that an intravenous infusion of 2 grams of magnesium chloride in patients having undergone coronary bypass surgery significanly lowers the incidence of severe ventricular arrythmias (J Am Assoc, 1992).

CONCLUSIONS

The numerous studies presented above lead to the conclusion that research in the field of magnesium and its role in cardiology are far from being exhausted. On the contary, they open the way to further research in the field of mechanisms of ventricular arrhythmias and should be completed by experimental studies and studies on cultures of human cardiomyocytes. Our small study confirms the favorable effect of oral magnesium orotate in the control of ventricular arrhythmias occuring in a subgroup of patients difficult to control, on chronic antiarrhythmic and cardioactive treatment after cardiosurgery.

In the near future of magnesium orotate research we will have to focus on following questions, in the attempt to find the answers:

1. Is orotate only efficient as transsarcoplasmatic carrier for Mg, while the increase of intracellular levels of Mg and consequent decrease of Ca is responsible for the antiarrhythmic effect?

2. Since orotic acid has been shown in several studies to have a protective effect on recently infarcted myocardium and is a key intermediate in the biosynthetic pathway of pyrimidines via respiratory-chain coupled DHODH (mitocondrially-bound dihydroorotate dehydrogenase), is the antiarrhythmic effect only due to metabolic supplimentation ?
3. Are both magnesium and orotate responsible for the effect, taking into consideration the previous questions?

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ASPECTS OF ESTABLISHING SOME MEASURES TO RECLAIM SOILS POLLUTED WITH HEAVY METALS IN COPŞA MICĂ

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ABSTRACT

This paper presents some results of a field experiment organized to establish the measures for reclamation of soils polluted with heavy metals in the Cospa Mica area (Romania).

Due to the processing activities of nonferrous ores and the carbon black production undertaken in the Copsa Mica industry, the soil and vegetation in this area were strongly affected by the polluting emissions resulted in these technological processes.

The main pollutants identified in this area were: heavy metals (Pb, Cd, Cu, Zn), sulfur oxides and carbon black. The accumulated action of these pollutants led to an accentuated degradation of the agricultural and forestry ecosystems.

The reclamation of soil polluted with heavy metals is difficult action due to their long-term persistence in soil. The technologies to reclaim the polluted soil aiming at the elimination of the heavy metals are very expensive. In the case of the agricultural lands in the excessively polluted areas, a practicable solution is the application of some reclamation measures which presume to diminish the availability of heavy metals for plants.

The total form content values, determined in the topsoil, exceed the maximum allowable limits (Pb – 2.7 times; Cd – 2.5 times and Zn – 2.0 times). Also, the mobile form content values of these heavy metals show a high availability degree which favors their accumulation in plants. The liming (5, 10 t/ha), the application of manure (100 t/ha) and materials with capacity toretain the heavy metals (bentonite – 10 t/ha and zeolotie tuff – 20 t/ha) had different effects on the mobility of heavy metals in soil.The influence of applied treatments on heavy metal contents in corn leaves was also obvious.

Key words: industrial pollution, heavy metals, Copşa Mică

INTRODUCTION

Soil pollution with heavy metals in Romania is mainly due to the activities concerning the non-ferrous ore metallurgy. For instance, around the most important factory for processing the non-ferrous ores, Copşa Mică, the area with soils affected by heavy metal pollution stretches up to about 180000 ha of which 31285 ha were forest resources and 149 465 ha agricultural lands. The severe polluted area where at least one pollutant exceeds the maximum allowable limit (100 mg . kg^{-1} Pb, 100 mg . kg^{-1} Cu, 300 mg . kg^{-1} Zn and 3 mg . kg^{-1} Cd) was 21 875 ha, of which 3 245 ha were forest resources and 18 630 ha – agricultural lands (Dumitru et al., 1994).

The main pollutants identified in this area were: heavy metals (Pb, Cd, Cu and Zn), sulphur oxides and carbon black. The accumulated action of these pollutants led to an accentuated degradation of the agricultural and forestry ecosystems. The pollution of soils in the area influenced by the industrial emissions is manifested not only by the increase of the heavy metal contents, but also by changing of some important soil properties (Dumitru et al., 1995).

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The reclamation of soil polluted with heavy metals is difficult action due to long-term persistence in soil of these pollutants. The technologies to reclaim the polluted soils aiming at the elimination of the heavy metals are very expensive. In the case of the agricultural lands in the excessively polluted areas, a practicable solution is application of some reclamation measures that presume to diminish the availability of heavy metals for plants.

MATERIALS AND METHODS

Experiments were organized near Copşa Mică city in order to establish the possibilities to reduce the translocation of heavy metals from plant, in a pilot field.

The treatments applied included:

- amendments (5, 10 t $CaCO_3$. ha^{-1})
- organic fertilisers (100 t manure . ha^{-1})
- mineral fertilisation ($N_{100}P_{100}K_{100}$)
- zeolitic tuff (20 t . ha^{-1})

The effects of these treatments on both the mobile contents of heavy metals (Cu, Zn, Pb and Cd) and the yield maize and its chemical composition were studied.

Total contents of heavy metals (Cu, Zn, Pb and Cd) have been measured in hydrochloric solution (soil digestion in HNO_3-H_2SO_4-$HClO_4$ mixture, at 2:1:0:2 ratio) and content of mobile forms have been extracted by NH_4NO_3 and Na_2EDTA solution. All the heavy metals have been determined by atomic absorption spectrometry.

RESULTS AND DISCUSSIONS

Main soil chemical properties influencing the heavy metal solubility are soil reaction, organic content and cation exchange capacity. By changing these characteristics, the process of blocking the heavy metal in soil may be amplified, reducing therefore the heavy metals accumulation in plant.

Investigation data referring to limiting effects, soil characteristics and contents of havy metals as well as effects of mineral fertilization and bentonite and zoolitic tuff are shown in Table 1, Table 2 and Table 3.

Data concerning the heavy metal contents (total and mobile forma), determined to characterise the soil profile in the demonstrative field, show a high accumulation of these pollutants in the first soil layer (0 – 18 cm), followed by an accentuated decrease in the lower soil layers. The total form content values, determined in the topsoil, exceed the maximum allowable limits (Pb – 2.7 times; Cd – 2.5 times and Zn – 2.0 times). Also, the mobile form content values of these heavy metals show a high availability degree that favours their accumulation in plants.

The data obtained reveal the significant influence of liming on soluble content of Cd, Cu, Pb and Zn in soil and accumulation level of these heavy metals in maize plants (see Table 1).

The best result in reducing the concentration of heavy metals soluble forms in soil (extraction in NH_4NO_3) were obtained with cadmium, the values being reduced from 0.3 mg/kg (control – non limited treatment) to 0.11 mg/kg (treatment limited with 5t/ha $CaCO_3$), Zn (from 0.414 mg/kg to 0.211 mg/kg), Pb (from 0.217 mg/kg to 0.112 mg/kg) and Cu (from 0.137 mg/kg to 0.122 mg/kg). Soil liming (10 t/ha $CaCO_3$) decreased the accumulation of heavy metals in maize leaves, as compared with the control such as: Cd – from 4.32 mg/kg to 2.28 mg/kg; Pb – from 148 mg/kg to 38 mg'kg; Zn – from 360 mg'kg to 173 mg/kg; Cu – from 20.2 mg/kg to 12.6 mg/kg.

Table 1 Liming effects on maize yield, some soil chemical characteristics and contents of heavy metals in soil and maize plant (FAO demonstrative pilot field, Copşa Mică, 2000)

Item			F-test significance levels [x]	Without lime	5 t·ha^{-1} $CaCO_3$	10 t·ha^{-1} $CaCO_3$	LSD 5% Tukey
Grain yield		t·ha^{-1}	**	3.5 a [y]	3.9 b	4.6 c	0.2
pH			NS	8.10 a	8.13 a	8.15 a	0.14
Organic C		%	NS	0.92 a	0.94 a	0.89 a	0.11
Total N		%	NS	0.121 a	0.126 a	0.123 a	0.014
Available P		mg·kg^{-1}	NS	52 a	42 a	37 a	11
Available K		mg·kg^{-1}	NS	198 a	202 a	205 a	37
Cd in soil	total	mg·kg^{-1}	NS	8.5 a	9.3 a	8.4 a	1.4
	soluble (Na_2EDTA)	mg·kg^{-1}	**	5.5 a	5.1 ab	4.7 b	0.6
	soluble (NH_4NO_3)	mg·kg^{-1}	**	0.30 a	0.14 b	0.11 b	0.05
Cd in plant	leaves	mg·kg^{-1}	**	4.32 a	3.88 a	2.28 b	0.94
	grains	mg·kg^{-1}	**	0.232 a	0.146 b	0.056 c	0.034
Cu in soil	total	mg·kg^{-1}	NS	50 a	46 a	49 a	10
	soluble (Na_2EDTA)	mg·kg^{-1}	*	9.0 a	8.4 ab	8.0 b	0.9
	soluble (NH_4NO_3)	mg·kg^{-1}	**	0.137 a	0.119 b	0.112 b	0.014
Cu in plant	leaves	mg·kg^{-1}	**	20.2 a	16.9 a	12.6 b	3.5
	grains	mg·kg^{-1}	*	6.1 a	4.1 ab	3.3 b	2.4
Pb in soil	total	mg·kg^{-1}	NS	235 a	231 a	232 a	39
	soluble (Na_2EDTA)	mg·kg^{-1}	*	149 a	134 ab	125 b	18
	soluble (NH_4NO_3)	mg·kg^{-1}	**	0.217 a	0.128 b	0.118 b	0.043
Pb in plant	leaves	mg·kg^{-1}	**	148 a	113 b	38 c	23
	grains	mg·kg^{-1}	NS	1.3 a	1.6 a	2.2 a	1.0
Zn in soil	total	mg·kg^{-1}	NS	763 a	733 a	716 a	103
	soluble (Na_2EDTA)	mg·kg^{-1}	**	102 a	93 ab	75 b	18
	soluble (NH_4NO_3)	mg·kg^{-1}	**	0.414 a	0.362 a	0.211 b	0.093
Zn in plant	leaves	mg·kg^{-1}	**	360 a	250 b	173 c	47
	grains	mg·kg^{-1}	NS	41 a	39 a	37 a	5

[x] Not significant (NS, $p > 0.05$), significant at $p \leq 0.05$ (*), significant at $p \leq 0.01$ (**).
[y] Values within a raw followed by the same letter are not significantly different at the p=0.05 level (Tukey's honestly significant difference procedure).

Table 2 Effects of organic and mineral fertilisation on maize yield, some soil chemical characteristics and contents of heavy metals in soil and maize plant (FAO demonstrative pilot field, Copşa Mică, 2000)

Item			F-test significance levels [x]	Without fertilisers	$N_{100}P_{200}K_{100}$	Manure 100 $t.ha^{-1}$	$N_{100}P_{200}K_{100}$ & manure 100 $t.ha^{-1}$	LSD 5% Tukey
Grain yield		$t.ha^{-1}$	**	3.4 a [y]	3.9 b	4.2 bc	4.5 c	0.3
pH			**	8.23 a	8.13 ab	8.11 ab	7.98 b	0.16
Organic C		%	**	0.92 a	0.96 ab	1.09 bc	1.12 c	0.14
Total N		%	NS	0.120 a	0.128 a	0.137 a	0.132 a	0.021
Available P		$mg.kg^{-1}$	**	34 a	39 a	42 a	59 b	15
Available K		$mg.kg^{-1}$	*	148 a	214 ab	203 ab	243 b	73
Cd in soil	Total	$mg.kg^{-1}$	NS	9.3 a	8.8 a	8.8 a	9.4 a	1.8
	soluble (Na_2EDTA)	$mg.kg^{-1}$	NS	4.9 a	5.1 a	5.2 a	5.3 a	0.8
	soluble (NH_4NO_3)	$mg.kg^{-1}$	NS	0.17 a	0.20 a	0.17 a	0.20 a	0.07
Cd in plant	Leaves	$mg.kg^{-1}$	NS	3.38 a	3.81 a	3.38 a	3.40 a	1.21
	Grains	$mg.kg^{-1}$	NS	0.154 a	0.150 a	0.123 a	0.151 a	0.044
Cu in soil	Total	$mg.kg^{-1}$	NS	44 a	46 a	46 a	44 a	12
	soluble (Na_2EDTA)	$mg.kg^{-1}$	NS	8.7 a	8.5 a	8.2 a	8.6 a	1.2
	soluble (NH_4NO_3)	$mg.kg^{-1}$	**	0.136 a	0.120 ab	0.118 ab	0.105 b	0.018
Cu in plant	Leaves	$mg.kg^{-1}$	NS	17.5 a	17.2 a	16.2 a	15.5 a	4.5
	Grains	$mg.kg^{-1}$	NS	3.7 a	4.5 a	5.3 a	4.6 a	4.4
Pb in soil	Total	$mg.kg^{-1}$	NS	256 a	243 a	247 a	264 a	49
	soluble (Na_2EDTA)	$mg.kg^{-1}$	NS	139 a	135 a	130 a	131 a	23
	soluble (NH_4NO_3)	$mg.kg^{-1}$	**	0.189 a	0.162 ab	0.109 b	0.158 ab	0.055
Pb in plant	Leaves	$mg.kg^{-1}$	NS	103 a	111 a	88 a	96 a	29
	Grains	$mg.kg^{-1}$	NS	1.9 a	1.5 a	2.3 a	1.1 a	1.3
Zn in soil	Total	$mg.kg^{-1}$	NS	782 a	752 a	758 a	791 a	132
	soluble (Na_2EDTA)	$mg.kg^{-1}$	*	103 a	95 ab	77 b	84 ab	23
	soluble (NH_4NO_3)	$mg.kg^{-1}$	*	0.420 a	0.310 ab	0.306 ab	0.281 b	0.119
Zn in plant	Leaves	$mg.kg^{-1}$	NS	246 a	281 a	239 a	277 a	59
	Grains	$mg.kg^{-1}$	NS	41 a	40 a	37 a	37 a	6

[x] Not significant (NS, $p > 0.05$), significant at $p \leq 0.05$ (*), significant at $p \leq 0.01$ (**).

[y] Values within a raw followed by the same letter are not significantly different at the p=0.05 level (Tukey's honestly significant difference procedure).

Table 3 Effects of bentonite and zeolitic tuff on maize yield, some soil chemical characteristics and contents of heavy metals in soil and maize plant. (FAO demonstrative pilot field, Copşa Mică, 2000)

Item			F-test significance levels [x]	Without bentonite and zeolitic	Bentonite 10 t.ha^{-1}	Zeolitic tuff 20 t.ha^{-1}	Bentonite 10 t.ha^{-1} & zeolitic tuff	LSD 5% Tukey
Grain yield		t.ha^{-1}	**	3.6 a [y]	4.0 b	4.1 bc	4.4 c	0.3
pH			*	8.01 a	8.08 ab	8.17 ab	8.18 b	0.16
Organic C		%	NS	1.05 a	1.02 a	1.04 a	0.97 a	0.14
Total N		%	NS	0.131 a	0.129 a	0.122 a	0.124 a	0.021
Available P		mg.kg^{-1}	NS	42 a	52 a	44 a	37 a	15
Available K		mg.kg^{-1}	NS	186 a	184 a	231 a	205 a	73
Cd in soil	Total	mg.kg^{-1}	NS	8.4 a	10.0 a	8.9 a	8.9 a	1.8
	soluble (Na_2EDTA)	mg.kg^{-1}	*	5.7 a	5.2 ab	5.0 ab	4.7 b	0.8
	soluble (NH_4NO_3)	mg.kg^{-1}	**	0.24 a	0.15 b	0.16 b	0.18 ab	0.07
Cd in plant	leaves	mg.kg^{-1}	NS	3.72 a	3.32 a	3.64 a	3.29 a	1.21
	grains	mg.kg^{-1}	NS	0.159 a	0.127 a	0.150 a	0.142 a	0.044
Cu in soil	Total	mg.kg^{-1}	NS	40 a	44 a	46 a	49 a	12
	Soluble (Na_2EDTA)	mg.kg^{-1}	**	9.3 a	8.6 ab	8.5 ab	7.5 b	1.2
	Soluble (NH_4NO_3)	mg.kg^{-1}	NS	0.128 a	0.120 a	0.124 a	0.119 a	0.018
Cu in plant	Leaves	mg.kg^{-1}	NS	16.7 a	16.6 a	17.3 a	15.8 a	4.5
	Grains	mg.kg^{-1}	NS	4.1 a	4.0 a	5.6 a	4.3 a	4.4
Pb in soil	Total	mg.kg^{-1}	NS	233 a	234 a	240 a	244 a	49
	Soluble (Na_2EDTA)	mg.kg^{-1}	**	153 a	139 ab	129 b	123 b	23
	Soluble (NH_4NO_3)	mg.kg^{-1}	NS	0.169 a	0.154 a	0.148 a	0.146 a	0.055
Pb in plant	Leaves	mg.kg^{-1}	NS	107 a	102 a	101 a	102 a	29
	Grains	mg.kg^{-1}	NS	2.0 a	1.8 a	1.5 a	1.6 a	1.3
Zn in soil	Total	mg.kg^{-1}	NS	724 a	804 a	760 a	796 a	132
	Soluble (Na_2EDTA)	mg.kg^{-1}	**	113 a	85 b	83 b	79 b	23
	Soluble (NH_4NO_3)	mg.kg^{-1}	NS	0.351 a	0.340 a	0.329 a	0.297 a	0.119
Zn in plant	Leaves	mg.kg^{-1}	NS	261 a	267 a	267 a	249 a	59
	Grains	mg.kg^{-1}	NS	41 a	38 a	37 a	39 a	6

[x] Not significant (NS, $p > 0.05$), significant at $p \leq 0.05$ (*), significant at $p \leq 0.01$ (**).

[y] Values within a raw followed by the same letter are not significantly different at the p=0.05 level (Tukey's honestly significant difference procedure).

The liming significantly decreased the Cd and Cu content in maize grains, but the modification of Pb and Zn content in maize grain was not statistically significant. The Cd values in maize grains decreased four times (from 0.232 mg/kg to 0.056 mg/kg) and the Cu values decreased two times (from 6.1 to 3.3 mg/kg). So it may be appreciated that liming ensures fixation of heavy metals in soil (Cd, Cu, Pd, Zn), decreases their translocation into maize leaves and grains.

Data presented reveal the significant influence of fertilization system on the kernel maize yield and content of organic carbon and available phosphorus and potassium in soil (see Table 2). The organic fertilization decreased the content of Cu, Pb and Zn soluble forms in soil(extracted in NH_4NO_3 and Na_2EDTA). Statistically significant decreases of Cd, Cu, Zn and Pb content in plants due to the fertilization were not observed, excepting only a tendency to decrease the content of these elements. These treatments also improved some chemical properties of the soil (pH, content of organic carbon, available phosphorus and potassium).

The treatments with bentonite and zeolotic tuff decreased the content of Cd, Cu, Pb and Zn soluble forms in soil, but the modification of heavy metal concentration in maize leaves and grains were not statistically significant (see Table 3). Due to the loading of vegetation with heavy metals from solid deposition, the significant effects of these treatments on heavy metal content in plant were not observed.

CONCLUSIONS

Research carried out within a pilot field organized in Copşa Mică pointed out that translocation of heavy metals can be reduced by liming, organic fertilisation, mineral fertilisation and by applying bentonite and zeolitic tuff having in view that:

- Soil liming reduces the mobility of heavy metals and their phytotoxic effect, stimulates bacteria development and ensures soil buffering capacity;
- Organic fertilisation stimulates the plant growing, fixation of heavy metals by organo-mineral bindings and improve soil biological activity;
- Bentonite and zeolitic tuff increase the soil cation exchange capacity and fixation of heavy metals;
- Mineral fertilisation, especially phosphorus, stimulates the plant development, fix the heavy metals (especially Cd and Zn) and decreases their translocation into plants.

In severely polluted area, a particular structure of crops should be so maintained to reduce as much as possible the impact on human life. Thus, it is not advisable for such areas to cultivate vegetables and other plants that are used directly in human's food.

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EFFECT OF DEUTERIUM TESTED ON DROSOPHILA MELANOGASTER

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ABSTRACT

Adult fruitflies (Drosophila melanogaster) were fed with 1% sugar solutions made in water with different concentrations of deuterium (D) set by mixing water samples containing 28 ppm and 99.6% of D, respectively. 100-300 flies were used for each D concentration tested. The flies were kept at constant temperature for a week in glass jars, the sugar solutions were changed for fresh every day. The number of dead animals was counted daily. We observed that the number of dead flies increased parallely with the concentration of D (from 28 to 300 ppm). After a week's treatment, the surviving flies were killed (and preserved) by freezing at -20 ^{o}C, and their protein content, superoxide dismutase (SOD) activity, catalase activity, as well as the values of lipid peroxidation (LPO) and free •OH radicals were determined. As a function of D concentration, the protein content showed a minimum type curve, while the Zn-Cu-SOD (SOD_{total}) a maximum-type curve, with the extreme values at 150 ppm D in both cases. All the other characteristics monitored (LPO, •OH, catalase, Mn-SOD) decreased momotonously with the increasing D content. The death rate of flies at D concentrations different from the natural value was the highest at the beginning.

Key words: *Drosophila melanogaster,* deuterium, free radicals, superoxide dismutase, catalase

INTRODUCTION

Deuterium is an essential but potentially toxic “micro-element”(Galbács et al., 1996). Numerous reports were published on the biological effects of the increased deuterium content in water. However, the effects of deuterium concentration significantly lower than the natural value (150 ppm D) were studied mainly by the present authors (Somlyai et al., 1993; Kiss et al., 1996; Kiss et al, 1996). These studies showed that, similarly to the effect of high deuterium concentration (Cope et al., 1965), low concentration also inhibited the schizogenesis and the growth of plant seedlings and tumors (Kiss et al., 1996).

The effect on the *Drosophila* lethality and sterility of a medium with 10-20% D_2O content was studied by Hughes (Hughes et al., 1964) The increased deuterium concentration had only a slight effect on the lethality but the sterility was significantly enhanced (30-80%). This finding could be connected to the formation of free oxygen radicals.

Free oxygen radicals can be formed in all cells and tissues during the process of photosynthetic and mitochondrial electron transport. Under normal conditions, 96-98% of the oxygen in the mitochondria forms water and the remaining 2-4% is concerted into free radicals. The oxygen radicals play an important role in the activity of phagocytes (Mehdy, 1994), in the destruction of microorganisms. An excess of

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parts/compounds of the tissues which are not properly protected. According to Szántay (Szántay et al., 1993), the lipide peroxidation (membrane damage) is caused by the depletion of thiols and NAD while the decrease of ATP concentration is due to the inhibition of ATPase (Kotyk et al., 1990). The oxygen free radicals also exert a damaging effect on sperm (Günther et al., 1995). This can be one factor responsible for the enhanced sterility of *Drosophila* at higher D_2O concentration.

Starting out from the above observations, we studied the effect of various D containing aqueous media (28-300 ppm) on *Drosophila*. The present paper summarizes the results of these investigations.

MATERIALS AND METHODS

All of the fundamental reagents used were of analytical grade purity (Merck, Germany; Calbiochem, Switzerland; Reanal, Budapest). The different D containing solutions were prepared by the mixing of a 28 ppm D and a 99.98% D_2O containing distilled waters obtained from HYD Ltd. (Hungary).

Maintenance and treatment of Drosophila. Adult flies of the Canton S wild type strain were used for the experiments. These flies were fed with 1% sugar solution made with distilled water containing 28, 150 and 300 ppm D. A 3 ml plastic vial was filled with the sugar solution and closed bubble-free with a tight cotton plug previously vetted with the same solution. In this way, the cotton remained always wet, so that the flies could drink the solution *ad libitum* but the liquid was not released. The vial was put into a horizontally laid milk bottle together with ca. 300 flies, which were previously anesthetized in carbon-dioxide. The container was then closed with a cotton plug and kept at a constant temperature selected in the range of 23-30°C. Daily the flies were anesthetized with CO_2, the dead one were removed and the total weight of the living organisms was determined with an accuracy of 0.1 mg. The living material was frozen at -20°C.

Sample preparation. The frozen flies were homogenized in a Potter homogenizer in 1:10 (w/w) of cold phosphate buffer (pH= 7.4). The homogenate was centrifuged at 2000 rpm, and the supernatant was used for most of the measurements. The LPO activity was determined from the raw homogenate before centrifugation.

Superoxide dismutase. SOD (EC 1.15.1.1.) activity was measured according to Misra and Fridovich (1972). Briefly, the enzyme inhibits the autocatalytic transformation of adrenalin into adrenochrom at pH= 10.2 in normal atmosphere. The color intensity of samples containing aliquots of the supernatant was measured by spectrophotometry at 480 nm. In paralel, Mn- and Cu-Zn-SOD activities were determined in the following way: the Cu-Zn-SOD activity was blocked with KCN so that the Mn-SOD (mitochondrial enzyme) was measured. The difference of SOD_{total} and Mn-SOD activities gave Cu-Zn-SOD activity.

Lipid peroxidation. LPO means the indirect quantity of active oxygen radicals acting in the metabolism of the multiply unsaturated fatty acids. The LPO was measured by the method of Plancer (Plancer et al., 1966): the reaction of malone-dialdehyde (MDA) with thio-barbituric acid (TBA) yield a reddish-yellow complex measurable at 532 nm.

Hydroxyl free radicals were determined according to the method of Cheeseman (Cheeseman et al., 1988). The OH radicals react with 2-deoxy-D-ribose and the product forms a reddish-yelow complex with thio-barbituric acid at low pH and elevated temperature. Similarly to the MDA-TBA complex (see above), the absorbance can be measured at 532 nm.

Protein concentrations were determined by the method of Lowry (Lowry et al., 1951).

Catalase activity determination was based on monitoring the decomposition of hydrogen peroxide through the extinction decrease measured at 240 nm and 25°C in every minute for 10 minutes (Beers and Sizer, 1952). The catalase activities were obtained in Bergmeyer units (BU), which is the amount of catalase that decompose 1000 mg H_2O_2 during 1 min.

RESULTS AND DISCUSSIONS

A part of the experiments aimed at establishing the way the decay of *Drosophila* flies depends on the D content of the feeding solution. We daily counted the living animals. Figure 1-3. show these numbers as a function of days passed.

If we connect the starting and ending points of the curves at each graph with a straight line, then the slope of this line gives the average rate of decay (DR). This value increases with the increase of the D content:

$DR_{28\ ppm\ D}$= 14.7 $DR_{150\ ppm\ D}$= 19.8 $DR_{300\ ppm\ D}$= 24.8

It is apparent from the figures, that the numbers of living organisms fed with sugar solution of unnatural D content always fall below the straight line, while the numbers for 150 ppm D containing solutions fall above the line. This suggests, that in the beginning the flies adapt to the change in the deuterium content uneasily, but after two days they manage the adaptation.

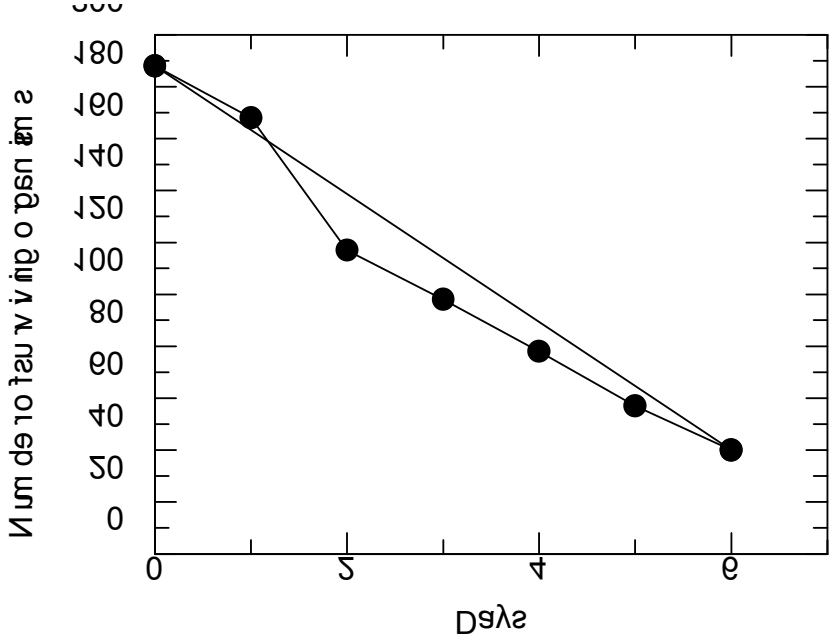

Fig. 1. Number of surviving *Drosophila melanogaster* flies as a function of the days passed. Deuterium content of sugar solution is 28 ppm, temperature 30°C

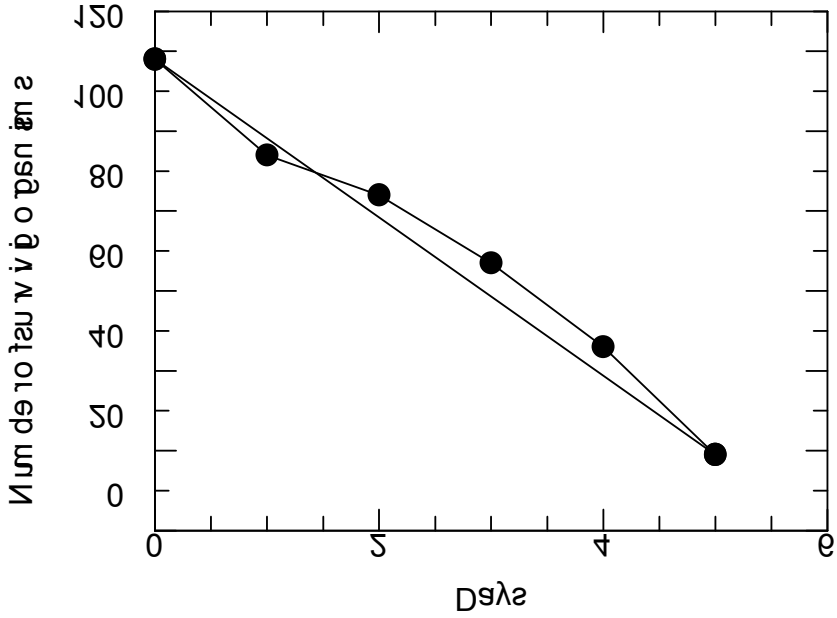

Fig. 2. Number of surviving *Drosophila melanogaster* as a function of the days passed. Deuterium content of sugar solution is 150 ppm, temperature 30°C.

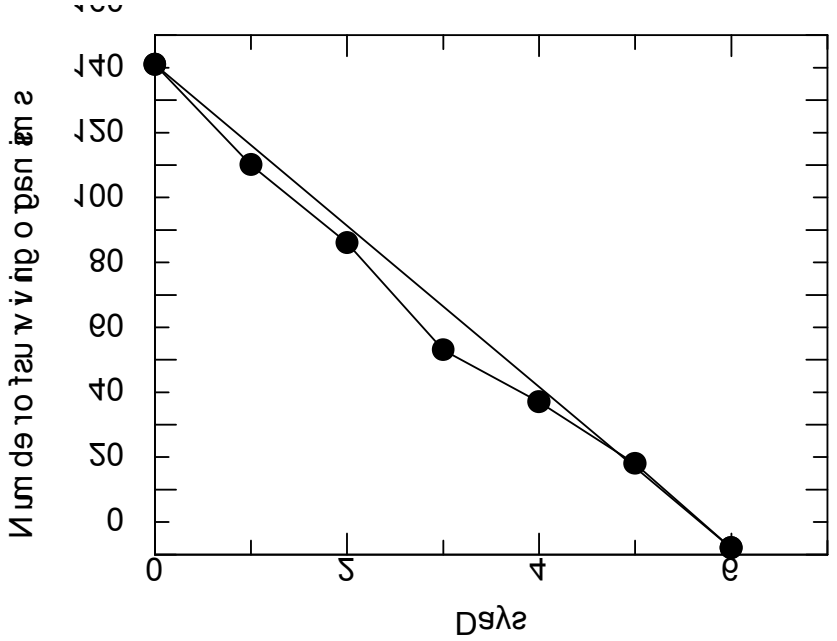

Fig. 3. Number of surviving *Drosophila melanogaster* flies as a function of the days passed.Deuterium content of sugar solution is 300 ppm, temperature 30°C.

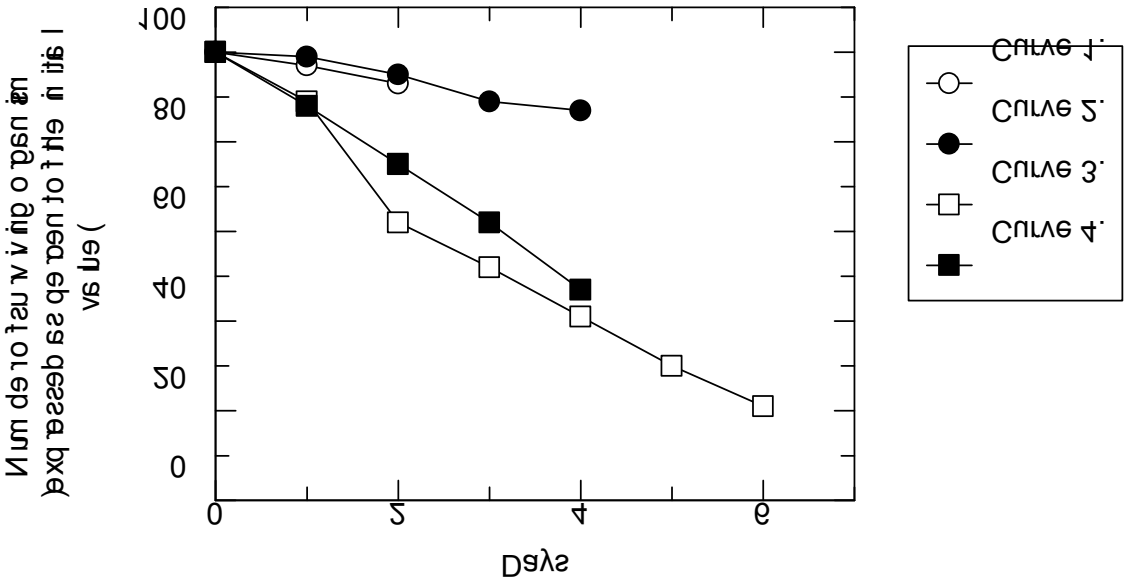

Fig. 4. Percent survival rate of Drosophila flies at different temperatures. Deuterium content of sugar solution is 28 ppm. *Curve 1.)* at 23°C, *Curve 2.)* at 23°C, *Curve 3.)* at 30°C, *Curve 4.)* at 30°C.

In other experiments, we intended to check reproducibility and temperature dependency. The results from these experiments are displayed in Figure 4. It can be seen, that the reproducibility is fairly good even for experiments conducted after many weeks. At the same time, it is apparent, that the rate of decay is strongly influenced by the temperature:

$$2.5 \cdot DR_{23°C} \cong DR_{30°C}$$

After 3 days of feeding, we determined some biochemical parameters of *Drosophila* flies (groups of ca. 300 organisms each). The results are shown in Table 1.

The data of this table clearly reflects, that the D content of feeding solution significantly changes the biochemical processes. The increase in D concentration causes the protein content to change according to a minimum type curve. At the same time, the SOD_{total} and Zn-Cu-SOD values follow a maximum type curve. All of these curves have their extremities at 150 ppm D. Other measured biochemical parameters (LPO, OH concentration, catalase, Mn-SOD) show a monotonous decrease with increasing D content.

Table 1. The effect of the deuterium-depleted water on the redox processes and parameters of adult Drosophila flies

Component measured	[D] 25 ppm	[D] 150 ppm	[D] 300ppm
Protein mg/g	34.91 ±0.86 n=3	30.2 ±0.49 n=3	33.92 ±1.86 n=3
LPO nM MDA/mg protein	3.69 n=1	3.32 n=1	2.80 n=1
•OH nM MDA/mg protein	0.885 ±0.05 n=3	0.700 ±0.054 n=2	0.551 ±0.07 n=2
Catalase (E/mg protein)x10^{-2}	0.33 ±0.029 n=3	0.26 ±0.01 n=3	0.19 ±0.01 n=3
SOD_{total} E/mg protein	8.396 ±0.174 n=3	9.423 ±1.63 n=3	6.319 ±0.313 n=3
Mn-SOD E/mg protein	0.436 ±0.049 n=3	0.362 ±0.061 n=2	0.131 ±0.043 n=2
Zn-Cu-SOD E/mg protein	7.96 ±0.132 n=3	9.04 ±1.54 n=2	6.17 ±0.28 n=2

CONCLUSIONS

Our experiments showed that the deuterium content of the feeding solution significantly influenced the biochemical processes of *Drosophila melanogaster*. The D content also had a significant influence on the redox processes. This effect is indirect and manifold. On one hand, the incorporation of deuterium into a compound increases the activation energy of the chemical processes involving that compound, therefore slowing down the biochemical processes (Simon and Palm, 1966). On the other hand, our measurements showed that the LPO value and the concentration of the •OH free radicals are the highest at 28 ppm D, and decrease with the increasing D content. The possible cause of this is that the production of •OH and LPO decreases with the increasing deuterium concentration/deuteration (Rys and Wang, 1992). Deuteration takes place already at room temperature at elevated D concentrations (Sacchi and Cocucci, 1992). Therefore, the decreasing amount of free radicals at higher D concentrations can not be caused by the enhanced effect of catalase and SOD because their values remain nearly the same at the tested deuterium concentrations.

Looking at the survival rates of the flies, we do not see dramatic differences between the different D concentrations but again, there is a definite tendency in the sense that the survival rate decreases with the increase in the D concentration. The energy production (ATP synthesis) in Drosophila likely decreases at higher degrees of deuteration (D content) (Simon and Palm, 1966; Sacchi and Cocucci, 1992). This can be a further cause of the enhanced decay rate of the flies.

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DEOXYRIBONUCLEIC ACID INTERACTION WITH CIS-PLATINUM: EVALUATIVE MODALITIES OF BIOCHEMICAL AND PHARMACOLOGICAL MECHANISMS

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ABSTRACT

In cytostatic chemotherapy the problem of the interaction between deoxyribonucleic acid (DNA) and the chimiotherapeutic drug (e.g. cyclophosphamide, vinblastine, cis-platinum etc.), reveals an outstanding importance. This is due to the fact that the limitation of the proliferative process is conditioned by the way the molecular fragment of the drug – the pharmacologic active group, binds to the DNA macromolecule.

In the case of cis-platinum (i.e. cis-diamminedichloroplatinum, noted cDDP) the interaction can be estimated by testing in vivo its action on the DNA biosynthesis at laboratory animals. There also exists the possibility of in vitro experiments of the interaction in the molecular system cis-platinum / DNA.

Experiments in vitro reveal various binding modalities of cis-platinum to diverse nucleotides from the DNA macromolecule and even to the amino acids from the constitution of the proteic macromolecule.

Key words: mechanism of DNA interaction with cDDP

INTRODUCTION

The class of platinum coordination complexes was discovered during the investigations of the lectric field effects on bacterial growth. Cisplatinum has the chemical denomination cis-diamminedichloroplatinum (abbreviated as cDDP) is the most important inorganic coordination compound.

Research on the cytostatic activity of cisplatinum implies the knowledge of biochemical, pharmacokinetic and toxicological aspects. In the case of cDDP / DNA system, the chemical structure – biological activity relationship and the pharmacon-receptor interaction is evidenced.

Studies dealing with the interaction of cisplatinum with DNA are of importance for the inorganic biochemistry of the nucleic acids, for pharmacology and toxicology.

Cisplatinum forms crosslinks with the two strands of the double helix DNA, it does not react with the sugar-phosphate backbone, but only with the nucleobase pairs adenine-thymine (A-T) and especially guanine-cytosine (G-C). It reacts most strongly with the G-C rich regions and can be used, in gradient centrifugation, for characterizing the relative G-C/A-T content of DNA.

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The interaction of cDDP and DNA leads to the biogenesis of a cDDP-DNA type adduct. This interaction is very slowly reversible in vitro and it can be removed more rapidly in the cell by the action of DNA repair enzymes.

1. BIOCHEMICAL ASPECTS OF THE ACTION OF PLATINUM COMPOUNDS

1.1. Specific interactions for the biogenesis of adducts

Peculiarities of the chemical structure and of the relation chemical structure-biological activity in platinic compounds originate in their interactions with the DNA biomacromolecule. These can be expressed as follows: the complexes exchange only some of their ligands during the reactions with biological molecules; the active forms may become charged after ligand exchanges; cis-monodentate or bidentate leaving groups are required as excheangeable ligands, corresponding trans-isomers are generally inactive; at a high reactivity, the cisplatinum reacts immediately with blood constituents and never acceeds to the tumor cells, while at a reduced reactivity it enters the cells but with no effect.

Cisplatinum (cDDP), as an anticancer drug, forms a variety of cDDP-DNA type adducts with covalent bondings.The consequences of the biochemical injury of DNA are mediated by proteins which bind to cDDP-DNA links or influence the cellular pathways as a response to the genotoxic stress. The adducts affect DNA repair, transcription, cell cycle responses.

Platinum binds at the N_7 position of purines forming 1,2-d(GpG) and 1,2-d(ApG) intrastrand crosslinks (in 90% of the adducts), 1,3-d(GpNpG) intrastrand, interstand and protein-DNA crosslinks.

Information provided by X-ray crystallography and NMR spectroscopy show that platinum induces a roll of 26-50° between guanine bases, platinum is displaced from the planes of guanine rings, the helix axes bends toward the major groove. The platinum atom lies in the minore groove, complementary cytosines are extrahelical, distorsions that unwind the duplex by 76-80°.Unusual conformations at the cDDP-DNA adducts sites show that these ones can serve as recognition elements for the proteins that process damaged DNA.These proteins can modulate cell sensitivity to the drug participating on various biochemical pathways. These aspects are presented in fig. 1 after Zamble and Lippard (1999).

The action of such proteins can determine a cell to repair the biochemical injury or can activate an irreversible program of cell death. It is most likely that these proteins contribute at the anticancer activity of this drug, being involved in the mechanisms of action and resistance.

1.2. Interaction with DNA: excision and repair

Natural substances as well as artificially introduced substances are some sources of DNA biochemical injury. In order to limit genetic mutations and to prevent malignant transformations derived from exposure to those agents, there are a lot of defence mechanisms of cells which remove DNA lesions and correct unwanted changes (Mathé and Muggia, 1908; Friedberg et al., 1995). One of the most versatile systems is nucleotide excision repair. By this means, more types of DNA injuries are excized as little, monochain oligonucleotide fragments and a new DNA is synthetised.

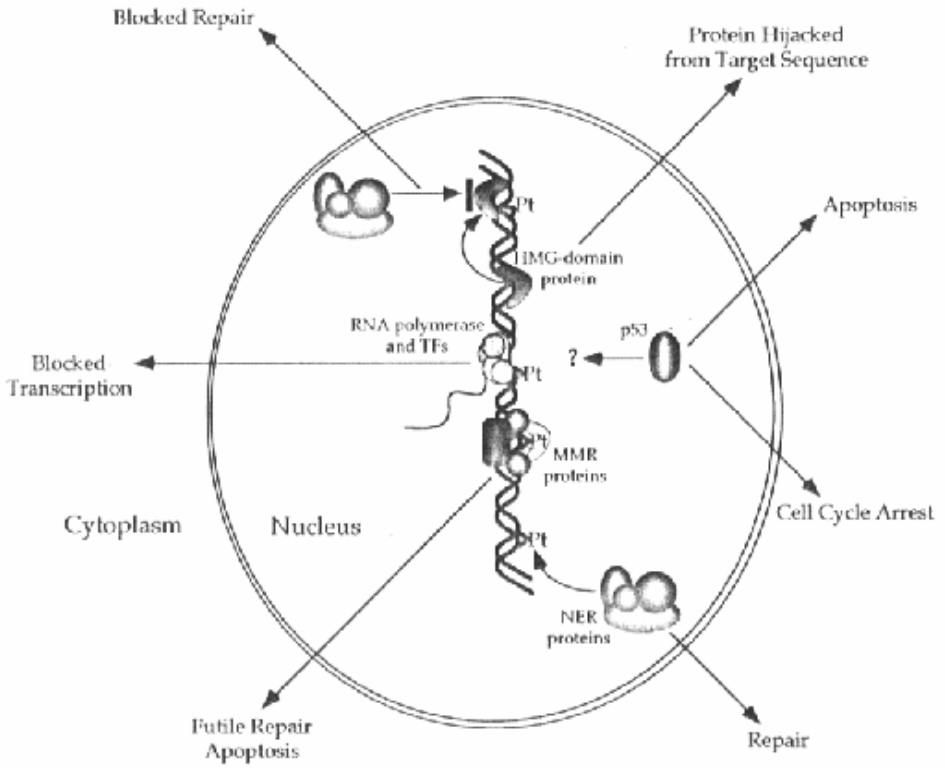

Fig. 2. *Effects of cisplatin-DNA adducts on some of the proteins in the nucleus that interact with the lesions*

Fig. 1. Effects of cisplatin-DNA adducts on some of the proteins in the nucleus that interact with the lesion (after Zamble and Lippard, 1999)

Low sensibility to cisplatin may be due to a replicative by-pass and not to excision repair. During time it was thought that antitumor activity of cisplatin is a consequence of some different cellular phenomena (Wu et al., 1992). DNA repair was investigated as a factor that can influence the sensibility of cisplatinum as long as the failure to replace DNA injuries permitted them to persist and to intefere with essential cellular systems. Trans-DDP have been inefficient because its adducts were more efficiently repaired than those of the cis isomer. Same time, trans-DDP forms predominantly monofunctional intrachain crosslinks that can be processed differently as intrachain adducts.

Atomic absorbtion spectroscopy studies and immunochemical techniques - used to demonstrate the relation between DNA adduct formation in blood cells and the response to disease - evidenced that building of adducts and their repair is the same in periferic and tumoral tissues. The levels of cDDP-DNA adducts are not always correlated with survival and can vary substantially between individuals.

2. MECHANISM OF ACTION OF PLATINUM COMPOUNDS

Interaction with cisplatinum. Cisplatinum is a well known distructive agent of DNA. The mechanism of action of the specific adducts is less known. Cell reparation can appear in each of the cell cycle phases (G_1, S or G_2). In case of failure, these

cells follow mithosis or apoptosis. Studies on apoptosis showed a lot of cell factors which decide over the cell's viability. One can mention the tumoral supressor p53, the family of proteins Bcl-2 and the intracellular pathways of transduction of the signals mediated by protein-kinases and phosphatidil-inositol-3-kinases.

In the case of cisplatinum appear interchain crosslinks with DNA and crosslinks between DNA-proteins. As a consequence the two complementary strands are not separated and the apparent molecular weight of denaturated monochain DNA increases.

The problem of the mechanism of action presented interest also because the formation of cDDP-DNA type adducts can be studied by computer (Holban, 2000). Research in this domaine can provide images of the spatial modifications of the nucleobases within DNA double chain. In such cases is made the evaluation of some steric parameters (linear and/or angular) which modify in the excision and biogenesis step of the adduct, but also in the circumstance of some DNA macromolecules repair.

The mono- and diadducts resulted in the reaction of cisplatinum with deoxyribonucleotides (dG-Pt; dG-Pt-dG) were used as cromatographic standard. In order to determine the structure of the adducts a radioactive analogue was used i.e. cisdichloro([3H]ethylendiamin)platinum (II) leading to the conclusion that certain DNA sites are preferentially modified and become saturated at a high saturation level. This analogue is an effective antitumoral agent in experimental models and produces DNA adducts on identical sites with cisplatinum.

Interaction with transplatinum. In the strategy of antigenes, oligonucleotides recognize specific sequences of double helix DNA containing sequences of homopurines. Research over oligonucleotides containing a single adduct trans-$[Pt(NH_3)_2(dG)Cl]^+$ or trans-$[Pt(NH_3)_2(dC)Cl]^+$ showed that the replacement of a weak acid proton N-H in a hydrogen bond, between two pairs of nucleobases and a metallic specia, and the binding between two pairs of nucleobases becomes much stronger.

Reacting with DNA, transplatinum forms monofunctional adducts which close in interchain links. One could correlate between clinical inefficiency of transplatinum and the long life time of monofunctional adducts combined with the great reactivity of glutathione. On the other hand, trans geometry compounds, such as iminoether, present in vivo antitumor activity against murine tumors. During in vitro reactions with DNA essential monofunctional adducts are formed which are less reactive with thioureea than monofunctional adducts of transplatinum.

Another remarkable aspect that could indirectly relate with cancer is the rearrangement of 1,3-intrastrand crosslinks in interstrand crosslinks. The adducts are stable in monochain oligonucleotides. In cells platinates oligonucleotides bind irreversible to target mRNA and inhibit the cell growth. Platinated oligonucleotides can also be used as "tools" in biotechnology.

3. ASPECTS REGARDING THE PHARMACOLOGICAL ACTION OF PLATINUM COMPOUNDS

3.1. Synoptic view on main platinum compounds

Specific differences between various types of tumors are influenced by the characteristics of cell pharmacology and of pharmacokynetic characteristics of drugs containing platinum (Cioga and Avram, 1978; Gârban et al., 1985; Lippert, 1999;

Readon ey al., 1999). Understanding of the pharmacology of these agents influenced the clinical use of platinum compounds.

Cisplatinum is administered in a chlorine solution for 0.5-2 hours. In order to prevent the risk of nephrotoxicity, pacients are prehydrated with at least 500 ml physiologic saline. Just before administering cisplatinum, manitol is injected parentherally to maximize the flux of urine and antiemetics. Cisplatinum can also be administered regionally to diminish secondary effects. The efficacity of administration in the peritoneal cavity is about 50 times higher than the intravenous one. In the case of hepatic tumors, melanoms or glioblastoms, intraarterial perfusion can be used, without considering it a standard method of treatment.

Cisplatinum nephrotoxicity was partially anhilated by introducing an aggressive hydration to prevent renal failure. Less toxic analogues were studied to avoid secondary effects as nausea, vomisment.

Secondary effects of cisplatinum (doses > 50 mg/m^2) include nausea, vomisment, nephrotoxicity, ototoxicity, neuropathia and mielosupression. In some cases appear arithmias, ischemic stroke, glucose intolerance and pancreatitis. Nephrotoxicity can be ameliorated by hydration, renal injury at the level of glomerules and tubules is cummulative, reason why serum creatinine is not longer a conclusive para,eter for the glomelular filtration rate.

As to ototoxicity, are recommneded audiograms after 2-3 cycles of treatment. Initially, acuity is lost at high frequencies (4000-8000 Hz). Peripheric neuropathy is cummulative, irreversible for a long period of time.

Carboplatinum is more simply to administer, extensive hydration is not neccesary because it does not present nephrotoxicity at standard doses.The duration of intravenous perfusion is over 30 minutes.

Carboplatinum is very toxic to platelets precursors, frequently appears neutropenia and anemia. The mimimum number of platelets can be observed after 17-21 days, and recovery appears in day 28. The effect depends on the dose but indivisual susceptibility varies in large limits. Nausea and vomisment are easy to control with standard antiemetics. Currently appears allopecia and the incidence of neurologic disturbances is cummulative.

Oxalilplatinum is clinically administered without complications, a short infusion of 500 ml, five times a day. There are mentioned studies on colonorectal cancer when oxalilplatinum was administered continously for 5 days, modifying the rate of dosage in order to observe the principles of chronopharmacologic administration. The maximum dose was injected at 4 p.m. The dependence of drugs activity on a certain time table needs supplementary studies.

Oxalilplatinum has a reduced nephrotoxicity. It produces short term and noncummulative neuropathies. Ototoxicity was not observed; mielosupression is not characteristic.

3.2. Pharmacodynamic peculiarities

Studies of clinical pharmacology on mild chimiotherapics in order to be used in therapeutics, must have in view the regression of the tumor, the extension of survival rate, of remission periods and/or slowing down of disease evolution.

Actual anticancer chimiotherapy with the available drugs can mark a significant increase of survival even healing in some generalised malignant diseases: acute lymphoblastic leukemia in children, coriocarcinoma, Hodgkin disease, testicular cancer. Good results (over 50%) are obtained in adults acute leukemia, chronic

leukemia, small cell lung cancer, ovarian cancer, sarcoma of soft tissues, neuroblastoma. In other malignant diseases – bronhial cancer, cervical cancer, gastrointestinal cancer, head and neck tumors, uterine cancer – the benefit is low and for a short period.

Chimiotherapeutics can be succcessfully used as an adjuvant modality of surgical inteventions or of radiotherapy. The effect of anticancer chimiotherapics is due to their action over cells biochemistry regarding nucleic acids and proteins. Cytotoxicity can be explained by: alteration of DNA preformed molecules and hindering or modification of DNA duplication, transcription and translation under the influence of alkylating agents or some antibiotics; interference of DNA, RNA and protein biosynthesis by antimetabolites.

At the beginning of anticancer treatment is followed the induction of remission by killing a great number of pathologic cells; further on the remission is consolidated and recurrencies can appear; during a maintenance treatment the immune, tumoral or cellular resistance can induce healing.

Usually, anticancer drugs are administered on systemic way, oral or parentheral. In some situations, when the localisation of the tumor allows it, intraarterial injections can be made. Administration schemes must take into onsideration pharmacological properties of the chimiotherapics used and the kinetic of cancer cells. For the great majority of anticancer drugs, the main limitative element at high doses is the supressive action on the hematogene marrow, with leucopenia, trombocitopenia and less anemia. Toxicity is manifested at the level of gastrointestinal mucosa, affecting the gonades etc.

The main target of platinum coordinative compounds is DNA. Platinated drugs contain electrostatic bonds and bind covalently to proteins. Interpretation of pharmacokinetic data is affected by biotranformation processes. Cisplatinum is metabolised at diverse aquated species which predominate in intracellular medium with low chlorine concentration. Platinum (IV) compounds are rapidly converted in plasma to platinum (II) derivatives, producing more distinct, circulating molecular species.

3.3. Pharmacokinetic peculiarities

Pharmacokinetic behaviour can influence the therapeutic potential of anticancer chimiotherapeutics. The great majority of anticancer substances are inactivated by metabolisation; in the case of cyclophosphamide active metabolites are formed.

Pharmacodynamic understanding of the action of platinum containing drugs can be approached investigating cellular pharmacology of these agents. Platinum compounds form numerous cDDP-DNA adducts and their repair in human cells is not easy to measure. Forming and repair of cDDP-DNA adducts were studied on human leucocytes.

The adducts from leucocytes can be quantified after 24 h from administration of 70-80 mg/m^2 cisplatinum. Maximum levels were registered at the end of perfusion, decreasing in next 18 h.

Drug kinetics interests absorbtion, distribution and purifying of the drug. These are determinant pharmacokinetic steps to obtain optimal therapeutic plasmatic and tissular concentrations.

Evaluation in time of concentration in various compartments of the body is made on some kinetic models important for correct administration in clinical conditions (pharmaceutic form, way of administration, dose, etc). The fluctuations of plasmatic concentration determined by each dose are proportional with the rate between half time and dose interval.Pharmacoreceptors are macromolecules able to bind specifically active substances with relative small molecule, forming complexes which “guide” some biological actions. A great number of receptors are proteins or nucleic acids; they can have enzymatic functions, can intervene as transporting mechanisms through membranes or represent important structural components for cell biology.

Receptors recognize in a specific manner the chemical signal presented by certain molecules due to the existence on the surface of the macromolecule of a binding site complementary with the drugs molecule.

Analysing the relation between chemical structure and biological activity of some drugs, there could have been established the characteristics for fixation, respectively for the way of action of receptors. The great majority of drug substances contain ionic groups and the pharmacoreceptors ionic surface groups; the two components attract electrostatically; ionic bindings consolidate the complex against thermal agitation.

Drugs that fix on receptors producing specific effects are of agonist type. Those that fix on receptors but do not action them are of antagonist type. A lot of drugs action over the cell membrane which they can influence directly or through some specific receptors resulting modifications on permeability, functionality of some membranar enzymes or desorganisation of the membrane.

Different intracell formations can be the place of action of some drug (or toxic) substances: the nucleus, rich in nucleic acids, is the target of some toxic substances. Alkylating agents form bridges on DNA chains hindering DNA synthesis and mithosis, implying the anticancer effect or in some cases cancerigene and mutagen effects; the ribosomes, the place of proteic synthesis, can be influenced by some drugs which inhibit formation of proteins or generate formation of anormal polypeptides; the mitochondria contains enzymes responsible for cell respiration, is the place where cyanides action; the lysosomes can be affected by hypoxia, acidosis or agressions generating inflamation.

Between the primary action of drug substances, at molecular level, and the appearance of global pharmacodynamic effects, appear numerous intermediary steps which affect cells, tissues, systems and organs, initiatory biochemical interactions.

Effects of the drug can be influenced by the interference of factors depending on the biological system such as weight, body dimensions, age, sex, physiologic state, as well as variables depending on conditions of administration. All these aspects can modify the pharmacokinetic profile and, in the same time, the pharmacodynamic one of the drug substances.

4.OVERVIEW ON ADDUCTS STRUCTURES

Among all platinum compounds the most active antitumoral agent was proved to be cis-platinum – characterized by a planar geometry, having two inert ligands ($2NH_3$) and two labile ligands ($2Cl^-$) which are released during the hydrolitic.

Hydrolisis, developed in two steps, implies the mono- or diaquated platinum species formation (fig.2). Both species can form bindings with the bases of DNA and in some cases with DNA and protein.. The bindings are made in the preferential order: guanine>adenine>cytosine, observing that thymine is not involved (Gârban et al., 1988).

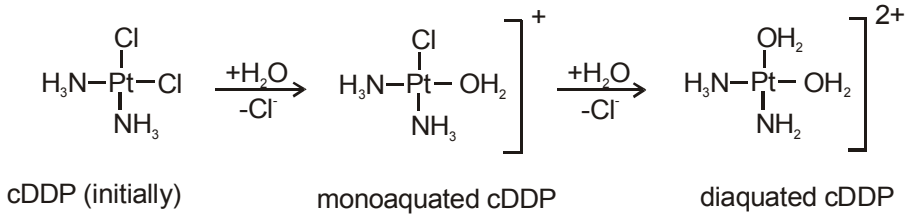

Fig. 2. Hydrolysis of cisplatinum (aquatation in steps)

Inside the cell, the neutral cisplatin molecule undergoes hydrolysis, in which a chlorine ligand is replaced by a molecule of water, generating a positively charged species. Hydrolysis occurs inside the cell due to a much lower concentration of chloride ion (~3-20 mM)—and therefore a higher concentration of water.

This hydrolysed product can bind to the spiral strands of DNA stopping the cancerous cells from growing.

In the cell membrane deactivation may be caused by the binding of platinum to intracellular thiols such as glutathione, thereby preventing the active hydrolysed species from reaching the DNA nucleus. It has also been seen that many cisplatin-resistant cells possess an enhanced capacity to remove platinum-induced adducts from their DNA.

Platinum(IV) complexes are very much slower in their hydrolysis reactions. This makes it unlikely for them to bind directly to cellular components such as DNA.

Cisplatin appears to enter cells by diffusion, the chloride ions are subsequently lost by hydrolysis, resulting in the formation of 2 active ligand sites.Interstrand and intrastrand cross linking of DNA then occurs in a manner similar to that produced by bi-functional alkylating agents.The primary binding site then appears to be the guanine base.The binding of platinum to complexes of DNA apparently disrupts and unwinds the double helix.

The strong preference of binding at the N7 site in purine bases may be attributed to the negative molecular electrostatic potential of the site.

At equilibrium, in the cell nucleus the chloro-hydroxo, aqua-hydroxo and dihydroxo complexes appear in comparable amounts for both isomers (cis- and trans)

The 1,2 intrastrand crosslinks are considered to play an important role in the anticancer activity of cisplatin, because they inhibit DNA replication and/or transcription.

Investigations on the DNA interaction with cis-platinum revealed the possibility of adducts formation which perturbs the secondary structure of the macromolecule. Stereochemical peculiarities of the binding to DNA imply the transition of macromolecule from B-DNA type (native state) to A-DNA and C-DNA. The conformational transitions facilitate the adducts formation. This interaction is

proceeded by the hydrolysis of cis-platinum: the two inert ligands (2 NH_3) are not modified, while the labile ligands (2 Cl^-) are released.

Thus mono- and diaquated platinum compounds can bind to bases of DNA or to DNA and protein. The binding may be homomacromolecular (DNA-cDDP) or heteromacromolecular (DNA-cDDP-Protein). As a result of the binding different adducts are formed by intercatenar or intracatenar cross-links, by monofunctionally binding and by DNA-cDDP-Protein cross-link (fig.3).

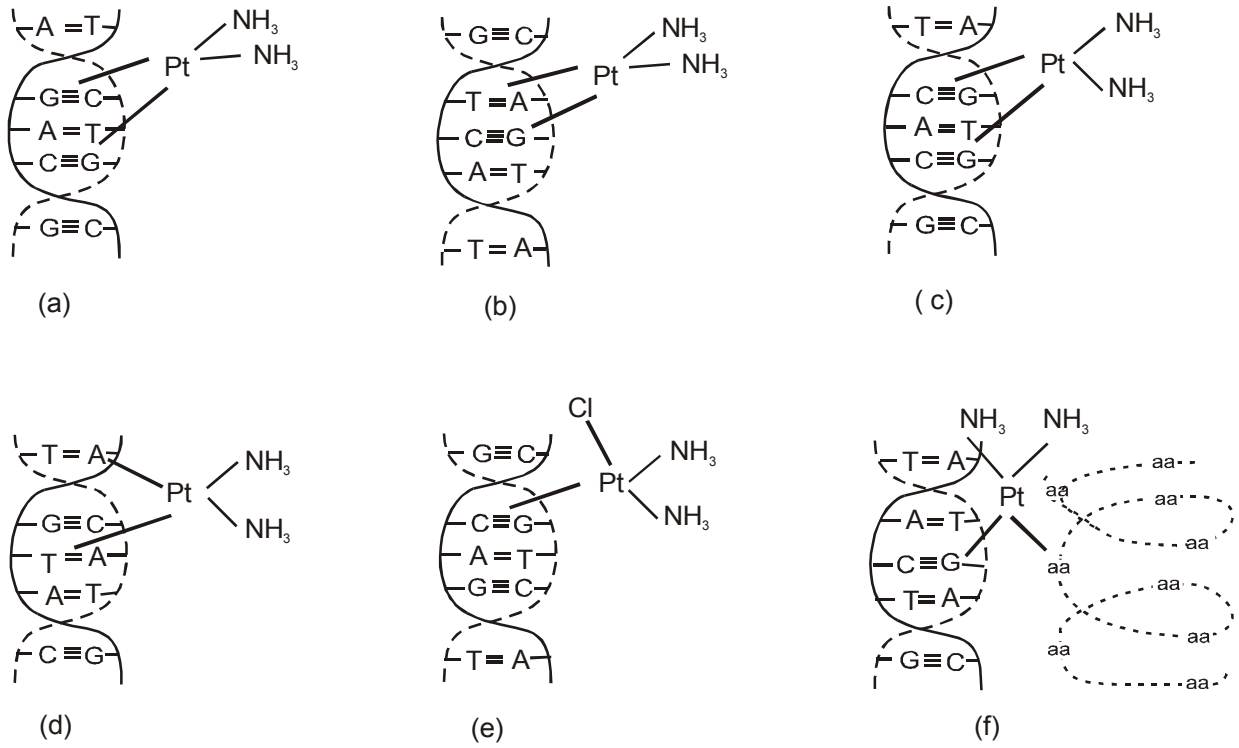

Fig. 3. Adducts of DNA with cisplatinum

Adducts of DNA with cisplatinum are of type: interstrand crosslink with identic nucleobases(a); interstrand crosslink with different nucleobases(b); intrastrand crosslink with identic nucleobases (c); intrastrand crosslink with different nucleobases (d); monofunctional binding (e); heteromacromolecular crosslink (DNA-cDDP-Protein) (f).

Forming of adducts attests the interaction between cisplatinum and DNA. Modern researches in this domains reveal that adducts' biogenesis can be monitored for bioanalytical investigations.

Adducts biogenesis is also possible in compounds with carcinogenic action, i.e. polycyclic aromatic hydrocarbons (PAH). Their quantum can be measured using performance liquid chromatography and mass spectrometry. Thus, it becomes possible to verify the modifications of DNA nucleobases.

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TOXIC METAL MICROELEMENTS (Cd, Pb, Ni and Cr) CONTENT IN THE SOILS OF BANAT

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ABSTRACT

The goal of the paper is to present the contents of total toxic metal microelements of the main types of soils in Banat, as well as 4 maps representing the distribution of this microelement in these soils.

Key words: toxic metal microelements content in soils, total forms.

INTRODUCTION

Soil liming and mineral fertilisers used in more and more quantities, reducing the weight of natural fertilisers in the total of nutritive elements, determines the necessity to know the total and mobile form of microelements from soil. The metal microelements from soil can bee natural source of nutrients to assure the nutrition of plants, on the contrary they can have a toxic effect.

Microelements are considered to be those chemical elements necessary for plants in very small quantities, of about 10^{-2} - 10^{-5} % related to dry substance. Excepting iron and manganese, which from geological respectively pedological point of view belong to the major elements, the other metal microelements (Cr, Mo, Co, Ni, Zn, Cd, V,) are found in soils in very small amounts (generally 0,0001%) being included in the group of minor elements (Bajescu et all 1984, Davidescu et al. 1988).

The total forms of metal microelements are present in the majority of the rocks of the earth crust in very small amounts. This fact makes difficult the laboratory analysis by classical methods. In roumanian literature are relatively little data about the abundance of these element in the soils of Roumania (Bajescu et al. 1984). Researches on metal microelements content as well as on other microelements, total and mobile forms, in soils from west part of Roumania (Banat county), started in 1978, by investigating the soils of the experimental fields of OSPA Timişoara, further on extended in Timiş, Arad and Caraş-Severin counties.

In this work is presented a review on the multitude of the obtained analytical data materialised in 4 maps of the repartition of the metal micro-elements in the soils of Banat.

MATERIALS AND METHODS

Metal microelements are difficult to determine directly by emission spectrography, being also necessary the extraction with solvent (Borlan and Hera, 1973). The most data referring to the abundance of these microelements were obtained by atomic absorption spectrometry methods, which suit best for routine determination (Jeffery, 1983).

The method by atomic absorption spectrometry in air-acetylene flame presents a sufficient sensibility for the direct determination in the acid solution resulted after mineralization and moving off of silica. It can be grown by concentration in steps,

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implying extraction with organic solvents of the complex (Edge et al, 1962, Gergen and Pusca, 1979).

Using electrothermic atomisation, the performances of the methods based on atomic absorption increases, but it implies more sophisticated apparatus. Passing of total microelements content from soil to solution, in the purpose of dosage, can be realised by (Jeffery 1983, Gergen et all 1985):

a) wet proceeding, which consists in soil disintegration at warm with a concentrated mineral acid or a mixture of mineral acids;

b) dry proceeding, which consists in the fusion of soil with a flux and the solubilisation of the fusion in a mineral acid;

c) combined proceeding, which consists in soil calcination and then the dissolving of the residue with a mixture of mineral acids.

In the analysis that were done we used the wet proceeding, with perchloric acid at warm, which presents the advantage that oxidases also the organic matter of the soils and makes insoluble, very good, the silica. Soil microelements, existing generally as salts absorbed by minerals and soils colloids or in the crystalline structure of some sulphoxidic minerals, is passed through the solution by soil digestion with perchloric acid 60%, at warm. At the soils with a superior humus content (> 5%) the previous oxidation with concentrated nitric acid is needed.

RESULTS AND DISCUSSIONS

To characterise the spreading of metal microelements in total forms there were analysed and interpreted over 100 soil samples, harvested from the most representative soils of west part of Roumania. The analytical results were statistically processed and mean value was used for interpretation and maps drawing.

Drawing the correlative maps of the repartition of metal microelements in total forms (figure 1) we made by extrapolation after the research on the over 100 analysed soil samples, which cover the most part of the soil types, of the relief and of the deposits of parental rocks from Banat. The graphic display on levels of metal microelements content was made by superposition of the geologic, soils, parental rocks, relief and climate maps.

Thus were created new areals characterised by analytical mean values. In the most cases these were explained through the interference of disintegration, alteration, migration and sedimentation processes with the bioaccumulative or eluvial-iluvial ones. For more data regarding the supplying states with microelements in mobile form, there were researched thoroughly, by analysis on agrochemical samples, some areals from two dominant pedoclimatic zones: the zone of low plane (Câmpia Torontalului) and the two zone of hills (Dealurile Lipovei and Bethausen) areal.

The rocks with metal microelements have a reduced resistance at mechanical disintegration and at chemical alteration. Metal compounds that result are carried through the solution and moved away from the origin zones (mountains and hills). A first modality of geogen accumulation of microelements in total form is realised in the depression basins (corridors, tectonic and erosional depressions) where the solutions of hypergene alteration mix with river waters, rich in calcium, accelerating the precipitation of salts (Janos 1994, Janos and Goian 1995).

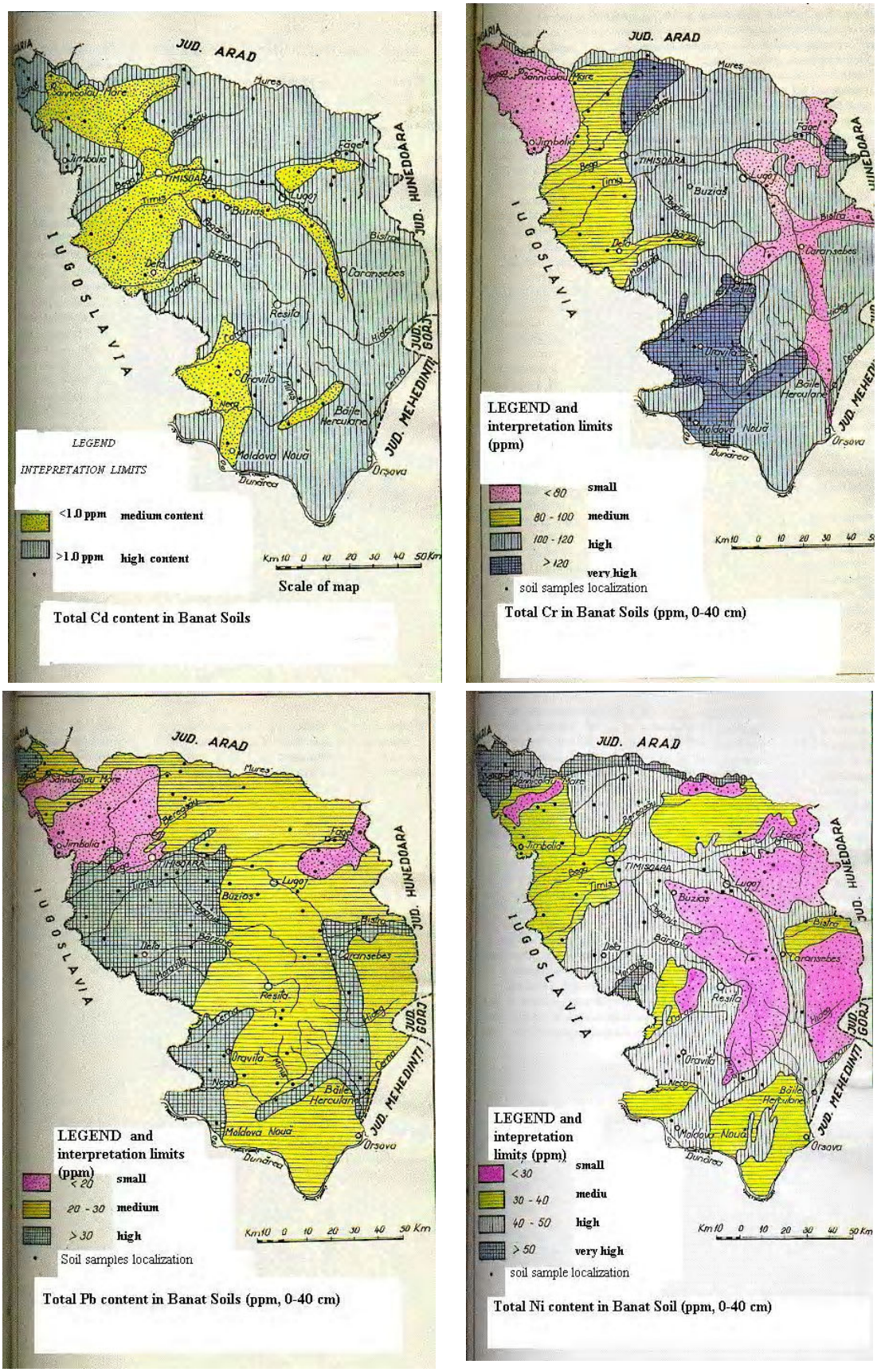

Figure 1. Map of toxic metal (Cd, Pb, Ni and Cr) in the soil of Banat.

In well-drained soils from the loess plane of Banat, microelements forms stable complexes with oxygen and calcium abundance maintains it enriching the sediment.

Researches made on the distribution of mobile microelements on profile indicate accumulations in the A horizon of all researched soils, with the tendency of decreasing of contents on profile at chernozem, vertisoils and reaccumulations in the B horizon at Illuvial brown clay soil or in the soils which suffer of excessive pluvial humidity depending on the intensity of eluviation. Generally, the eluvial horizons are very pored in these elements.

Lăcătuşu (1986,1987,1992) asserts that the humus, quantitatively as well as qualitatively, has a benefice role in metal microelements accumulations and adding organic fertilisers contributed to this accumulations. In this context it can be concluded that the little highest values of mobile microelements content in the low plane and in a part of the high plane can be associated with the greatest humus contents from these areals, generally occupied by chernosioms. There also exists a strong binding between microelement contents (mostly total form) and clay content, the clay soils, especially the verticsoil ones, having higher quantities of microelements.

CONCLUSIONS

After analysing more than 100 soil samples of the most representative soils of Banat, for maps were realised representing the distribution of metal microelements in total forms in that soils. The obtained maps have inherent limitations due to the work scale, the lack of some experimental data and the reduced volume of analysis and fulcrums. Anyway, they can direct the future researches, especially in the zones where the obtained values raise pollution or desequilibrium problems in the nutrition of plants.

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THE INFLUENCE OF SOME HEAVY METALS (Cd AND Pb) ON PLANTS

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ABSTRACT

The accessibility of heavy metals for plant depends of soil reaction; organic matter content, mineral colloids, soil humidity and microbiological activity. Organic matter content, especially humus compound can form organo-mettalic compounds with high mobility in soil solution and availability for plants. The essential metal microelements for plants: Fe, Mn, Cu, Zn, Mo can bee toxic in high quantities. Another metallic microelements Hg, Ag, Cd, Cr, Ni, Pb are very toxic for green plants. These metallic microelements can affect the biological and biochemical processes in plants: nutrition, photosynthesis and respiration, yield of crops. It were investigated many aspects of heavy metals influence over: a) the influence of Cd and Pb from soil and culture medium over the development of plant; b) the accumulation of Cd and Pb from soil and culture medium in the plant tissue; c) the influence of Cd and Pb over the nutrition elements; d) the influence of Cd and Pb over the protein synthesis; e) the influence of Cd and Pb over the photosynthesis; f) the influence of Cd and Pb over the respiration; g) the influence of Cd and Pb at cellular level; h) tolerance mechanism of plants at toxicity stress.

Key words: Cd and Pb – effects on plants.

INTRODUCTION

The access for plants of heavy metals is conditioned by the soil's reaction, by the content of the organic matter, especially by the organic compounds not so strong to humification, by the quantity and the nature of the mineral colloids, by the conditions of the soil's humidity and by the intensity of the activity of the microorganisms.

The organic matter has and important role in the restrained of the metals through the forming of the chelat complexes from which these have different degrees of access for plants (Lixandru et al., 1990, Maciejwska, 1997).

The essential heavy metals (Fe, Mn, Zn, Cu, and Mo) even they are indispensable for the growth and the development of the organismus, they are toxic in case of theirs accumulation in very big concentrations. The others heavy metals (Hg, Ag, Cd, Pb, Cr, Ni, Co) are toxic for plants, affecting a number of physiological and biochemical processes, as nutrition, photosynthesis breathing, growth, the development and the level of the harvests for the crop plants.

The toxic degree of the heavy metals has been estimated depending on the critical concentration to which the plants have a normal development (table 1).

It has been ascertained that Hg, Cr, Cd, Co, and Pb are the most toxic heavy metals for plants.

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Table 1: The critical concentration of the heavy metals for plants (Kastori, 1997).

Metal	Cr	Hg	Cd	Co	Pb	Cu	Ti	Ni	Zn	Mn	Fe
Critical conc. (μg/g s.u.)	1	2	5	10	10	15	20	20	150	150	200

DISCUSSIONS OVER THE INFLUENCE OF HEAVY METALS, Cd AND Pb FROM LITERATURE DATA

a) **The influence of Cadmium and Lead from soil or crop medium on the development of plants.**

Cadmium has destructive effects on germination and initial development of plants, even to very little concentrations (0,0001M). It has been ascertained an inhibition of the development of the roots and coleoptil and at the same time a subtraction of the percent of the dry substance (Kiss et al..,1997). Being analyzed the toxicity of cadmium on the whole cycle of life of the plants, it has been ascertained that the destructive effect appeared in the first stages of development, it blurs step by step in time (Gay et al.;1997).

The destructive effect of Lead on germination and the initial development of plants, it has been more pronounced in comparation with the destructive effect of cadmium when the concentration was 0,0001M.

Being analyzed 22 species of plants it has been ascertained a reduction of the production with 10% when the cadmium's content accumulated was about 4-200 μg/g dry substance (Macnicol and Beckett, 1995, quoted by Kastory, 1997).

b) **The influence of Cadmium and Lead from soil or crop medium on the content of metals in vegetable tissues.**

The presence of cadmium in crop medium reduces the content of calcium and zinc in plants while the content of aluminum and iron grows (Kiss et al.., 1997).

The presence of cadmium and lead in medium dukes to an accentuated accumulation in plants.

The accumulation is bigger in roots comparatively with the one from leafs, and grows at once with the growth of the metal concentration in the crop medium (Kiss et al.., 1997; Kastori, 1997).

c) **The influence of Cadmium and Lead on the transport of the nutritive elements.**

Heavy metals presented in the nutritive substratum can stimulate or inhibit the absorption of others elements. Also they affect the transport of the elements from root to the airy parts of the plants (Kastori, 1997).

d) **The influence of Cadmium and Lead on proteins biosynthesis.**

Heavy metals inhibit protein biosynthesis and favors hydrolysis. Also inhibit enzymatic activity which metabolized the nitrogen: e.g. : nitrate reductaze, glutamate dehidrogenaze, glutamin-sinthetaze (Kastori, 1997).

In the presence of some big concentrations of lead the nutritive value of the products is reduces because of the diminution of the crude proteins content, calcium and phosphorus (Avram and Medrea, 1944).

The plants which presents tolerance to the heavy metals possess the skill to synthesize one or more peptides rich in cistein which may fix the metals (Jackson et al., 1985).

It has been elucidated the structure of a group of peptides rich in cistein, able to tie the ions by the heavy metals through a co-ordination of thyolato type (Grill et al., 1985).

These peptides, called phytochelatins with general structure $[\gamma Glu\text{-}Cys]_n\text{-}Gly$ (n = 2-8) are induced by a various scale of metallic ions Cd^{2+}, Zn^{2+}, Pb^{2+}, Ag^{+}, AsO_4^{3-}. Such peptides comparatively with metalothyonins specifically to the mammals are more efficacious in the process of bundle of heavy metals ions.

e) **The influence of Cadmium and Lead on photosynthesis process.**

Heavy metals have a typical effect so on the photosynthesis process as on the content of pigments. The content of the photosystem I and II is reduced and are inhibit:

- Hill reaction, oxygen casualness, electron transport and oxidative phosphorilation of glucides and lipids. Also big concentrations of heavy metals inhibit the reactions of Calvin cycle to the C3 plants, while to the C4 plants is inhibit phosphoenol-piruvat phosphorilation (Kastori, 1997).

f) **The influence of Cadmium and Lead on breathing process.**

The presence of heavy metals in cytoplasm and mitochondria, inhibit the cellular breathing. In cytoplasm is inhibit activitatea the activity of glucose-oxidase and in mitochondria is inhibit the Krebbs cycle reactions (Kastori, 1997).

g) **The influence of Cadmium and Lead at the cellular level**.

Heavy metals reduce the plasticity, the resilience of the cellular walls and cellular hydration and often inhibit the cellular division. Very big concentrations of heavy metals can be mutagenic (Kastori, 1997).

h) **The mechanisms after which is manifested the tolerance of the plants at the heavy metals.**

The tolerance of the plants at the heavy metals depends on the species of the plant and also by the type of the metal.

The mechanism of the tolerance, depending on the place of action can be extern (apoplastic) or intern (symplastic).

The extern mechanism consists in the avoid of heavy metals penetration in the root cells, fixing them on the cellular wall, or through the creation on the roots surface, of some pH or redox barriers (Kastori, 1997).

The intern mechanism of tolerance of the plants at the heavy metals is manifested after theirs penetration in the interior of the cell. May be fixed in the time of transport through plasmalema, may be accumulated in vacuole, or may be tied through some chelats by phytochelatins (Grill et al..,1985; Kastori, 1997).

GENERAL CONCLUSSIONS

The tolerance of the plants at the heavy metals has a genetically base, being obtained in the time of the phylogenetic development. Because of the relation genotype – medium, it has been produced the adaptation to unfavorable condition.

The different behavior of the species in the presence of some big concentration of heavy metals is utilized in the creations of new genotype obtaining plants, which have a big tolerance to the heavy metals. In the selection process we have to know the content of heavy metal in the vegetable tissue. The selection of the plants with big tolerance which in the same time incorporates heavy metals in the vegetable tissues dukes to the introduction of these in the nutrition cycle, producing sickness to the human-being, and also to the animals.

Is important to be selected those forms which incorporate in their tissues minimum quantities of metals.

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STUDY REGARDING THE DETERMINATION OF METALLIC ELEMENTS FROM VARIOUS SORTS OF VEGETABLES AND FRUITS BY EMISSION AND ATOMIC ABSORPTION SPECTROMETRY

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ABSTRACT

The paper presents the working conditions at the analysis of some metalic elements from various vegetables and fruits used in nutrition.

There were analysed by the method of atomic emission and absorption spectrometry (AAS) in air-acetylene flame the following metal elements: Na, K, Ca, Mg, Mn, Fe, Co, Ni, Zn, Cu, Cd and Pb. Our motivation of this experiment is related to the relatively small amount of information regarding heavy metals distribution in different classes of products.

Obtained results show the non-uniform repartition of metallic elements in the analysed sortiments. However the analysed products represent an important source of nutritive elements but in the same time, due to the very high content of some metals (Cd, Pb, Cu, Zn) they can become potential sources of contamination with toxical substances.

Key words: metal elements in vgetables and fruits

INTRODUCTION

Metallic elements accomplish in the organism extremely important functions: regulation of osmotic pressure and of acidic-basic equilibrium in biologic fluids; metabolic effectors (activators or inhibators) in diverse enzymatic reactions; presence as bioconstituents in the composition of some chemical effectors (tissues, vitamines, enzymes) etc. Practically there is no biologic phenomenon which does not implie the direct or indirect participation of metals. Their presence in insufficient quantities or the exceeding of some limits generate disfunctions of the organism with very dangerous consequences. There also are some elements which, even in very low concentrations, present a toxycogen potential.

Because the large majority of metals originate from food products, there is imposed a rigorous control in order to evaluate their quantum. For the vegetal products used in nutrition the method used is the spectrometry of emission or absorption in flame.

The paper presents a study regarding the determination of some metals from various sorts of fresh vegetables and fruits. Using flame emission spectrometry were

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analysed Na and K; Ca, Mg, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb were analysed by absorbtion spectrometry in air-acetylene flame. We decided to undertake this experiment because literature data are incomplete and the Roumanian legislation imposes some restrictive limits in food consumption.

MATERIALS AND METHODS

There were used fresh vegetables and fruits from the agromarket Josephin-Timisoara, bought from various producers from Banat. The working steps were the following:

- preparing of samples for analysis by removing mechanic rests through washing, or peeling. The quantity used from every product was 100 g, taken from a part used as food; three distinct samples for each range
- calcination of samples, in order to solubilize the metallic cations, followed by treatment of the resulted ash with concentrated solution of HCl. The operation of desagregation lasted 6 hours at 550^{o}C, after a previous drying at 100^{o}C. Obtained acidic solutions evaporated on a water bath and after cooling the residues were treated with a HCl solution (1:2) and maintained on a water bath for half an hour. After cooling the acidic solutions have been filtrated quantitatively and brought at the level using distilled water.
- measurement of extinction and respectively of the absorbance out of the solutions or of their dillutions was made using a Carl Zeiss Jena type FLAPHO-4 spectrophotometer for Na and K, and AAS-1 for the other elements. Working parameters were selected and maintained constant as long as the determination took place for each element. For calibration pure reactives were used. The estimation of concentrations from the analysed samples was made by the help of the graphic callibration method. Graphics were drawn computerized using the program “ORIGIN 30”
- obtained results have been verified and confirmed using an atomic absorption spectrophotometer “Spectr 110” - 2000, equipped with a computer to select the working parameters, the work up and to display the results.

RESULTS AND DISCUSSIONS

Obtained results are presented in Table 1 and Table 2.

Each result is the mean value of three determinations from the same product. One can observe that repartition of the studied elements in the two classes of compounds (vegetables and fruits) is different, their concentration varying in large limits. Comparing the concentrations of different metals we can conclude that from the whole of elements the highest quantum (about 99%) is represented by Na, K, Mg and Ca; the rest of microelements are only in the quantum of 1%.

In fresh vegetables Na, K, Ca and Mg and even Zn, Mn are much better represented as the rest of microelements, present in much less concentrations, but close values in vegetables as well as in fruits.

Analysing the repartition of metallic elements in various sortiments of products after their concentration, experimentally determined, we can observe: ***Sodium*** is present in vegetables in very high concentrations in dill, cellery and parsley leaves (700-1200 ppm) as compared to green beas, cauliflower, cellery (R); decreased contents of Na, under 100 ppm, are obtained in potatoes, marrow, tomatoes or onion. In fruits sodium is met in higher.

Table 1. Distribution of some metals in vegetables

Nr. crt	Fresh product	Element (ppm)											
		Na	K	Ca	Mg	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
1	Cellery (R)	263	2743	249	162	1.50	4.60	0.27	0.19	0.47	3.10	0.04	0.25
2	Cellery (F)	939	2937	3902	1409	9.90	114.00	0.59	0.65	1.40	6.50	0.11	1.10
3	Parsley (R)	294	1765	323	470	2.50	8.20	0.47	0.69	1.10	5.90	0.04	0.39
4	Parsley (F)	725	3861	1319	711	6.90	46.70	1.50	0.62	1.40	21.00	0.09	1.10
5	Dill	1223	2292	1528	565	4.10	18.30	0.61	0.52	1.20	8.30	0.06	0.61
6	Cucumber	102	1334	85	153	0.27	2.10	0.17	0.05	0.22	2.10	0.02	0.08
7	Paprica	95	1110	128	123	0.43	4.90	0.11	0.22	0.11	6.40	0.02	0.11
8	Tomatoes	81	1319	91	107	0.35	2.20	0.11	0.25	0.12	2.90	0.03	0.11
9	Carrot (R)	501	636	260	241	1.30	5.70	0.19	0.11	0.67	2.10	0.02	0.19
10	Carrot (F)	1356	4070	1804	196	4.80	48.20	0.81	0.05	1.30	3.90	0.11	1.40
11	Garlic	105	1997	145	241	1.70	12.20	0.21	0.25	1.50	11.00	0.04	0.21
12	Onion	67	1853	192	202	1.10	3.70	0.15	0.11	0.75	4.60	0.03	0.08
13	Potatoes	61	3271	21	180	1.70	5.60	0.26	0.28	0.71	2.10	0.05	0.11
14	Aubergine	92	2088	27	310	0.86	2.90	0.05	0.08	0.81	4.10	0.02	0.07
15	Cauliflower	321	2313	82	60	1.30	3.90	0.22	0.02	0.51	1.70	0.03	0.23
16	Marrow	32	1649	35	39	0.66	2.70	0.13	0.02	0.36	1.40	0.01	0.32
17	Cabbage	180	1800	180	29	1.10	4.10	0.18	0.02	0.95	1.20	0.01	0.36
18	Grean peas	611	3727	1952	74	6.39	35.50	0.57	0.02	0.85	4.30	0.09	0.68

Note : R - root; F - leaves

Table 2. Distribution of some metals in fruits

Nr. crt	Fresh product	Element (ppm)											
		Na	K	Ca	Mg	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
1	Apples (B)	3.20	871	25.6	41.3	0.18	8.36	0.22	0.24	0.40	1.15	0.02	0.11
2	Pears (B)	3.31	689	19.3	50.5	0.25	4.68	0.33	0.25	0.28	0.96	0.02	0.12
3	Plumps (B)	8.40	1389	278	94.7	0.81	3.79	0.23	0.24	0.48	1.11	0.02	0.12
4	Peaches (B)	8.79	1400	38.1	131	0.38	3.85	0.35	0.70	0.14	1.08	0.02	0.12
5	Raisins (B)	19.60	1398	554	144	1.89	7.87	0.27	0.25	0.54	0.97	0.02	0.12
6	Raisins (R)	5.90	1415	861	200	1.04	3.77	0.28	0.21	0.92	1.25	0.02	0.17
7	Nectarines	8.15	1234	148	105	0.25	5.42	0.26	0.42	0.27	0.94	0.02	0.13
8	Oranges	5.59	1221	444	163	0.57	5.60	0.35	0.26	0.49	1.14	0.02	0.15
9	Lemons	11.30	1038	660	99	0.51	1.88	0.19	0.23	0.42	0.94	0.02	0.12
10	Bananas	20.90	1289	18.20	307	0.99	4.97	0.33	0.17	0.51	1.72	0.02	0.16
11	Melon	20.30	1279	151	122	0.38	6.75	0.23	0.42	0.42	1.60	0.02	0.20
12	Water melon	6.17	1016	99.00	103	0.23	4.90	0.11	0.11	0.12	0.54	0.02	0.20

Note: B-Borlova, R-Recas

concentrations in grapes (B), bananas, lemons and melon. There are poore in sodium: apples, pears, oranges, water melone (concentrations of 3-6 ppm Na)

Potassium is present in high concentrations in the large majority of vegetables, some of them (cellery, parsley, dill, green peas, potatoes etc.) exceeding 2,000 or even 3,000 ppmK. In fruits potassium is well represented in concentrations close to 1,000 pm; poorer in potassium are apples and pears.

Calcium in the highest concentrations (3,000-4,000 ppm) is present in cellery and parsley leaves. There are poore in calcium, under 100 ppm, cucumbers, aubergines, potatoes, tomatoes, cabbage and marrow. The fruits best supplied with calcium are raisins, oranges, nectarines and lemons. As compared to these, apples, pears, water melone and bananas contain much less quantities of calcium, close to 20 ppm.

The microelements Mn, Fe, Co, Ni, Cu and Zn, considered as essential elements for the organism are ununiform distributed and in much less quantities than Na, K, Ca and Mg in all sortiments.

Iron is present in sensible increased concentrations as the other essential microelements in all analysed products. In vegetables it is well represented in cellery and parsley leaves, carrot, cauliflower and grean peas. Very low contents of iron are registered in cabbage, cucumbers, aubergines, tomatoes, paprica etc. In fruits, iron is present in less concentrations than in vegetables, in the range 2-8 ppm.

Manganese, with little exceptions (paprica, tomatoes, cucumbers, marrow) when it does not exceed 1 ppm, presents values under 10 ppm in most vegetables. In fruits, excepting raisins (1-2 ppm) Mn is present in low concentrations, under 0.5 ppm.

Cobalt and **nickel** were detected in close and very low concentrations under 1 ppm, in all analysed products.

Copper *and zinc* are considered elements with toxycogen potential if their concentrations exceed the limits established by L. 268/1999 (Table 3)

Table 3. Maximum admitted values (after L. 268/1999)

Product	Cd (ppm)	Pb (ppm)	Zn (ppm)	Cu (ppm)
Fresh or freezed vegetables	0,1	0,5	15,0	5,0
Vegetables - leaves	0,2	0,5	-	-
Fresh or freezed fruits	0,05	0,5	5,0	5,0

Experimental data show that Cu and Zn contents are sensibil inferiour to the safe ones in all products, being recommended as foods.

Lead and cadmium are toxic metallic elements for the organism, their content in vegetables and fruits is with rigurosity limited. All analysed products, with a few exceptions, do not present risks for consumption. Overdoses are observed in cellery and parsley leaves (1.1 ppm Pb), cellery root (0.4 ppm Cd).

A systematization of metals content on sortiments, respectively a diagramme of the supply with various metallic elements is difficult to build because, in the same time, are products good supplied with nutritive elements but containing apreciable quantities of heavy metals.

CONCLUSIONS

Flame atomic spectrometry is a method compatible with the exigencies imposed by the specific of metallic determinations in vegetale food products (complex, matrix, low concentrations - sometimes traces, rigurosity of tolerance limits etc.).

Obtained results show that the repartition of metallic elements is different in the analysed products. Generally, this products constitute an important source of nutritive elements. Due to the unexpected high content in some metals, vegetables and fruits can become contamination sources with toxic substances.

In order to elliminate this difficulty a rigorous analytic control is imposed at their consumption and processing.

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PROTECTION OF WELL WATERS FROM HEAVY METAL POLLUTION

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ABSTRACT

The concentration of iron and other heavy metals are the most important characteristics of the quality of the well water on the territory of Vojvodina Province. The origin of pollutants of well waters is corrosion of the metallic construction of the wells and the unfavourable hydrogeological conditions at the exploitation of drilled and sub drilled sources. Iron and heavy metals besides of the pollutions of drinking water also causes formation of colmatants in filter areas of well. Following the changes of the concentrations of iron and other heavy metal ions in drinking water could be diagnosed the sate the well and on that base will be taken preventive action to prevent colmatation or undertake revitalisation atrophied wells. Monitoring with contemporary instruments followed the concentration variation of iron and other heavy metals in this work. The revitalisation of well was carried out by reagent method, which improved the quality of drinking water what was the aim of this work.

Key words: well water, heavy metal pollution

INTRODUCTION

Artesian and sub artesian wells very often don't fits to regulative of drinking water. At the choice of sources maks some of problems, especially in suburbs and around industrial capacities. Very often happens mixing the waste water with drinking water in sub drilled wells, in the period of high height of the water level in March-May and September-December. The situation is difficult in the case of artesian wells at low height of the water in the period June-September. The mentioned periods are critical for some of categories of habitants and the water supply in suburbs is problematic. That for must be protected the sources and the well in that locations.

One of the problems in the estimation of the pollution of water in water resources on individually locations, in this case on the territory of Novi Sad in direction northeast-southwest. On south line of the city, named Liman I-IV, includes 5-6 wells from Ribarsko Ostrvo-Kamenjar. An this location are lot of pollutants for example the shipyard. The Liman I-IV is compact settlements with lot of smaller pollutants of water, soil and air.

Near Novi Sad is Beocin, on Danube riverside with big factory of cement, which is also one of the pollution sources. Also big problem present the neglected wells. This paper deals with research of revitalisation of wells by means of decrease of iron and other heavy metal-ions concentrations in drinking water. All research was carried out in Institute of Chemistry and DOO Aquatic in Novi Sad.

MATERIALS AND METHODS

For AAS measurements was used apparatus SHIMADZU AA-670, acetylene/air flame. The sample impute was 0.55cm^3/min trough capillary. The furnace is made from steel and ceramic. The flame is on 1cm distance from optical line. All standard solution were Merck, Darmstadt, Germany, p.a.. grade 0.01-10mg/dm^3.

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ICP-OES measurements were carried out on Perkin Elmer OPTIMA 3200 with CETAC 500 AT ultra sound atomiser.

Regeneration of the capacity of wells applied reagent method is accommodated to the temporary conditions and states of wells. The substances for regeneration were used HCl ω^{v}=10% and sulphamic acid ω =10%, at 333-353K temperature. For heating the solutions were used electrical heaters in hermetical closed wells. The decolmatation was carried out by means of airlift method. The estimation of the end of regeneration based on decrease of the internally pressure of the high of water Column.

The electric conductivity was measured by instrument Iskra MA 5962. The pH-value was measured on Iskra MA 5703 pH meter. The pH-value was measured on Iskra MA5703 pH -METER. The parameters turbidity-hardness etc. Were determined by methods JUS ISO 3696 (Sl. list SRJ, No 54/94 and No 42/98).

RESULTS AND DISCUSSIONS

In this work was solved the problem of the diagnosis of the state of wells by means of relevant parameters. There were choised the detection or determination of the presence of two metals in water Fe and Mn (Dimitrijevic, 1989.). It is important for restaurants, which supplied directly from wells with water on the Danube riverside. In this case were choised precise methods for regeneration of wells by means of reagent methods.

The concentration of Fe and Mn was carried out by means of atom absorption spectrophotometer (AAS) (Gaspar, 2000.) and with inductive coupled plasma emission spectrometer (ICP-OES) (Bozsai, 2000.). In the case of ICP-OES apparatus were used ultra sound atomiser (USN), and axial visualiser of plasma. There were use both AAS and USN ICP-OES methods. The characteristic lines for spectral analysis for Fe and Mn are shown in Table 1.

Table 1 - Analytical lines for Fe and Mn

Fe, λ (nm)	Mn, λ (nm)
259.400	259.372
238.204	294.920
239.569	257.610

The structures of colmatante and the soil under wells were determined by means of XRD with apparatus Philips PW 1710, using camera CuKα and the chemical content with apparatus XRFS Philips PW 2404 with LiF 200 analyser.

The sampling was done by methods EPA 3015 with HNO_3 in microwave apparatus for degradation. There is a more contemporary method beam injection flame furnace atomic absorption spectrometry (BIFF-AAS) in this case applied the jet impact vaporization (JIV), which injects whole sample into atomiser, making longer retention time of atoms.

The thermo spray - flame furnace (TSFS-AAS) method is more rational than thermo spray (TS) method because required only a simple peristaltic pump for injection of the sample instead of high-pressure pump and a complicated electronic apparatus.

Apply of mentioned methods could make some of troubles, when the condition of experiment can not be changed. That for is useful the chemometric methods and different corrections of the results of measurements. Often the analytical results by

means of AAS and ICP methods are extremely high or the curves shows hyper fine structure and than they cannot be used for expertizing. The software material is necessary, with adaptable EXCEL. That for take into, series of standardized concentrations values of absolute dispersion, or estimated value of dispersion, correction of the concentration of ions, which interferes with measurements, the sensitivity of the measurements, the concentration limits at investigated ions.

On the base of obtained data starts the regeneration of wells. For regeneration a relevant data is the concentration of iron under 0.3mg/dm^3. Higher concentration than the water with the 0.3mg/dm^3 is not drinking water and cannot be use in household. In most cases after the regeneration of wells the concentration of the iron-ions decreasing ten times. For recognition the state of wells as the chemical content of colmatantes and their structures very effective method is the XRD, which used also for geological investigation of soil around the source. Without that no sense for hydrogeologic and hydrochemical measurements. It makes possibility to recognise the origin of the iron, witch is necessary for exactions for the regeneration of the wells.

On the base of the change of the structure of colmates around filters and of change of the structure of the soil in hole, we could recognise processes causing ageing the wells. There could be influenced of this process as privacy by complex dissolving colmates, which is more economical than the use of drastically pneumo-reagens method to destroy colmate or deposits.

At the regeneration process of Wells one of basic criteria is to obtain lower MAC values for the metals, also for Fe and Mn. The MAC values are shown in Table 2 predicted by ISO/CEN standards by EU/98/83/EC, Annex I, part B (Lazic, 1994.). Comparing MAC values (Dalmacija, 2000.) with the obtained parameters of the investigated well waters, shown in Table 3 a - 3 i, comes out that in further wells from Danube, the average Fe concentration is higher.

Table 2 - MAC values of drinking water

Parameters	Unit	MAC
Turbidity	NTU	<1
pH	pH unit	6.5-9.5
El. conductivity	μS/cm	<2500
Dry remnant, 378K	mg/dm^3	<1200
Hardness	mg$CaCO_3$/dm^3	10-500
Total Fe	mg/dm^3	0.3
Total Mn	mg/dm^3	0.05

Exception makes locations at the enter on the "Ribarsko ostrvo" which is hydrogeologically the same with territory of Novi Sad (Halasi, Andric, 1999.) as seen in Table 3 b and 3 d. In this part Danube riverside is more compact what shows the analysis of the sediments and colmates of the wells "Ribarsko ostrvo K No 83" Table 4 and 5.

Table 3a- Results of analysis of well waters, "Ribarsko ostrvo I"

Parameters	Unit	Before regeneration	After regeneration
Turbidity	NTU	1.7	0.9
pH	pH unit	7.249	7.1343
El. Conductivity	μS/cm	829.1	791.9
Dry remnant, 378K	mg/dm^3	547.2	504.3
Hardness	$mgCaCO_3/dm^3$	421.4	341.4
Total Fe	mg/dm^3	0.6828	0.0811
Total Mn	mg/dm^3	0.1714	0.0231

Table 3 b- Results of analysis of well waters, "Ribarsko ostrvo II"

Parameters	Unit	Before regeneration	After regeneration
Turbidity	NTU	2.4	0.1
pH	pH unit	7.223	7.114
El. Conductivity	μS/cm	836.5	802.7
Dry remnant, 378K	mg/dm^3	552	521.4
Hardness	$mgCaCO_3/dm^3$	435.7	390.3
Total Fe	mg/dm^3	0.968	0.1931
Total Mn	mg/dm^3	0.1572	0.0212

In the well of "Ribarsko ostrvo III" the content of Fe is lower, but high in comparison with MAC value. This well is near shipyard. This area of, "Ribarsko ostrvo" which is territory of Liman is soundly and is less polluted what is shown on Table 3 e and Table 3 f. The well in the "Atuocamp" should be emphasizing with the constant quality of water even the colmates occurred. It refers to the higher content of SiO_2 in colmates and in sediment. In the Industrial area in Novi Sad the state of well water are alarming. In the table 3 g is seen a higher pH value and high Fe content $0.72mg/dm^3$. After the well regeneration the Fe content decreased to $0.2mg/dm^3$, which is on the limit of strictly regulative. In several countries the MAC values for Fe $0.2mg/dm^3$ in distinction from Yugoslavia, where is $0.3mg/dm^3$. In the circle of Dairy in Novi Sad are two wells Mlekara I and Mlekara II shown in Table 3 h and 3 i. Un fortunately in Mlekara II even after the well regeneration, the content of the Fe did not decrease under 0.3 mg/dm^3 (Halasi, et.al., 1999.).

Table 3 c- Results of analysis of well waters,"Ribarsko ostrvo III"

Parameters	Unit	Before regeneration	After regeneration
Turbidity	NTU	2.3	0.8
pH	pH unit	7.2	7.125
El. conductivity	μS/cm	680	664.1
Dry remnant, 378K	mg/dm^3	453	404.5
Hardness	$mgCaCO_3/dm^3$	401.8	388.7
Total Fe	mg/dm^3	0.55	0.092
Total Mn	mg/dm^3	0.05	0.01322

Table 3 d- Results of analysis of well waters, "Ribarsko ostrvo K No 83"

Parameters	Unit	Before regeneration	After regeneration
Turbidity	NTU	1.7	
pH	pH unit	7.249	7.148
El. conductivity	μS/cm	829.1	823.2
Dry remnant, 378K	mg/dm^3	547.2	543.3
Hardness	$mgCaCO_3/dm^3$	421.4	442.9
Total Fe	mg/dm^3	0.6828	0.0804
Total Mn	mg/dm^3	0.1714	0.2584

Table 3 e- Results of analysis of well waters, "Ribarsko ostrvo K No 86"

Parameters	Unit	Before regeneration	After regeneration
Turbidity	NTU	1.4	0.8
pH	pH unit	7.148	7.114
El. conductivity	μS/cm	823.2	802.2
Dry remnant, 378K	mg/dm^3	543.3	495.3
Hardness	$mgCaCO_3/dm^3$	428.6	402.2
Total Fe	mg/dm^3	0.0804	0.08
Total Mn	mg/dm^3	0.2584	0.031

Table 3 f- Results of analysis of well waters, "Ribarsko ostrvo, autocamp"

Parameters	Unit	Before regeneration	After regeneration
Turbidity	NTU	2.4	0.9
pH	pH unit	7.104	7.001
El. conductivity	μS/cm	846	840.2
Dry remnant, 378K	mg/dm^3	453.2	418.6
Hardness	$mgCaCO_3/dm^3$	392.9	380.3
Total Fe	mg/dm^3	0.0521	0.0476
Total Mn	mg/dm^3	0.128	0.1226

In the case of Mlekara I by regeneration the Fe content changed from 0.875mg/dm^3 to 0.0931 mg/dm^3. The mentioned two well are near to each other, but their water are different. That for well waters for the use in dairy industry must be treated.

Table 3 g- Results of analysis of well waters, "GRAAS"

Parameters	Unit	Before regeneration	After regeneration
Turbidity	NTU	4.2	
pH	pH unit	7.516	7.52
El. conductivity	μS/cm	630.5	612.4
Dry remnant, 378K	mg/dm^3	416.1	402.2
Hardness	$mgCaCO_3/dm^3$	400	388.5
Total Fe	mg/dm^3	0.7179	0.2001
Total Mn	mg/dm^3	0.0498	0.0161

This results shown in Table 3 h and 3 i. After regeneration in the well water in Mlekara II the content of Fe remained above $0.3mg/dm^3$, but in Mlekara I by regeneration resulted a decrease from $0.875\ mg/dm^3$ to $0.0931\ mg/dm^3$.

Table 3 h- Results of analysis of well waters, "Mlekara I"

Parameters	Unit	Before regeneration	After regeneration
Turbidity	NTU	0.75	0.7
pH	pH unit	7.951	7.212
El. conductivity	μS/cm	981.4	783.8
Dry remnant, 378K	mg/dm^3	647.7	563.8
Hardness	$mgCaCO_3/dm^3$	210.8	206.4
Total Fe	mg/dm^3	0.875	0.0931
Total Mn	mg/dm^3	0.0635	0.0144

Table 3 i- Results of analysis of well waters, "Mlekara I"

Parameters	Unit	Before regeneration	After regeneration
Turbidity	NTU	0.72	0.72
pH	pH unit	7.764	7.661
El. conductivity	μS/cm	1003.7	987.2
Dry remnant, 378K	mg/dm^3	662.4	620.2
Hardness	$mgCaCO_3/dm^3$	207.1	203.8
Total Fe	mg/dm^3	0.7779	0.3135
Total Mn	mg/dm^3	0.0141	0.0141

In the Table 4 are dates for sediments from 140m depths, under wells. The chemical content of colmates in filter area of the same well is shown in Table 5. In this case is seen clearly the considerable participation of Fe_2O_3, CaO and MgO while SiO_2 prevailed in hole.

There is a conclusion that every well must be regenerated periodically after 18 months (Halasi, 2000.). In the case where the Fe content is above MAC value for regeneration recommended the aggressive HCl reagent but for lower Fe content the

sulphamic acid. The process of regeneration is also a good preventive method to save the well waters

Table 4 - Chemical content of the sediments of the wells (XRD and XRF)

Substance	%
SiO_2	58.3
Al_20_3	14.2
Fe_2O_3	6.2
CaO	12.8
MgO	1.4
Others	7.1

Table 5 - Chemical content of colmates in filter area of wells (XRD and XRF)

Substance	%
SiO_2	12.8
Al_20_3	6.9
Fe_2O_3	43.6
CaO	28.8
MgO	4.5
Others	3.4

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SOME CONSIDERATIONS ON THE LOADING WITH HEAVY METALS IN THE CITY OF TIMISOARA

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ABSTRACT

The great majority of the soils from urban agglomerations are constituted of important or processed materials and deposited of appreciate thickness on that place or on other lands. Most of these materials have a mineral origin, made of rocks and building residuald, different industrial or house garbage's or other garbage's which come from different sources, some of them with a high risk of toxicity. Part of these materials have been deposited in mediums which favored polluant accumulation, others were deposite in conditions of natural epuration so that finally the antrosoils have evoluted in different qualitative conditions. The work also has in view the study of these situation in some towns from the south-west part of Romania with the suggest and agree for the introduction of some new taxonomic divisions in the case of the highly antropic remaniated soils. The geogenous fund of the area plays an important role in the accumulation of heavy metals, but the physical and chemical characteristics of the rocks and soils condition the spreading of the chemical elements on the horizontal and vertical space.

Key words: heavy metals, antroposols, urban area, Timisoara, Romania

INTRODUCTION

In the urban areas, the soil represents the place where several polluting agents meet: solid residues-thrown away or deposited; residual waters used for irrigating areas where an intense garden activity takes place; harmful powder emitted by industrial agents, toxic gases disolved by the rain and deposited on the soil etc. At least twice a day, the atmosphere of the big cities is characterized by high concentration of gas polluants and sedimentary dust from the vehicles. These polluants are deposited again in an area with the influence of environmental factors (the aspect of the relief, the wind, the cloudiness) as well as that of the urbanistic ones (high blocks, green area). Therefore, the soil is loaded with a series of toxic, polluting elements. The different polluting elements that enter the soil will be integrated in numerous natural cycles or in artificial ones, depending on the characteristics of the climate and of the soil. This can lead either to the diminution (leaching or transformation into inaccessible forms) or to the increase (mobile form accumulations) of their toxicity.

MATERIALS AND METHODS

To determine the level of loading with heavy metals in Timisoara there were gathered samples from about 20 stations considered representative both for the concen-tration level as well as for the possibilities of spreading of the pollutants in immision sources.

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The analysis method relied in the weathering of the prevailed samples with a mixture of acids nitric, sulphuric and percloric in a 2,0 : 0,1 : 1,0 (I.C.P.A., 1986) proportion and spectometrical atomically absorbtion dosation in variant of air-acetylene flame atomisation.

RESULTS AND DISCUSSIONS

In order to illustrate with examples the statements presented above we chose a large agglomeration for the context of the urban agglomeration existing in Romania today. Timisoara is a large city, with industrial traditions but with no factories that have high-polluting activities. For this city, Florea and Ianos (1995) estabilshed a research methodology regarding the vulnerability of the counterurban land to pollution, without including in these activities the land from the areas where people live. Later the some authors (1996) identified the lands that present the risk of pollution within the counterurban areas. They also identified the agents that could be ressponsible for these actions. This investigation will extend the research to the intraurban areas of Timisoara, establishing a series of factors and agents of pollution, as well as the consequences of their actions.

The configuration of the relief within the limits of the city is much blurred by the anthropic activities developed throughout the centuries. In the nouth-west of the city lies the only area that has not been affected by the excess of water (fig.1a). This strip of higher land (99m) is continued in the north of the city, but it winds a lot and it is penetrated by numerous sand banks and areas of small depressions or low plain gulf, formed by the present day movements of local subsidence (Tufescu, 1957). To the north of the Bega riverbed and to the south of this river, up to the Bega-Timis interfluve there lies an old alluviations field, well stabilized as the result of the regularization of the two water streams two hundred years ago. The old meanders and the secondary streams of the two rivers can be observed only in unbuilt areas (parks, recreation areas, vacant lands).

The lithology and especially the solification rocks in the higher areas of the eastern and nord-eastern region of Banat have played an important role in the accumulation of toxic elements, especially heavy metals. It is known that the western mountain area of Romania presents banatitic intrusions that contain large qualitities of polisurphurs. The weathering and alteration of theserocks, as well as their transportation to the west, towards the center of the Panonic Depression led to the depositing and sorting of elements according to the morphological and hydrological conditions of the sedimentation epochs. The whole northern area of the city is covered by a loess materials cover: Some of them are older and more alterized towards the north-east, in the high plain of Vinga and more recent, intensly transformed in lacustrian condition towards the north-west (the south-east of the Torontal Plain) (fig. 1b). The great majority of the loess powders come from the eastern mountain areas and their chemical characteristics have favoured the accumulation in time of some toxic elements (lead, cadmium). The southern and central part of the city is covered with a thick

cover of alluvial deposits, two hundred years old, period in which no significant over flowings or accumulations of sediments have taken place. These materials also come from the eastern mountain and piedmont areas but they were brought and deposited here by another transporting agent: the water.

The depth of the phreatic level is conditioned by the altitude of the land form, as well as by the permeability and the thickness of the horizons that bear the water.In the north-east of the city, under the southern terminations of the high plain, the phreatic level is positioned at 3-5-10 meters deepth. In the east and the sout-east of the city, under the packets of permeable rocks, the phreatic level can be found at a depth between 2 and 3 meters. Finally, in the central and the western part of the region, the phreatic level oscillates between 0,5 and 2 meters, depending on the configuration of the minor landforms and on the granulometric characteristics of the rocks.

The soil cover is defined by the bioclimatic characteristics, and its differentiation is made according to the depth of the phreatic level and to the nature of the solification rocks. Due to the numerous and energetic antropic interventios throughout time, the genetic types of soil cannot be reconstituted today but the sense of the evolution of soils can be inferred with the help of the solification material and that of some fragments of soil; the distribution of soils in the peripheral areas can also indicate the evolution of the soil (fig, 1d).

The oldest land, situated in the high plain, is covered by argiluvisols and molisols (the later in the transition zone). On the loess covered lands, chernozems have developed-they were found in different gleization stage and affected by alkalization, especially in the depth. The estern corner of the perimeter is covered by various soils. The predominant are vertisols, phreatic hidromorphyc soils, alluvial soils, halomorphic soils. In the center and the south of the perimeter, the cambisols dominate: cambic chernozems in the north and argillic brown soils in the center and rhe south, associated with alluvial soils.

The particular caractersitics of the soils are determined by the solification materials and by the stage of the evolution of soils in the bioclimatic area. The above mentioned soils have been intensly modified by man in time (they are antrosols today) and they have a great capacity for adsorbtion in the north and in the east, and a moderate one in the center and in the south. The reaction is the mild acid interval (pH= 5,8-6,8) and the degree of base saturation is higher in the north and lower in the south.

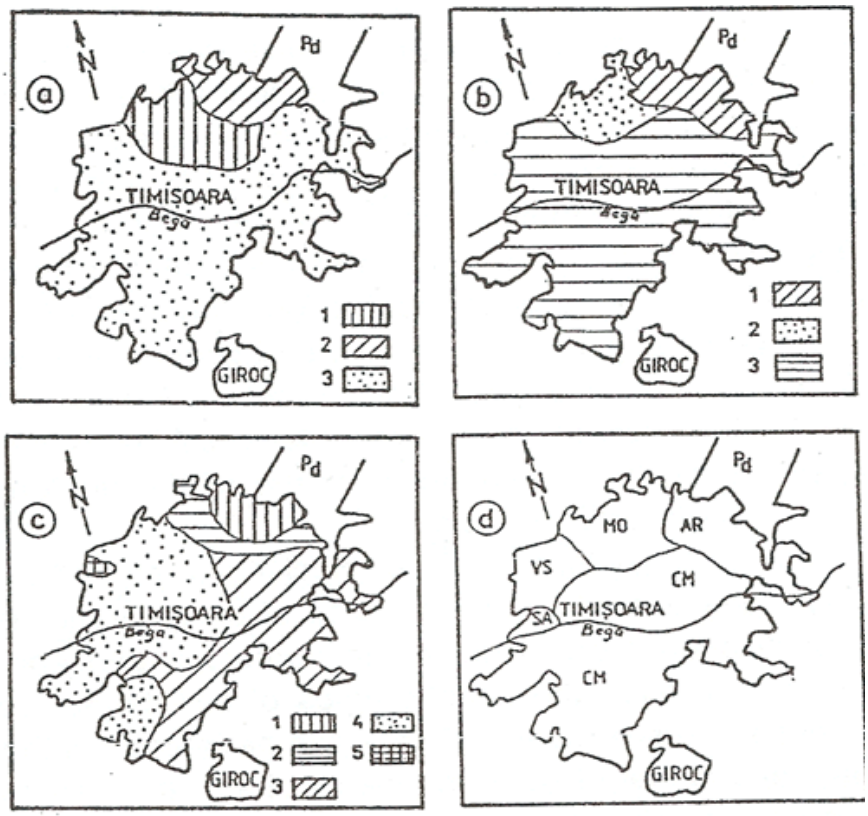

Fig. 1 The pedogeographical characteristics of the Timisoara City area
a) *The main landforms (1. The High Plain of Vinga; 2. The tabular plain of Torontal; 3. The low Gulf-Plain of Timis-Bega);* ***b)*** *Materials of Solification (1. old limonated loess deposits; 2. reshuffled loess doposits; 3. old alluvial deposits);* ***c)*** *The depth of phreayic level;* ***d)*** *The original soil cover (AR-argiluvisols; MO-molisolss; VS-vertisols; SA-fluvisols; CM-cambisols).*

The heavy metals in the antrosols in the Timisoara city. Some of the chemical elements that are part of the heavy metals cattegory are naturally present in the sedimentar rocks and in the Timisoara city soils as a consequence of the way in which the sedimentation took place and of the geochemical characteristics of the deposited materials (Ianos and Lacatusu, 1995).

Other elements are introduced in the soil as a result of various antropic activities (irrigation with waste waters, silt spreading, fertilizers industrial processes, substances carried by air and proceeded from traffic or industrial emissions).

The lead is present is concentrations that go far beyond the average value, in the whole city. The values go over 100 and even over 150ppm and therefore they go beyond the tolerable concentration. The highest values, of over 100ppm are to be found on the north- west-south east axis of the city; it starts in the north-western industrial zone, it goes through the center of the city and it ends in the south-east (fig.2). The lead comes, in this case, from the Solventul Chemical factory. The dominant direction of the wind (north-west) send the toxic gases in this direction. To this source, we can add the numerous vehicles that use combustibles based on tetramethil lead or other lead components not to mention the poor technical condition of the vehicles and and the far from perfect fluidization of the traffic that generates numerous blockings and frequent startings in the crossings.

Table 1 *The heavy metals content of antrosols in the perimeter of Timisoara*

Nr crt	*Element*	Pb ppm	Cd ppm	Zn ppm	Cu ppm
	The medium allowed concentration	***20***	***0,3***	***50***	***20***
	Tolerable value	***100***	***3***	***300***	***100***
1	Arad Street	100	1,4	200	30
2	Torontal Street	85	1,5	210	48
3	Lipova Street	139	0,8	185	70
4	Circumvalațiunii Street	100	1,7	200	60
5	City Hall	110	30	180	136
6	Eroilor Street	100	3	200	105
7	Republicii Street	290	4	175	90
8	M.Viteazul Street	80	1,5	200	40
9	Cluj Street	215	0,5	255	70
10	Buziaş Street	115	1,5	110	30
11	Şag Street	100	1,2	185	30
12	Fountain (rotary)	102	1,1	115	40
13	Thermal (rotary)	66	2,8	125	60
14	Children's Park	10	0,5	135	50
15	Park of Justice	90	0,5	105	34
16	Central Park	40	1,5	70	40
17	Botanic Park	55	1,5	100	30
18	Park of the Rozes	10	1,5	100	20
19	6 Martie Kindergarten	123	1,2	116	
20	V.Lucaci Kindergarten			135	

Compared to other areas of the country (Bucharest for instance that according to Rautta & colab., 1986 – has a natural concentration of Pb of less than 10ppm) the geogenic fund of the region is high, that is found in 30ppm Pb (Ianos, 1995). This fund is found in its natural value only in the south-western districts of the city. In all other areas, the concentrations go over the admisible limit: 30-40ppm in the eastern peripheral districts; 40-60-100ppm in the northen and southern areas of the city. The anthropic source of the lead excess found in antrosols epipedones is confirmed by the quantities of lead found in the deeper horizons. These quantities decrease slowly to half, and then enter within the limits of the normal values (30 ppm).

The cadmium pollution doesn't warry us, although the values are far above the average admisible limit, getting close to or going over the tolerable limits (Tietjen, 1977, Kloke, 1981, Adriano, 1987). There weren't found any secondary accumulations of cadmium from antropic sources that can be harmful. The values that were found in the soils that are situated in the neighbourhood of

intensly circulated areas are close to those found in remote areas or at depth (40cm). Therefore the accumulation fund is geogenous.

The copper is found in concentrations that are lower than the tolerable limit (100ppm) as the Timisoara soils and rocks. Compared to the geogenous fund of the plain area where Timisoara lies (20 and 30ppm)(Ianos, 1994), the urban soils have double or triple loads, especially in the perimeter of the old industrial platform (fig. 2). In the depth, the cooper concentrations are constant or they decrease within low limits with the exception of a new district, that was built on an old garbage-depositing area. Here the copper concentrations increase with the depth (83ppm at 40cm deep).

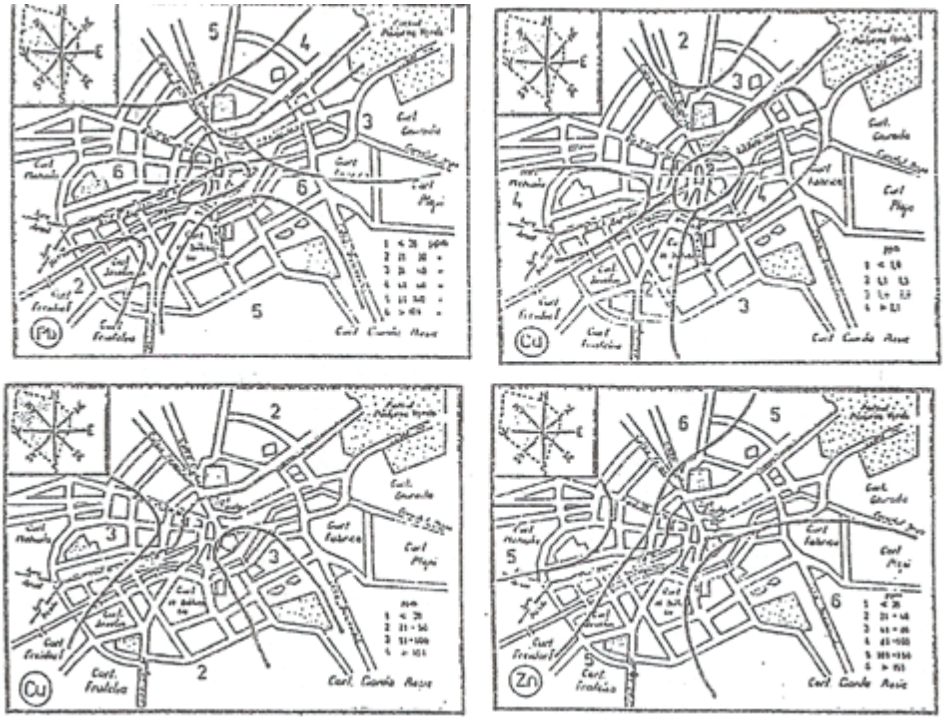

Fig. 2 The areas of Timisoara, acording to the content of heavy metals

Element with a great afinity for sulphur, **zinc** is concentrated in high amounts in the silts of the old moors that surrounded in north-west & west the old fortres of Timisoara surpassing even in this case thecharacteristic value of the pedolitological area (50-70ppm)(Ianos, 1995). More reduced values were found in the north-eastern corner of the city, a higher area that functioned a long time in conditions of exondations and the south-western corner, partially the western one, fields with soils more coarse-textured, in which waters left behind coarse-textured sediments. The same tendency of maintaining the concentrations in depth is observed in the case of zinc to.

Knowing that the heavy metals from the soil, from the dust of the streets, or from aerosols, taken through lungs, are reabsorbed in proportion of over 50% (value dependent on the dimension of the inhaled particles). The question is over dust and other toxic elements can there be accumulated in also other toxic elements. In this context, Ilis et al (1995) analysed samples of soil and dust that come from some places of leasure as the sandy materials from crowded places (schools, nursery schools, sport grounds), establishing some critical points, but which really raise problems (sand from the playing groups with over 120ppm lead, with great loads of coliformi (over 22,336 g. dry substance), with streptococus (1,626 g. dry substance) etc.

The activity of depuration of waist urban waters leads to the daily storage of tens and hundreds of tones of dry substance. If since 1990 a part of those silts were used as fertilizers in agriculture and spread on large areas of fields, nowadays nobody seems to want them any longer. These residues were gradually accumulated on large areas and considerable depths, froming the solification material for the soils which will be formed once with the shetting of the spoil bank or sources of dust which will risc at a certain intensity of the wind. The same thing hapens in the case of the sterile material from electric centrals or from other forms of processing other rocks (braking sorting). Neglecting the problems connected to the degree of toxicity of the soils from urban perimeters will have negative effects on human heath, knowing that a great part in the delivering in one city's markets is done by inhabitants of the city which owe gardens and which we other cultivate them on fields with soils affected by toxic substances (which have been area of storing the residues) or fertilise with uncontrolated substances, or irigate with waist waters with a great degree of toxicity.

CONCLUSIONS

1.The basic concentrations of the solic and subsolic matherials are of geogenous origine, and they come from the eastern Banat mountain range.

2. The values over the normal limit for the researched area coming from the polluting industrial activities, activities that stopped lately.

3. The distribution of the oligoelements in Timisoara urban area was conditioned by a series of natural factors (geology and lithology, relief, climate, phreatic water, the state of evolution of the soils.

4. When the study was done there were not determined pollution concentrations of heavy metals in Timisoara urban area.

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URIC ACID LEVEL IN SUBJECTS WITH DUCHENNE MUSCULAR DYSTROPHY

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ABSTRACT

Free radicals, a reactive group of chemical compounds produced by one-electron reduction or oxidation reactions, are normally generated by both enzymatic and nonenzymatic pathways in biological systems. The interaction of free radicals with cellular components results in damage to DNA, proteins, and lipids. Defense systems against this free radical damage are critical to the survival of a cell.

Free radicals cause peroxidation of unsaturated fatty acids in cellular membranes of skeletal muscle, and could allow leakage of cellular proteins, even though physical membrane damage is not detectable by conventional methods of analysis. Such release of creatinine phosphokinase and other muscle proteins is characteristic of Duchenne muscular dystrophy. Oxidative damage also reduces the ability of calcium-ATPase to concentrate calcium in the sarcoplasmic reticulum. The same inability to accumulate calcium occurs in the sarcoplasmic reticulum prepared from dystrophic muscle of humans. Other symptoms of muscular dystrophy that can be explained in terms of free radical damage include: enzyme inactivation, increased protein turnover, altered collagen synthesis, preferential depletion of unsaturated fatty acids, increased activities and decreased latencies of lysosomal proteases and elevation of antioxidant enzymes.

The critical questions remain as to the source of free radicals within dystrophic muscle cells and in what way this source might be related to the genetic defect.

The answers to these questions lie in a clearer understanding of cellular processes which involve free radical intermediates and of the antioxidant systems important to muscle.

From our facts is possible to accept a secondary implication of uric acid in the pathogenesis of Duchenne muscular dystrophy.

Key words: Duchenne muscular distrophy – uric acid levels

INTRODUCTION

Duchenne muscular dystrophy, a progressive degenerative muscle disorder inherited as an x-linked recessive trait, is associated with proximal muscle weakness and atrophy, as well as with involvement of different organs (Radu, 1978; Walton et al, 1974).

Elevated levels of muscle enzyme activities in the serum and their depletion in muscle tissue implied an abnormality depletion in the plasma membrane as the probable site of the genetic defect, although the primary inherited metabolic defect is unknown. The elucidation of the primary biochemical defect by studies of muscle tissue obtained by biopsy is made extremely difficult by presence of atrophy, fibrous tissue, the change in innervation that may cause multiple secondary biochemical

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changes, and by small yeld. It is paradoxical that fast-twitch muscle fibers have a lower concentration of antioxidant enzymes than slow-twitch fibers, but also have a greater proportion of polyunsaturated lipids susceptible to free radical damage. Vitamin E and Uric Acid, which are present in both types of muscle fiber, may therfore be relatively more important to fast-twitch muscle fibers as the antioxidant defense for their membranes. This conclusion would explain the greater susceptibility of fast-twitch fibers to nutritional myopathies, and evidence that electrically stimulated fast-twitch fibers in models are more susceptible to ischemic damage. More importantly, this pardox may also explain the fast-twich fiber type specificity of inherited muscular dystrophy. If the underlyng defect causes the production of excessive levels of free radicals, then the fast-twich muscle fibers, with their lower overall antioxinant capability and higher proportion of unsaturated lipids, would be less able to scavenge the free radicals before they damage the cell.

Malondialdehyde (MDA), pentane, conjugated dienes, and lipofucsins are produced as minor by-product of free radical damage to unsaturated fatty acids. MDA is measured after a reaction with thiobarbituric acid and, although other compounds can interfere with this assy, thiobarbituric acid-reactive substances (TBARS) are significantly elevated in muscle samples from dystrophic humans. Increased amount of pentane are expelled by patients with Duchenne Muscular Dystrophy, and a study has reported a significant elevation of TBARS, conjugated dienes and lipofuscins in the plasma of such patients. On the other hand we raported sidnificantly variations of fatty acids in some types of muscular distrophies (Ionescu et al, 1985; 1998).

It is very important to point out that hypoxanthine is converted to xanthine and subsequently to uric acid by enzyme xanthine oxidase (xantine: O_2-oxido-reductase). This emzyme, like many which employ molecular oxygen as oxidizing agent, is a flavine, but in this instance a most complicated one, containing Fe, Mo, and perhaps coenzyme Q as additional cofactors.

MATERIALS AND METHODS

The diagnosis of Duchenne Muscular Dystrophy was based on: the clinical history, neurological and electromyographical examinations; biochemical, enzymological, bioptical tests. The group was selected aleatory from our patients. Reagents were from "Sentinel"-Italy. Automatic Analyser Electa 216-E from "BPC BioSED"-Italy were used. Samples of 3 - 4 ml. of venous blood were added to heparinized test tubes and were immediately centrifuged at high speed. All tests are performed currently in our laboratories in the same configuration. Samples were obtained before breakfast.

RESULTS AND DISCUSSIONS

Our results are presented in the next table and figures.

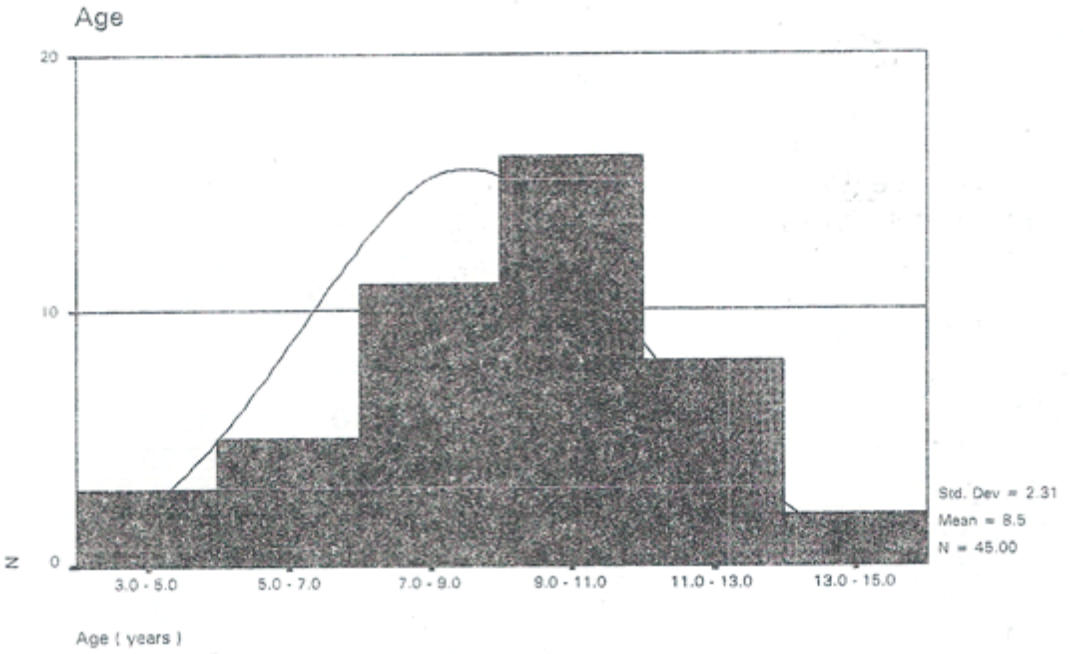
The activities of serum enzymes are remarkably constant for any given healthy individual, a fact which probably indicates a dynamic balance between production and removal. It is often inferred, therefore, that rapid destruction of tissue will temporarily overwhelm the serum clearance mechanisms for those enzymes which are present, and perhaps peculiarly abundant, in that tissue, thus raising their activities in the serum, as, for instance, in the well-known rapid rise of GOT, activity shortly after myocardial infarction. This simple and attractive hypotesis, however, fails to explain many relevant clinical and experimental findings. The distribution of the enzymes values were "normal" for patients affected by Duchenne Muscular Dystrophy.

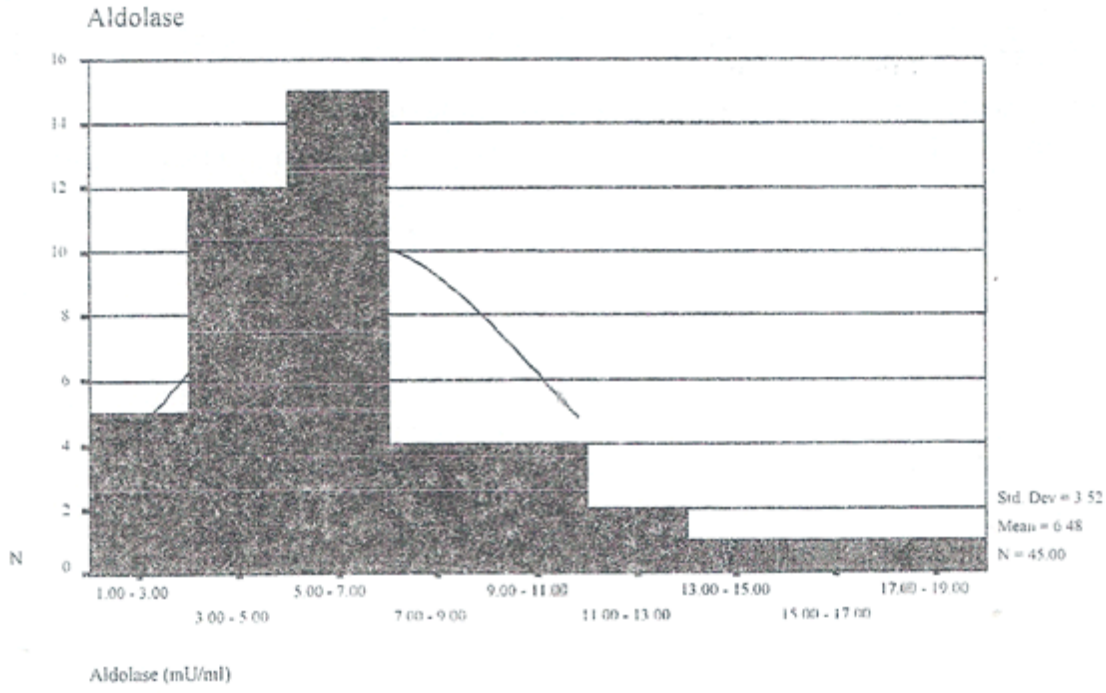
Table1. Statistical terms for 45 subjects with Duchenne muscular dystrophy

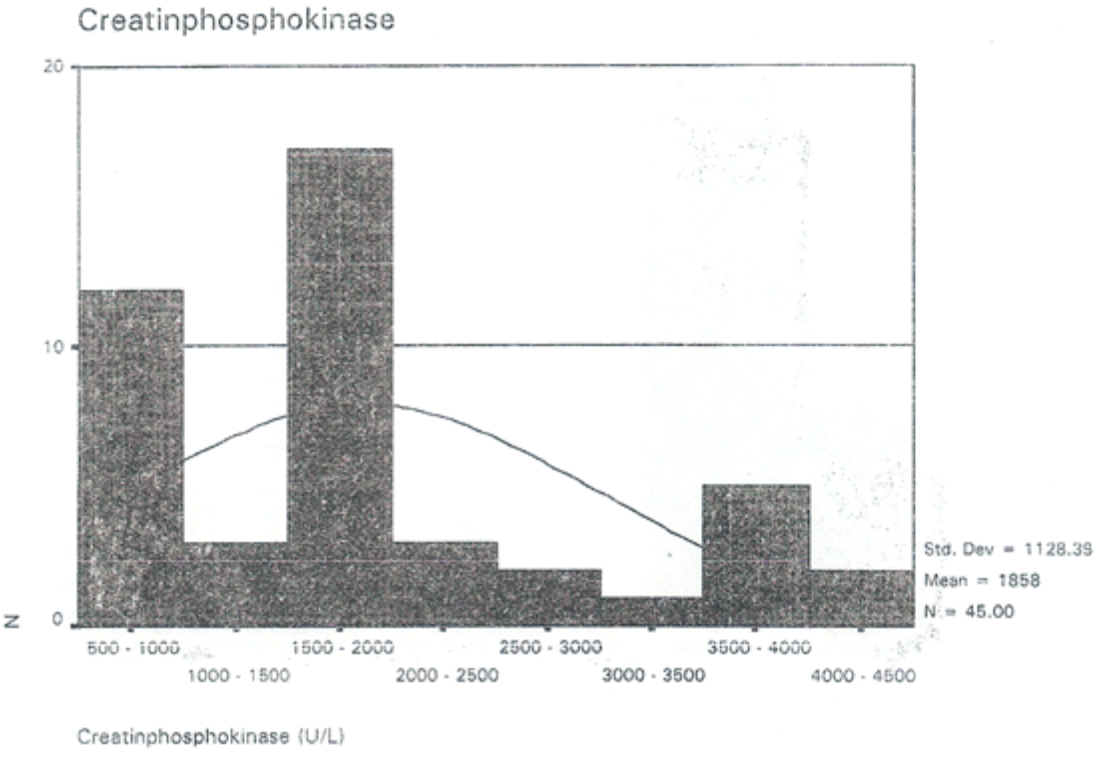
Specification	Count	Range	Mean	Std. Deviation
Age (years)	45	3.00 - 13.00	8.51	2.31
Alanine Aminotransferase (U/L)	45	2.00 - 352.00	104.02	83.64
Aldolase (mU/ml)	45	1.35 -18.00	6.48	3.52
Aspartate Aminotransferase (U/L)	45	3.00 - 390.00	100.27	96.06
Cholinesterase (U/L)	45	1200 - 18232.0	6165.0	2478.9
CreatinphosphoKinase (U/L)	45	45.00 - 4120.0	1858.0	1128.40
Glucose-6-Phosphate Dehidrogenase (mU/ml)	45	0.07 - 0.80	0.33	0.08
GOT/GPT	45	0.51- 2.10	1.04	0.38
LDH/TGO	45	2.36 - 264.33	27.39	46.91
α-HBDH/LDH	45	0.30 - 0.99	0.78	0.20
α-Hydroxybutyrate Dehydrogenase (U/L)	45	267.00 - 2284.	760.69	341.14
Cholesterol (mg/dl)	45	127.0 - 182.0	127.00	6.01
Free Cholesterol (mg/dl)	45	37.00 - 65.00	52.73	3.65
HDL-Cholesterol (mg/dl)	45	16.00 - 36.00	26.25	2.26
LDL-Cholesterol (mg/dl)	45	45.00 - 123.00	92.75	8.91
Nitrogen Urea (mg/dl)	45	5.81 - 14.10	10.23	0.98
Phospholipids (mg/dl)	45	184.00 - 241.0	217.62	6.54
Triglycerides (mg/dl)	45	82.00 - 128.00	82.00	5.49
Urea (mg/dl)	45	12.37 - 30.00	21.77	2.09
Uric Acid (mg/dl)	45	2.60 - 6.13	4.15	0.55

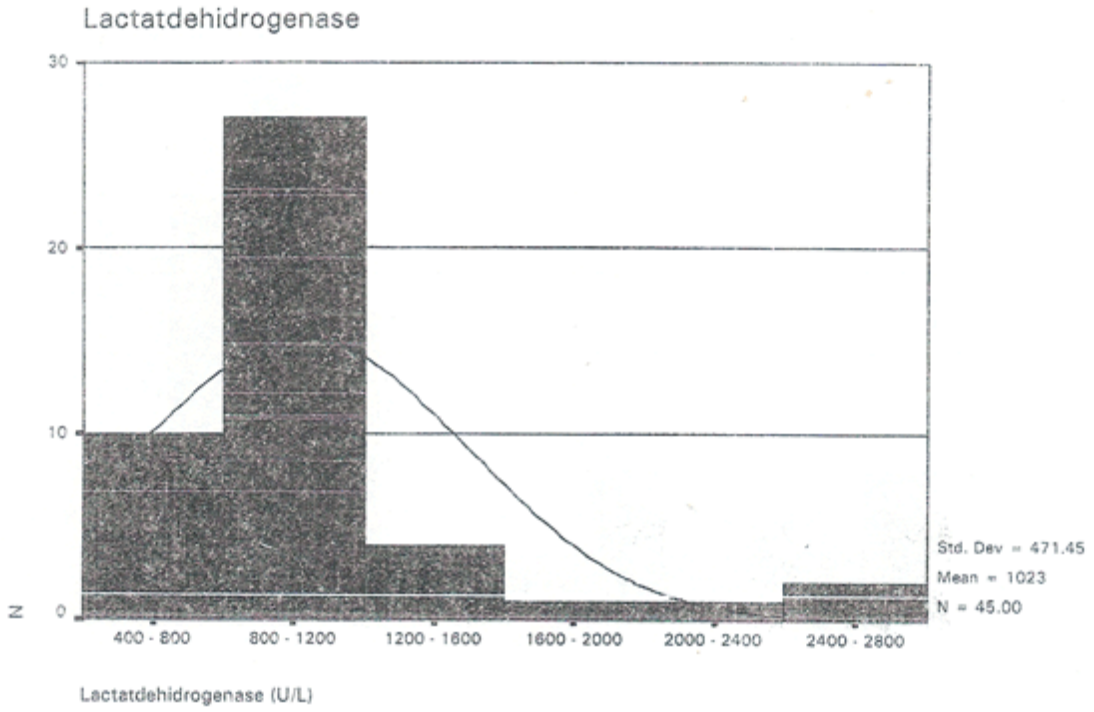
We point out also other investigations on ours subjects.
The normal distributions of these parameters are presented in the next figures.

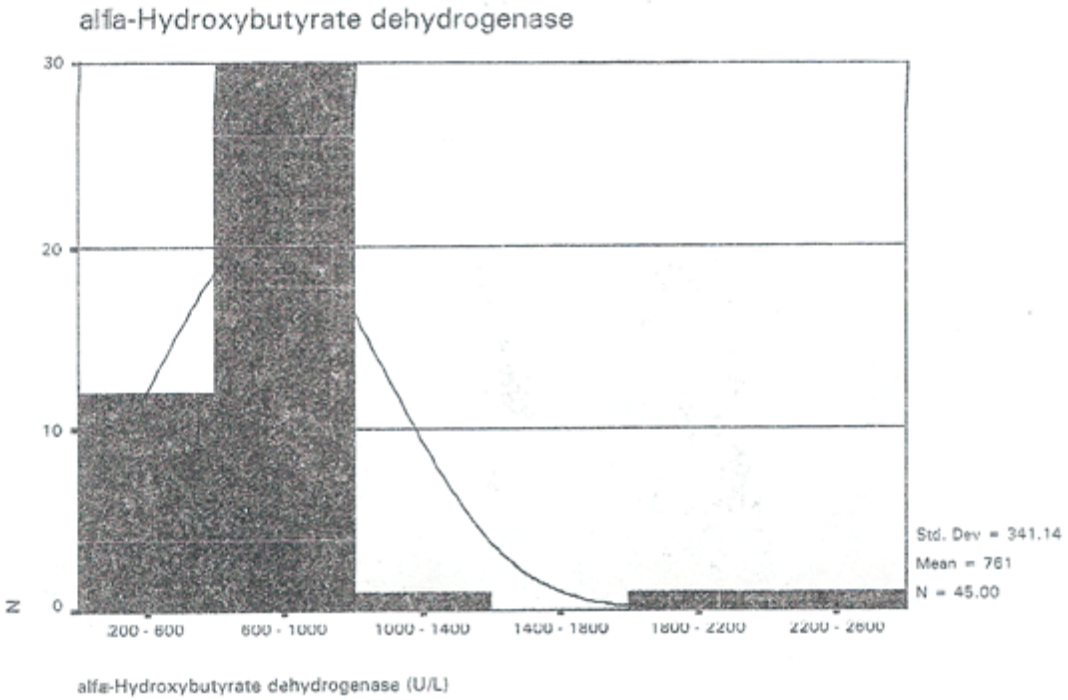
STATISTIC ANALISIS OF BIOCHEMICAL DATA FROM 45 PATIENTS WITH DUCHENNE MUSCULAR DYSTROPHY

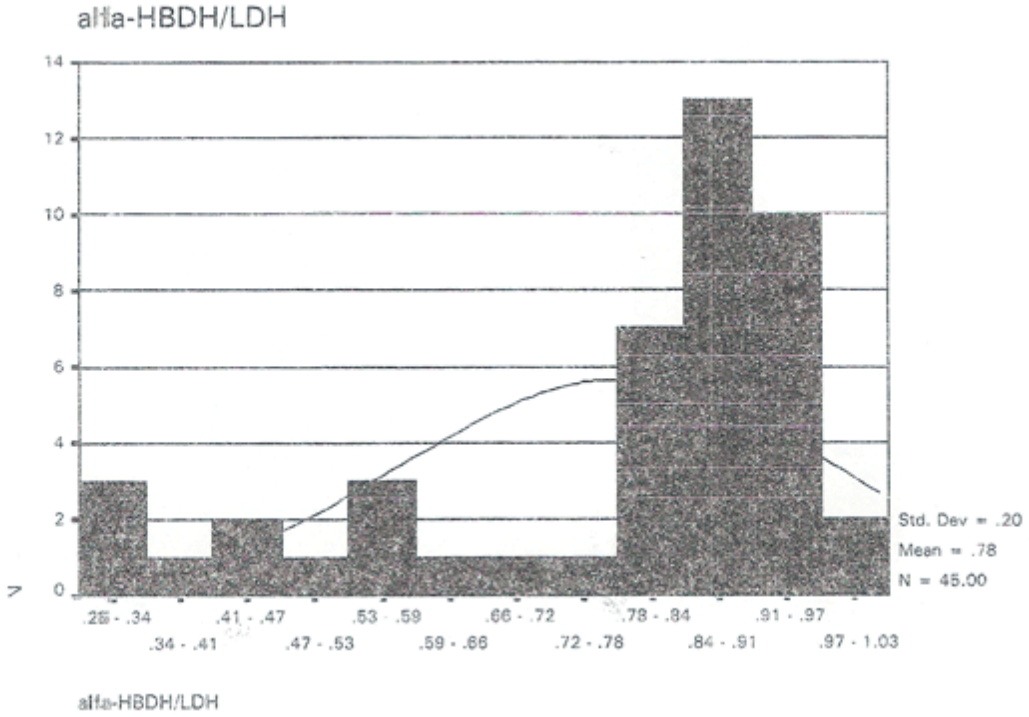


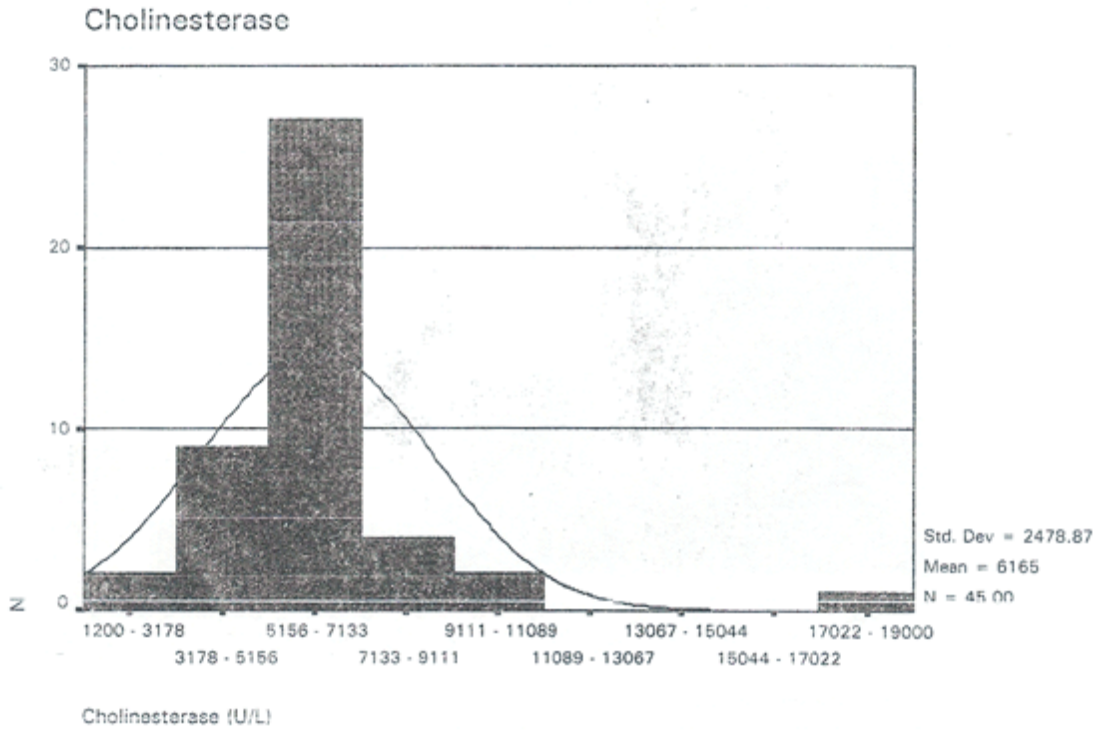


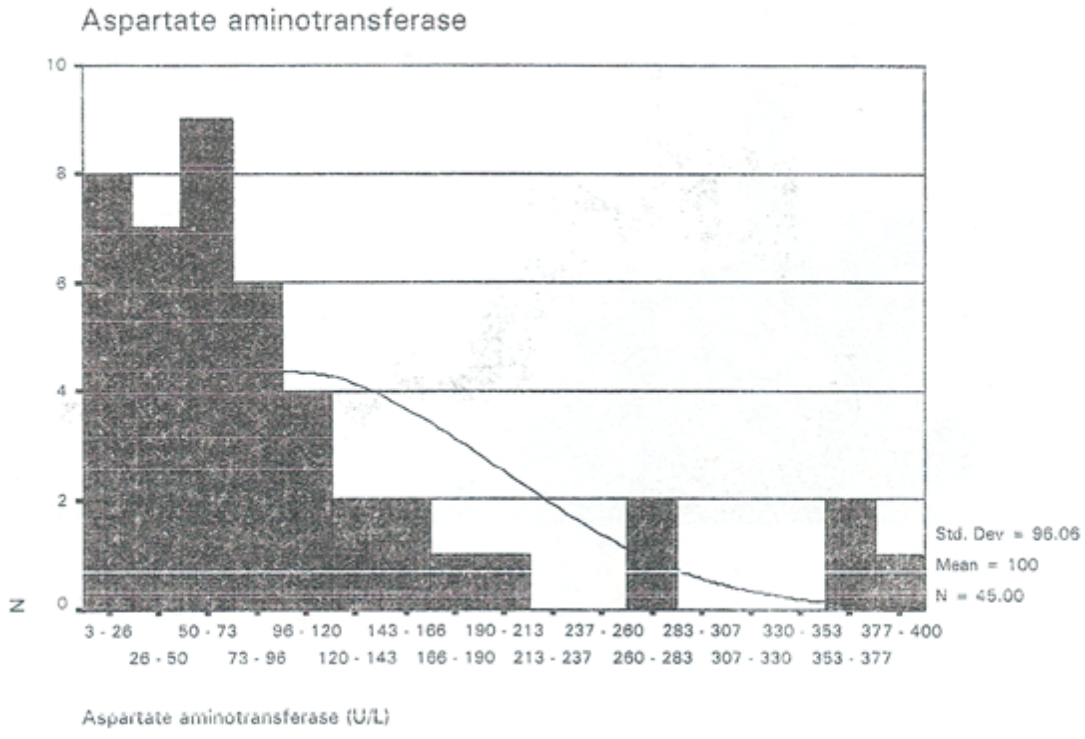

Creatinphosphokinase
20
10
0
N
500 - 1000
1000 - 1500
1500 - 2000
2000 - 2500
2500 - 3000
3000 - 3500
3500 - 4000
4000 - 4500
Std. Dev = 1128.39
Mean = 1858
N = 45.00
Creatinphosphokinase (U/L)

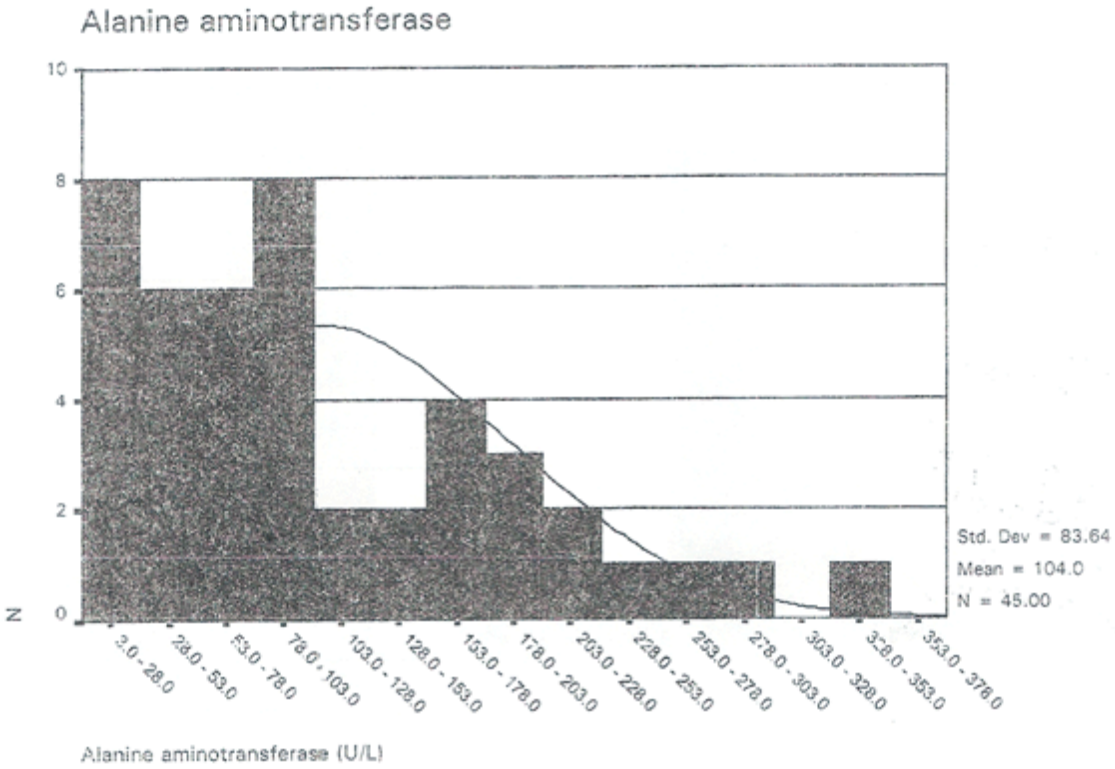

Lactatdehidrogenase
30
20
10
0
N
400 - 800
800 - 1200
1200 - 1600
1600 - 2000
2000 - 2400
2400 - 2800
Std. Dev = 471.45
Mean = 1023
N = 45.00
Lactatdehidrogenase (U/L)

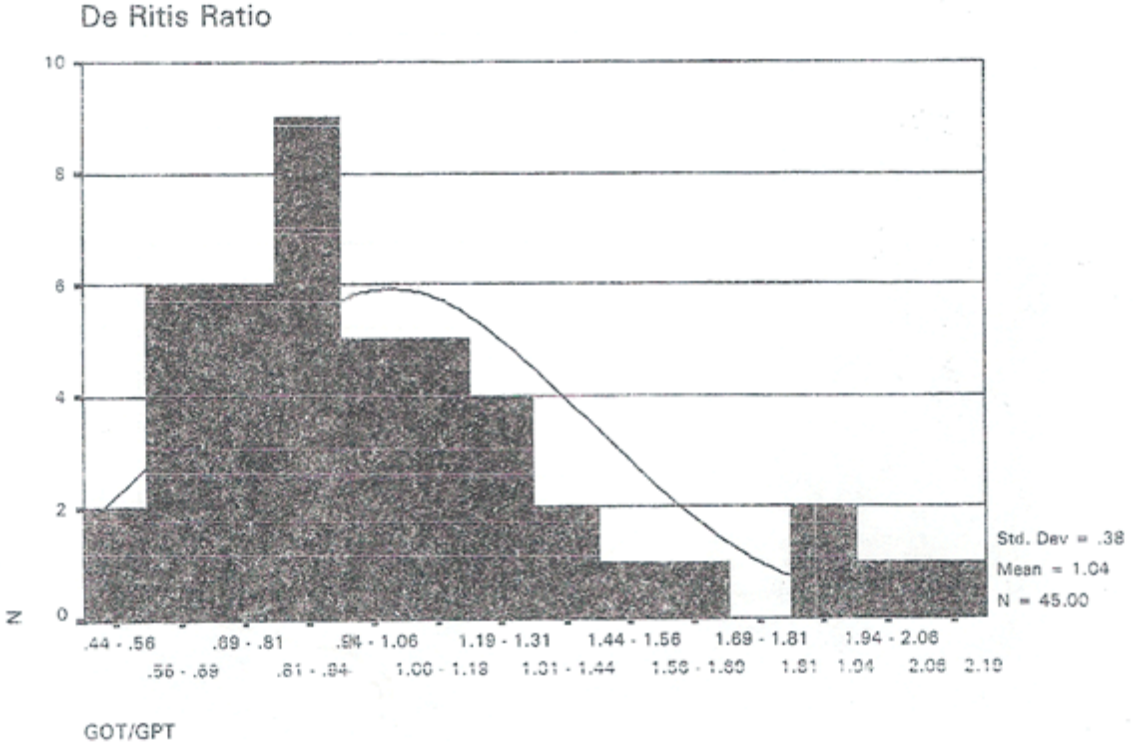

alfa-Hydroxybutyrate dehydrogenase
30
20
10
0
N
200 - 600
600 - 1000
1000 - 1400
1400 - 1800
1800 - 2200
2200 - 2600
Std. Dev = 341.14
Mean = 761
N = 45.00
alfa-Hydroxybutyrate dehydrogenase (U/L)

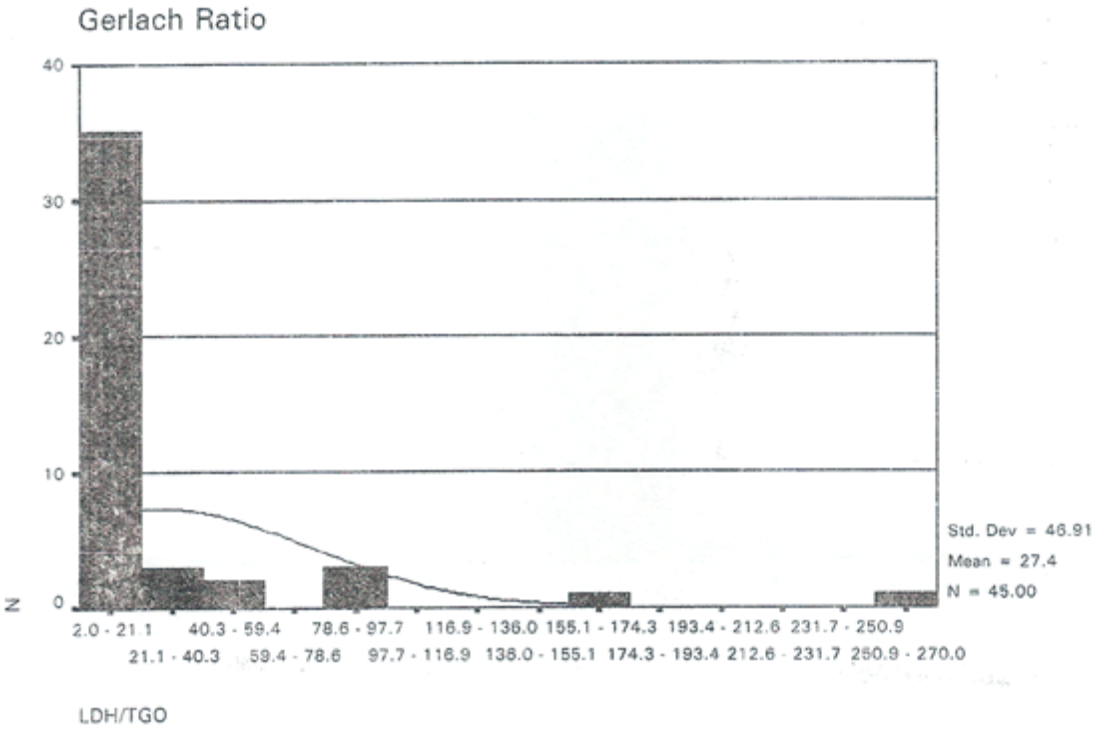

alfa-HBDH/LDH
14
12
10
8
6
4
2
0
V
.25 - .34
.34 - .41
.41 - .47
.47 - .53
.53 - .59
.59 - .66
.66 - .72
.72 - .78
.78 - .84
.84 - .91
.91 - .97
.97 - 1.03
Std. Dev = .20
Mean = .78
N = 45.00
alfa-HBDH/LDH

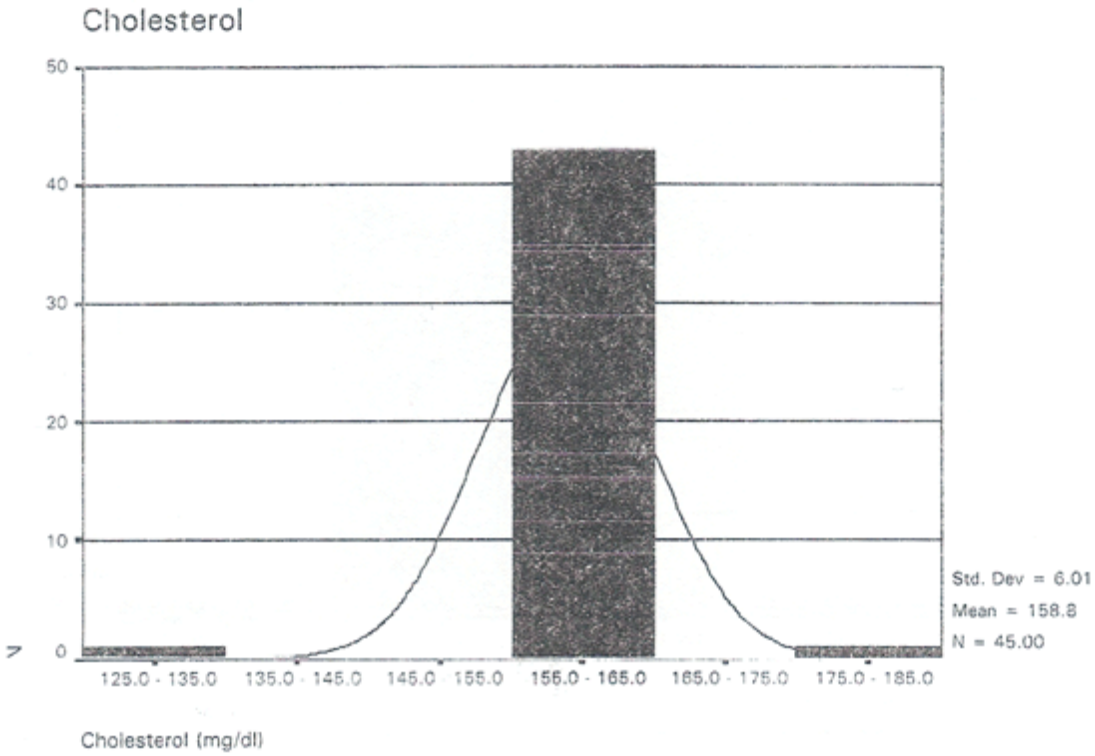

Cholinesterase
30
20
10
0
N
Std. Dev = 2478.87
Mean = 6165
N = 45.00
1200 - 3178
3178 - 5156
5156 - 7133
7133 - 9111
9111 - 11089
11089 - 13067
13067 - 15044
15044 - 17022
17022 - 19000
Cholinesterase (U/L)

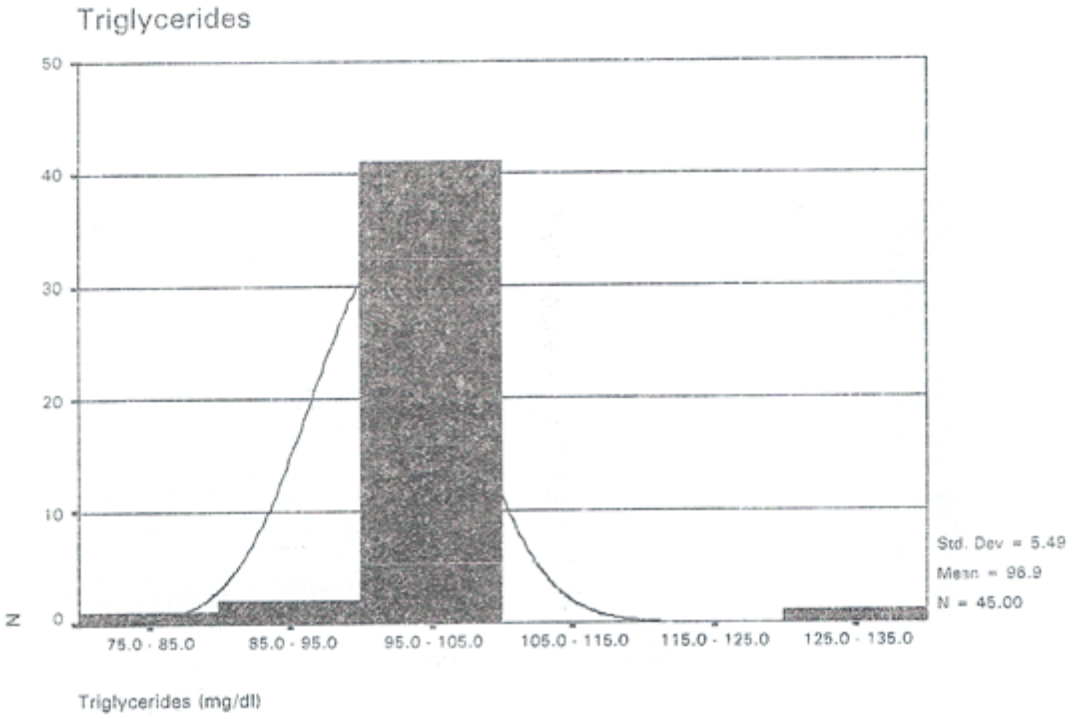

Aspartate aminotransferase
10
8
6
4
2
0
N
Std. Dev = 96.06
Mean = 100
N = 45.00
3 - 26
26 - 50
50 - 73
73 - 96
96 - 120
120 - 143
143 - 166
166 - 190
190 - 213
213 - 237
237 - 260
260 - 283
283 - 307
307 - 330
330 - 353
353 - 377
377 - 400
Aspartate aminotransferase (U/L)

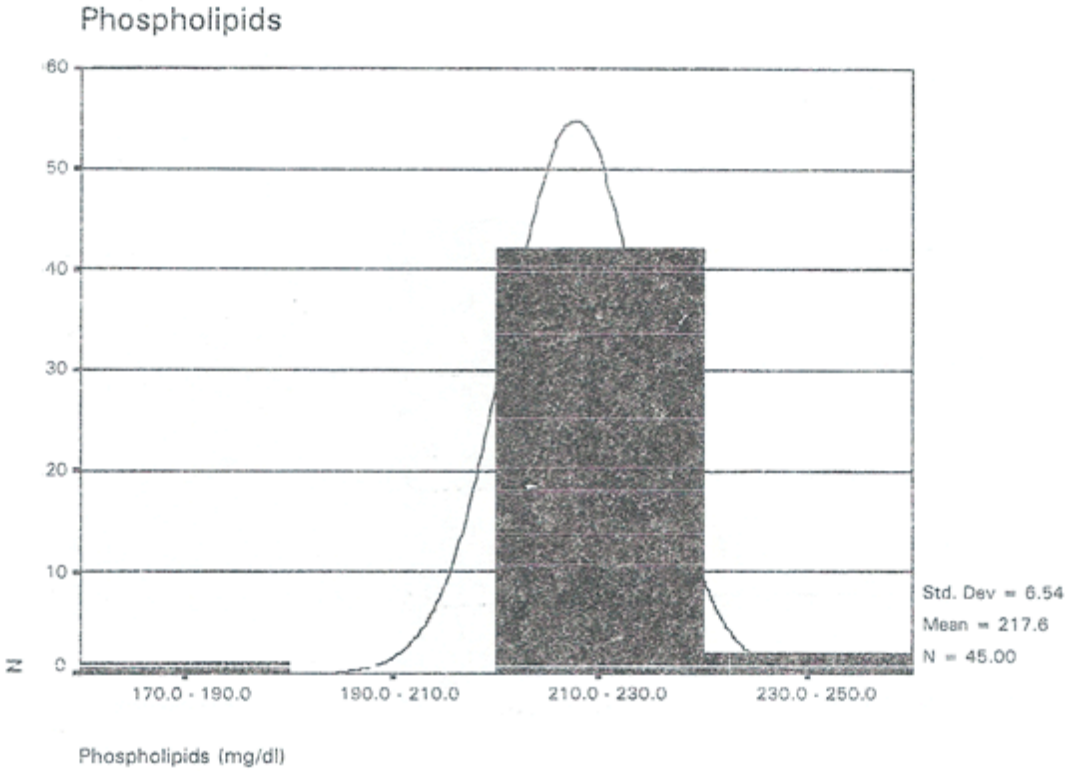

Alanine aminotransferase
N
Std. Dev = 83.64
Mean = 104.0
N = 45.00
3.0 - 28.0
28.0 - 53.0
53.0 - 78.0
78.0 - 103.0
103.0 - 128.0
128.0 - 153.0
153.0 - 178.0
178.0 - 203.0
203.0 - 228.0
228.0 - 253.0
253.0 - 278.0
278.0 - 303.0
303.0 - 328.0
328.0 - 353.0
353.0 - 378.0
Alanine aminotransferase (U/L)

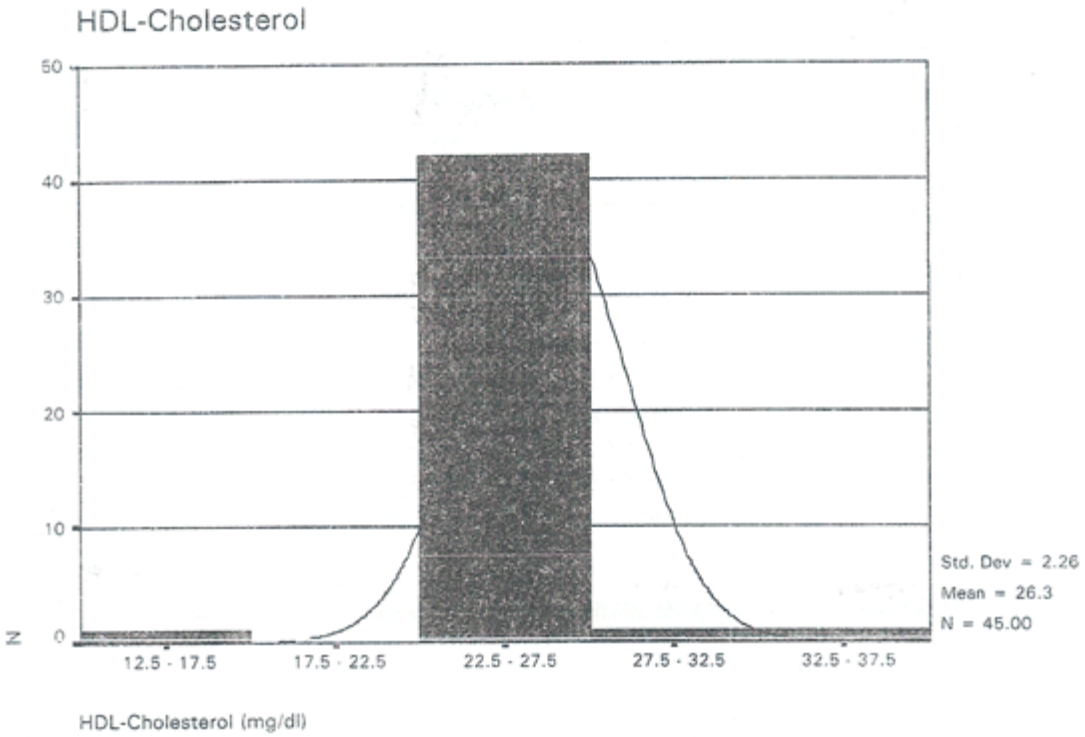

De Ritis Ratio
N
Std. Dev = .38
Mean = 1.04
N = 45.00
.44 - .56
.56 - .69
.69 - .81
.81 - .94
.94 - 1.06
1.00 - 1.18
1.19 - 1.31
1.31 - 1.44
1.44 - 1.56
1.56 - 1.69
1.69 - 1.81
1.81 - 1.94
1.94 - 2.06
2.06 - 2.19
GOT/GPT

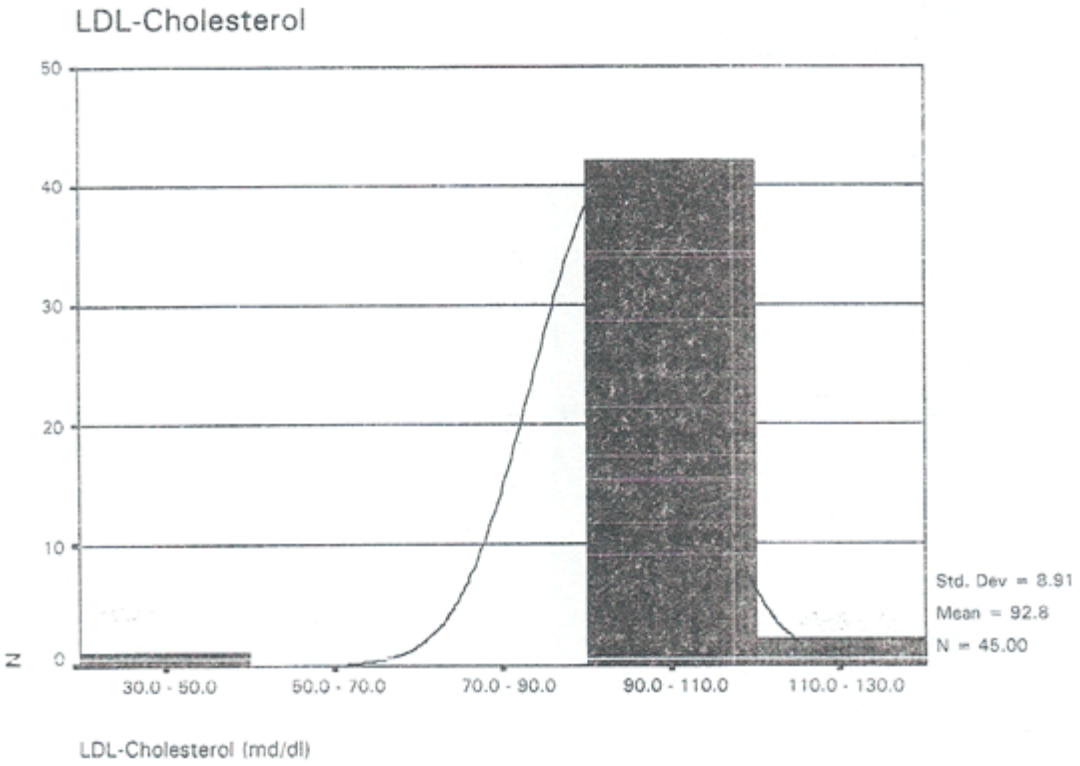

Gerlach Ratio
40
30
20
10
0
N
Std. Dev = 46.91
Mean = 27.4
N = 45.00
2.0 - 21.1
21.1 - 40.3
40.3 - 59.4
59.4 - 78.6
78.6 - 97.7
97.7 - 116.9
116.9 - 136.0
136.0 - 155.1
155.1 - 174.3
174.3 - 193.4
193.4 - 212.6
212.6 - 231.7
231.7 - 250.9
250.9 - 270.0
LDH/TGO

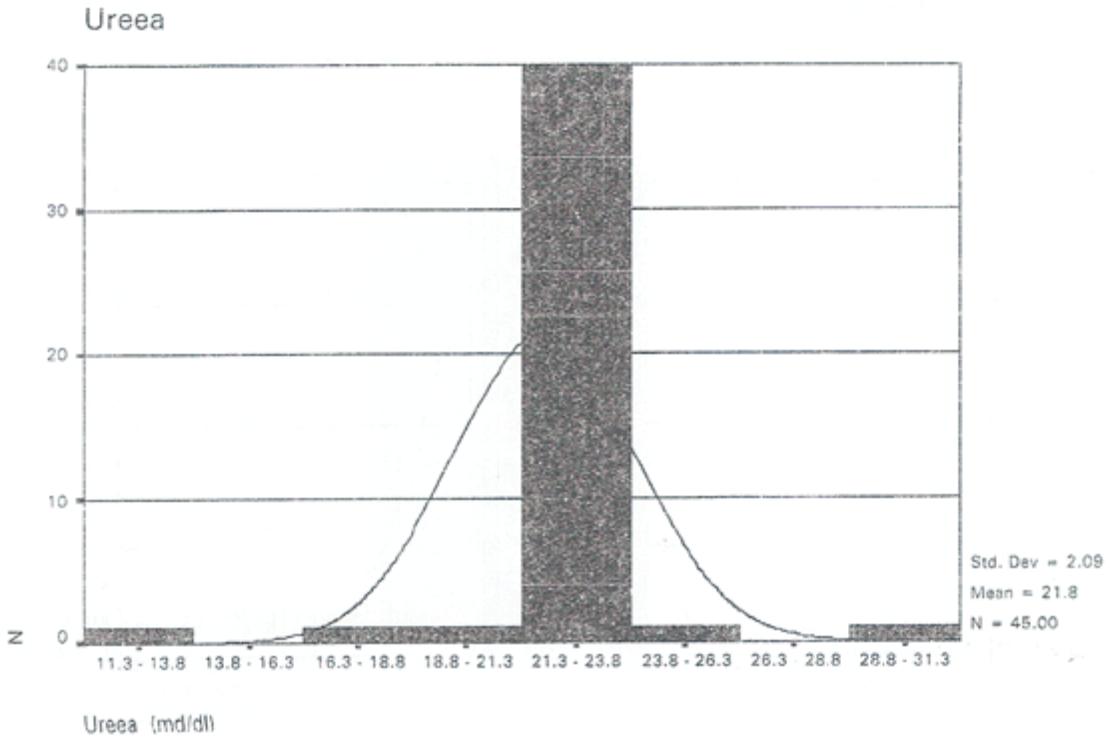

Cholesterol
50
40
30
20
10
0
N
Std. Dev = 6.01
Mean = 158.8
N = 45.00
125.0 - 135.0
135.0 - 145.0
145.0 - 155.0
155.0 - 165.0
165.0 - 175.0
175.0 - 185.0
Cholesterol (mg/dl)

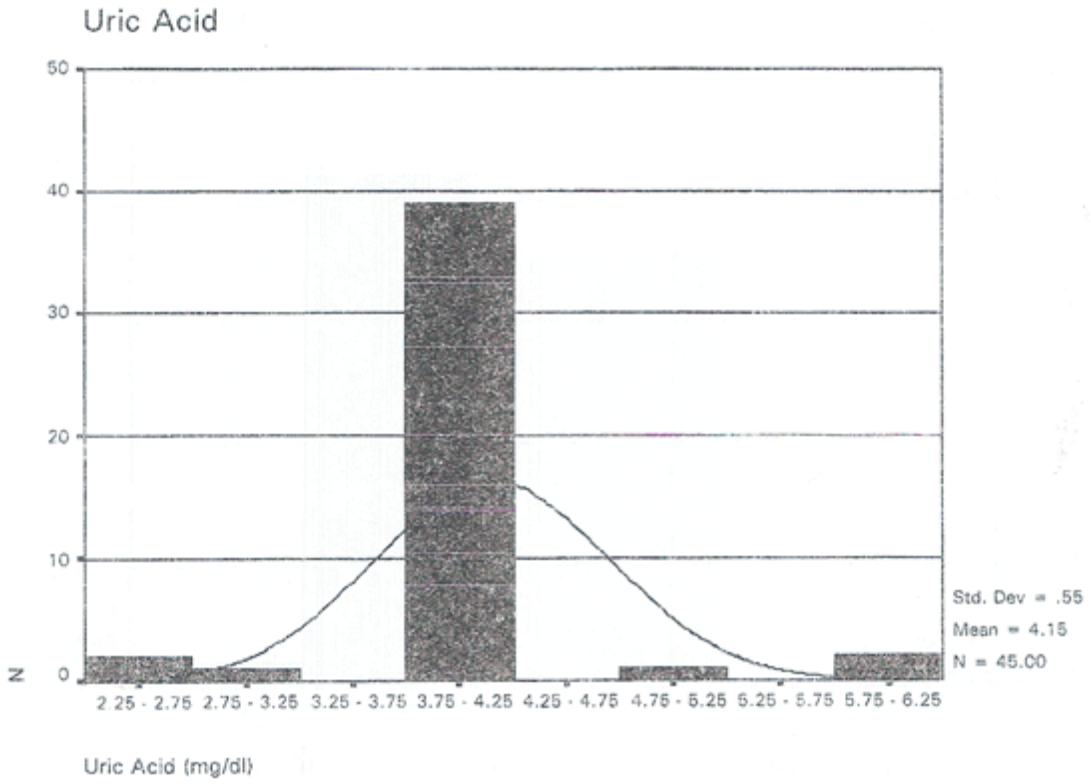

Triglycerides
50
40
30
20
10
0
N
75.0 - 85.0
85.0 - 95.0
95.0 - 105.0
105.0 - 115.0
115.0 - 125.0
125.0 - 135.0
Std. Dev = 5.49
Mean = 96.9
N = 45.00
Triglycerides (mg/dl)


Phospholipids
60
50
40
30
20
10
0
N
170.0 - 190.0
190.0 - 210.0
210.0 - 230.0
230.0 - 250.0
Std. Dev = 6.54
Mean = 217.6
N = 45.00
Phospholipids (mg/dl)


HDL-Cholesterol
50
40
30
20
10
0
N
12.5 - 17.5
17.5 - 22.5
22.5 - 27.5
27.5 - 32.5
32.5 - 37.5
Std. Dev = 2.26
Mean = 26.3
N = 45.00
HDL-Cholesterol (mg/dl)


LDL-Cholesterol
50
40
30
20
10
0
N
30.0 - 50.0
50.0 - 70.0
70.0 - 90.0
90.0 - 110.0
110.0 - 130.0
Std. Dev = 8.91
Mean = 92.8
N = 45.00
LDL-Cholesterol (md/dl)


Ureea
40
30
20
10
0
N
11.3 - 13.8
13.8 - 16.3
16.3 - 18.8
18.8 - 21.3
21.3 - 23.8
23.8 - 26.3
26.3 - 28.8
28.8 - 31.3
Std. Dev = 2.09
Mean = 21.8
N = 45.00
Ureea (md/dl)


Uric Acid
50
40
30
20
10
0
N
2.25 - 2.75
2.75 - 3.25
3.25 - 3.75
3.75 - 4.25
4.25 - 4.75
4.75 - 5.25
5.25 - 5.75
5.75 - 6.25
Std. Dev = .55
Mean = 4.15
N = 45.00
Uric Acid (mg/dl)

DISCUSSIONS

We present in this paper few observations made on a group of 45 subjects with Duchenne muscular dystrophy. The age of the patients was 8.51± 2.31 years old.

All parameters are generally "normal" for this disease excepted the concentration of uric acid with a mean of 4.15 ± 0.55 (very closely).

Reference values accepted range between 3.5 - 7.0 mg/ dl. (208 - 416 µmol/l).

It is possible to accept a secondary implication of uric acid in the pathogenesis of Duchenne Muscular Dystrophy.

An increased flux of free radicals occurs in inherited muscular dystrophy and leads to subsequent pathological changes in muscle tissue.

Evidence collected since that time has reinforced the hypothesis that free radical damage may be the initial mechanism of disease pathogenesis. Nevertheless, critical questions remain as to the some of free radicals within dystrophic muscle cells and in what way this source might be related to the genetic defect.

The answers to these questions lie in a clearer understanding of cellular processes, which involve free radical intermediates, and of the antioxidant system important to muscle.

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SERUM CALCIUM CONCENTRATION IN SOME NEUROMUSCULAR DISEASES

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ABSTRACT

The neurological effects of altered calcium metabolism may be prominent and varied. With low serum concentration, skeletal muscle is highly irritable, and carpopedal spasms and spontaneous contraction may occur. With marked hypocalcemia, the chief clinical sign is tetany, presumably owing to dysfunction in the peripheral neuromuscular system. Central nervous system manifestations are less common but may include abnormal movements. With high serum calcium concentration, reported central nervous system effects include the relatively non-specific symptoms of fatigue, weariness and weakness, which may be less severe than those symptoms that accompany hypocalcemia. It has been demonstrated that bivalent cations, and especially calcium, bind to negative charges in cell membranes. The requirement for calcium for neurotransmitter release in both the peripheral and central nervous system may reflect this action. We report in these paper particular observations on the serum calcium level in certain neuromuscular diseases.

Key words: Neuromuscular diseases – serum calcium

INTRODUCTION

The pathogenesis of Duchenne muscular dystrophy is unfamiliar. Even the nature of the weakness in the disease is not clear, for while usually attributed to necrotic loss of muscle fibers, it could also be due to a disorder in any system, including that of the contractile proteins within the muscle cell. The pathogenesis of Duchenne muscular dystrophy may be related to an abnormality of the muscle surface membrane, which could explain the leak of intracellular enzymes and the ingress of calcium. Accumulation of calcium may be important in the evolution of muscle degeneration. A molecular model of an excitatory membrane has been proposed, in which the functional unit is a macromolecular complex of Ca-ATP-phospholipid-protein. The Ca^{2+} is linked to ATP and the phosphate group of phospholipid which in turn interacts hydrophobically with a structural protein moiety. In addition to having a high affinity for ATP, the protein may have certain catalytic functions. The initial chemical event in excitation is believed to be the displacement of Ca^{2+} followed by the release of ATP and its eventual hydrolysis. Associated with the chemical events are the conformational changes in membrane structure responsible for the increased Na^{+} permeability. Restoration of the membranous structure requires the resynthesis and recombination of ATP, which in turn promotes the reuptake of Ca^{2+}. In addition to interfacial and other studies demonstrating the interaction of Ca^{2+}, ATP, and phospholipids, the model derives some support from studies on synaptic and other neural membranes that demonstrate the presence of high concentrations and combinations of the various constituents. This abnormality is possible to be reflected in serum. We think that it is significant to study the distribution of the concentration of calcium in a group of patients with Duchenne Muscular Dystrophy.

MATERIALS AND METHODS

The diagnosis of Duchenne Muscular Dystrophy was based on clinical history, neurological and electromyographic examinations, biochemical and enzymological tests Bioptical examination. The Group was selected allegory from our patients. Samples were obtained before breakfast. Samples of 3-4 ml of venous blood were added to heparinized

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test tubes and were immediately centrifuged at high speed. Reagents for Total protein were from "Sentinel"-Italy. Automatic Analyser Electa 216-E from "BPC BioSED"-Italy. Total Calcium was determinated using Atomic Absorption Spectroscopy (AAS1, Carl Zeiss Jena, Germany. Lamp Current: 8 mA, Air/Acetylene: Stoichiometric, Aspiration rate: 5 ml/min., Wavelength: 422.7 nm. Slit width: 320 μ. Lanthanum diluent: 1% La. Ionised Calcium was determinated with Ion Selective Electrode (ISE): Electrolyte analyser AVL 9180 U.S.A. All tests are performed currently in our laboratories in the same configuration.

RESULTS AND DISCUSSIONS

The results of our study are presented in the next pictures.

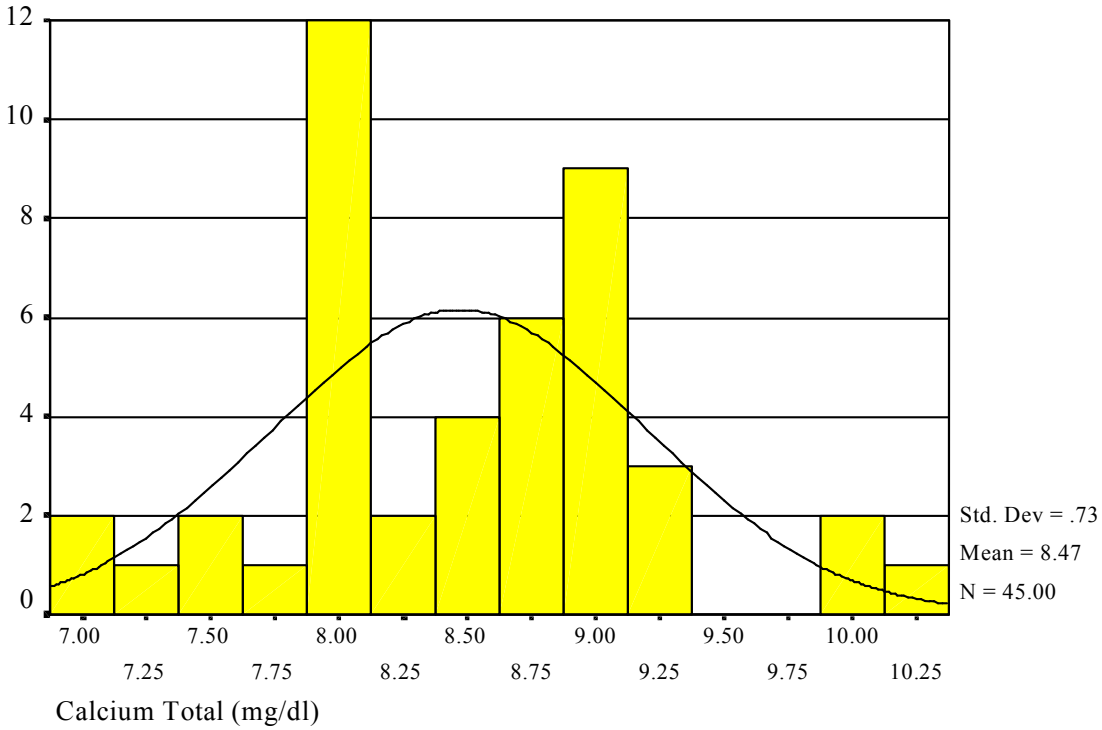


Fig. 1. Distribution of total calcium in subject group

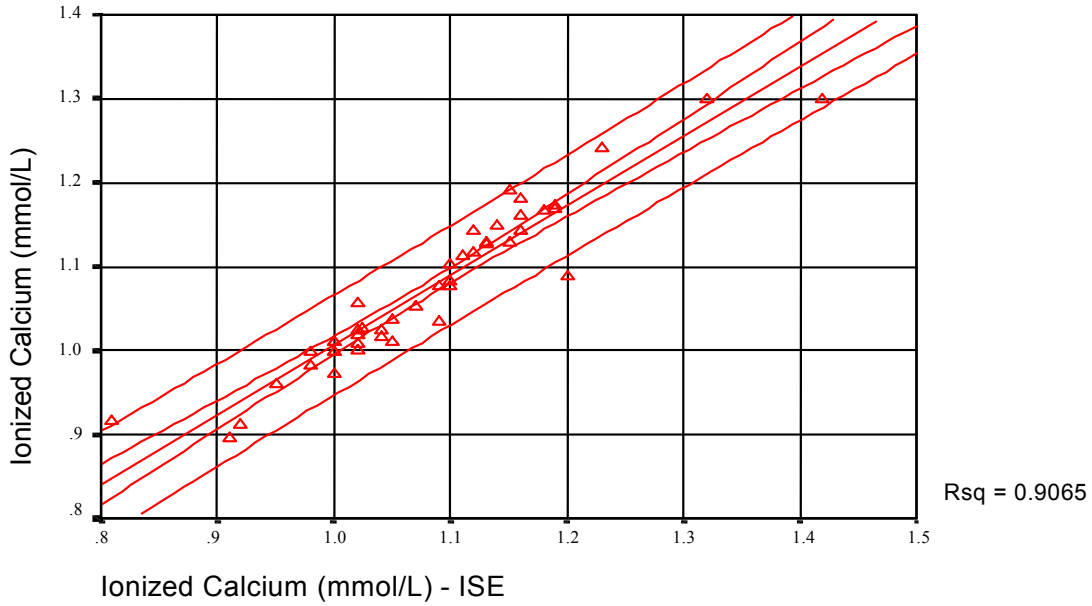


Fig. 2 Correlation of the ionized calcium vs. ionized calcium

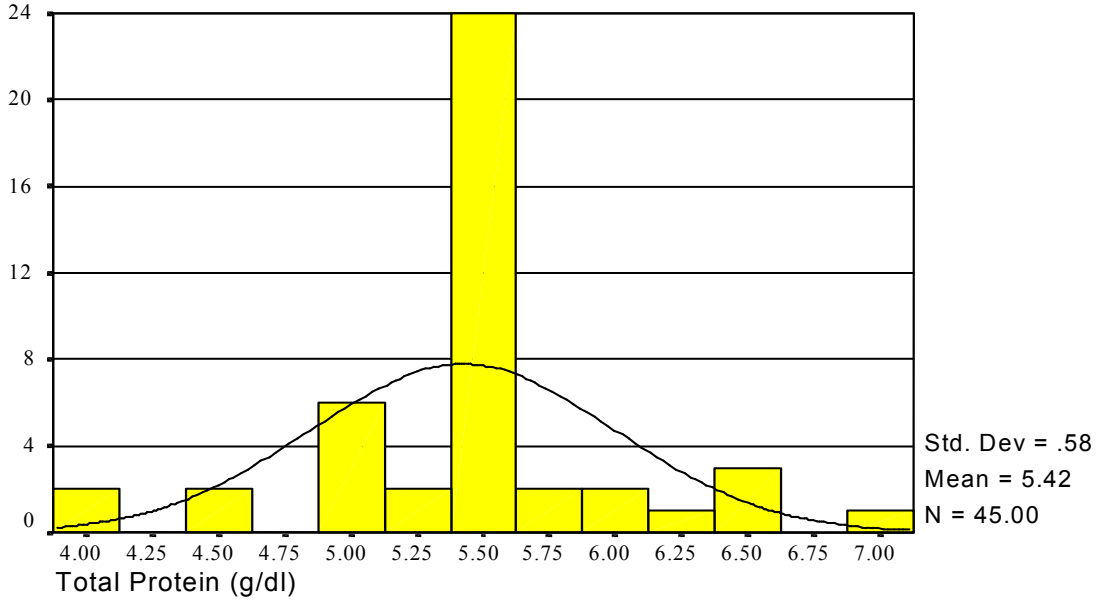
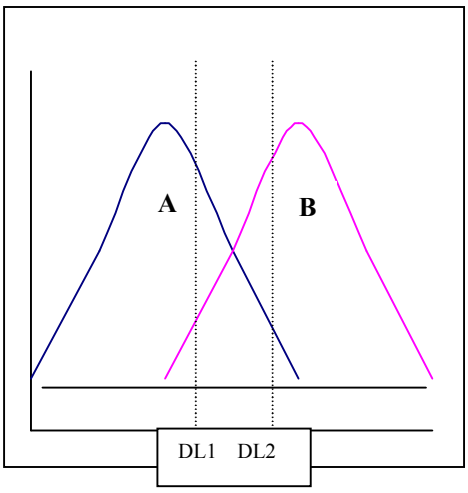


Fig 3 Distribution of total protein

Gräsbeck and Saris introduced the concept of reference values. The word "normal" is ambiguous, meaning, inter alia, usual frequent, gaussian, non-pathological. Normal values were traditionally associated with "health", which however, was regarded in a simplistic fashion and usually meant only subjective health. Briefly, both health and disease are relative concepts and absolute health as defined in the World Health Organisation Constitution is Utopian. "Health is characterised by a minimum of subjective feelings and objective sings of disease, assessed in relation to the medical activity, and is in the absolute sense an unattainable ideal state" after Gräsbeck[2] .

Health can be compared to temperature, it may be higher or lower but it is always present as long as the person is alive.

Everybody has at least some slight defect, e.g. a scar or is a heterozygote in respect to a congenital error of metabolism.



The next figure presents a situation, which should serve to illustrate the basic decision levels. Let us assume there are two relevant clinical classes that are called "A" and "B". The class of individuals represented by "A" is a group of healthy subjects while the individuals in "B" have some medical problem for which a particular therapy is suggested. Note that the distributions are symmetrical, the prevalences are equivalent, and that there is a certain degree of overlap in the values. A particular value can be in one of three intervals: definitely A, definitely B, or uncertain. The threshold value below which all measurements are definitely A is labelled as "DL1" while the threshold value above which all values are definitely B is labelled "DL2". Obviously, the abbreviation DL represents DECISION LEVELS. A decision is made depending upon

what side of the threshold a value of a particular measurement is found. When the value is below DL1, the possibility that the individual is a member of group B is excluded. Thus, DL1 is used as an exclusion limit. What is the action? The action is the clinician stating to the patient, "Your prognosis is not affected by the possibility of you being a member of class b".

The following statements made by clinician illustrate. Examples of this case:

1. Your child does *NOT* have Duchenne Muscular Dystrophy, as the serum creatinphosphokinase value is above the exclusion limit for Duchenne Muscular Dystrophy to consider as a possibility.
2. Your baby will *NOT* have the respiratory distress syndrome, as the amniotic fluid surfactant assay is above the threshold value.
3. You do NOT have evidence of diabetes as your plasma glucose value is below the threshold value.

If the measured value is above DL2, the diagnostic of being a member of class B is confirmed - assuming there are no other classes than A and B to consider Here. In terms of predictive value model, the value of DL1 gives 100% diagnostic sensitivity, that is, all individuals who are members of class B will have *ABOVE* DL1. In an analogous manner, the value of DL2 can be considered as a value having 100% diagnostic specificity, that is all individuals who *NOT* have the disease (who are members of class A) will have values *BELOW* DL2." It is assumed that a certain therapeutic and/or diagnostic action will take place if the patient is definitely a member of class B. Note that there is also an uncertain interval DL1 and DL2. Values here could indicate being in either class A or class B .We made the same observations on a group of 45 subjects with Duchenne muscular dystrophy concerning the Calcium Concentration in serum. In Duchenne muscular dystrophy there is an alteration of calcium levels in serum.

All our results concerning the serum calcium concentration are in the range of 7.00 - 8.50 mg /dl, with a mean of 8.47 mg/dl. and SD 0.73. The total protein was ranging between 4.25 - 6.00 with a mean 5.42 mg/dl and SD 0.58. We found also a good correlation between ionised calcium calculated and ionised calcium determinate with ISE, (R = 9065). The age of the patients was 8.51± 2.31 years old. Our results are importants for the biochemical aspects of Duchenne Muscular Dystrophy.

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PLASMA CONCENTRATIONS OF ACID – SOLUBLE THIOL (- SH), AND URIC ACID IN MULTIPLE SCLEROSIS AND MYASTHENIA GRAVIS

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ABSTRACT

The complex biochemical pathways described in the human body are strictly controlled by an array of regulatory mechanisms. Many of these biochemical reactions involve energy transfer and release and can result in the production of free radicals, unstable and highly reactive molecules with unpaired electrons which are important for normal cellular function and as a line of defense by macrophages for the destruction of foreign materials. Free radical levels in the body must be tightly controlled as their highly reactive nature makes them a potential threat to healthy body tisues. The body has an integrated antioxidant system to control free radical levels and neutralise excess by several components including enzymes, metal binding proteins etc.

When antioxidant reserves of the body become unable to control free radical levels they can cause serious damage at the cellular level. Clinical treatment of disease generally involves treating the symptom and not the cause of the problem.

The concentration of reduction equivalents in serum was investigated in an assembly of healthy individuals, in a group of multiple sclerosis pacients and another group of subjects with myasthenia gravis. We studied the evolution of the levels of the acid soluble thiols and uric acid.

Key words: multiple sclerosis, myastenia gravis, plasma concentration of acid soluble thiol and uric acid

INTRODUCTION

Multiple sclerosis (MS) is a major cause of neurologic disability among young and middle-aged adults. The disease is rarely diagnosed in persons under age 15 or over 55 years, with a peak incidence of onset around the age of 30. MS is characterised by demyelination in the white matter of the brain (usually periventricular), brain stem, or spinal cord. Sharp-edged demyelinated patches, ranging from 1 mm. to 4 cm., are macroscopically visible throughout the white mater of central nervous system. These lesions, which represent the end result of acute myelin breakdown, are called plaques. Oligodendrocytes are decreased in number and may be absent, especially in older lesions. The sequence of myelin breakdown is not well understood although it is known that the lesions contain small amounts of myelin basic proteins, increased amounts of proteolytic enzymes, macrophages, lymphocytes, and plasma cells. Acute, subacute, and chronic sclerotic lesions are scattered throughout the central nervous system.

Many believe that the disease has an immunological basis, but this has not been confirmed. The demyelination process in MS is marked by prominent lymphocytic invasion in the lesion. Both T4, helper cells and T8 suppressor cells are

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present. In some persons a sharp decline in suppressor T-cell population in the blood accompanies exacerbations of the disease.

Myastenia gravis (MG) is a disorder of transmission at the myoneural jonction that affects communication between the motoneuron and the innervated muscle cell. The disease may occur at any age, but the peak incidence of onset is between 20 and 30 years and is about three times more common in women than men. A small second peak occurs in later life and affects men more often than women. The disorder appears transiently and lasts for days to weeks in about 10% in infants born to mothers with MG. Myastenia gravis is through to result from a decrease in ACh receptor sites at the myoneural jonction that leads to decreased muscle function. Evidence indicates that the reduction in ACh receptor results from an autoimmune response. Recent research has demonstrated the presence of an ACh receptor antibody in 85% of persons with MG. This receptor antibody is thought to cause receptor degradation and inhibition of receptor synthesis. The exact mechanism that triggers the autoimmune response is unknown but is thought to be related to abnormal T-lymphocyte characteristics.

Popoviciu L (1963) reported first time, some abnormalities regarding the -SH concentration in MS.The sulfhydryl group is one of the most reactive and ubiquitous ligands in biological systems. It is found in most proteins and also in a few low molecular-weight substances such as glutathion, CoA, lipoate, thioglycolate, and free cysteine. It is perhaps the most studied of ligands, particularly in relation to the role in emzymic activity and properties of proteins. It is also involved in many membrane functions, since chemical agents with a degree of specificity for sulfhydryl can disturb many functions attributed to cell membrane. There was showed that the cysteine supply and the intracellular glutathione levels have a strong influence on the T cell system.

We found a substantial alteration of plasmatic –SH groups concentration in subjects affected by multiple sclerosis vs. normal and myasthenia gravis.

MATERIALS AND METHODS

Blood was taken from 10 patients with multiple sclerosis. 10 patients with myasthenia gravis and 10 controls. All of patients and controls were aged between 40-43 years. The diagnosis of multiple sclerosis and myasthenia gravis was made on clinical findings. Blood was taken from an antecubital vein into a sterile vacutaner. skin sterilisation, minimal venous stasis.

SH groups were measured spectrofotometrically, using a particular Ellman method, according Suzuki (1990) reversed Cavrini (1989) method and HPLC. Uric acid was measured enzimatically according Trinder (1969), Batham (1972), Fossati (1980) and Sanders (1980).

RESULTS AND DISCUSSIONS

The results of our study are presented in table1.

Table 1. Thiol and uric acid concentrations in control and experimental groups

Specification	SH (µMol / L)	p	Uric acid (µMol / L)	p
CONTROL (n =10)	290.15 ± 45.40		311.08 ± 19.25	
Multiple sclerosis (n =10)	198.45 ± 30.78	< 0.001	250.02 ± 75.64	< 0.05
Myasthenia gravis (n =10)	250.26 ± 65.44	< 0.1	270.00 ±85.12	< 0.2

We have investigated two components of the oxidative stress in plasma of 10 patients affected with multiple sclerosis. 10 patients with myasthenia gravis and 10 healthy age matched controls.

Cysteine (free thiol groups) is represented at the lowes concentration among all proteinforming amino acids in the blood plasma. Complementary laboratory experiments have shown that the cysteine supply is indeed limiting for important lymphocyte functions. Proliferate responses of mitogenically stimulated limphocytes and T-cell clones and the activation of cytotoxic T cells in allogeneic mixed lymphocyte cultures are strongly influenced by small variations in the extracellular cysteine concentration even in the presence of relatively high and approximately physiologic concentrations of cystine. Cysteine can be substituted by N-acetylcysteine but not by cystine. The more detailed analysis revealed that the extracellular supply of cysteine influences strongly the intracellular level of glutathione (GSH) and also the activity of the transcription factor. NFk B that regulates the expression of several immunologically relevant genes. A large body of evidence indicates that certain aspects of the T cell response require the action of active oxygen derivatives while other aspects of the response require the action of antioxidants such as cysteine and glutathione (GSH). The prooxidant and antioxidant states may be required sequentially at different times during T cell activation. The extremely weak cystine transport activity of T cellls together with oxidising metabolites from inflammatory microenvironments appear to be important factors that support the prooxidant state The relatively high cystine transport activity of the antigen-presenting macrophages, in contrast, provides these cells with a “cysteine pumping” function that antigen binding. T cells in their vicinity to shift to the antioxidant state. The difference between the membrane transport activities for cysteine of T cells and macrophages thus appears to be the key element of a mechanism that facilitates both the prooxidant state of T cells and their regulated shift to the antioxidant state. When T cells do not receive sufficient amounts of cysteine, the intracellular GSH levels and rates of DNA synthesis activity decrease

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UPTAKE OF EXOGENIC MAGNESIUM INTO LEAVES

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ABSTRACT

We studied the uptake rate of Mg through the membranes of plant leaves. The uptake rate was found to differ from plant species to species, e.g. it was smaller for sorrel than for bean. It was also established that the Mg taken up was fixated immediately, probably by proteins. The protein and Mg content of plant leaves was also found to depend on the age of the leaves; younger leaves contained 10-20% more of these.

Key words: magnesium, plant leaves, protein, age

INTRODUCTION

Former studies conducted by Mackov (1957) showed that the mechanism of uptake of nutrients from solutions through leaves is basically identical to that through roots. Therefore it seemed to be a logical choice to study the effect of exogenic Mg on plants in the form of leaf (aerosol) fertilizer.

According to Rousselet and Durlach (1971) there is an equilibrium existing between the free and protein-binded Mg. The value of the equilibrium constant for this process was estimated by Copeland and Sunderman (1952) to be K=33.77 which means a strong preference of the protein-binded Mg. Starting out from this we assumed that the Mg sprayed onto leaves and taken up is binding to proteins quickly and consequently 1) it can hardly be washed out similarly to endogenic Mg, and 2) it takes part in the metabolism.

MATERIALS AND METHODS

This study was conducted using sorrel and bean plants. The increase of Mg content of leaves after spraying was determined by the atomic absorption method after rinsing with distilled water, ashing and dissolution in hydrochloric acid. The spraying treatment was conducted on fully grown, young plants using a 1% $MgSO_4$ solution (250 ml/m^2). The Mg content was measured after 24, 48 and 72 hours.

The extractability of Mg was investigated by soaking the leaves, intact or cut lengthwise and crosswise, in distilled water at 25 and 90°C. The duration of the soaking was 5 hours at 25°C and 10 minutes at 90°C. The remaining Mg content was determined by using the atomic absorption method.

The relation between the Mg content and the age of plants was established through studying the results of the above process for leaves of paprika, brussels sprout, sorrel and bean plants. Protein contents, measured using the conventional Kjeldahl method, were also determined.

RESULTS AND DISCUSSIONS

The results showing the effect of Mg spraying on sorrel and bean leaves are displayed in Table 1. It can be seen that Mg uptake is complete after 48 hours. The uptake rate is different for different plant species, e.g. it is smaller for sorrel than for

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bean. The extractability of Mg from intact or cut sorrel leaves can be seen in Table 2 and 3. It can be stated that the results are basically the same in both the treated (exogenic Mg) and control (endogenic Mg) cases. This finding led us to the conclusion that exogenic Mg is incorporated into proteins very rapidly. Finally, Table 4 illustrates the finding valid for various plant species that younger leaves generally contain 10-20% more Mg and proteins than older ones.

Table 1. Effect of spraying a Mg solution on the Mg content of leaves (mg Mg/100 g)

Sampling after treatment (hrs)	Sorrel		Bean	
	mg	*%*	*mg*	*%*
0	660	100	464	100
24	669	106	650	138
48	735	111	702	150
72	740	112	695	148

Table 2. Extractability of Mg by water from intact sorrel leaves at different temperatures (mg Mg/100 g)

Soaking		Control		Treated	
Time	Temp.	mg	%	mg	%
0	-	660	100	464	100
5 hrs	25	669	106	650	138
10 min	90	740	112	695	148

Table 3. Mg content of intact and cut sorrel leaves after 5 hours of leaching by distilled water at 25°C (mg Mg/100 g)

	Control		Treated	
	Mg	%	Mg	%
Unleached	1260	100	1485	100
Intact leaves	1259	92	1391	94
Cut lengthwise	1094	87	1260	85
Cut crosswise	1010	80	1143	77

Table 4. Mg- and protein-content of leaves of various plant species, as the function of their age (mg Mg/100 g)

	Protein			Mg		
	Young	Old	Diff. %	Young	Old	Diff. %
Paprika	24.1	22.3	-7.5	1001	880	-12
Brussel sprout	33.6	27.8	-14.0	190	150	-20
Sorrel	37.0	29.8	-19.4	1120	910	-19
Bean	30.3	29.9	-1.3	350	310	-11

CONCLUSIONS

The stimulating effect of Mg on the protein synthesis and consequently on maintaining a juvenile condition can be explained in terms of its impact of the structure of ribosomes. Measurements by McCarthy (1962) proved that the extent of protein synthesis is proportional to the number of ribosomes. Protein synthesis only takes place on poly-ribosomes according to Wettstein (1963). Ricciardi (1974) stated, using ^{14}C measurements, that less protein synthesis is conducted in older cells in spite of the fact even if the total number of ribosomes was unchanged. His further studies revealed that while in young (actively protein synthesizing) cells 80% of ribosomes is in the poli-ribosome form, in older cells this portion is 15% only. Mg plays an important role in maintaining the poli-ribosomal structure. Palade (1956) applied electron microscopy to show that ribosomes are attached to each other pearlchain-like in poliribosomes but only if Mg concentration exceeds a certain 10^{-3} M limit. If Mg concentration fails to reach this level , the poliribosomes get reversibly depolarised. This explains why in older cells, which have a decreased Mg content, protein synthesis drops too. Furthermore, Goldberg (1966) provided proof of that the role of Mg in ribosomes is to bridge RNS to proteins.

We found the uptake of Mg sprayed onto leaves to be quite rapid. Adopting the proposal of Rousselet and Durlach (1971) according to which Mg is binded to proteins is supported by the finding that no extra Mg can be extracted at neither 25 not 90°C with water after treatment. There are more than one publications suggesting that Mg penetrates both plant and mammal membranes, and therefore binds to proteins, relatively quickly. In the experiments by Jovanovic and Maksimovic (1998), drinking 1 dm^3 550 mg/dm^3 Mg-containing mineral water caused a significant increase of Mg in erytrocita and a decrease in blood pressure just two hours after drinking. A publication by Bara et al. (2000) describes the dependence of Mg uptake through human amniotic membranes on the type of the accompanying anion.

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MODIFICATIONS OF SUPEROXID DISMUTASE ACTIVITY AT DIFFERENT PERIODS OF MECHANICAL TRAUMA

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ABSTRACT

Mechanical trauma, as any other extremal factor, significantly amplify lipid peroxidation and activate the antioxidant system in the human organism. Different tissues respond to trauma in manners corresponding to their specific chemical composition and metabolism. The dynamic of SOD activity was studied in liver, kidneys, brain and erythrocytes at different periods of muscles compression and after their decompression. Only in liver SOD activity is lower then the normal levels during either the compression or decompression. In the compression period it decreased slowly for 30 min with 37% and returned almost to initial values after 240 min (88% from the control), the decompression starts a new diminution but it is not so fast and profound as the first one. The dynamic of kidney's SOD activity is similar to hepatic one, except an important rise to 150 % above the control level after 60 min of compression. SOD activity in erythrocytes and brain also decreased during the compression with a rise after 30 min in the brain and 60 min in the erythrocytes. In brain and erythrocytes (unlike liver and kidneys) SOD activity was increased by the decompression. The amplitude of SOD activity modifications and their speed in erythrocytes are bigger then in rest of the tissues.

Key words: superoxide dismutase activity – mechanical trauma

INTRODUCTION

The superoxide anion radical is generated by a wide range of enzymatic oxidation reactions in respiring cells as an intermediate of the reduction of oxygen. It has been reported that the superoxide anion may damage the cell membranes (Tyler D.D., 1975; Lynch R.E., Fridovich I., 1978) and DNA (Hemmen J.J., Meuling W.J.A., 1975) and inactivate various enzymes (Boehme D.E. et al.,1976).

The enzyme superoxide dismutase (SOD, EC 1.15.1.1), which catalyses the dismutation of the superoxide anion radicals to peroxide and oxygen, has an important role in the defensive mechanism against the oxygen toxicity in the cells. Mammalian cells contain Cu, Zn-SOD in the cytoplasm and Mn-SOD in the mitochondria. The contents of superoxide dismutase are different among various organs and tissues (Marklund S.L. et al., 1982).

Mechanical trauma, as any other extremal factor, significantly amplifys the production of oxygen radicals and lipid peroxidation, also it activates the antioxidant system in the human organism. The equilibrium between this two systems determined the resistance of the cell to oxidative stress and its survival.

MATERIALS AND METHODS

The trauma was modelled by the compression of the back feet muscles of Wistar rats, males with the weight of 180-200 g (V.Kulagin's method, 1977). SOD

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activity was evaluated in erythrocytes, liver, kidneys and brain at different periods of compression – 15′, 30′, 60′, 240′ (1, 2, 3, 4 group) in comparison with its activity in intact animals (control group). A special group (group 5) includes animals whose muscles were decompressed after a 240′ traumatization.

SOD activity was determinate in erythrocyte haemolysate and liver, kidneys, brain tissue omogenizat according to S.Cevari method based on the inhibition of neotetrazol reduction in the presence of $NADH.H^+$ and fenazinmethasulfate (S.Cevari et al., 1985). The activity of the enzyme was referred to haemoglobin content in erythrocytes and protein content in the rest of the tissues.

Statistical analysis was performed by Student's t-test.

RESULTS AND DISCUSSIONS

The dynamic of SOD activity in different tissues at different stages of compression and after the decompression is not the same, the respond to the trauma corresponding to their specific composition, metabolism and function.

Only in liver the SOD activity is lower than the control level in all the groups that were studied. The compression determined a slow and moderate decrease for 30′ of SOD activity with 37% ($p<0,01$). The prolongation of the compression induced the mechanisms of adaptation to trauma and SOD activity increased to 70% of the normal values at 60′, and to 88% at 240′. SOD is inactivated for the second time by decompression, but not so significant than for the first time.

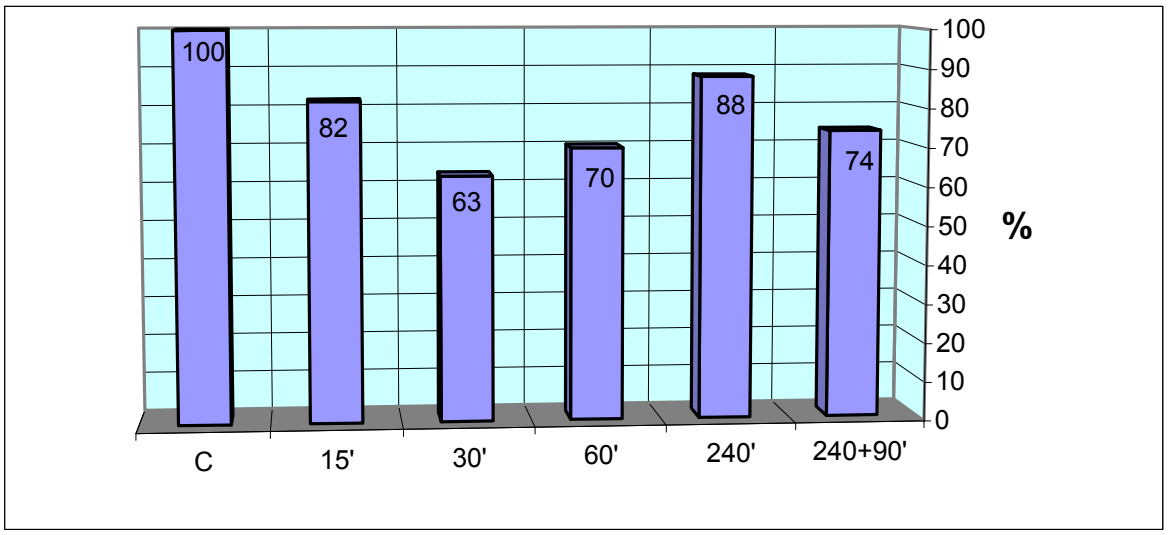

Fig.1.The dynamics of the SOD activity in the liver at different periods of the mechanical trauma

The modifications of SOD activity in kidneys are mostly similar to the liver one. The enzyme activity also decreases in 1 and 2 groups and in the decompression period. But in this case SOD inhibition is more profound as the minimal level had been registered in the 5^{th} group – 40%. In the bilated stages of the mechanical trauma - 60′ and 240′, SOD activity is higher than the control levels (150% and 105%).

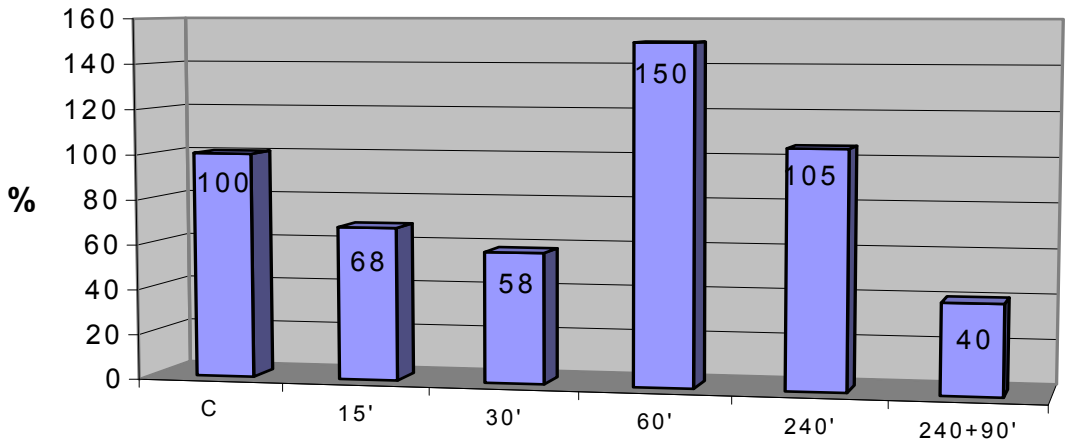

Fig. 2. The dynamic of the SOD activity in the kidneys at different periods of the mechanical trauma

There are analogies in the dynamic of SOD activity in erythrocytes and brain , having been decreased in the same periods of compression – at 15′ and 240′. IN the later stage SOD activity is significantly lower than at the beginning of the traumatization. So in the 1st group SOD activity in brain is decreased with 18% and in erythrocytes with 50%, in the 4th group its activity falls to 50% in brain and 22% in red blood cells. In the last group (240′ compression + 90′ decompression) SOD activity outruns the control in the both tissues. The amplitude of SOD activity modifications and their speed in erythrocytes is higher than in the rest of the tissues.

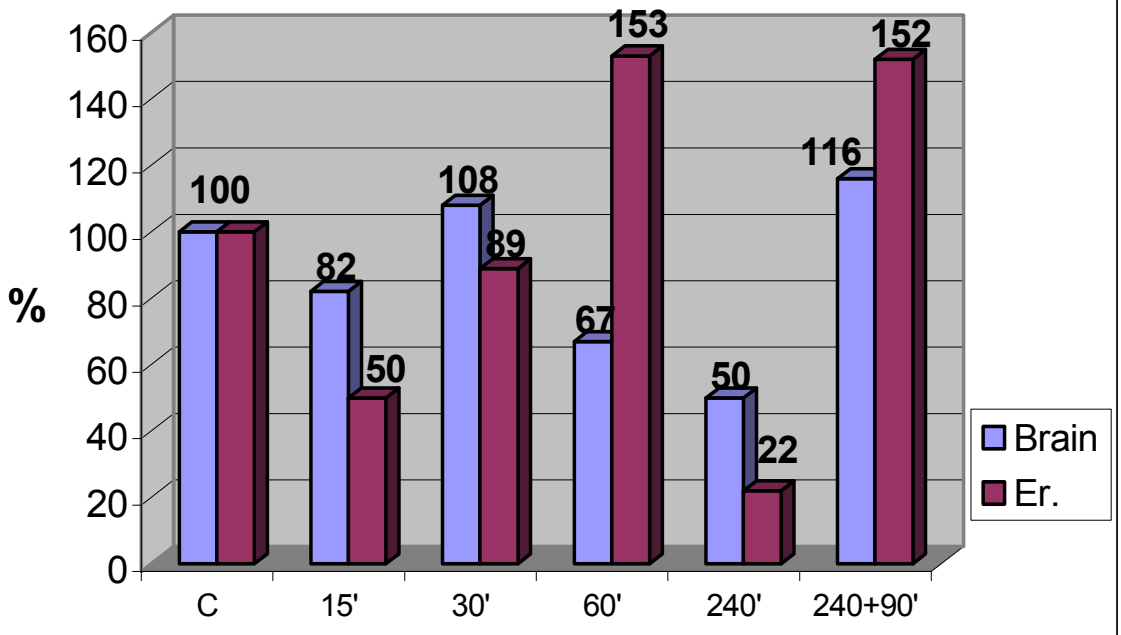

Fig.3. The dynamic of the SOD activity in the brain and erythrocytes at different periods of the mechanical trauma

CONCLUSIONS

Our results allow us to make the following conclusions.

- Severe mechanical trauma decreases SOD activity in liver, kidneys, brain and erythrocytes in the earliest stage (15′ of traumatization).
- In the middle stages – 30-60′, SOD activity increases in kidneys, brain and erythrocytes. Their levels reach the highest one in comparison with the other periods. That shows an adaptation of the metabolism of that tissues to extremal conditions.
- The liver, possible because it is the main place of detoxification in organism and has the ability to scavenge free radicals from blood, requires a longer period for restoring SOD activity – 240′.
- A long traumatization requests are too hard for tissues and their capacity of adaptation is exhausted, so SOD activity repeatedly decreases in kidneys, brain and erythrocytes.
- The decompression is followed by the reoxigenation of the former ischaemic muscles and the efflux into blood of a series of toxic products that affect organs and tissues that weren't traumatised.

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THE SYNTHESIS AND CHARACTERIZATION OF BIOLOGICALLY ACTIVE ANTIMONY COMPOUNDS

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ABSTRACT

A new compound was obtained by reacting antimony-trichloride with sulphatiazole. The resulting complex was characterized through elemental analysis, IR spectroscopy, thermogravimetric analysis, X-ray diffraction.

The biological activity of sulphatiazole-antimony complex was tested at the Transilvanian Ethnography Museum. The complex was successfully used for protecting wood against bacteries, fungi and insects.

Key words: Synthesis of Sb(III)- sulphatiazole complex-structure, activity

INTRODUCTION

The interest for metal organic compounds of antimony appeared out of the necessity to obtain new biologic activ substances and was stimulated, at least formally, by the analogy with arsenium compounds whose therapeutical importance was almost known by 1910.

For the first time a tertiary antimony-organic combination was prepared in 1850 (C. Lowig and E. Schweizer). It followed a period of sustained interest till the XX century.

Last years as a result of analysis means, interest over this compounds increased due to the great structure variety [1-7] as well as due to the practical utility in medical domain or museology.

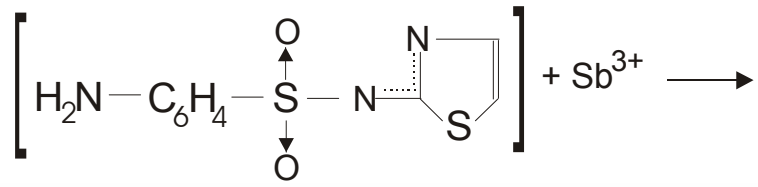
This paper presents our research regarding the synthesis and charaterization of the complex Sn-sulphathiazole.

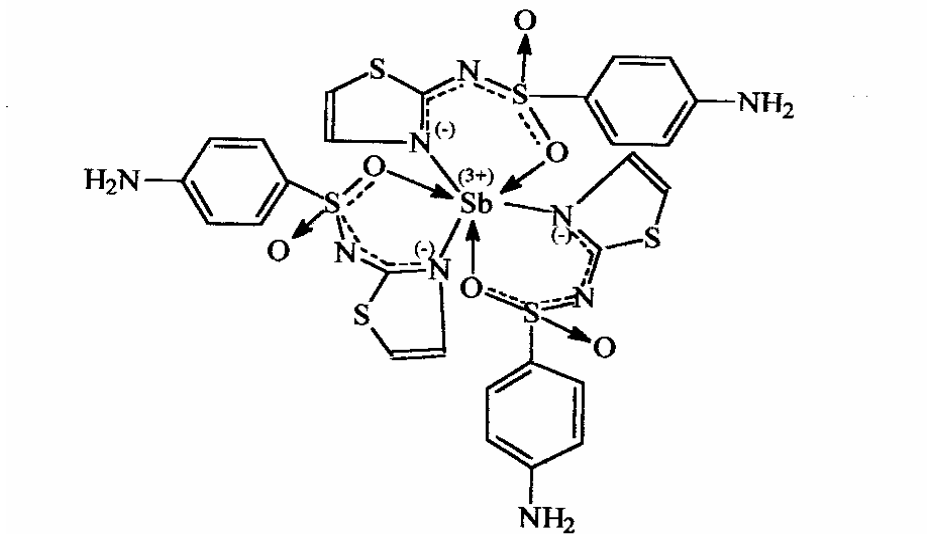
MATERIALS AND METHODS

In order to prepare the Sn(III) - sulphathiazole complex we used 5g pharmaceutic sulphathiazole which was dissolved at heat in 150 mL acetone and 3g $SbCl_3$ also dissolved in heat in acetone. The mixture was refluxed for 3 h at 55 ^oC. The solution was kept for 24 h at cold after correcting its pH from 4.5 to 6 using Na_2CO_3 solution 10%. It was obtained a white colour compound which was filtrated, washed with acetone and dried at 50 ^oC.

RESULTS AND DISCUSSIONS

The obtained product Sb(III) – sulphathiazol was characteized using elemental analysis, IR spectrometry and thermogravimetry. The corresponding reaction is:





Tabel 1 presents the result of elemental analysis and confirms the formation of a complex in which the recombination rate Sb(III) : sulphathiazol is of 3 : 1 g/g.

Table 1. Elemental analysis of the Sb(III) : sulphathiazol complex

Element	Sb	C	H	S	N
Calculated	13.88	36.86	2.73	21.84	14.33
Found	14.23	37.69	2.82	21.35	14.28

Data of thermogravimetric analysis of the Sb(III) – sulphathiazol complex are presented in Table 2.

Table 2. Results of thermogravimetric analysis of Sb(III) : sulphathiazol complex

Temperature domain (^{O}C)	TG	DTA				Observations Phenomena
	Temperature (^{O}C)	Losts		Temperature (^{O}C)		
		Calculated (%)	Found (%)	Effects		
				endothermal	exothermal	
20-110	90	3.93	3.84	Medium		Complex is dehydrated
110-460						Stability domain
460-880	540 610 830	83.27	83.32	Strong (540) Weak (610)	Medium (830)	Formation and loss of NH_3, CO_2, H_2O, SO_2, NO, NO_2
880-1000		13.33	12.82			Analyzed residue Sb_2O_3

Due to the total loss of water up to 90^{O}C the weight decreases (two water molecules to one molecule of complex). Up to 460 ^{O}C is considered the stability domain of the anhydric compound and between 460-880 ^{O}C decomposition takes place with emission of volatile compounds such as ammonium, carbon dioxide, water, sulphur dioxide, nitrogen oxides with a strong endodermal effect at 540°C. Beyond 880 ^{O}C the final product was obtained Sb(III) oxide.

Main vibrations from IR spectra of sulphathiazol and Sb(III) - sulphathiazol complex are given in Table 3.

Table 3. IR spectra of sulphathiazol and Sb(III) - sulphathiazol complex

Compound	$\nu_{(SO2)as}$	$\nu_{(SO2)sim}$	ν_{NH}	$\nu_{thiazolic\ ring}$	$\nu_{(CH)plane}$	$\nu_{(NH2)as}$	$\nu_{(NH2)sim}$	$\nu_{CC(para\ benzen)}$
Sulphathiazol	1322.9	1137.8	3279.8	929.5	1090.1	3353.1	3320	1531.6
Sb-(Sulphathiazol)$_3$	1327.3	1137.8	-	937.7	1087.1	-	-	1526.8

One can observe the disparition of sulphonamidic NH vibration band from the IR spectra as a fact that the hidrogen atom from sulphonamide nitrogen shifts by tautomerie to the nitrogen from the thiazolic cycle, being thereafter replaced with the metalic ion Sb^{3+}. After the reaction of complexation the assymetric vibration of SO_2 group is also affected because a hidrogen atom of this group takes part at the binding of the antimony ion in complex.

CONCLUSIONS

1. We obtained a Sb(III) compound having sulphathiazol as a ligand;
2. By physico-chemical studies (elemental analysis, IR spectroscopy, thermogravimetric analysis, the structure of the complex antimony-sulphathiazol was determined

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INVESTIGATION ON SUPEROXIDE DISMUTASE IN ALOE ARBORESCENS

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ABSTRACT

Superoxid dismutase (SOD, EC.1.15.1.1.) are a family of metallo-enzymes (Mn-SOD, Cu-Zn-SOD) present in all aerobe living beings, having a role in the catalysis of disproportionating superoxid anion radicals in hydrogen peroxide and molecular oxygen.

The based material used was haves of Aloe sp. from the Botanical Garden of the University of Medicine and Pharmacy, Tg.Mures. From fresh and dry leaves, through a method applied and modified by us, we obtained Aloe I and Aloe II extracts, in which superoxid dismutase activity and constituent parts which contribute to form the active center of the SOD enzyme were followed-up. The elements Zn, Cu and Mn were determinated with ICP-AES method.

The extracts obtained are characterized by an appreciable superoxid dismutase activity (Aloe I 435 UE/g, Aloe II 330 UE/g), and this activity is more explicit in the case of enzymes in which the Cu and Zn content is higher (Aloe I Cu=0,99, Zn=5,7 ppm; Aloe II Cu=0,44; Zn=5,3 ppm).

A limited correlation between the Cu and Zn content of the enzymes and enzymatic activity can be established.

The results obtained are comparable with those with SOD-natural product, made by Cantacuzino Institute in Bucharest.

The extracts can be used in reducing free radicals in the human body, formed in oxidant stress.

Key words: superoxide dismutase – aloe species

INTRODUCTION

The biological function of superoxide dismutase (SOD, EC.1.15.1.1.) wich can be found in every aerobe tissue, is to transform the highly reactive superoxide-anion into a lesser reactive hydrogen-peroxide and oxygen molecule. Thus the superoxide-anion transform quichly, preventing the formation of the much more reactivehydroxile-radical. The increase of the oxygen concentration in some tissue speeds up the formation of the reactive oxygen derivates. It is the red-blood cells and chloroplasts (exposed to oxadative-stress) that contain enzymea with antioxidant effects in larger quantities (superoxide-dismutase, catalese, peroxidase).

Superoxide-dismutases are o part of the group of metallo-enzymes. On the basis of their cofactors there can be distingushed several types of SOD: Cu-Zn-SOD; Mn-SOD; Fe-SOD. Cu-Zn-SOD can be found in the cytoplasm of every eucariotic cell, the Mn-SOD in mithocondrias, meanwhile Fe-SOD can be found manly in procariotes.

The structure of the active site of Ca^{2+} in the active site, meanwhile Zn^{2+} is responnsable only for structure stability, and it can be replaced with other metallic ions. It is interesting, that two metallic ions are bound to HIS-61, both being its ligand.

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The Cu ion undergoes reversable oxidatve and reductive process during catalytic cycle.

In our work we izolated a lesser known antioxidant enzymes of the Aloe species (SOD EC.1.15.1.1.) and tried to determine its metallic ion concentration which is responsible for the activity and structure of the enyzme. he purpose of our study was to show connection between the metallic ion content and enzyme activity in case of Aloe extract.

MATERIAL AND METHODS

The izolation and purification of SOD

SOD was isolated and purified from Aloe arbotrescens Mill. (Liliaceae) herb, grown in the bothanical garden of the University of Medicine and Farmacology in Marosvasarhely. The main ponts of the procedure were the following:

- the homogenisation and centrifugation of newly cut leaves (25 g leaves + 7 ml TRISS buffer, pH=8.8);
- precipitation of SOD with $(NH_4)_2SO_4$ from the supernatant;
- the removal of precipatete (by centrifugation) then placing it in TRIS-HCl buffer solution;
- salt-removal on Sephadex G-10 column (1.4x22 cm);
- purificatin on DEAE cellulose columns (3x24 cm);
- concentration of the extacts.

Determining SOD-activity:

The determination of SOD-activity was based on the adrenaline-adrenochrom autooxidant transformation. The enzyme inhibits the adrenaline-adrenochrom autocatalytic transfromation at pH=10.2 and normal pressure. The measurments were made by the VSU 2-p DDR apparatus. After blocking Cu-Zn-SOD with KCN (1.5 mM), we measured mithocondrial Mn-SOD activity. Cu-Zn-SOD activity was calculated from the difference beteen total SOD and Mn-SOD activity. The results were expressed in enzyme-unit/g. As a control solution we used SOD-natural, a product of the Institute I. cantacuzino-bucarest.

Determination of metallic ions:

Metallic ions were determinated with the ICP-AES method. The measurments were made in JATE ICP-AES Laboratory in Szeged, With Atomic Scan 25 ICP (Thermo Jarell Ash Coorporation) (8).

The parameters of ICP-AES determinations are: ICP AES (950 W, 0.4 l /min sheathgas, 12 l/min plasmagas babington, Gilson minipuls III pump). The multicell calibrating solution were prepared from millipore water, through Merck multielemental standard dilution. The wave-leghs and concentrations of the measurments werethe following: (0.2 + 10 ml water, 1 + 10 ml water, 5 + 5 ml water) Zn 206.2 nm, Mn 260.5 nm, Cu 324.7 nm.

RESULTS AND DISCUSSIONS

The extracts of Aloe arborescens contain Mn-SOD and Cu-Zn-SOD. After the inhibation of Cu-Zn-SOD activity with KCN, first we measured the Mn-SOD activity. The cytoplasmic Cu-Zn-SOD activity was calcutated from the difference between total SOD activity and Mn-SOD activity. The results of our study are shown in the table 1.

Table 1 Mn-SOD activity, the cytoplasmic Cu-Zn-SOD activity

Name	Mn-SOD	Cu-Zn-SOD	Total-SOD
Aloe I	85	350	435
Aloe II	82	248	330
SOD-natural	125	195	320

Note: the data represent the averaghe of three parallel measurements

The extracts obtained from Aloe herbs showed a higher SOD activity than that of control (SOD-natural – I. Cantacuzino, Bucharest). This applies only to total-SOD and Cu-Zn-SOD and Cu-Zn-SOD activity; Mn-SOD activity proved to be higher in SOD-natural, than in the case of Aloe I and Aloe II. The ICP-AES mesurement results of the elements are sumarized in table 2.

Table 2 . Concentrations after ICP-AES measurements

Name	Element	Measured concentration (1:11) ppm	Concentration of the samples ppm
Aloe I	Cu	0.09	0.99
	Zn	0.52	5.70
	Mn	0.10	1.10
Aloe II	Cu	0.04	0.44
	Zn	0.48	5.30
	Mn	0.05	0.55
SOD-natural	Cu	0.05	0.55
	Zn	0.57	6.30
	Mn	0.28	3.10

Note: the data represent the averaghe of three parallel measurements

The Cu, Zn and mn contents of Aloe extracts are not same, the difference can be explained with the diferent origine and purification method. A strong correlation was shown between the quantity ration of the elements and SOD enyzmes activity. The Cu content is the highest in Aloe I natural. However, Mn-SOD content is higher in SOD-natural, wich perhaps determines its higher enzyme activity.

CONCLUSIONS

The SOD activity of Aloe I and Aloe II are significant, fact that makes them suitable for the elimiation of free radiacls from the body. Strong correlation was observed between the quality of the elements and SOD activity. Aloe I extracts with higher metallic ion concentration showed stronger enzyme-activity.

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IMPLICATIONS OF SOME CATIONS IN GLYCEMIA AND GLUCIDIC METABOLISM REGULATION

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ABSTRACT

An important number of macro and trace elements, especially cations, have important influences on glycemia regulation and implications in glucidic metabolism. There are important involvements of disbalance in this elements in the pathology of glucidic metabolism, especially in diabetes mellitus and its complications. Zinc, magnesium, vanadium, copper, chromium and other cations influence both pancreatic hormone secretion and their peripheral action. Zinc and magnesium decreasing formation of free radicals are involved in antiradical protections of Langerhans islet. Vanadium and their compounds have an important hypoglycemiant effect .The second level for these cations to exert their influence is the liver. Here they influence glycogenolysis, gluconeogenesis, glucose hepatic flow, etc. The third cation field of action, with involvement in glucidic metabolism regulation, is the peripheral tissues. At this level the influence is on the insulin actions, to their receptors too and in some intermediary stages of glucidic metabolism. A group of trace elements like Va, Zn, Cr have a favorable influence on the insulin tissular action. The wider use in therapy of cation products in different diseases is a supplemental motive for an increase attention toward their influence in glucidic metabolism.

Key words: diabetes mellitus, zinc, magnesium, vanadium, chromium, copper

INTRODUCTION

Macro and trace elements play major roles at the cell level and they are involved in the normal cell function. Among these, cations play a predominant role. Disturbances in concentration balance of these cations, inner or outer cell, are involved in pathogenesis of many diseases from human clinic.

One of the serious diseases often met in human pathology is diabetes mellitus. The influences of different cations in diabetes is multiple, at the level of synthesis and secretions of pancreatic hormones and also at the peripheral actions of hormones involved in glycemia regulation and at the level of different cell levels of glucidic metabolism. The main involvement of cations in glucidic metabolism are shown in this paper.

VANADIUM

Vanadium is one of the most studied trace elements regarding implications and therapeutic potential in glucidic metabolism. Vanadate compounds mimic a lot of metabolic actions of insulin (Sun et al., 2000). The vanadium organic compounds are 2-3 times more active regarding insulin like actions vs. inorganic vanadium (Verma et al 1998). Oral ammonium dipicolinat orto-oxo-vanadium is a chemically useful hypoglycemic agent (Craw DC, 2000). Vanadium acetyl-aceto-acetonat and 3-ethyl-2,4-pentanedionat are compounds with insulin like hypoglycemic effects in streptozotocinc diabetes in Whistar rats. The organic compounds with vanadium have less side and toxic effects at the hepatic and kidney level vs. inorganic compounds. They don't give gastrointestinal discomfort. (Srivastava, 2000). Meyerovitch et al 2000 have shown that experimental administration of sodium metavanadate 3,92 mmol/L (in drinking water) in non-obese

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diabetic rats for 8 weeks produced significant decreased of glycemia vs. control group (3.83±0.67 mmol/L vs. 4.44±0.83 mmol/L in control group). It was not observed (at the end of treatment) hystologic differences at the level of Langerhans islets.

We enumerate some of the vanadium organic compounds realized for therapeutic use (found in different trial stages for a possible use in NIDDM patients):

-bis (maltolato) oxovanadium (BMOV)
-bis (cysteinamide N-octyl) oxovanadium (NAGLIVAN)
-bis (pyrolidine-N-carbodithiolate) oxovanadium
-vanadyl-cysteine methyl ester
-bis-glycinato oxovanadium (BGOV)
-peroxovanadate (Brand RM and Hamel FG, 1999)
-sodium metavanadate
-sodium ortovanadate,etc.

Vanadium problem is that therapeutic index LD_{50}/ED_{50} is small (Christiansen et al, 1999).

There are some side and adverse effects of vanadium compounds: oral treatment in rat with maltol or bis (maltolato) oxovanadium (BMOV) produces biochemical changes in activity of some hepatic enzymes linked with Golgi complex (e. g. galactosyl transferase) and morphopathologic changes (shown in electronic microscopy) of Golgi complex.(Daleros W. et al.,.2000)

MAGNESIUM

Magnesium is the most important bivalent cation and has many implications on glucidic metabolism. Among these we mention:

-Increases hypoglycemic insulin action
-Decreases presynaptic adrenaline release (hyperglycemic)
-Decreases free radicals formation (exacerbated in diabetic patients)
-Decreases insulin resistance (Molis KW, Mc Mullen JK, 1982)
-Decreases neuromuscular, cardiac (especially arrhythmia) manifestations from diabetic severe keto-acidosis (Mc Mullen JK, 1977)
-Decreases platelet hyper aggregation in diabetics
-Delays the appearance of crystalline cataract (hypomagnesemia in diabetic patients accelerates cataract appearance)

Plasmatic and tissular Mg^{2+} concentration decreased in diabetes mellitus due to:

- lack of insulin decreases magnesium intestinal absorption (Durlach J, 1980, Leeuw et al, 1992)
- osmotic diuresis with an increase of urinary Mg^{2+} elimination which produces phosphodependent hypermagnesemia (Mather et al, 1979)
- vit. D possible deficiency in some forms of diabetes mellitus in human and in experimental diabetes
- biguanides frequently used in NIDDM therapy decreased intestinal absorption of Mg^{2+}
- intra/extra cellular repartition of magnesium is disturbed in diabetes mellitus

McCarthy, 2000 considers that magnesium might have a preemptive effect toward of diabetes mellitus emergence and that it would be recommended for this disease. The main reason is that hypomagnesemia produces insulin resistance. Hausmann et al, 1997 observed extracellular magnesium decrease in IDDM patients. The level of circulated ionic magnesium decrease is more important than total circulated magnesium decrease, underlining the role of ionic magnesium (not sufficient defined). Balan et al, 1995 have shown that Mg^{2+} supplementation in diet in male obese Zucker fatty rats (a model of non-insulin-dependent diabetes mellitus) prevent the deterioration of glucose tolerance.

Hypomagnesemia is a factor which aggravates and speeds the emerge of complications in diabetes mellitus. For this idea plead the following data:

Nephropathy- Albumin excretion rate is higher in hypomagnesaemic children (IDDM) compared with normomagnesemic children (Arslanoghi et al., 1995, Pickup JC et al, 1994)

Retinopathy- Diabetic patients (Type II diabetes) with rethinopathy have a lower Mg^{2+} levels vs. patients without rethinopathy

Patients (NIDDM) with proliferative rethinopathy have a significantly decreased magnesemia than those with non-proliferative rethinopathy (Mc Naur P et al., 1978, Hatwal A et al., 1989)

Angiopathy- Mg deficiency produced hyperlipidemia with high level of plasmatic tryglicerides in IDDM patients (Corica et al., 1994)

Mg^{2+} deficiency increases platelets aggregation and thrombotic potential in diabetic patients (Eibl N., Schernthaner G., 1997) Mg^{2+} deficiency (in animal experimental models) accelerates the development of atherosclerotic lesions

Mg^{2+} supplementation (4,5g magnesium pidolate/day) in NIDDM patients for 4 weeks increased significantly statistic insulin secretion (Paolissa et al, 1989; Nechifor M. et al., 2000).

Many from the enzymes involved in glucidic metabolism are (in different ratio) magnesium dependent. E.g: 6-phosphofructokinase, aldolase, hexokinase, pyruvate kinase, glucose-6-phosphatase, synthase phosphatase (Gilboc D.P.,Daniel P. 1986; Shils ME, 1997). ATP-dependent enzymatic reactions request Mg^{2+} presence (Hikava JK, 1971).

Intracellular Mg^{2+} decreases in patients with diabetes mellitus (Mather H et al, 1979; Delva P et al 1997)

Cell incubation with relative higher glucose concentrations (10mM) decreases Mg intracellular concentrations (without significant variations in intracellular concentrations of ATP or Ca).

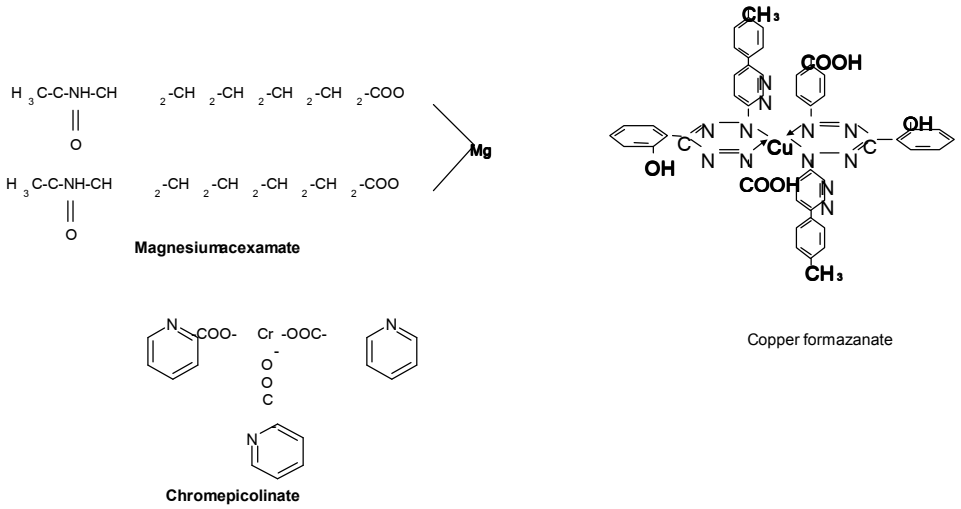

Fig. 1. Chemical structures of MgAcz , CrPi , CuFm

Personal research about the influence of magnesium (magnesium acexamate, MgAcz 0,5 mEq/kg i.p) and copper (copper formazanate CuFm 0,5 mEq/kg i.p, Nechifor et al, 2000)
(chemical structure of MgAcz, CrPi, CuFm are shown in Fig. 1) administrated for 14 days in alloxan-induced diabetic rats have shown that :

1. MgAcz decreases glycemia in alloxan-induced diabetes rat (Fig. 2)

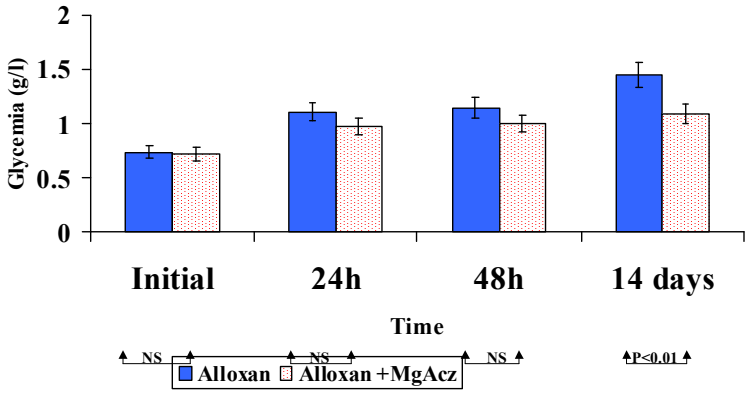

Fig. 2. MgAcz 0,5 mEq/kg/day i.p. influence on glycemia in alloxan-induced diabetes in rat (Nechifor et al., 2000)

2. MgAcz determines a moderate decrease of MDA level (Fig. 3) and increases GSH level (Fig. 4)

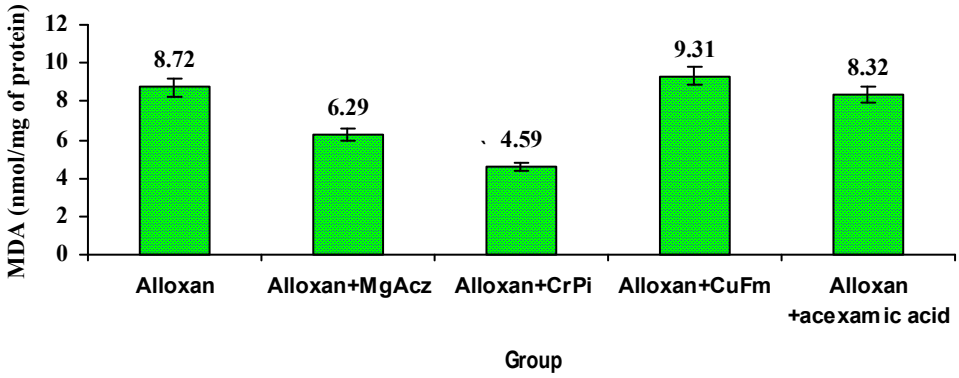

Fig. 3 Influence of MgAcz, CuFm CrPi and acexamic acid on MDA level in rat liver homogenate (Nechifor et al., 2000)

3. CuFm doesn't influence significantly glycemia evolution in alloxan-induced diabetes but increases moderately mortality and MDA level in liver homogenate (Fig. 3)

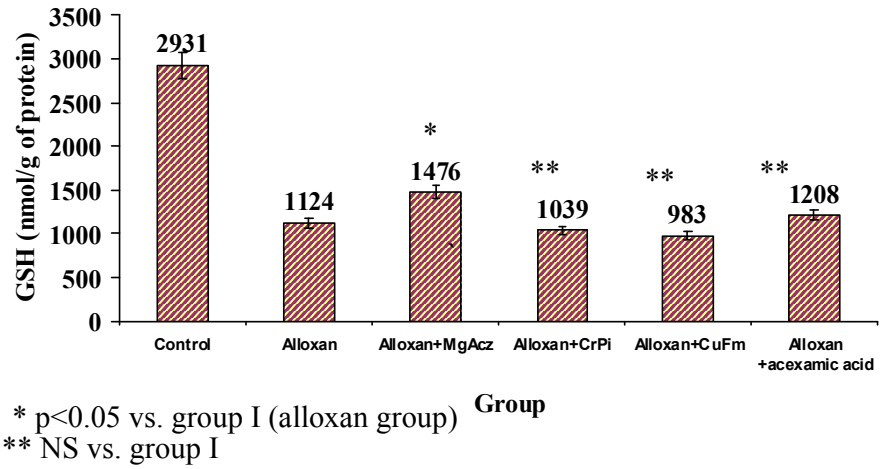

Fig. 4. Influence of MgAcz, CuFm ,CrPi and acexamic acid on **GSH** level in liver homogenate (Nechifor et al., 2000).

4. MgAcz (0,5 mEq/kg i.p) decreases mortality in alloxan-induced diabetes in the first 72 h (with 20%) due to multiple influences on alloxan general toxic effect
5. Efects are due to Mg^{2+} ion , acexamic acid having no efect on glycemia and peroxidic radicals generation (Nechifor M. et al, 2000). Our data are in agreement with other authors who reported that CrPi and MgAcz decrease glycemia and positively influence diabetes mellitus evolution. The alloxan-induced diabetes being an experimental method with the mechanism of action based mainly on free peroxidic radicals production we consider that MgAcz decreases mortality due to this anti radicalic effect of Mg cation.

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COPPER

The copper roles in diabetes mellitus are controversy. This cation level increases in patients with NIDDM (Zargar AH et al., 2000, Walter et al, 1991) and renal copper increases in rats with streptozotocine diabetes. Fructose administration at these rats significantly decreased concentration of renal copper.
Copper increases lipid peroxidation.

Another problem is represented by relations (not enough established) between the level of trace elements and diabetes mellitus complications. In diabetics with microvascular complications and rethinopathy the level of plasmatic copper is a significantly higher vs diabetic without these complications. Cu-Zn superoxide dismutase activity in erythrocyte of diabetic patients doesn't differ of normal subjects.

ZINC

Walter et al 1991 observed also that in diabetes mellitus zinc plasmatic level is lower vs. normal subjects at descendents of niddm patients was observed a significant decreased level of zinc vs. non diabetic persons of the same age. The insulin hypoglycemic action is enhanced by zinc. This cation has also an effect of peroxid reduction. Hyperzincuria associated with hypocopperuria was detected in patients with niddm (tanega et al, 1998).

Zn^{2+} (20 mg Zn/day p.o. 3 months) reduced mean fasting blood glucose level in NIDDM patients from 202 to 169 mg/dl and glycosylated hemoglobin (HbA_1C) decreases from 12.2% to 9.5% (Song MK et al., 1998). The mean fasting plasma insulin slightly decreased (15.5 to 13.8 μM/ml) in patients with NIDDM who get Zn (Proderyl PRO-Z 20 mg/day).

Lower consumption of dietary Zn and low Zn levels were associated with an increased prevalence of coronary artery diseases and diabetes mellitus (Singh R. B et al., 1998).

Zn decreases glucocorticosteroid plasmatic level (hormones which stimulates hepatic gluconeogenesis) (Brando-Neto J et al, 1999).

Eventually, zinc seems to be one of the most important cation involved in glucidic metabolism regulation.

MANGANESE

Diabetics not treated with insulin excreted in urine much more Mn vs. diabetics treated with insulin or normal subjects (El-Yazigi et al 1991). The optimum level of manganese and also the daily recommended intake for diabetics remain to be established, but the daily diet brings about 1-10mg/day and the Mn deficiency is associated with changes in cholesterol and glycemia level ,delays in growth and disturbances in reproduction area (Greger, 1999)

SELENIUM

Selenium is known for its effect in reduction of free radicals formation.

Low level of selenium dependent glutathion peroxidase was observed in IDDM patients (Holler et al, 1997). Plasmatic selenium level is significantly decreased in diabetic patients vs. normal subjects (64 $\pm$ 2.2 μg/l vs. 74.9 $\pm$ 2.7 μg/l in control group), the same as urinary selenium (Navarro-Alarcom et al, 1999). The antioxidant effect of selenium suggests a deficit in antioxidant factors in plasma of patients with diabetes mellitus.

CHROMIUM

there is a certain effect of chromium to induce hypoglycemia and to increase cell sensitivity to the insulin action. this cation increases also glucokinase expression in hepatocytes. we consider chromium as an essential trace element. steams, 2000 considers that cr^{3+} is a therapeutic agent with an interesting potential but not an essential element. in the case of treatments with chromium it has to be noticed that cr^{6+} is cancerous and there is a genotoxic and mutagen potential of chromium.

Chromium decreases the total quantity of lipids from the blood and from the body (anderson 1998). among chromium products used in practice we mention chromium picolinate and chromium polynicotinate. Chromium enhances insulin receptor number, increases insulin binding to receptors, activate insulin receptor kinase and finaly increase insulin sensitivity. Anderson, 2000 considers chromium an essential nutrient involved in glucidic metabolism and in regulation of plasmatic levels of lipids and traw et al, 2000 have shown that chromium is essential for regulation of peripheral insulin action. Hepatocyte glucokinase is an important enzyme in glucidic metabolism. their activity is decreased in niddm. the decreased activity determines a greater hepatic glucose output in diabetic patients. Chromium increases genic expression of these enzyme and also their hepatocyte activity (Mc Carty 1999). Feng et al 1999 have shown that in normal and diabetic rats the highest Cr^{3+} quantity in the liver was found in the nucleus, but in diabetic rats the percentage of chromium retained by the mitochondrial and lysosomal fraction is greater than in normal rats. in niddm patients, Cr plasmatic concentration is with 33% lower than in normal and urinary excretion is almost double vs. normal subjects (Morris et al 1999). It is considered that daily chromium dietary intake in normal person has to be 50-200 μg and a lower daily intake would facilitate the appearance of diabetes mellitus (Prens and Anderson, 1998). Some cr implications in glucidic metabolism are shown in Fig. 5.

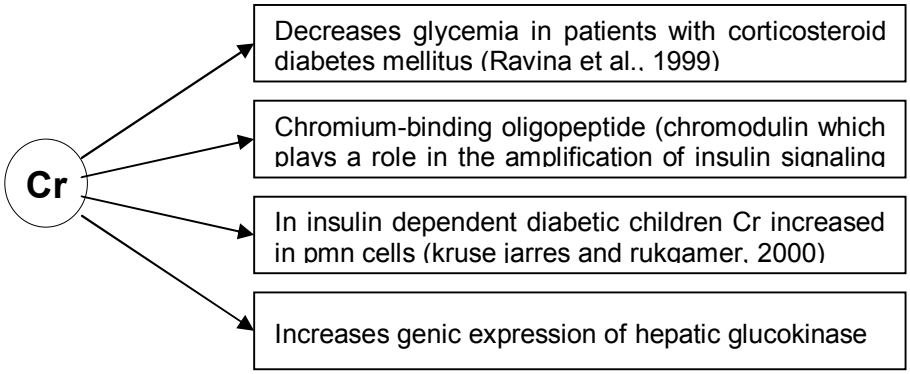

Fig. 5. Some implications of chromium on glucidic metabolism

Raving et al, 1999 have shown that chromium administration (as chromium picolinate) in the therapy of diabetes mellitus leads to decrease in blood fasting glucose values) from 13.9 mmol/l (250 mg/dl) to 8.3 mmol/l (150 mg/dl). Administration of chromium picolinate allows the reduction with about 50% of oral antidiabetic drugs administrated in these patients.

Personal research about the influence of chromium (chromium picolinate, CrPi 1 mEq/kg i.p administrated for 14 days in alloxan-induced diabetic rats have shown that CrPi decreases significantly statistic glycemia, MDA and GSH level in liver homogenate (see Fig. 3 and Fig. 4)

NA^+/K^+ ANTIPORT SYSTEM

An interesting implication of monovalent cations in proliferative rethinopathy in patients with IDDM is that sustained by DeFaria et al 2000 which show that erythrocyte co-transport Na^+/Li^+ in these patients is significantly increased than in normal. Activity of Na^+/H^+ antiport system in skin fibroblasts at IDDM patients with diabetic nephropathy is increased. Fibroblasts from diabetic patients with albuminuric nephropathy are significantly more alkaline compared to non-albuminuric diabetic patients or normal controls (6.9 vs. 6.82)(Davies et al, 1992).

This increased activity of Na^+/H^+ antiport system in diabetics followed by an increased entrance of Na^+ into the cell is considered to be one of the explanation of diabetic predisposition for blood hypertension.

Semplicini et al 1992 have shown in IDDM patients, at the erythrocyte membrane and at the hearth myocardium level, a counter transport Li^+/Na^+ and Na^+/H^+ greater than in normal subjects and consider that these growth might be facilitated by cell hypertrophy and hyperplasia. It is considered that there is a direct correlation between the amplitude of Li^+/Na^+ and Na^+/H^+ exchange and the thickness of interventricular septum.

Researches performed on vascular smooth muscle cells show that the high level of glucose concentration in medium decreases Na^+-K^+ membrane exchanges and Ca^{2+} transport and increases Na^+ and Ca^{2+} intracellular concentrations (Kariyama et al 1994)

Diabetes mellitus complications are also associated with disturbances in the balance of these cations. We consider that intra and extra cellular cations have a vital influence both on evolution and diabetes mellitus complications. The therapeutic

potential of magnesium, vanadium and zinc compounds (and maybe other cation compounds) need to be more valued in therapeutic practice.

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ALTERATIONS OF THE PLASMATIC CATIONS LEVELS IN PACIENTS WITH MAJOR DEPRESSION

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ABSTRACT

There are numerous data regarding trace elements and their influences in normal functionality of the brain system. The aim of the study is to investigate the serum levels of trace elements(Ca^{++}, Mg^{++}, Cu^{++}, Zn^{++}, Fe^{++}) using atomic absorbtion spectrophotometry in pacients with major depression(M.D.) hospitalized for 4-6 weeks in the Hospital of Psychiatry "Socola", Iaşi. Pacients with alcohol dependence or pharmacological treatments (diuretics, antacids) were not included. These cases were examined in order to investigate the modified serum levels of cations and to compare them to those from normal subjects. Correlations between the severity of the acute episode of M.D.and serum cations were established. The results were statistically interpreted. Our findings suggest that the serum zinc level is significantly lower(with approximately 30%) in pacients with severe episode of M.D. compared to normal subjects or pacients with mild episode of M.D.

Keywords: major depression, trace elements.

INTRODUCTION

The trace elements (bivalent cations mainly) exert many influences on normal functionality of the Central Nervous System (CNS) and there are known many mechanisms of altering the neuronal function by the intra and extra-cellular cations.

Different cations (macro and oligoelements) are involved in the delivery of neuro-transmitters into the CNS synapses and in the functionality of the receptors of the central synapses or those coupled to the ion channels (Calabresi et al. 1000, Bastian, 1998).

The CNS functions abnormality could be a result of these influences and some correlations with the effect of pharmacological treatment in psychiatry (anti-depressants, anti-psychotics and others) could be established.

On the other hand, modified levels of extra and intracellular cations were found to the patients with serious psychiatric disorders but there is no certitude that these variability of cation concentrations could be involved in the pathophysiological mechanisms of these disorders (Kamel et al., 1998, Beik et al., 1998).

Major depression is a frequent psychiatric disorder and many of trials of pharmacological treatment for this disorder are made.

The aim of this study was to investigate serum levels of different cations in patients with major depression (mild to severe episodes) hospitalized in the Universitary Hospital of Psychiatry “Socola” Iasi, during year 2000.

MATERIALS AND METHODS

In the present study, blood samples from 19 patients (of female sex) with major depression were studied and compared to normal (the control group of 11 suspects, female sex) (Fig.1, 2).

The serum levels of trace elements (Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}) were measured by spectrophotometry.

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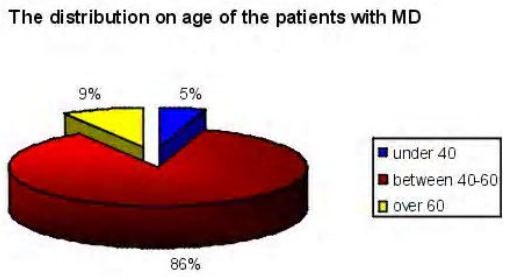

Fig.1

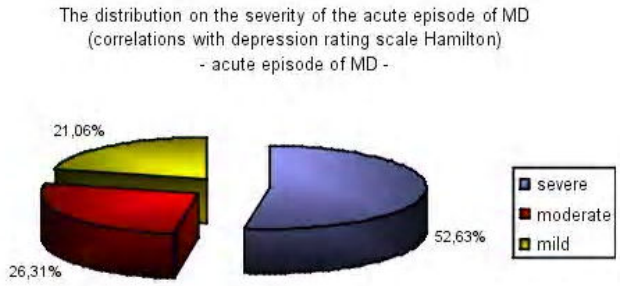

Fig.2

Table 1. Hamilton Rating Scale for Depression

1.Depressed mood (sadness, hopeless, helpless, worthless)
2.Feelings of guilt
3.Suicide
4.Insomnia early
5.Insomnia middle
6.Insomnia late
7.Work and activities
8.Retardation (slowness of thought and speech, impaired ability to concentrate, decreased motor activity)
9.Agitation
10.Anxiety psychic
11.Anxiety somatic
12.Somatic symptoms gastrointestinal
13.Somatic symptoms general
14.Genital symptoms
15.Hypocondriases
16.Loss of weight
17.Insight
18.Diurnal variation
19.Depersonalization and derealization
20.Paranoid symptoms
21.Obsessional and compulsive symptoms
22.Helplessness
23.Hopelessness
24.Worthlessness (ranges from mild loss of esteem, feelings of inferiority, self depreciation to delusion notions of worthlessness)

Including criteria for the group of patients with MD

1. Major depression, acute episode (mild to severe). The diagnosis was checked at least twice before the start of the study.

2. At least 30 days of hospitalization in the University Hospital of Psychiatry “Socola”, Iasi, in each case in the study.

Excluding criteria

1.Age under 18
2.Alcohol dependence
3.Pharmacological treatment with diuretics or other medication consisting of cations
4.Chronic heart failure
5.Complicated cirrhosis (with ascitis)

Drugs used in the treatment of the patients with MD

Severe episode
•Tricycle antidepressants
•Tetracyclic antidepressants
•Serotonin reuptake inhibitors (SSRI)
•Trozodone, Nefazodone
•Antipsychotics: Haloperidol

Moderate episode
•Tricyclic antidepressants
•Tetracyclic antidepressants
•Serotonin reuptake inhibitors (SSRI)
•Carbamazepine

Mild episode
•SSRI
•Tricyclic antidepressants
•Benzodiazepine receptor agonists
•Diazepam

RESULTS AND DISCUSSIONS

Table 2. Results

Serum trace element mg/l	Media ± SD		P
	Patients with MD N=19	Normal subjects N=11	
Mg	20,8 ± 3,8	21,9 ± 3	SN
Ca	81,9 ± 6,6	91,09 ± 21	SN
Cu	0,80 ± 0,15	0,63 ± 0,20	0,016
Zn	0,25 ± 0,025	0,293 ± 0,04	0,04
Fe	0,51 ± 0,15	0,50 ± 0,08	SN

SN = statistically non-significant

The results of this study show a significant increase ($p<0,016$) of serum copper in depressive disorder and a significant decrease ($p<0,04$) of serum zinc level in these patients (Zn: 0,25 ± 0,025 vs. 0,293 ± 0,04 mg/l) compared to normal.

Statistically nonsignificant changes of other serum cations (Mg, Ca, Fe) were found in these patients (Mg and Ca plasma levels suffer a mild decrease in depressive disorder).

Mas et al., 1999, noticed a low Zn serum level in patients with MD

Basarky et al., 1998, find a moderate decrease of serum Mg in depressive disorder and an increase of intracellular Ca (the serum Ca being not modified or at a slight lower level)

Alterations of serum trace elements and a modified cellular response was noticed in MD when cations were administered to the patients.

Delisi et al., 1998, found exaggerated rise in platelet cytosolic calcium produced by serotonin in patients with mood disorders.

Abnormalities of serum level and distribution of calcium were noticed in patients with bipolar disorder.

Our findings suggest a reduced intensity of the withdrawal syndrome animals with induced morphine dependence when magnesium was administered.

Magnesium (magnesium aspartate) administered to the chronic users of benzodiazepine receptors agonists (alprazolam, lorazepam, bromazepam) reduces the intensity of the withdrawal syndrome (Hantouche et al., 1998)

The data in this study do not allow a correlation between the alterations of serum trace elements and the antidepressive treatment in patients with MD

These results represents the first phase of a continuing experiment.

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ZINC STATUS AND SOME INFLUENCING FACTORS IN NORMAL PREGNANCY

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ABSTRACT

The aim of the study was to investigate zinc level in pregnant women in connection with various factors that could influence this level. The investigated group consisted in 167 apparently healthy women, being in different trimesters of pregnancy. Serum and erythrocyte zinc levels by atomic absorption spectrophotometric method were determined. The relationships between these indicators and the weekly consumption frequency of some foods, the socioeconomic status (income, education), age, gestational age, parity, residence, smoking habit were investigated. Serum and erythrocyte zinc levels were strongly related with the pregnancy stage. Stratified analysis by trimesters emphasised associations of zinc deficit with low eggs and organs consumption and with high sweets, bread, alcoholic beverages, dairy produces consumption ($p<0.05$). Unfavourable socioeconomic status didn't associate with zinc level, but it associated with the alcoholic beverage consumption and exaggerated bread consumption ($p<0.05$). Residence in rural area significantly associated with erythrocyte zinc deficit in the first trimester.

Key words: zinc, affecting factors, normal pregnancy.

INTRODUCTION

Zinc (Zn) deficit is a risk factor in pregnancy; it can be associated with spontaneous abortion, high frequency of infections, prematurity, prolonged travel, low birthweight of the new-born, foetal abnormalities etc. (Jameson, 1993; Scholl, 1993).

Zn is an essential micronutrient which arrives in organism mainly by means of food. Meat, organs, eggs, dairy products, cereals, bean, nuts, cacao, spices are the richest food (Westin, 1983). Metal bioavailability varies in very large limits, being enhanced in the presence of animal proteins and reduced by alimentary fibers and phytates from vegetables, but also by the presence of other bioelements like calcium from dairy products. According with the supplementary need of the placental and foetal tissues, the recommended daily intake of pregnant women rises with 25% (Luke et al., 1993).

The aim of the study was to follow the Zn level in relationship with different characteristics of the pregnant women.

MATERIALS AND METHODS

Investigated group involved 167 apparently healthy, by chance elected women which presented for routine control in the Outpatient Department of University Hospital Iasi. They were 18-41 years aged, were in different stages of pregnancy and were not mineral supplemented (Table 1).

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Table 1. Subjects data

Subjects number	**1st Trimester 46** **2nd Trimester 50** **3rd Trimester 72**
Age (years)	1st Trimester 25.7 ± 4,7 (18-37) 2nd Trimester 25.3 ± 5,5 (18-41) 3rd Trimester 25.8 ± 4,2 (19-41)
Gestational age (weeks)	1st Trimester 9.2 ± 2,3 (6-13) 2 nd Trimester 19.7 ± 3,9 (14-26) 3 rd Trimester 34.5 ± 2,5 (27-40)

Serum and erythrocyte Zn levels by atomic absorption spectrophotometric method were determined (normal ranges: 70-120 μg serum Zn /dl and 10-14 μg erythrocyte Zn /dl). The relationships between Zn level and age, gestational age, parity, nutrition, educational level, income, residence, smoking habit were investigated. In regard to nutrition, the weekly consumption frequencies of some food with a high Zn content (meat, liver and kidneys, dairy products, eggs), others which break metal absorption (sweets, bread, coffee) or food which determine its increased elimination (alcoholic beverages) were recorded on a questionnaire. The income was calculated per family member.

There are, of course, many other factors like genetic factors, environmental pollution, Zn content of drinking water, stress, some drugs intake, etc., that can influence body Zn level. So, the problem is complex and need caution in results evaluation.

The following of the maternal variables in relation with Zn level was performed by means of Pearson correlation coefficient (r) or " χ square" (χ^2) test for nonparametric variables.

RESULTS AND DISCUSSIONS

Zn level (Table 2) was tightly connected with gestational age ($r = 0.26$, $p<0.01$ for serum Zn and $r = 0.35$, $p<0.001$ for erythrocyte Zn).

Table 2. Zinc level (Mean ± Standard deviation)

	1st Trimester	**2 nd Trimester**	**3rd Trimester**
Serum Zn (μg /dl)	74.6 ± 16.4	68.6 ± 12.7	65.0 ± 12.7
Erythrocyte Zn (μg /ml)	13.0 ± 1.89	12.0 ± 2.1	11.4 ± 1.4

Zn deficit frequencies gradually increased (Figure 1), majority being marginal.

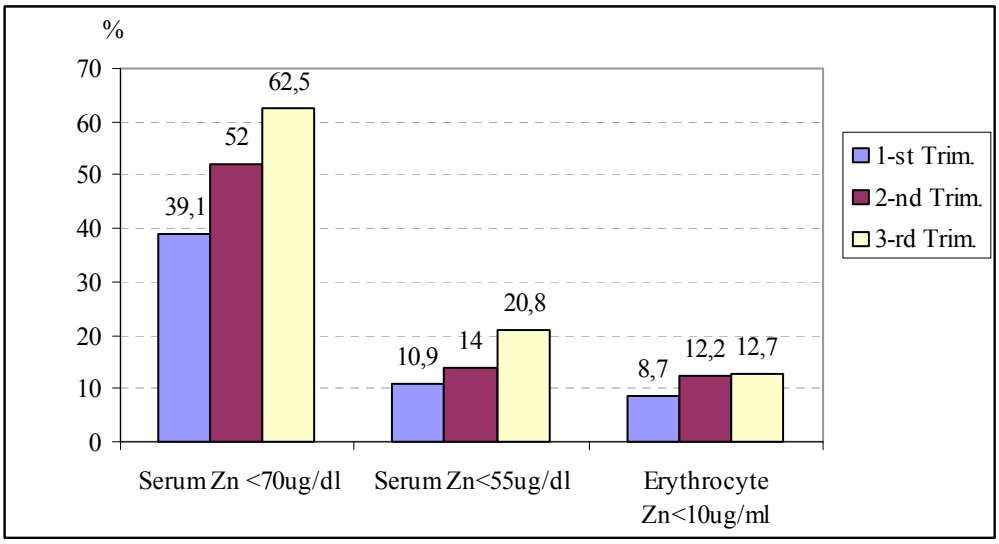

Fig.1. Frequencies of serum and erythrocyte zinc deficits

Taking into account this dependence Zn-gestational age, the statistical analysis was stratified by trimesters.

The age of pregnant women did not correlated with Zn level (Table 3).

Table 3. Investigation of the correlation between the zinc level and the age

		r	p
1st Trimester	Serum Zn Erythrocyte Zn	+0.09 +0.15	>0.05 >0.05
2nd Trimester	Serum Zn Erythrocyte Zn	+0.05 +0.30	>0.05 >0.05
3rd Trimester	Serum Zn Erythrocyte Zn	+0.20 +0.32	>0.05 >0.05

The majority of women were primigravida (Table 4).

Table 4. Parity of the investigated pregnant women

Number of births	Number of pregnant women	%
0	118	70.7
1	33	19.8
2	11	6.6
3	2	1.2
4	2	1.2
5	1	0.6

The parity did not correlate with Zn level (Table 5), although an inverse correlation was expected.

Table 5. Investigation of the correlation between zinc level and parity

		r	p
1st Trimester	Serum Zn	+0.02	>0.05
	Erythrocyte Zn	-0.04	>0.05
2nd Trimester	Serum Zn	-0.14	>0.05
	Erythrocyte Zn	-0.12	>0.05
3rd Trimester	Serum Zn	+0.09	>0.05
	Erythrocyte Zn	+0.18	>0.05

As regard to the food consumption, it was found that the pregnant women were relatively well fed, about 80 % of them, consuming at least 2 times on a week meat, eggs, dairy products (Table 6).

Table 6. The subjects distribution depending on the weekly frequency consumption of some foods (%)

Consumption frequency	Meat	Organs	Dairy	Eggs	Sweets	Alcoholic beverages	Coffee
-	4.4	35.5	0	5.9	2.9	50.4	52.4
+	4.4	64.5	17.7	10.3	30.9	45.8	22.9
++	38.2	0	27.9	39.7	25.0	1.8	5.4
+++	52.9	0	54.4	44.1	41.2	2.4	19.3

-: No consumption; +: Sometimes; ++: 2-3 times /week; +++: At least 4 times/week.

56.3% from the investigated women reported an exaggerated bread consumption. The statistical analysis emphasised association of Zn deficit with low eggs and organs consumption and with high sweets, bread, dairy products and with the at least occasionally alcoholic beverages consumption (Table 7).

Table 7. Investigation of the association of zinc level with some food consumption

		1st Trimester		2nd Trimestre		3rd Trimestre	
	Food	χ^2	p	χ^2	p	χ^2	p
Serum Zn	Meat	0.95	0.62	2.67	0.26	0.27	0.87
	Organs	0.53	0.46	0.57	0.75	0.0	0.97
	Dairy	2.12	0.34	1.54	0.46	1.11	0.29
	Eggs	1.12	0.57	4.71	0.09	**8.35**	**0.01***
	Sweets	**5.44**	**0.01***	**8.30**	**0.01***	2.96	0.22
	Coffe	0.66	0.71	3.59	0.16	3.94	0.13
	Alcohol	0.0	1.0	2.98	0.08	**6.59**	**0.01***
	Bread	1.81	0.17	1.21	0.27	**5.72**	**0.01***
Erythrocyte Zn	Meat	1.25	0.74	0.68	0.71	1.38	0.50
	Organs	0.02	0.87	**5.65**	**0.03***	0.04	0.84
	Dairy	1.30	0.25	**6.05**	**0.01***	2.46	0.29
	Egs	0.03	0.86	1.07	0.30	0.32	0.57
	Sweets	3.37	0.06	2.64	0.10	0.30	0.58
	Coffe	0.61	0.43	1.92	0.16	0.43	0.51
	Alcohol	0.0	1.00	1.31	0.25	0.87	0.35
	Bread	**3.68**	**0.05**(*)	1.92	0.16	0.02	0.90

Relating to the socio-economic status, the distribution of the subjects depending on the educational level was balanced, while 72% of women had low incomes (Table 8).

Table 8. Socio-economic al status of the subjects

		%
Educational level	Elementary	43.3
	Middle	32.5
	High	24.0
Income	Low	72.9
	Middle	23.0
	High	3.6

Unfavourable socio-economical status associated with Zn deficit only in the third trimester at significance level (Table 9). But both educational level and income associated, in whole group, with alcoholic beverages consumption and exaggerated bread consumption (Table 10).

Table 9. Association of socio-economical status with some food consumption

		1st Trimestre		**2nd Trimestre**		**3rd Trimestre**	
		χ^2	**p**	**χ^2**	**p**	**χ^2**	**p**
Serum Zn	Education	2,26	0,32	1,68	0,43	**5,81**	**0,05(*)**
	Income	0,13	0,71	1,62	0,20	**3,68**	**0,05(*)**
Erythrocyte Zn	Education	1,52	0,21	0,07	0,78	0,22	0,89
	Income	1,07	0,30	1,54	0,21	1,41	0,23

Table 10. Association of the socioeconomic status with some food consumption

Association	**χ^2**	**p**
Education – Alcohol consumption	**11.8**	**0.027***
Income -Alcohol consumption	**6.6**	**0.010***
Education – Bread excess	**20.9**	**0.000*****
Income – Bread excess	**9.8**	**0.001****

These foods associated, as it was shown, with low Zn level. 66.3% of subjects were from urban area. Erythrocyte Zn deficit statistically associated with residence in rural area in trimester III (Table 11).

Table 11. Association of zinc level with the residence in rural or urban area

	1st Trimestre		**2nd Trimestre**		**3rd Trimestre**	
	χ^2	**p**	**χ^2**	**p**	**χ^2**	**p**
Serum Zn	2.48	0.11	0.30	0.58	0.00	1.00
Erythrocyte Zn	2.05	0.15	0.03	0.87	**4.44**	**0.03***

Only 6.7% of pregnant women smoked and they were in the 1st or 2nd trimester. Smoking habit did not associate with Zn level (Table 12).

Table 12. Investigation between zinc level and smoking habit

	1st Trimestre		2nd Trimestre		3rd Trimestre	
	x^2	p	x^2	p	x^2	p
Serum Zn	0.37	0.54	1.79	0.18	-	-
ErythrocyteZn	0.42	0.51	0.95	0.32	-	-

Other researches (Naggers et al., 1996) which studied factors influencing plasma Zn levels in low income pregnant women, found that race, parity, and pregnancy weight were significantly associated with maternal plasma Zn levels, adjusted for gestational age.

CONCLUSIONS

Our study on pregnant women, emphasised that Zn level significantly correlated or associated with:

-gestational age

-nutrition (eggs, organ, alcoholic beverages, bread, sweets consumption)

- residence in urban or rural area.

Socio-economic status indirectly influenced Zn level, as consequence of the association with some unhealthy alimentary habits.

Our study allowed elaboration of some recommendations:

- gradually physiological decrease of Zn level during pregnancy makes necessary the increase of the Zn intake (by diet or supplements),
- the nutrition of pregnant women has to be various, including animal proteins, without alcoholic beverages and excess of sweets and bread,
- education for health of the childbearing women, especially of the socioeconomic unfavourable women would improve the structure of their nutrition with beneficial effects for mother and foetus.

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A COMPLEX STUDY ON THE NEUROPHY-SIOLOGICAL AND BEHAVIORAL EFFECTS OF INORGANIC LEAD EXPOSURE IN RATS

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ABSTRACT

Among all toxic heavy metals, lead is one of the most abundant as environmental pollutant. Prolonged lead exposure was shown to impair the mental development of children and to induce a variety of central and peripheral neural symptoms. Our was to conduct a series of experiments demonstrating the effects of inorganic lead on the nervous system of rats the neuronal to the behavioral level. Rats were treated with ionic lead (acetate salt), 5,50 or 500 mg/kg b.w. for 10-12 weeks. The animals' behavior was tested in a maze and an open field and then their spontaneous and evoked cortical activity was recorded and analysed. In other rats, treated with 2000 mg/kg lead in a single dose, cortical single activity and hippocampal population spikes were recorded. The memory performace of the treated rats was singnificantly below that of the controls and there were alterations in the frequency distribution of the cortical activity. The hippocampal population spikes were reduced by acute lead treatment and their potentiation was partially abolished. Taken together, these effects constitute a suitable model of the human neuvous system effects of lead.

Key words: inorganic lead, ne rvous, memory, rat.

INTRODUCTION

Heavy metals, including lead (WHO, 1995) are widely used in industry and agriculture, thus ill effects due to them deserve special attention. Lead is readily obsorbed via inhalation (Kehoe,1987) and ingestion (Chamberlain et al. 1978). Lead penetrates the blood-brain barrier and is deposited preferentially in the neocortex and hippocampus (Grandjean, 1978). Onace in the nervous system, lead primarily interferes with Ca-regulated functions (Sandhir and Gill, 1993). Its effect on NMDA channel function (Marchioro et al, 1982) can explain the adverse effect of chronic lead exposure on metal power and mental development of children. Needleman et al (1990) found impaired IQ and different behavioral difficulties in children with elevated body lead content. Otto et al (1985) foud characteristic EEG and auditory evoked potential alterations in schoolchildren after several years of expositions to lead. Observing a series of acute cases, Pach et al. (1991) described functional and morphological alterations corresponding to a lead-induced organic brain lesion. In animal models, postnatal lead treatment induced EEG disorders and learning disability in rats (Kumar and Desiraju 1992; Nagymajtenyi et al, 1997). Lead-exposed rhesus monkeys had a diminished discriminative auditory performance (Molfese et al.1986). Most effects of lead are seen in the higher order functions of the nervous system, their mechanistic explation, however, must corne from the mechanisms. To this purpose, a complex neurophysiological and behavioral study on rats treated with inorganic lead was perfomed.

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MATERIALS AND METHODS

The experiments were done on young adult male Wistar rats (10 weeks old at start), kept under standard conditions. Groups of rats (12 animals) were treated by daily doses of lead (see table 1) Control animals received saline.

During the **behavioral tests**, the adult rats had, after the lead aministration, a limited access to food (for 1hour/day) to keep their body weight at 80-85% of that of the controls (Beatty et al, 1980). From the 7th treatment day on, spatial working and reference memory testing was performed in an 8-arm radial maze. The animals were first accustomed with the maze and the food searching task. Duuring acquisition in the next week, witk, one training per day, the animals imprinted that they must visit the farthest points of each arm. Run time was limited and pellets found were never replaced.

This way, the rats were forced to learn a win-shift food search strategy. In the working memory test they had to find, in a limited time, the four arms which they did not visit 2 hours and 4 hours ago ("event-to-be-remembered"). the test was continued until the rat succeeded in finding all the pellets or until 10 minutes elapsed. In the reference memory test, the animals were to reach day by day the same 4 arms of the maze where pellets were found on the 1st day. The arms were determined accorting to the typical movement direction of each animal shown during working memory test. Long-term retention was investigated following a 14 days rest period with no food deprivation or testing. During memory return after this rest period, the information gained previouly in the acquisition was recalled in daily one session under indentical circumstances. Then, 2 and hours spatial working memory was tested as before.

Electrophysiological recordings on the subchronicallly treated animals were a few days after the last behavioral test. All electrophysiological work was carried out on acutely prepared animals. In urethane (1000 mg/kg) anaesthesia, the animal's head was fixed in astereotaxic frame, the skull was opened and the left hemisphere exposed. Macroscopic cortical activity was recorded from the surface of the primary somatosensory, visual and auditory area with silver electrodes in subchronically pretreated rats (see Table 1). First, spontaneous activity (electrocorticogram, EcoG) was recorded for 15 min. The, evoked potentials were elicited by a series of 50 sensory stimuli each: electric shocks to the whiskery skin (ca. 4V, 0.05 ms, 1 Hz), flashes (60 lux, 1 Hz) into the contralateral eye via an optical conductor, and clicks (40 dB, 1 Hz) delivered through the hollow ear bar into the contralateral ear of the rat. Recording and evaluation was performed on a PC after digital conversion. From the EcoG, relative power spectrum, according to the classical bands, was automatically calculated. Latency and duration of the evoked potential waves measured off-line manually operated cursors.

Activity at he neuronal level was assessed by recording cortical single unit activity and hipoocampal population spikes in acutely treated rats (Table 1). Single unit activity was recorded by a glass microelectrode (15-25 M, 3M NaCl) inserted into the somatosensory area using an electromechanical servo manipulator. Having reached an electrode position yielding a clear and stable single unit record (depth 200-800 m), a few 30s samples of control activity were recorded. After that, lead acetate was injected i.p. and single unit activity was then recorded in the 2nd ,5th, 10th, 15th, etc. minute after administration. To record stimulus-evoked activity, whiskery skin area stimulation was used as above. Histograms and mean values of the principal parameters of the activity to be analyzed, i.e. interspike interval (IS) and poststimulus time (PST), were generated electronically.

Hippocampal population spikes (POPSPs) were recorded from the CA1 zone of the hippocampal pyramidal layer by glass microelectrode. For stimulation (1-4 mA, 0.05 msec, 0.1 Hz), a bipolar electrodewas placed in the perforant path. Twenty evoked POOSPs were averaged and stored, followed by a 10 min interval. After at least 4 controlrecords, lead was administered i.p.and further records were taken in the same pattern for 2 hours or longer. Hippocampal POPSPs were characterized by their amplitude, latency and duration.

Tabel 1. Treatment doses and schemes for the electrophysiological and behavioral investigations.

Subchronic administration	Behavioral investigation (maze) Spontaneous and Stimulus-evoked cortical Activity recording	50 and 500 mg/kg Pb^{2+} (lead acetate). 5 days a week for 10-12 weeks.	p.o.by gavage
Acute administration	Cortical single unit Recording. Hippocampal population spike recording.	1500 mg/Kg Pb^{2+} (lead acetate). Given after control recording.	i.p.

The primary data were tested for normality the Kolmogorov-Smirov test and then treated with one-way ANOVA. $p<0.05$ was accepted as limit of significance.

RESULTS AND DISCUSSIONS

The lead doses applied in the present investigation had no general toxic effect. In the behavioral study, acquisition was affected in the higher dose lead group (Fig.1). Both doses caused significant ($p<0.05$) memory deficit in the 2 and 4 hours

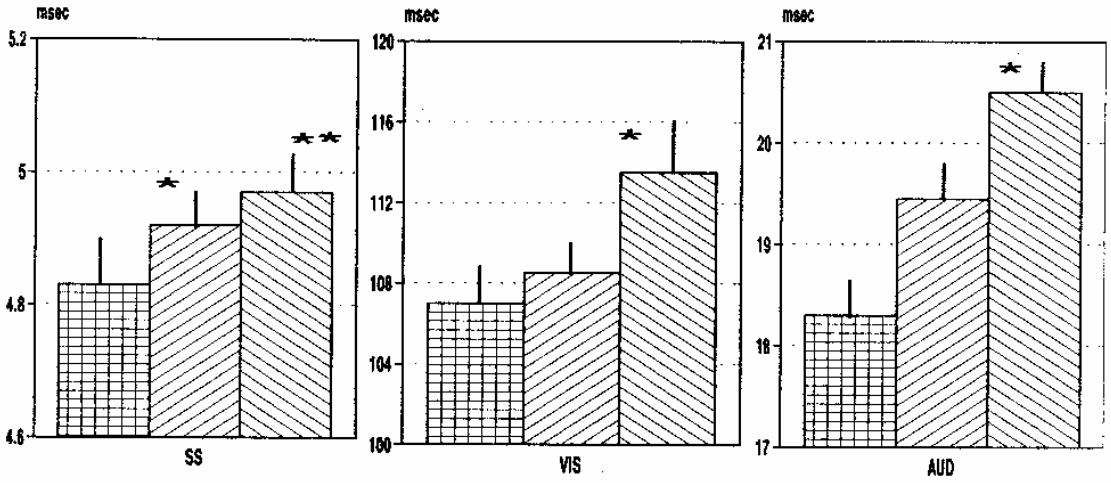

Fig. 1. Performance (correct responses / all responses, %) of control and lead-treated rats in the open field test during the treatment period.

working memory performance from the 14th day on. On the 21st day, reference memory was significantly impaired by both lead doses. Long-term memory tested from the 8th week of lead administration after a 2 weeks rest period, had also a significant deficit. In the 2 hours working memory return test, control rats seemed to regain some knowledge while rats treated with lead acetate had but a minimal (ca. 10%) ability to recall.

The cortical electrical phenomena of subchronically lead-treated rats showed also a clear, albeit less marked, effect. The change EcoGs was quite similar in all three cortical areas. There was an increase in the average frequency whereby the effect of the higher dose was significant (Fig. 2) The band-by-band evaluation (not shown) indicated a relative power increase in the bate band and a decrease in the theta and delta bands.

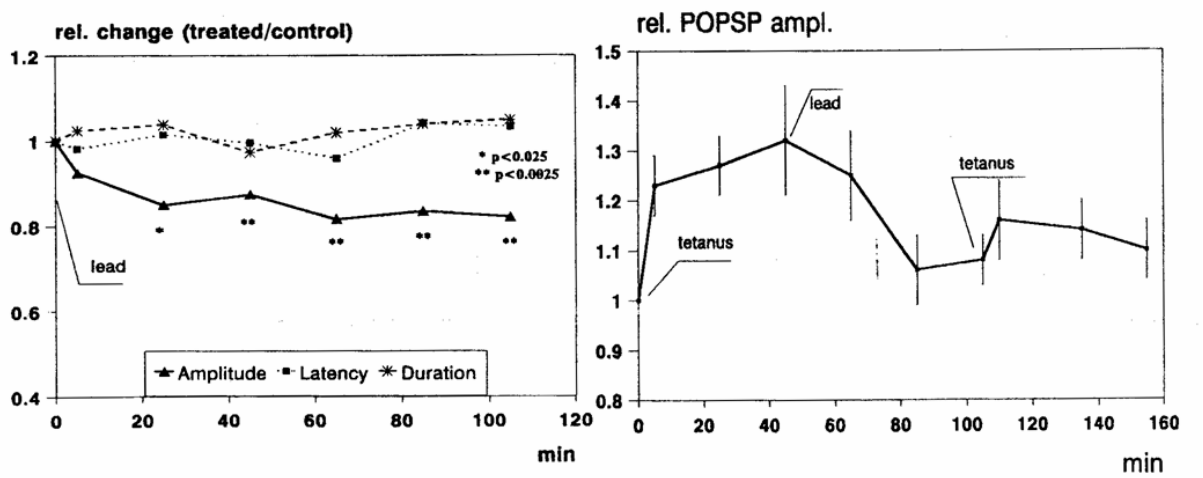

Fig.2. Changes in the EcoG average frequency on lead treatment in the three (SS, somatosensory; VIS, visual; AUD auditory) cortical areas, p <0.05

From the parameters of the cortical evoked potentials, the latency of the first

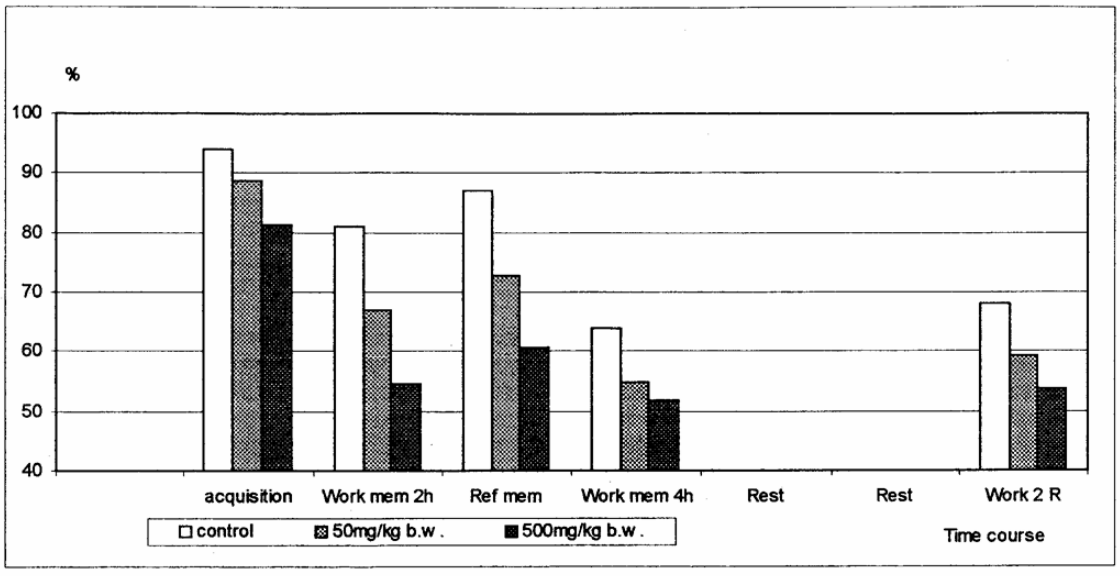

Fig.3. Changes of the peak latency (ms) of the evoked potentials on lead treatment.

main wave showed the best correlation with lead treatment. The lengthening of the latency was dose-dependent and was in some cases significant.

For legends, see Fig.2.

In case of the POPSP, amplitude was the only parameter significantly influenced by lead. Amplitude was the only parameter of the population spikes being singnificantly influenced by leadf. We saw a gradual decrease of the amplitude which could already be observed at 5 min following lead administration and became more expressed up to ca. 1 hour. At the same time, the temporal parameters remained practically the same (Fig.4).

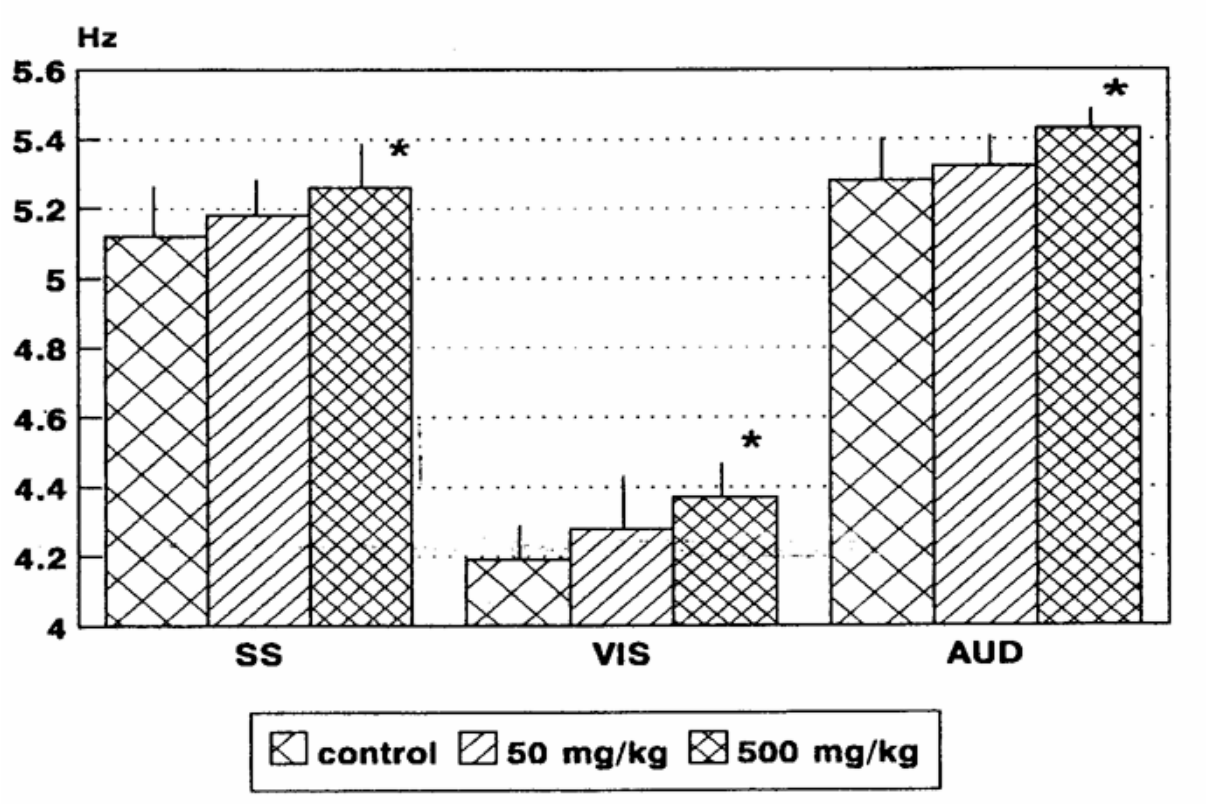

Fig.4. Change of the parameters of POPSP on acute lead administration (left). Interaction of tetanic stimulation and lead (right)

A typical feature of hippocampal POPSP is tetanic potentiation induced by a strong stimulus train. A lead-treated POPSP could hardly be potentiated by a tetanus and the amplitde increase brought about by tetanization was reduced or abolished by a subsequent lead administration. On the cortical single unit activity, the main effect of acute lead treatment was the gradual loss of temporal patternes.

The results show that subchronic or acute lead exposure affected the higer order functions of the rat brain.In rats, spatial memory depends on the sensory perception of the environmental stimuli (Olton and Papas, 1979). Short-term memory, keeping information for ca. 8 hours, determines the quality of acquisition retention and recall and depends highly on fast transmitter and modulator systems. These are known to be affected by lead (Busseelberg, 1995; Cory-Schlechta, 1991).

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CHEMICAL ELEMENTS AS REGULATORS OF THE BIOSYNTHESIS OF LIPIDS AND CAROTENOID PIGMENTS IN THE YEAST

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ABSTRACT

The paper gives the patterns of the influence of the metal complexes $[Zn(Benz)_2]$, $[Mn(Gly)_2Cl_2]$ and $[Co(NH_3)_5(H_2O)](NO_3)_3$ on the biosynthesis of intracellular lipids, fatty acids and carotenoid pigments in the yeast Sporobolomyces pararoseus. It was shown that the response to their effects is related to the nature of metal complexes, the concentrations applied, and the period of incorporation. The evident stimulation of lipidogenesis and carotenogenesis as well as the high quality of fatty acids demonstrates the importance of the application of coordinative compounds to the microbial oleobiotechnology.

Key words: metal complexes, biosynthesis of lipids and carotenoids

INTRODUCTION

The development of microbial biotechnology emphasises the importance of the search of the new ways for the increase and regulation of the producent activity. The application of chemical elements, especially in form of coordinative cpmpounds, is crucial for the growth and metabolism of microbial cultures. While assesing a possible role in the biosynthesis of such elements as nitrogen, zinc, manganese iron, it is assumed that their lack of excess in the cultivation medium for microorganisms indirectly effects the lipidogenesis through the changes in the synthesis of proteins (Kujavsky et al., 1992; Gadd G., Laurence O.,1996).

It cannot be exluded that the ions of the metal have specific effects on the enzymatic systems involved in the lipid synthesis. Naganuma et al., (1987) found that zinc ions influence the activity of nine enzymes from the tricarbonic cycle, glycolisis, and the synthesis of fatty acids. Lomascolo et al., (1996), while studying the δ12 – desaturase system in the microsomes of Lypomyces Starkey – an enzymatic system involved in the transformatuion of oleic acid into linoleic acid - observed that the ions of Mg^{2+}, Mn^{2+}, and Zn^{2+}activate this enzimes. In contrast, Co^{2+} which can trigger the microsome aggregation completely inhibits the activity of these enzime.

The studies of many researchers proved high efficiency of trace metals in form of coordinative compounds which are used in microbiology for the regulation of the biosynthesis of different physiologically active substances. By their nature, metal complexes closely resemble the biological compounds involved in the vital activities of microorganisms, due to the presence ofcoordinate bonds in theit molecules. This type of structure of coordinative compounds enables their interaction with the amino groups of the protein molecules from the cellular membranes and, especially, with the molecules of the transportation proteins. The application of coordinative compounds results in the positive effects of antibiotics, cyancobalamine, pectinolytic and hidrolytic enzymes, the production of algal preparatios (Shishkov, 1987; Rudic, 1993, Gerbeleu et al., 1999).

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It is known that metal complexes are less toxic and more readily assimilated that the salts of metals. Keeping this in mind, the authors conducted the research which reveals the effects of the coordinative compounds of zinc, manganese and cobalt on the growth, lipidogenesis and carotenogenesis of the yeast.

MATERIALS AND METHODS

The efficiency of the chemical elements was evaluated under the conditions of the periodicval cultivation of the yeast *Sporobolomyces pararoseus* CNM-ys-01 which is patented and deposited in the National Collection of Microorganisms, Instutute of Microbiology (Academy of Sciences of Moldova) (Usatâi et al., 1997). The evaluation criteria were the output of dry biomass, the cell lipid content, qualitative and quntitative content of fatty acids, and the output of carotenoid pigments. The coordinative compounds studied were $[Zn(Benz)_2]$, $[Mn(Gly)_2Cl_2]$ and $[Co(NH_3)_5(H_2O)](NO_3)_3$ in the concentrations 5.0, 10.0 and 20.0 mg/l.

The lipid separation was carried aut by usual methods with modified prcedure of cell wall disintegration and the utilisation of extraction agents. Chromatography plates “Sulifol” were developed in the mixture of solvents of different polarity and scanned with a densitometer. Fatty acids were determined with gas-liquid chromatography.

RESULTS AND DISCUSSIONS

At present**,** biological functions were studied only for a part of trace elements. Data abound on the role of zinc ions in the metabolism of microorganisms. It was found that the zinc deficit negatively influences the majority of the microbial cell components, including the levels of DNA and RNA, the synthesis of protein, saccharides, and the phosphate metabolism (Chatterjee et al., 1988).

It is of special interest that the application of cobalt can compensate the deficit of the enzyme system, changing in this way the metabolism of yeast.Dedyuhina and Eroshin (1992) found that in case of substitution of zinc with cobalt, the decrease of biomass and increase of intracellular lipides occur. In contrast, changes in the composition of fatt acids are not observed. Another element which is known to as a chemical element with the direct effects of lipidogenesis enzymes is manganese. This element is crucial for the activity of acetyl-CoA-carboxilas – an enzyme which is involved in the synthesis of malonil-CoA. As a result, the importance of the aforementioned metals is remarkable in the physiological studies on the regulation of the yeast growth process and the synthesis of bioactive substances.

The goal of the present research was to study the effects of the coordinative compounds of Zn (II), Mn (II), Co (III) on the synthesis of substances from the lipid class by a stem of the oleaginous yeast *Sporobolomyces pararoseus* CNM-ys-01. The research done shows (tab.I) that zinc benzoate is an important factor in the cultivation medium for the synthesis of lipids and carotenoid pigments. The quantity of intracellular lipids varies from 44.7 – 46.9 mg/100g DB, the reference culture yields 38.5 mg/100 mg DB. Under these cultivation conditions the biomass output is lower by 10 – 19 % than in the reference culture. In case of the application of small concentration of zinc compound the yeast cultivationmedium and namely , of 5.0 and 10.0 mg/l, the content of carotenoid pigments – β – caroten, toruline, and toruloidine – grows in comparison to the referenxce and measures 118 – 140 %.

Table 1. The effects of metal complexes of Zn (II), Mn (II) and Co (III) on the productivity of the yeast *Sporobolomyces pararoseus* CNM-ys-01.

Concentration of a metal complex (mg/l)	Period of incorporation	Dry biomass		Intracellular lipids, mg/100mg DB		Carotenoid pigments (β-caroten, torulin, torularodin) (μg/gm DB)	
		M±m	% R	M±m	% R	M±m	% R
$Zn(Benz)_2$							
Reference		17.42+1.87		38.5+0.5		191.2+2.5	
5.0	start	15.84+0.99	90	45.9+1.1	119	225.7+2.8	118
	48 hours	16.68+0.46	96	45.2+5.5	117	243.4+3.1	128
10.0	start	14.40+0.08	83	45.1+3.5	117	267.6+5.2	140
	48 hours	15.36+0.47	88	45.5+3.4	118	177.4+7.6	93
20.0	start	14.15+0.63	81	46.9+2.1	122	186.2+7.1	97
	48 hours	15.61+0.39	89	44.7+4.0	116	177.4+6.4	93
$Mn(Gly)_2Cl_2$							
Reference		11.97+1.27		42.7+0.2		167.6+14.8	
5.0	start	12.20+0.88	102	41.2+3.3	96	173.9+11.6	104
	48 hours	11.74+0.64	98	40.3+2.1	94	164.4+10.1	98
10.0	start	13.13+0.72	110	42.5+2.9	99	191.5+5.3	114
	48 hours	12.96+0.28	108	41.6+2.6	97	187.3+4.8	112
20.0	start	13.44+0.33	112	40.9+1.3	96	197.8+8.0	118
	48 hours	13.00+0.31	109	39.3+1.0	92	196.9+1.0	117
$[Co(NH_3)_5(H_2O)](NO_3)_3$							
Reference		14.00+1.34		38.9+0.4		413.8+8.1	
5.0	start	13.65+0.80	97	40.3+1.1	103	368.6+6.1	89
	48 hours	13.76+0.59	98	38.0+1.8	98	376.8+2.9	91
10.0	start	13.35+0.40	95	40.8+0.7	105	400.3+9.2	97
	48 hours	13.10+0.60	94	39.7+0.9	102	447.6+3.3	108
20.0	start	11.07+0.27	79	43.1+1.0	111	445.2+3.0	107
	48 hours	12.55+0.52	90	41.5+0.5	107	588.2+1.2	142

Besides the determination of the lipid output, the quantitive composition of lipid fraction was also studied. The experiments show (Table 2) that the composition of lipids resulting from the cultivation in the prersence of 10.0 mg/l $[Zn(Benz)_2]$ does not differ significantly from the composition of the lipids in the yeast cultivated under references conditions, except for the content of triglycerides, which increase by 27%. Metal complex $[Mn(Gly)_2]Cl_2$ stimulates the biosynthesis of stearines and triglycerides, and the amino complex $[Co(NH_3)_5H_2O](NO_3)_3$, of mono- and diglycerides.

Table 2 The effects of metal complexes of Zn (II), Mn (II) and Co (III) on the fractional composition of lipids in the yeast *Sporobolomyces pararoseus* CNM-ys-01 (% to reference).

Lipid fractions	$[Zn(Benz)_2]$		$[Mn(Gly)_2]Cl_2$		$[Co(NH_3)_5\ H_2O](NO_3)_3$	
	start	after 48h	start	after 48h	start	after 48h
Phospholipids	103	96	100	120	108	82
Sterines	96	94	123	123	103	121
Unidentified fractions	92	111	-	-	72	98
Monoglycerides	89	90	96	107	127	126
Triglicerides	127	108	115	118	84	80
Eters of Sterine and wax	92	94	-	-	113	102

Keeping in mind that a large part of yeast species in consideretd to be producers of valuble fatty acids, we studied the potential of biosyntesis of fatty acids by *Sporobolomyces pararoseus* CNM-ys-01 using the same set of coordinative compounds. The foundings of the research prove (Table 3) that the most valuble range of

fatty acids yields with $[Zn(Benz)_2]$ – 8.66 % essential fatty acids, the next efficient complex was $[Co(NH_3)_5\ H_2O](NO_3)_3$ – 4.18 % of the total content of acids. However, the essential acid biosyntasis rates decrease under effects of the metal complexes after 48 hours since the beginning of experiments.

Table 3 The effects of metal complexes of Zn Zn (II), Mn (II) and Co (III) on the content of fatty acids in the yeast *Sporobolomyces pararoseus* CNM-ys-01 (% of the total amount).

Fatty acids	Reference	$[Zn(Benz)_2]$		$[Mn(Gly)_2]Cl_2$		$[Co(NH_3)_5\ H_2O](NO_3)_3$	
		start	after 48h	start	after 48h	start	after 48h
$C_{14:0}$	0.97	3.59	4.4	4.0	5.18	traces	1.46
$C_{15:0}$	0.13	-	-	1.26	traces	0.73	0.09
$C_{16:0}$	25.31	17.05	26.6	10.9	13.38	17.02	26.23
$C_{16:1}$	3.78	5.13	12.1	13.08	13.65	5.53	2.43
Unedentified	-	-	-	traces	traces	1.75	0.14
$C_{18:0}$	8.45	15.2	11.7	10.92	10.86	20.09	11.35
$C_{18:1}$	56.2	50.3	45.1	56.32	54.53	50.84	56.28
$C_{18:2}$	-	2.03	traces	1.54	traces	2.58	1.06
$C_{18:3}$	-	-	-	-	2.4	0.7	0.42
$C_{20:0}$	1.8	1.68	traces	1.64	-	0.9	0.4
$C_{20:4}$	3.24	4.95	traces	-	-	-	-
Saturated	36.6	37.52	42.7	28.72	29.42	38.4	39.64
Unsaturated	63.22	62.41	57.2	70.94	70.58	61.4	60.19
Essential	5.04	8.66	-	3.18	2.4	4.18	1.88

CONCLUSIONS

The research conducted demonstrates that coordinative compounds $[Zn(Benz)_2]$, $[Mn(Gly)_2]Cl_2$ and $[Co(NH_3)_5\ H_2O](NO_3)_3$ have different effects on the synthesis of lipids and carotenoids in the yeast *Sporobolomyces pararoseus* CNM-ys-01. The influence is related to the nature of the nature of metal complex used, the concentration appied, and the period of incorporation. The mechanism of action of the coordinative compounds on the lipidogenesis, carotenogenesis and the synthesis of fatty acids in studied insufficiently and additional research is required for its complete understanding. However, the stimulating effects of metal complexes on the microbial syntesis of the compounds from the class of lipids is of broad prospective in terms of their use in the microbial oleobiotechnology.

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THE BIOTECHNOLOGICAL PROCESSING EFFECTS UPON THE NUTRITIONAL EFFICIENCY OF MINERALS CONTAINED IN CEREALS

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ABSTRACT

Previous investigations demonstrated that calcium, magnesium and iron contained by wheat seeds are released from phytates during germination. The present studies try to prove that calcemia, magnesimia and siderimia is improved by germinated wheat consumption at children who had these physiological indices at the lower normal limit.

Keywords: nutritional efficiency, germinated wheat flakes, calcemia, magnesimia, siderimia

INTRODUCTION

Cereals are foods that represent an important part of the human diet because of their nutritive principles density and diversity. But, all these principles can not be assimilated with high efficiency due to the cereals content in anti-nutritious compounds. This is the case with mineral salts, whose bioavailability is affected by their existence as phytates (Abdulah, A. and Baldovin, R.E, 1994; Urbano, G. et al., 1995; Lolas, G.M. et al, 1996)..

Our previous "in vitro" experiments confirmed the fact that mineral level is increased during cereal germination. For this reason we considered necessary to check weather "in vitro" results are confirmed "in vivo", in growing organisms (Segal, B. et al. , 1987; Dallmann, P.R. et al., 1990).

This paper present the nutritional effects appreciated as calcium, magnesium and iron content in the blood serum, on subjects who consumed cereal flakes produced from germinated seeds. Into the germinated seeds the phytates were split-up by the germination activated phytase (Thompson, L.W. and Serraino, M.R., 1985).

MATERIALS AND METHODS

Germinated wheat was taken as flakes, commercialised under the name of GRANOVIT. Produced after an original method (SC Granovit SA, Braila, RO), which preserve the biological and nutritive value of wheat.

GRANOVIT is produced from wheat grains that are steeped in water until they reach a humidity of 40-45% and germinated at 15 – 17°C, during 4 days. During germination the grains are periodically aerated to prevent their overheating and alteration due to microorganisms growth (Segal, B. and Segal, R., 1991).

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Germinated grains (radix of about 1 – 1.5 cm) are rolled and the resulted flakes are dried in a tray kiln till their humidity reach 11-13%.

During drying, it is not allowed for the flakes temperature to exceed 50°C, to not damage the biological active compounds (vitamins, amino-acids) through thermal destruction or compounds interactions (ex., Maillard reactions).

The biochemical content of GRANOVIT is presented in Table 1.

The biological test, which last 21 days, was done on a group of 9 boys (B) and 12 girls (F), who were 7 years old. During the test period, children consumed every morning, on empty stomach, 50 g of germinated wheat, while the individual regime have not been changed.

Calcium, magnesium and iron analyses were performed using standard clinical methods.

Table 1. Nutrition information (per 100 g GRANOVIT)

Energy	kcal	308
Protein	g	13
Carbohydrates	g	58
of which:		
sugars	g	19
starch	g	39
Fat	g	1.8
Dietary fibre	g	9.0
Minerals		
of which:		
Magnesium	mg	110
Iron	mg	5.0
Phosphorus	mg	270
Calcium	mg	5.0
Zinc	mg	3.0
Vitamins		
of which:		
Vitamin B1	mg	0.6
Vitamin B2	mg	0.8
Niacin	mg	7.0
Tocopherols	mg	13.0

RESULTS AND DISSCUTIONS

Figure 1, 2 and 3 present the calcemia, magnesimia and siderimia variation for the 21 tested children.

Calcemia, magnesimia and siderimia presented normal initial values for all the children but for 10 of them these values were situated at the lower normal limit.

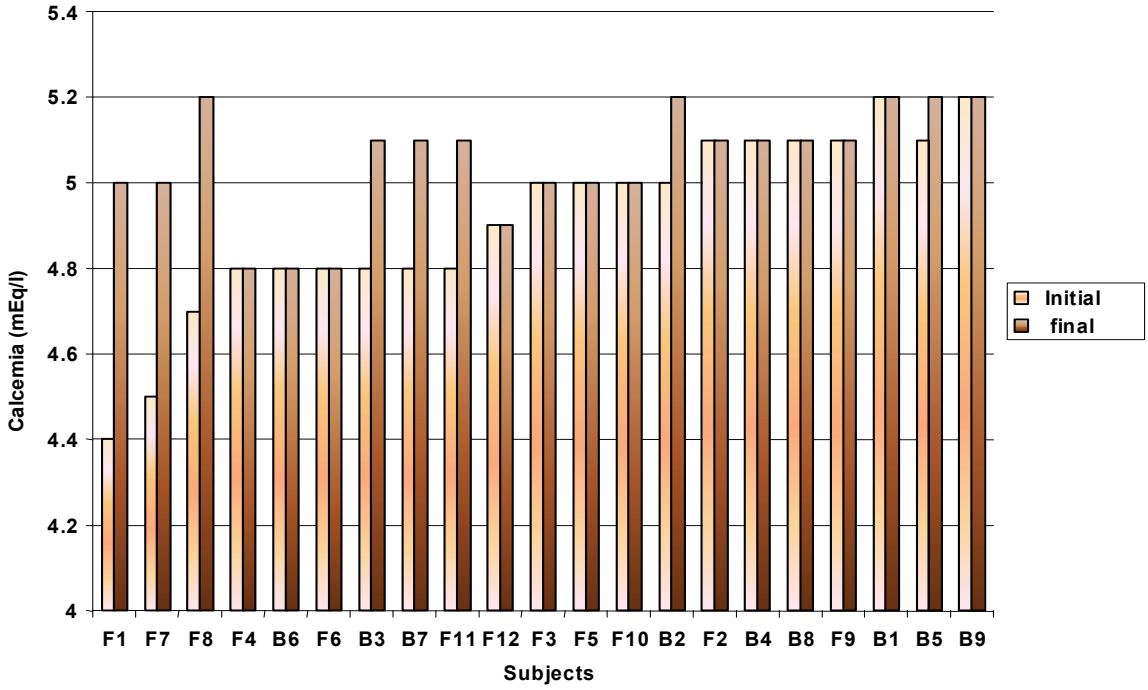

Figure 1. The influence of germinated wheat consumption upon children calcemia

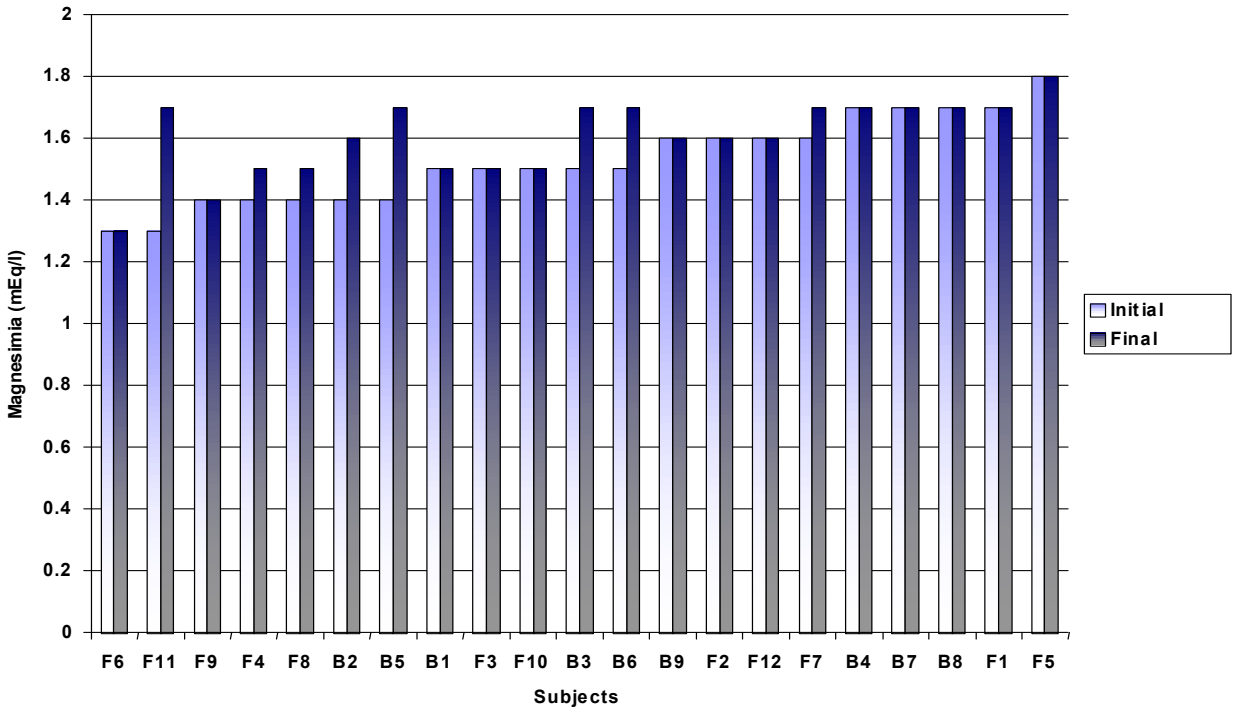

Figure 2. The influence of germinated wheat consumption upon children magnesimia

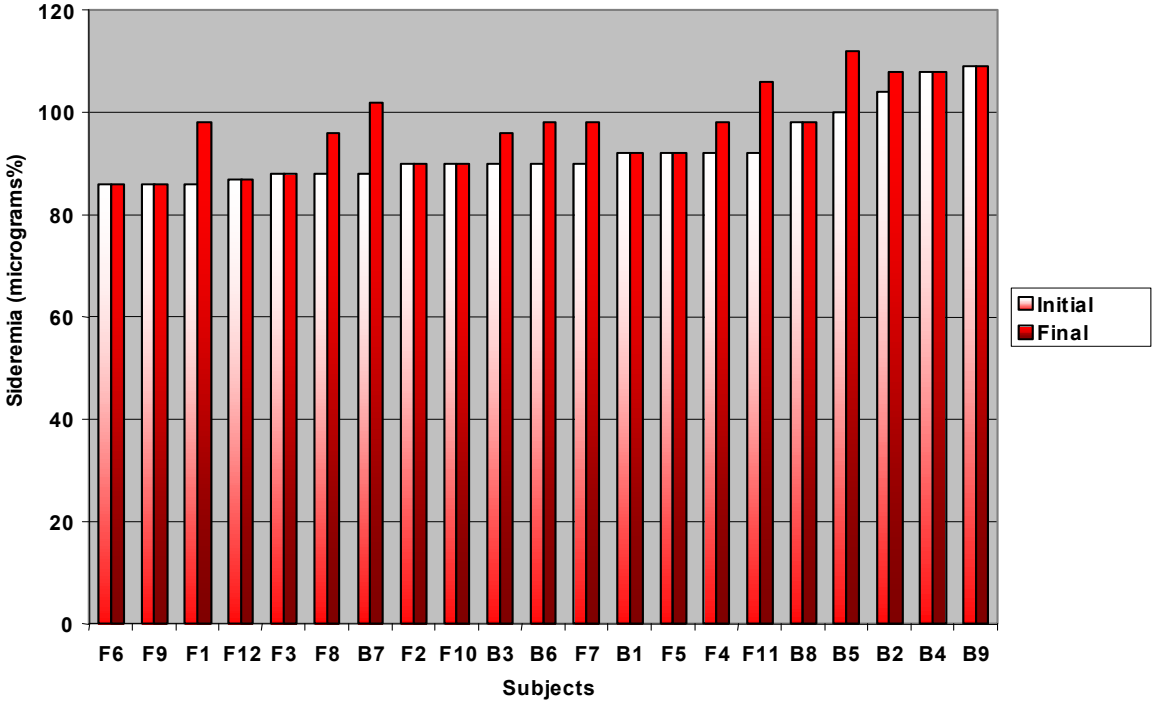

Figure 3. The influence of germinated wheat consumption upon children siderimia

The study demonstrated that germinated wheat administration determines the rise of calcium, magnesium and iron level (statistic interpretation of the data are presented in Table 2). To the subjects whose initial values of the physiological indices were situated closer to the lower normal limit it was noticed a significant increase.

A sensitive increasing of calcemia it is noticed for the three girls whose initial calcium level was initial situated at lower normal limit. For these girls, calcemia significantly increased (10,6 - 13,6%), as result of germinated wheat consumption (Johnston, C.C. et al., 1992).

Table 2. Statistical analysis of calcium, magnesium and iron levels in blood serum

	Normal limits	**Subjects**	**Mean value X**	**Dispersion of selection S^2**	**Index of band Including I_B**	**Medium increase %**
Ca level	4.5-5.5 mEq/l	Initial point	4.92	0.049	0.72	13.3
		Final point	5.05	0.019	1.24	
Mg level	1,25-2.0 mEq/l	Initial point	1.53	0.020	0.59	10.2
		Final point	1.61	0.015	0.90	
Fe level (girls, F)	80-110 γ %	Initial point	88.92	3.54	0.42	16.1
		Final point	93.75	2.87	0.85	
Fe level (boys, B)	90-120 γ %	Initial point	96.89	5.11	0.30	16.3
		Final point	101.78	4.18	0.65	

It is supposed that biological active compounds contained by the gourmand wheat regulate the ionic homeostatic mechanisms and do not produce a chaotic increasing of calcium, magnesium and iron ions.

The study was carried out at the end of the winter period, when the vitamins and minerals intake is reduced because fresh fruits and vegetables are less consumed. We mention that children, who were not used to eat this kind of food, happily accepted the germinated wheat flakes and well tolerated them. Neither digestive troubles nor colds were registered during the diet.

It could be concluded that germinated wheat represents a natural food supplement, which is recommended for prevention and fighting against mineral imbalance, carential and malabsorbtion anaemia, spasmophilia and prolonged stress situations.

CONCLUSIONS

1. Foods obtained from germinated cereals could make their efficient contribution to the mineral salts assurance in human organism. This is a consequence of the bioavailability of minerals in germinated seeds.

2. The systematic presence of germinated cereals in children food ratio improved the level of iron, calcium and magnesium in the blood stream.

3. The physiological effect of germinated cereals does not exceed normal limits. Thus, the peril of overdosing them does not exist.

4. Due to their peculiar nutritional effects, food produced with germinated seeds act as real functional foods.

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THE EFFECT OF TRINUCLEAR CLUSTER WITH Fe^{3+} AND Co^{2+}-DIFECODEN ON EASY SOLUBLE PROTEIN PATTERN FROM ZEA MAYS L. PLANTS IN DROUGHT CONDITIONS

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ABSTRACT

The influence of trinuclear cluster with Fe^{3+} and Co^{2+} - difecoden, in condition of water deficiency, on quantitative and qualitative changes of easy soluble proteins from organs of Zea mays L. hybrid M456 plants was studied, in order to elucidate its biochemical and physiological role in achievement of plant resisting potential. In the laboratory and vegetation experiments the stabilizing effect of difecoden on protein pattern was stand out. The easy soluble proteins concentration in organs of treated maize seedlings at initial stages of ontogenesis is higher than in organs of untreated ones. In condition of water stress the difecoden induce the maintenance of protein content at level of that from control plants both in seedlings and mature plants. Coomassie blue staining of sodium dodecil sulfate polyacrylamide gels indicate, that difecoden not induced the appearance of new polypeptides in protein spectrum from organs of water stressed plants. Only content modification of certain protein components was observed. The trinuclear cluster with Fe^{3+} and Co^{2+} also, have the stabilizing action on peroxidase and cytocrom oxidase izoenzyme spectrum in dehydration maize plants. So, the functional role of the difecoden, probably, consist in changing of the kinetic characteristics of the easy soluble protein synthesis and less in their synthesis de novo, provides the proteolysis minimization of proteins at drought action.

Key words: trinuclear clusters, protein content

INTRODUCTION

One of the important way of lasting agriculture development and ensurance of population with food products is the increasing of crop productivity on basis of more complete realization of variety genetic potential. For solution of this task becomes more evident the utilization of bioactive substances, because, besides of their optimizing effect on cultivars productivity and resistance, they ensurance the increasing of work cost (many making). On the other hand, the foliar treatment or soil administration of bioactive substances, especially, of hormonic type, may have negative results on human health. But the increasing of dry years frequency, the deficiency of nutrition elements, the perpetual increase of energy cost, the making of bumper crops, advance the necessity of new bioactive substances prospecting, which are ecological harmless, of nonhormonic type. The elucidation of action mechanisms of these compounds represent one of the notorious objective of contemporary science. Because the protein heterogeneity serve as index of genetic subprogram modifications induced by stress factors, the study of the content and the electrophoresis spectrum present the interest. It is known, that in the plants, resisting

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to stress action, there are substitution of some enzyme systems and activation of stable enzyme izoforms. Until now it is not clear the peculiarities of coordination compound action, which contain metal-microelements on easy soluble protein pattern (ESPP) in organs of agriculture plants. As it proceeded from above mentioned, during several years, the complex studies of the effect of the seed treatment with trinuclear claster with Fe^{3+} and Co^{2+} on the biochemical and physiological peculiarities, growth, development, productivity of *Zea mays L.* plants.

MATERIALS AND METHODS

Zea mays L. plants, hybrid M456, high productive, bur receptive to drought, were grown in laboratory, vegetion condition in order to elucidate the dozes with optimum physiological effect and to clarify the peculiarities of coordination compounds action on ESPP. Plants grown in optimum humidity conditions serve as control. In laboratory condition a part of plants after 3 days of germination were transferred on PEG (6000) solution, in order to create the physiological drought conditions. In vegetation condition the drought was modulated through differential watering of plants (70%-30% of total water content for soil (TWC). The parallel variant foreseen plants seed treated with aquatic solution of difecoden.

The biochemical analyses were done in organs of *Zea mays L*. seedlings at initial stages of ontogenesis and in leaves and roots of maize plants at VI th phases of ontogenesis (11-12 leaves). The easy soluble proteins (ESPs) were extracted in conformity with recommendation of Safonov V. and Safonova M. (1971). The content of proteins were estimated by the method of Bradford M (1976). The polypeptides were separated using gradient SDS-PAGE according to the method of Laemmly (1970). To stand out the peroxidase izoenzymes was used benzidine. The relative molecular weight (Mr) of proteins was estimated using calibration curve. For enzyme the migration coefficient (Rf) was calculated.

RESULTS AND DISCUSSIONS

It was established, that before sowing treatment of seed with aquatic solution of difecoden, optimize the release o start reaction of germination, water absorption, the mobilization and utilization of assimilates from endosperm, the velocity of embryonic root growth at initial stages of ontogenesis. The acceleration of root formation and growth at initial stages of ontogenesis ensures the subsequent increase of foliar surface growth index. Seeds treated with difecoden were distinguished by their assimilation surface, biomass accumulation, the efficiency of water utilization for synthesis of one yield unity etc. The preparation influences the realization of productivity potential, especially in optimum conditions (N857 MD) and contributes to diminish of drought impact on plants (N1131MD). The antistress effect of difecoden is realized through the modification of metabolic reaction intensity, the inhibition of hydrolytic processes, the stabilization of biosynthesis.

The content of ESPs from embryonic roots and coleoptiles of *Zea mays L*. seedlings from treated seeds exceed their concentration in control seedlings, especially in optimum condition of humidity. The increase of protein concentration under influence of preparation was established in cells from growth zones of embryonic roots. A higher content of protein, as comparison with control seedlings, was stand out in meristeme and elongation zones of root. In cells from mature zone of root the action of trinuclear clusters with Fe^{3+} and Co^{2+} is less meaningful that in those above mentioned. The seed treatment of seedlings with difecoden ensures the

maintaining of ESPs content in coleoptiles in embryonic roots at the considerable higher level in condition of physiological drought too. On drought phone in coleoptiles and in cells from elongation zone of root of treated seedlings a less decrease in comparison with the diminish of ESPP from untreated seedlings was observed (Table1, Table 2). In meristeme cells and in cells from mature region of root the treatment with trinuclear clusters causes the enhancement of ESPs content in water stress condition (Table 2.)

Table 1. The trinuclear cluster with Fe^{3+} and Co^{2+} effect on content of ESPs ($\mu g \cdot g^{-1}$ dry weight) in coleoptiles and embryonic roots of water stressed *Zea mays L.* seedlings at initial stages of ontogenesis.

Variant	Coleoptile	Embryonic root
Control	21422,9 ± 30,51	51770,2 ± 41,52
Physiological drought	19220,2 ± 30,53	44877,2 ± 36,4
Ph. d.+ difecoden	20,864, 9 ± 10,67	55306,8 ± 27,31

Table 2. The influence of trinulear cluster with Fe^{3+} and Co^{2+} on ESPs ($\mu g \cdot g^{-1}$ dry weight) content in cells from growth zones of embryonic roots of *Zea mays L.* seedlings in water stress conditions.

Variant	Meristeme zone	Elongation zone	Mature zone
Control	80663,6 ± 87,9	34807,4 ± 90,1	20698,0 ± 85,7
Physiological drought	88122,1 ± 70,6	23358,2 ± 96,7	14785,6 ± 90,2
Ph. d + dofecoden	101955,0 ±89,1	31071,3 ± 92,6	21857,1 ± 81,5

The peculiarities of protein synthesis, caused by difecoden influence are keeping, as well, at mature plants (12 leaves phases). The effect of water deficit on ESPs content in organs of treated plants is weakly that at plants from untreated seeds (Table 3). The preparation action is correlated with stabilization of protein complex in roots and leaves during desiccation period. In roots of treated plants the content of ESPs is higher than in control plants, what permit the supposition, that trinuclear clusters with Fe^{3+} and Co^{2+} encourage their synthesis.

Table 3. The action of seed treatment of *Zea mays L.* plants with difecoden on ESPP ($\mu g \cdot g^{-1}$ dry weight) from leaves and roots during dehydration period.

Variant	Dehydration period		
	The Ist day of water stress	The IIIrd day of water stress	The VIIth day of water stress
Control (70% TWC)	roots		
	3192,9 ± 30,4	3055 ± 10,8	3167,9 ± 16,9
The drought (70-30%TWC)	2906,2 ± 19,6	2914,3 ± 9,3	2182 ± 9,3
The drought+ difecoden	3223,1± 53,4	3144,9 ± 87,9	2606,8 ± 26,7

The VII-VIII th leaves			
Control (70%TWC)	788,5 ± 18,5	8143,9 ± 37,5	8625,7 ± 49,6
The drought (70--30% TWC)	7741,7 ± 31,8	6794,6 ± 49,2	6269,6 ± 21,8
The drought +difecoden	8521,7 ± 46,2	8271,3 ± 23,4	8170,1 ± 41 1
The XI-XII th leaves			
Control (70% TWC)	8835,9 ± 58,3	6768,8 ± 18,8	8935,8 ± 67,9
The drought (70--30%TWC)	7868,5 ± 66	6369,8 ± 31,5	7785,2 ± 40,6
The drought +difecoden	8585,3 ± 11,3	7826,7± 84	8166,6 ± 63,1

The modification under influence of difecoden were established in different regions of leaf (Table 4.). Thus, in condition of water deficiency, in leaves with finishd growth, the seed treatment of plants with studied compound causes the increasing of ESPs content in meristeme and elongation zone of leaf base and stabilization of their concentration in central zone of leaf. In apical zone the higher content of ESPs than in untreated plants, but less than in control plants was established. The enhancement of protein level in cells from meristeme and elongation zone and stabilization of their content in middle and apical region were stand out in young leaves of treated plants on drought phone.

Table IV. The contenr of ESPs ($\mu g \cdot g^{-1}$ dry weight) in cells of foliar limb growth zones of *Zea mays L.* difecoden treated plants at water stress action .

Variant	**The meristem and elongation zone**	**The zone of mature cells (the middle part of leaf)**	**The zone of senescent cells (apical)**
	The VIII th leaf		
Control (70% TWC)	5647,83 ± 5,69	6473,62 ± 5,3	7219,1 ± 17,7
The drought (70%--30% TWC)	5206, 7 ± 6,8	5671,6 ± 2,5	5578,6 ± 2,46
The drought +difecoden	6424,2 ± 3,18	6241,66 ± 3,43	6388,4 ± 3,77
The XII th leaf			
Control (70% TWC)	5634,78 ± 10,1	6563,13 ± 9,83	7063,76 ± 5,5
The drought (70%--30% TWC)	5538,66 ± 2,66	6190,47 ± 4,47	5884,62 ± 0,2
The drought +difecoden	6325,66 ± 3,04	6534,96 ± 9,1	6378,6 ± 5,34

Therefore, trinuclear cluster with Fe^{3+} and Co^{2+} causes the increase of protein content in optimum conditions and stabilize their concentration in condition of water deficiency. A higher enhancement of protein level was observed in young

tissues with unfinished growth. This allow the presumption, that difecoden can conditioned the formation of certain plant phenotype, which is in accordance with dates of Bogdanova E. (1986), Polimbetova L. (1991) about nicotinic acid and nicotinamide (B_5 vitamin) capacity to induce the activation of reply reaction of plants to stress factors action.

The bioactive substances action, particularly of auxine, on protein pattern is manifested not only by content modification, but also through the changes of their composition (Melanson D., Thewavas A.J., 1982; Meyer Y. et al., 1984; Leguay J., Jounneu J.P., 1987). In this work it was established, that electrophoreses spectrum of ESPs from *Zea mays L.* seedlings, seed treated with trinuclear cluster with Fe^{3+} and Co^{2+} and exposed to water stress action, at initial stages of ontogenesis is similar to that of control ones. At all studied variants it is observer a wide spectrum of polypeptides with molecular weight 93 kD- 14 kD. The detailed analysis of obtained protein spectrums shows some differences between studied variants. Thus, in electrophoreses spectrum of ESPs from coleoptiles of untreaded and desiccation seedlings in comparison to that of control ones, the appearance of some protein components with relative molecular weight (Mr) 70 kD and 50 kD were trace out. The electrophoreses of ESPs from coleoptiles of treated seedlings in the same conditions (water deficiency) stand out the similar composition to that of the ESPs from control seedlings (Fig.1). The water stress influence at these seedlings not induces the appearance of protein components above mentioned. It was established the intensification of color of proteins components with Mr 50-40 kD and 20-14 kD, what indicate to increase of content of these polypeptides. The polypeptide composition of protein extracts from different zones of embryonic roots of treated seedlings was similar with the electrophoreses spectrum of ESPs of control seedlings too. On physiological drought phone in meristeme cells of treated seedling roots the presence of polypeptides with Mr: 94; 43; 14 kD, iduced by water stress in untreated plants is not trace out. The same influence of difecoden on ESPs electrophoreses spectrum from root elongation zone of *Zea mays L*. seedlings was established in condition of water deficiency (Fig.1). At comparative analysis of ESPs spectrum from root|'s mature zone of control, untreated and treated stressed seedlings no important differences were observed.

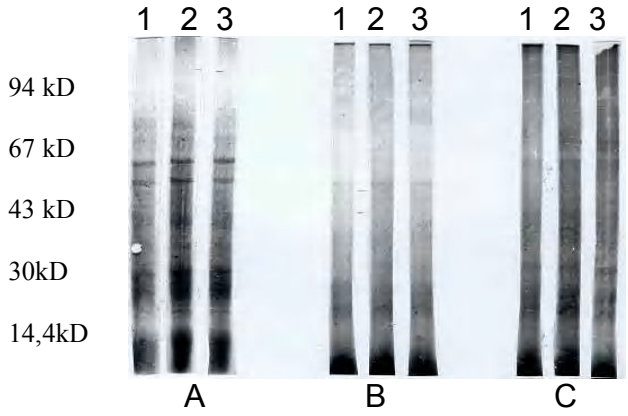

Fig. 1. The electrophoregrame of an SDS-polyacrilamide gel, comparasing the effct on ESPs spectrum of untreated and treated (with difecoden) *Zea mays L*. plants in coleoptile (A); in cell elongation zone of embryonic root (B) and in meristem cells of embryonic root (C). The lane 1 represent the protein extract from control plants;

lane2- ESPs spectrum under action of physiological drought; lane 3- ESPs spectrum from treated seedlings.

Therefore, at initial stages of ontogenesis the trinuclear cluster with Fe^{3+} and Co^{2+} effect on ESPs composition was achieved by stabilization of their spectrum. Only the content modifications, particularly, the increase of certain protein component concentration was recorded.

In roots and leaves at VI th stage of ontogenesis of *Zea mays L.* plants the difecoden action is characterized by its stabilizing effect on ESPP in condition of short time action drought (Fig. 2).

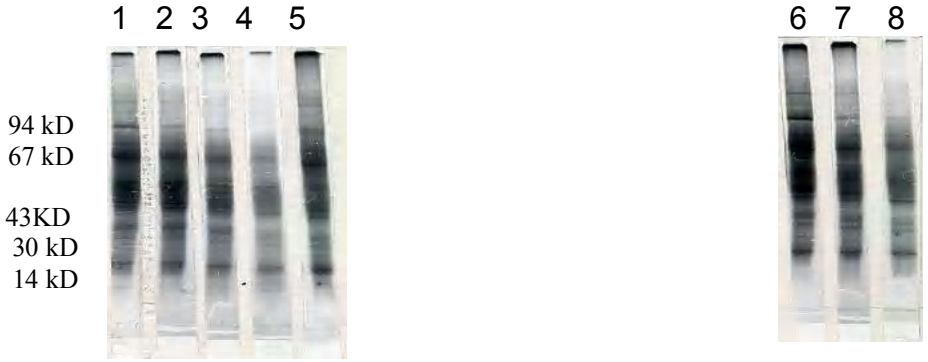

Fig. 2. Electrophoregrames of an SDS- polyacrilmide gel, showing the time action of drought on ESPs spectrum from leaves with finished growth and the effect of difecoden on their composition in water stress deficiency conditions.

1. the electrophoretic spectrum of ESPs from control (70%) plants;
2. the ESPs spectrum from plants after 1d of dehydration;
3. the ESPs spectrum after 3 days of dehydration;
4. after 7 days of dehydration;
5. the ESPs composituion from rehydration plants;

- the lines 6; 7; 8 represent the protein spectrum from treated plants after 1; 3; and 7 days of dehydration, respectively.

But, at time evolution of water stress, in ESPs spectrum of treated plants the same changes, that in untreated ones were established. In first 1-3 days of desiccation, at comparative analysis of ESPs electrophoreses spectrum from leaves with finished growth of control plants and treated plants the same polypeptide composition was established. Particularly, under influence of difecoden the decrease of polypeptide content with Mr: 112kD-85kD and the enhancement of the concentration of polypeptides with Mr about 20kD-14 kD during the three days of drought action were established. In ESPs specrum from young leaves no significant modifications during this time action of water stress have been observed. After seven days of dehydration in protein spectrum from senescent leaves the stabilizing effect of difecoden is not stand out. It was observed the decline of polypeptide content with Mr: 112kD; 101 kD; 97,7 kD; 89 kD and 28 kD; 26 kD..

The protein composition from young leaves of treated plants was not significantly altered by severe water stress (Fig.3) in comparison with their spectrum in untreated plants. This indicates, that the stabilizing effect of difecoden on protein spectrum is more obvious in leaves with unfinished growth, that genetic systems are more labile.

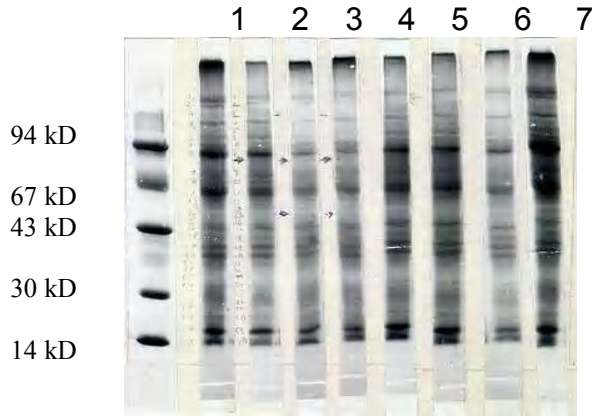

Fig. 3. The electrophoregrames of ESPs from leaves with unfinished growth of *Zea mays L* which represent the drought action and the influence of plant treatment with trinucleaer clusters with Fe^{3+} and Co^{2+} on their specrum.
The line 1 represents the protein spectrum from control plants; the lines 2; 3; 4 -protein composition during 1; 3 and 7 days of water stress,l ine5; 8 –protein spectrum from rehydration untreated and difecoden treated plants respectively; lines 6 and 7 – the ESPs spectrum of treated plants on drought phone

Thus, the seed treatment of plants with trinuclear clusters with Fe^{3+} and Co^{2+} can somehow influence the protein synthesis apparatus in cells. But the concrete mechanism of their action is not clear. It is known, that Co^{2+} induces the auxines accumulation in tissues, what conditioned the RNA synthesis and subsequently the protein pattern in cells.

The treatment of plants with bioactive substances induces modifications on activity and izoenzyme spectrum. It is known the action of coordination compounds on plant resisting through the medium of metal-enzime synthesis. From these reasons, the study of trinuclear clusters with Fe^{3+} and Co^{2+}(difecoden) on peroxidaze and cytocromoxidaze activity and izoenzyme spectrum in organs of *Zea mays L.* plants in condition of water deficiency present interest. The biochemical effect of difecoden depends on duration and intensity of stress factor. In water stress conditions the seed treatment of maize plants conditions the stabilization of peroxidaze activity in roots and leaves in comparison with untreated plants (Table 5).

Table 5. The influence of trinuclear cluster with Fe^{3+} and Co^{2+} and water deficiency on peroxidase activity (the modification of optic density during 1 $s \cdot g^{-1}$ fresh weight) in leaves and roots of *Zea mays L* .

Variant	Dehydration period		
	The Ist day of water stress	**The IIIrd day of water stress**	**The VIIth day of water stress**
Control (70% TWC)	**roots**		
	555	555	555
The drought (70-30%TWC)	991	1223	2418
The drought+ difecoden	807	893	1395

The VII-VIII th leaves

Control (70%TWC)	138	134	133,8
The drought (70--30% TWC)	157	210	331
The drought +difecoden	131	151	163

The XI-XII th leaves

Control (70% TWC)	69	69	103
The drtought (70--30% TWC)	99	168	134
The drought +difecoden	77	83	107

The study of the molecular spectrum of enzymes is very significant, because, their heterogeneity serve as index of the modification in genetic apparatus of cell and have influence on the molecular conformation of the proteins.In the first days of drought action the izozyme spectrum of peroxidase from leaves and roots of treated plants is similar to that of control plants(Fig. 4). Only a some modifications of certain peroxidase izoforms were established. In condition of middle water stress in roots of plants, treated with difecoden the activity enhancement of izoenzymes with Rf 0,36 and 0,39 was stand out. At time evolution of drought (after 7 days of water deficiency), in roots of treated plants, the induction of new molecular izoforms of peroxidase with Rf 0,26 and 0,49 was established (Fig.4). In roots of untreated plants these izoenzymes come out after three days of water stress. In leaves, both with finished and unfinished growth, of *Zea mays L.* treated plants no induction of new izoperoxidases appearance was observed in drought conditions. However, the increase of activity of enzyme components with Rf: 0,08; 0,125; 0,33 was established in leaves of treated plants (Fig.4). Particularly, in senescent leaves, the izoenzymes with Rf: 0,05; 0,18; 0,28; 0,29; 0,32 and 0,35 are characterized with relative higher peroxidase activity under influence of difecoden. In the electrophoreses spectrum of peroxidazse from leaves with unfinished growth of treated plants the increase of activity of molecular form with Rf: 0,175; 0,19; 0, 28 and 0,295 was observed. The enzyme components with Rf 0,175 and 0,295 have the relative higher activity in untreated plants too. Thus, under influence of the difecoden stabilizing effect on activity and izoenzyme spectrum of peroxidase from roots and leaves of *Zea mays L*. plants, as well as, on ESPP, was established. Probably, the difecoden influence the changes of activity of those izozymes, which fulfill the most functional role in condition of reduced water supply.

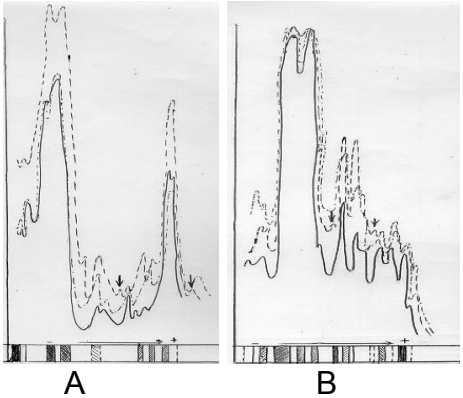

Fig. 4. The electrophoregrames and densigrames of peroxidase izoenzyme from roots (A) and leaves (B) of *Zea mays L*. plants exposed to water stress and trinuclear cluster seed treatment.
— control plants
---- drought stressed plants
-· -· plants treated with difecoden

The treatment effect of trinuclear clusters Fe^{3+} and Co^{2+} on drought phone, under izoenzyme spectrum of cytocrom oxidase from leaves and roots of *Zea mays L*. plants as comparison to untreated plants is achieved by significant increase of relative activity of izoenzyme components (Fig.5). In roots of *Zea mays L.* treated plants the stabilization of izozyme composition and intensification of their cytocrom oxidazs activity were trace out. The izoforms with Rf: 0,16; 0,22; 0,24 and 0,3 from electrophoreses spectrum of cytocrom oxidase are characterized by relative higher activity, which is keeping during dehydration period. The difecoden action on enzyme spectrum also, is characterized by the activity enhancement of certain enzyme components with Rf: 0.22; 0,1; 0,12; 0,156. On basis of obtained results the antistress effect of difecoden, which is achieved through intensification of cytocrom oxidase catalytic properties is discussed.

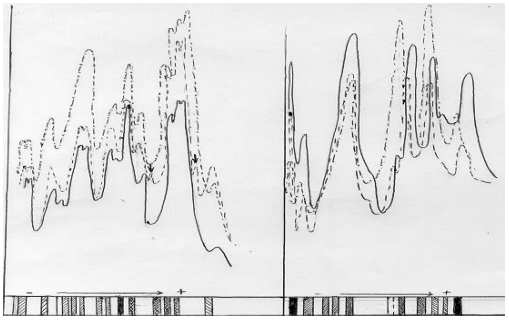

Fig. 5. The izoenzyme spectrum and densigrames of cytocrom oxidase from root (A) and leaves (B) of *Zea mays L*. plants treated with trinuclear cluster and exposed to drought action.
— control plants; ---- drought stressed plants; -· - · treated plants

Thus, on basis of obtained data, the functional role of trinuclear cluster with Fe^{3+} and Co^{2+}, particularly, difecoden, probably consists in changing of the kinetic characteristics of ESP synthesis. A less action it have on *de novo* synthesis of stress induced proteins. Difecoden, maybe, provides the proteolysis minimization of ESPP an a less altered metabolism rections at drought, considering by its stabilizing effect on protein content and composition from water stressed plants and in this way conduct to higher resistance potential of the plants at stress factor action, such as drought.

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DISTRIBUTION OF METALS IN SOIL AT THE OIL REFINERY SITE

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ABSTRACT

During the operation "Allied Force" large quantity of crude oil and its products has been burned and spilled out onto the soil at the Oil Refinery Novi Sad. Consecutive soil pollution with heavy and inorganic metals presents one of the greatest environmental risks due to the very close vicinity of the city water wells to the Refinery. The aim of this work is to present the information concerning the pollution level of the soil with heavy and inorganic metals taking into consideration the total contents of these ones. It was found that surface soils concentrations of metals were greater than in background soils. Therefore, special attention is paid to the metals distribution in the soil as a function of depth and in consequence migration of these ones as it represents the great environmental risk due to possible pollution of the groundwater and especially drinking water system supply.

Key words: soil pollution, metals

INTRODUCTION

Crude oil represents a complex mixture of both organic and inorganic components. Trace metals are one group of elements amongst the inorganic component present in crudes. These elements have been found in different proportions in different crudes. Although other metals such as Fe, and Zn, may be importantly present, frequently Ni and V are found in the largest concentrations in crude. It is expected that, upon extraction of the crude oil and accidental deposition on surface of surrounding soil and sediments, bacterial decomposition, dissolution and oxidation of most of the organic components and remineralisation of the organic matrix, trace metals are incorporated in the soil and sediment load, increasing the background levels of metal content of the local soil and sediment.

During the period of NATO aggression, from March 24 to June 9, 1999, the Oil Refinery of Novi Sad was twelve times bombed, and 348,700 m^3 of storage tanks with more than 100,000 t of crude oil and its products were destroyed. It is estimated that about 90% of these were burned, 10% leached and 130 t recovered. Various substances with carcinogenic, mutagenic, toxic and perilous effects to human, plants and animal life were released into the air, water and soil. About 50,000 t of crude oil with a total sulphur quantity of 1 to 2% was incompletely burning after the strikes between the night of 1-2 May. Fires raged for 10 days. The cloud of smoke was 1.5 km wide, 3 km height and visible about 20 km downwind from the source, i.e., it was

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over the town, by Danube River and in opposite direction depending of the weather conditions. The sun was blotted out for a day. Also, due to the conflict, many pipelines have been broken or seriously damaged (approximately 3 km) and there is still considerable leakage from these damaged pipes. Uncontrolled leakage from the pipe system can contribute considerably to the contamination of the soil and groundwater, and thus, regarding to the Refinery location, can be considered as a serious threat to the drinking water sources (infiltration galleries) of Novi Sad.

Supporting the idea that airborne fallout from oil fires was deposited in limited area, this study was carried out to determine the concentration of trace metals in soil samples taken from several fields of the Refinery. Two types of soil samples have been taken in the post conflict period: (a) surface zone, and (b) soil core samples, in an effort to quantify the deposition and migration of heavy and inorganic metals. The following elements: V, Cr, Ni, Co, Cu, Zn, Cd, Sn, Tl, Pb, Sb and Hg were analyzed, because of the known, or suspected importance of atmospheric impacts to the concentration in the soil.

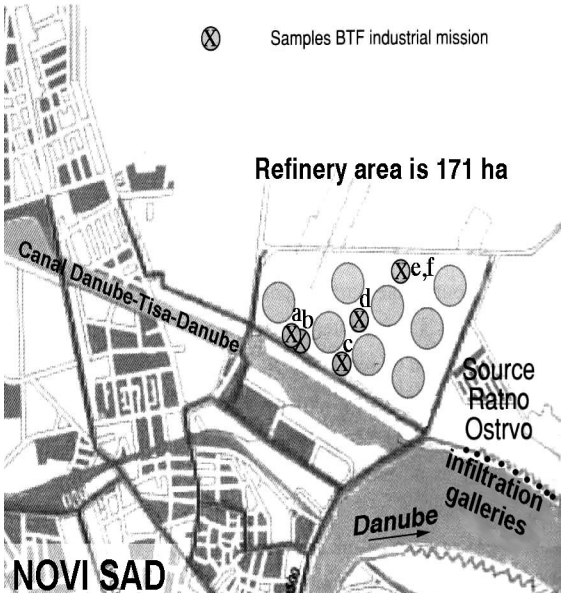
REFINERY LOCATION

The Oil Refinery of Novi Sad is on the bank of canal Danube-Tisa-Danube which is directly connected with Danube River and is located not more than 1.5 km from downtown Novi Sad. The Oil Refinery is built on gravely sandy slit deposits with a thickness of up to 8 m. The groundwater table is located only 1-2 m below ground level of the Refinery.

Within the Petroleum Industry of Serbia, the Oil Refinery of Novi Sad is the unique refinery that produced motor and industrial fuels, motor and industrial lubricating oils, and road and industrial bitumens. The refinery's products are being produced inside the process units, which were developed through the four investment programmes in the period 1968 to 1985.

MATERIALS AND METHODS

The soil core and surface zone samples were taken with a hand-held coring device and a garden trowel, respectively, from several sites of the Refinery, depending on the position of the destroyed plant units. The position and description of field sites in the Oil refinery, from which the soil samples were taken, is presented in Figure 1. The samples coded NS, X2 and X3 were stored frozen in dark jars until analysis.



a-NS S1 0-30 and NS S1 80-100 - damaged storage tanks with leaded motor gasoline and destroyed pipeline
b-NS S1 0-10 - about 10 m opposite of the first one
c-NS S4 0-10 - reservoir of bitumen, just opposite of the additive storage
d-NS S4B 0-10 – in the very close vicinity of vacuum oil distillate
e-X2 0-20, X2 20-50 and X2 50-100 - in the vicinity of three totally destroyed crude oil storages with the capacity of 10,000 t each
f-X3 0-10, X3 10-45 and X3 45-55 - about 10 m distance from the taken sample signed X2

Fig. 1. Position of Oil Refinery in Novi Sad and locations from which soil samples for pollutant analysis were taken

Total concentrations of metals (V, Cr, Ni, Co, Cu, Zn, As, Cd, Sn, Tl, Pb, Sb and Hg) were determined by thermal ionization mass spectrometry and optical emission spectrometry. Analysis of total mercury content was done by cold vapor atomic absorption spectrophotometry. Acid digestion procedure (HCl, HNO_3, and HF in platinum crucible) was applied as a method for the preparation of samples (Boumans, 1987). Appropriate calibration curves were prepared with the series of multielement reference solutions. Results are concerned to dry matter of sample. Detection limits for the employed analytical methods are 0.04 mg/kg for Cd, 0.2 mg/kg for V, Cu, Zn, As and Cr, 0.06 mg/kg for Sn, Tl, Pb, Sb, Hg and 2 mg/kg for Ni and Co.

The pH measurements were carried out in deionized water (50 ml) after stirring the sample portion of 20 g for an hour (Chon et al., 1998).

RESULTS AND DISCUSSION

Soil sample content of heavy metals in μg/g dry matter is presented in the Table 1. Content of these compounds is given as a function of the depth.

Table 1. Content of heavy metals in soil samples as a function of depth, μg/g dry matter

	NS S1[a] 0-30 cm	NS S1[a] 80-100 cm	NS S1[b] 0-10 cm	NS S4[c] 0-10 cm	NS S4B[d] 0-10 cm	X2[e] 0-20 cm	X2[e] 20-50 cm	X2[e] 50-100 cm	X3[f] 10-45 cm	X3[f] 45-55 cm
pH	8.06	7.51	8.05	7.34	8.3	7.38	8.09	8.59	7.78	7.92
V	43.7	72.7	45	66	66	57.4	23.8	27.3	50.1	59.2
Cr	34.3	77.7	34	50.1	44	63.8	31.9	29.8	47.9	58.1
Ni	109	43	51.2	68.8	159	36.8	12.2	6.7	18.9	21.9
Co	152	224	179	184	175	181	128.5	116	154	223.5
Cu	39.7	27.6	9.7	23.7	39.2	21.7	23.5	14.5	21.2	36.6
Zn	38.1	78.4	62.1	66.9	63	76.7	56.4	31.5	54.7	53.5
As	6.9	7.9	3.3	7	5.9	2.8	3.9	3.2	4.1	3.95
Cd	8.2	4.7	2.35	3.2	3.28	4.2	3.05	2	3.95	3.09
Sn	1.24	1.86	1.88	2.72	2.36	1.13	1.25	2.24	1.45	1.61
Tl	41.2	95.9	49.9	35	42.9	52	96.3	33.9	50.3	87.6
Pb	70.2	100.1	76.6	77.8	81.6	112.2	74.6	62.1	80.4	87.7
Sb	3.06	9.7	0.51	6.8	1.18	4.27	2.61	1.21	2.95	3.09
Hg	1.4	0.1	0.41	0.11	0.11	0.12	1.03	0.94	1.2	<0.01

[a]NS S1 0-30 cm and NS S1 80-100 cm – damaged storage tanks with leaded motor gasoline
[b]NS S1 0-10 cm – about 10 m opposite the storage tank with leaded motor gasoline and destroyed pipeline
[c]NS S4 0-10 cm – reservoir of bitumen, just opposite the additive storage
[d]NS S4B 0-10 – very close to vacuum oil distillation facilities and storage tanks without protecting lining
[e]X2 – in the vicinity of three totally destroyed crude oil storages with the capacity of 10,000 t each
[f]X3 – about 10 m distance from the taken sample signed X2

The obtained concentration of heavy and inorganic metals (V, Cr, Ni, Co, Cu, Zn, Cd, Sn, Tl, Pb, Sb, Hg) showed their migration down to the 1 m depth. In the neutral and slightly alkaline range (8.05-8.59) metal mobility is low, but only a slight soil acidification is necessary to enhance their mobility. A decrease of pH results in an increase in adsorption and in a corresponding increase of the mobile fraction of heavy metals in the soil. Since Cd and Zn are more mobile in comparison with other metals increasing of their content is evident at pH=7 and depth of 80-100 cm where the concentration of former exceeds the assumed permissible level of 3 mg/kg.

Taking into consideration the average mean concentrations of Cd, Pb, Hg, As, Ni, Cr, Cu, Zn (in μg/g) in arable soils with neutral pH reaction (0.48; 10.2; 0.01; 2.19; 2.4; 4.2; 10.8; 10.6) determined by analysis of 1600 samples collected from representative sites distributed in a regular pattern across the Province of Vojvodina (Ubavić et al., 1993a, 1993b) and the ones from Table I, it is obvious that in some instances the concentration is higher 140 times in the case of Hg, 45.4 times for Ni, 17.1 for Cd, 11 for Pb and 3.6 times for As.

Also, it should be emphasized that the contents of metals in arable soils (Ubavić et al., 1993a, 1993b) were determined by the technique different than the OOne used in this investigation indicating possible variations in the obtained concentrations and the calculated numbers. Namely, the concentration of arsenic is determined with silver-diethyldithio carbamate by spectrophotometry, mercury content is done by cold vapor atomic absorption spectrophotometry, while the other heavy metals are extracted by concentrated nitric acid and determined by atomic absorption spectrometry.

The concentration found in this analysis can be considered high with respect to those reported for other areas, taking into consideration very toxic and carcinogenic metals like Ni, Cd, Hg, As and Co. For example, Lorenz et al. (1997) studied 10 contaminated European soils and found for Ni average concentration of 25.6 μg/g. In this study the average value obtained taking into consideration both surface and soil core samples was 52.75 μg/g. The mean content of Ni in the examined surface soil layers (0-30 cm) was 84.96 μg/g, while the reported content of this one in polluted Bulgarian surface soil (Bojinova et al., 1996) was 63.8 μg/g. The obtained value for Cd (4.25 μg/g) in surface soil layer corresponds to the found one in Bulgarian (3.59 μg/g) and European (5.01 μg/g) contaminated soils. Level of Hg (0.43 μg/g) in Refinery surface soil is more than four times higher than polluted Bulgarian soil (<0.1 μg/g). The average values found in this study in accordance of their distribution to the 1 m depth for the enlisted metals were 52.75; 25.67; 58.13; 47.6; 3.87 and 88.54 μg/g, respectively.

As the Oil Refinery is located on a layer of gravely sandy slit deposits (high permeability and vulnerability) with the groundwater table at shallow depth (1-2 m below ground level), the contamination with heavy metals indicates a great environmental risk especially for drinking water due to potential migration of identified pollutants by groundwater. Because of high groundwater, migration of these pollutants takes place to the river Danube, and their presence in sediment is evident (UNEP/UNCHS, 1999). Knowledge of presence of these contaminants is needed, for water wells are located on the bank of Danube, nearly 0.5 km from the Refinery and there is no protecting barrier at the downstream end of the Oil Refinery preventing any possible contamination to flow with the groundwater towards the infiltration galleries. Therefore, this location should be treated for removal of identified pollutants because they are presented in almost all of the area due to protecting the health of 350,000 inhabitants of Novi Sad.

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DATA CONCERNING THE MINERAL COMPOSITION OF THE SOILS FROM BRETAGNE – FRANCE AND FROM BANAT – ROUMANIA BASED ON GEOLOGY AND SOIL SCIENCES

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ABSTRACT

This paper presents some general aspects regarding the pecularities of chemical composition related to the geological substrate, which originated the formation of soils in two regions: one from western Europe (Bretagne – France) and the other from eastern Europe (Banat-Roumania)

Approaching this subject has a historical connotation, the two regions belonging to latinity, respectively to the Roman Empire, by the antique Galia (today territory of France) and the antique Dacia (today territory of Roumania).

This study has been initiated due to the cooperation between Department of Soil Science – University Rennes, France and Department of Soil Science - University of Agricultural Sciences and Veterinary Medicine Timişoara, Roumania in the years before.

From the point of view of soil sciences, in a context which reffers to the chemical composition, generally remarks are made regarding geochemical aspects. These are correlated with data referring to the chemical composition of soil decelated by methods specific for agrochemistry.

The work deals with mineral compounds of cationic nature, i.e. metals as Ca, Mg, Na, K, Zn, Fe, Mn, Cu etc. and with compounds of anionic nature i.e. sulphates, carbonates, phosphates etc.

As a general view there are also presented the most cultivated plants in the mentioned geographic areas.

Key words: geological substrate, mineral compounds

1. COMPARATIVE CONSIDERATIONS REGARDING PEDOGENESIS

Soils of Bretagne (France) and Banat (Roumania) have similar peculiarities regarding the pedogenesis process. The rocks on which soils have been formed in the two regions are identical from the point of view of mineralogic composition, with less differences due to climateric conditions.

Trace elements are considered those chemical elements which the plants need in small amounts (10^{-2} – 10^{-5} %), related to dry substance. Excepting Fe and Mn which geologically and pedologically belong to major elements, the other microelements: B, Cr, Na, Ca, Mo, Zn, Cd and Pb are found in very small quantities in soils and rocks (generally under 10^{-2}%) being included in the category of minor elements.

Bretagne is situated in the north-west of France, mean annual precipitations are 800-850 mm and the mean annual temperatures range between 8.5 – 9^{o}C.

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Banat region is situated in the south-west of Roumania where the mean annual precipitations vary between 550-680 mm and mean annual temperatures range between 9.5 -11.5 ^{o}C.

Regarding the soil types in the studied regions, these are likely from the point of view of the rocks and in a less extent on clima conditions.

The soils formed in the cold and wet clima from Bretagne are of reduced variety (brown argilic luvial, luvic brown, pseudogleised etc.). In Banat there is a great diversity of soils due to climateric conditions and the variations of forming rocks in the territory (granite, granodiorite, loess and loess deposits, argillic, sands, calcareous).

2. PECULIARITIES OF THE TRACE ELEMENTS COMPOSITION

In soil, trace elements are found, in principal, in the structure of primary and secondary minerals as well as retained during various physico-chemical and chemical mechanisms by inorganic colloids. Important quantities of trace elements are associated with organic matter from soil, in forms that are accessible for plants.

Total reserve and mobility rate of trace elements from soil are conditioned by the nature of solifying rock and the processes of pedogenesis (elluvial-illuvial and bioaccumulative process). The action of these factors can be differently manifested from one soil to another, depending on sollification conditions.

Table 1 presents the content in trace elements of the main rocks formed in the soils of Bretagne.

Table 1. Trace elements content of parenthal rocks (Bretagne)

Parenthal rock	Trace element (ppm)				
	Cu	Mn	Zn	B	Mo
Acid magmatique rocks	3.7-20	170	86	3-10	2
Alkaline magmatique rocks	58-140	1000-2200	112-148	1-5	1-1.4
Crystaline shiver	15-25	561	107	100	2.6
Gnais	9-15	490	100	80-100	0.6
Granodiorites	25-39	115	60	60-80	0.8

Content of main trace elements of the soils of Bretagne are given in Table 2.

Table 2. Content of trace elements in some soils from Bretagne

Soils	Trace element (ppm)				
	Cu	Ca	B	Mn	Mo
Podsoils on granite and granulite	3-5	0.10-0.98	-	-	-
Brown soils on calcar	-	-	0.31-0.91		
Soils on different rocks	-	-		24-998	0.30-1.00

One can observe that the soils from Bretagne region have a low content in trace elements as compared with parenthal rocks. This difference is due to pedogenesis processes in which alteration is reduced.

The soils of Banat were formed on a multitude of rocks due to varied pedoclimatic conditions corresponding with those of Bretagne region. The trace elements content of rocks is alike as it can be seen in Table 3.

Table 3. Trace elements in the rocks of Banat (Roumania)

Parenthal rock	Trace element (ppm)					
	Cu	Mn	Ca	Zn	B	Mo
Acid magmatique rocks	10-20	170-231	5-10	86	3-10	2
Alkaline magmatique rocks	100-200	1000-2200	30-45	112-148	1-5	1.0-1.4
Crystaline shiver	23	561	3.7	107	100	0.3-1.5
Loes and loes deposits	19-20	580-720	5.5-5.8	57-71	35-47	-
Gley	26	230-1200	2.4-68	50-76	34	2.8-3.5
Sands	3,6-8	20-500	3	16	2.5	0.4
Calcar	9	400-500	0.06-2.8	24	8-20	0.4

Content in trace elements of main Banat soils is given in Table 4.

Table 4. Content of trace elements – total forms – in main soils of Banat

Types of soils and localization	Trace elements – total forms – in A horizon (ppm)								
	Cu	Pb	Zn	Co	Ni	Mn	Cr	Cd	Fe
Gleized chernozems - Lovrin	23.5	18.5	56.5	20.0	44.5	685	101	1.0	25000
Gleised cambic chernozems – Comloş	44.0	21.0	49.5	18.5	25.5	575	68.5	1.5	17400
Gleised cambic chernozems – Jebel	19.5	30.5	42.0	17.5	38.0	575	84.5	1.0	19000
Argilic chernozems – Fântânele	24.0	20.0	53.0	23.5	47.5	760	133	1.5	29500
Brown argilic-iluvial soil - Fibiş	22.5	25.0	59.5	22.5	46.5	1150	100	1.5	23600
Brown argilic-iluvial soil - Şagu	25.5	31.0	85.0	27.5	27.5	955	131	1.0	35600
Brown argilic-iluvial soil – Moldova Nouă	25.0	35.5	97.0	23.0	48.5	650	166	1.0	27700
Pseudogleised brown luvic soil - Coşova	9.0	17.5	22.5	13.5	21.0	375	59	1.5	10100
Pseudogleised brown luvic soil – Prigor	17.0	31.5	170.5	20.0	42.0	530	146	1.0	21600
Pseudogleised brown luvic soil - Darova	16.5	27.5	44.5	18.0	32.0	490	113	1.0	16900
Pseudogleised albic luvisoil – Sistarovăţ	19.0	34.0	47.5	21.0	29.5	1015	97	1.0	19600
Pseudogleised albic luvisoil – Fârdea	15.0	17.5	34.5	13.0	31.0	360	48.5	1.5	11200
Brown soil – Recaş	18.5	30.0	103.0	23.0	43.5	560	107	1.0	30300
Brown soil – Gătaia	22.5	34.0	69.0	18.5	45.0	540	95	1.0	23300
Salined soil – Foeni	15.0	28.0	37.5	10.0	28.5	265	84	1.0	12600
Gleised vertisoil – Cheglevici	62.5	41.0	101.0	27.0	69.0	320	84.5	1.5	36000
Gleised alluvial soil – Coştei	26.0	29.5	64.0	20.0	52.5	710	115	1.5	35300
Gleised alluvial soil - Dalboşeţ	22.0	31.0	112.0	21.5	49.0	510	150	1.0	26200

It is clear that Banat's soils are rich in trace elements. This may be due to the native richness in elements of these soils that build the mountains of Banat. The wet and warmy climate favourised the primary alteration and subsequently bioaccumulation.

CONCLUSIONS

1. In the soils of Bretagne the wet and colder climate (oceanic type) did not favourise the alteration of primar minerals and the content in trace elements is more reduced. The concentration in metallic elements in second phase increased due to organic sulphures, permanently released in a predominantly reductive environment.
2. The soils of Banat have the greates contents of metal elements in the western size (vertisoils) where they were formed on gley.
3. All soils of Banat present high levels of heavy metals but under the limit of pollution. Any supplementation by chemical fertilisers, town mud or vaste products from zootechnical complexes can brake the existing balance and provoke pollution with one or more elements.

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EFFECT OF CADMIUM STRESS ON GLUCOSE AND FRUCTOSE CONTENT IN WHEAT (TRITICUM AESTIVUM L.) SEEDLINGS

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ABSTRACT

Water-soluble carbohydrates contributing in the response to Cd^{2+} stress in wheat (Triticum aestivum L.) seedlings were studied. The hydroponically grown wheat seedlings were exposed to different concentration of cadmium (10^{-10}, 10^{-7}, 10^{-5}, 10^{-3}, 10^{-1} M Cd^{2+}) stresses for 80 and 104 hours. Treatments were completed with titanium ascorbate (5μg/L). At the same time we have determined the effect of cadmium treatments at two cadmium concentrations (10^{-5}, 10^{-3}, M Cd^{2+}) when the plants were treated at 6, 15, 18, 22 and 39 hours after the beginning of the treatments. The cadmium induced changes were measured in glucose and fructose contents by overpressured layer chromatography (OPLC). Our results suggest that the glucose and the fructose showed considerable response to Cd^{2+} treatments. We have no found significant differences in glucose and fructose content when the cadmium concentrations were low. The cadmium dependent characteristic changes were detected from the 10^{-5} M Cd^{2+} to the higher cadmium concentration. The level of glucose and fructose were continuously increased with increasing cadmium stress at the two sampling times. In the both cases glucose showed more sensitive stress effect than fructose. In all cases titanium ascorbate influenced the effect of cadmium treatments.

Keywords: cadmium, titanium ascorbate, glucose, fructose, wheat, stress

INTRODUCTION

Nowadays the toxic element contamination is one of the most serious problems with strong effect on human health and the environment. These harmful pollutant are found largely in water and soil, where they can accumulate. Heavy metals taken up by plants and they can reduce plant grow, destruct photosynthetic and respiratory mechanisms, damage membrane structures (Somasshekaraiah et al., 1992; Neumann et al., 1994; Moral et al., 1994).

Cadmium as a non essential element was often chosen as a probe metal ion because it is wide spread trace pollutant of high toxicity with a long biological half life (Himly et al. 1985). It is well known that the cadmium stress has an effect on several metabolic processes and various enzymatic activities in plants (Wollgiehn and Neumann, 1999; Van Assche and Clijster, 1990; Mattioni et al., 1995). Biochemical studies have revealed similarities in processes induced by stress that lead to the accumulation of metabolites (McKersie and Leshen, 1994) as amino acids, polyamines and water-soluble carbohydrates which can protect membranes and proteins againts dehydratation. Therefore the water-soluble carbohydrate contents are to be found a very sensitive trait to indicate environmental stress effects (Housley and Pollok 1993, Kerepesi at al. 1998).

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The aim of our preliminary experiment was to investigate the effect of cadmium stress on glucose and fructose content in a wide range of cadmium, from 10^{-10} to 10^{-1} M Cd^{2+} on wheat seedlings. We determined the effects of cadmium treatments that were completed with ascorbic acid and titanium ascorbate. Our further aim was to characterize the initial section of the cadmium stress with frequent sampling after the treatments.

MATERIALS AND METHODS

Plant samples and treatment: The experiment was performed in hydroponic culture under optimized nutrient element conditions (Knopp solution). The seeds of wheat (*Triticum aestivum* L.) were swollen in distilled water for 24 hours. Then the seeds were placed into Knopp-solution (Suba 1978) for one week. The one-week-old seedlings were exposed to cadmium stresses: A) 10^{-10}, 10^{-7}, 10^{-5}, 10^{-3} and 10^{-1} M Cd^{2+} for 80 and 104 hours and cadmium treating completed with titanium ascorbate (5μg/L), B) 10^{-5} and 10^{-3} M Cd^{2+} for 6, 15, 18, 22 and 39 hours. The control samples were in Knopp-solution without any treatment. After the treatment the seedlings were washed in distilled water and dried immediately.

Preparation of plant samples for chromatographic analyses: Leaf tissues were frozen with liquid nitrogen, powered and suspended in dimedone solution (0,05 % dimedone in methanol). This suspension was centrifuged at 1500 g for 10 minutes at 4 ^{o}C. The clear supernatants were used to OPLC.

OPLC separation: Separations were carried out with a Liquochrom Mode 2010 liquid chromatograph equipped with UV 308 detector, OH-814 recorder, Digint-180 Integrator and a 20 μ l loop.

RESULTS AND DISCUSSIONS

Changes of glucose and fructose contents induced by cadmium stress (10^{-10},10^{-7},10^{-5}, 10^{-3} and 10^{-1} M Cd^{2+}) at the different sampling times (80 and 104 hours) are shown in Fig.1 and Fig. 2.

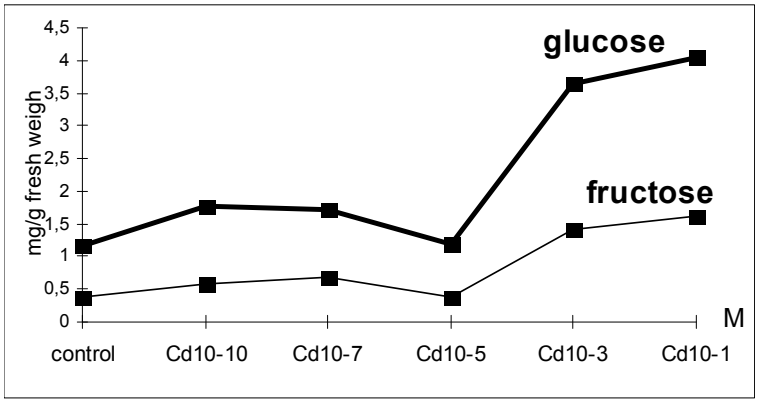

Fig. 1 Effect of cadmium stress on glucose and fructose contents 80 hours after the treatment

At the two sampling times in our preliminary treatments we have no found significant changes in the cadmium induced glucose and fructose contents compared to the control at the low (10^{-10}, 10^{-7},10^{-5} M) cadmium concentrations. Water-soluble carbohydrate components significantly increased from 10^{-5} M cadmium concentration to the higher ones.

In both cases (80 and 104 h) when the glucose and fructose contents increased the glucose content was higher in all cases, than that of the fructose. Both water-soluble carbohydrates were higher in the samples treated cadmium for 104 hours than 80 hours.

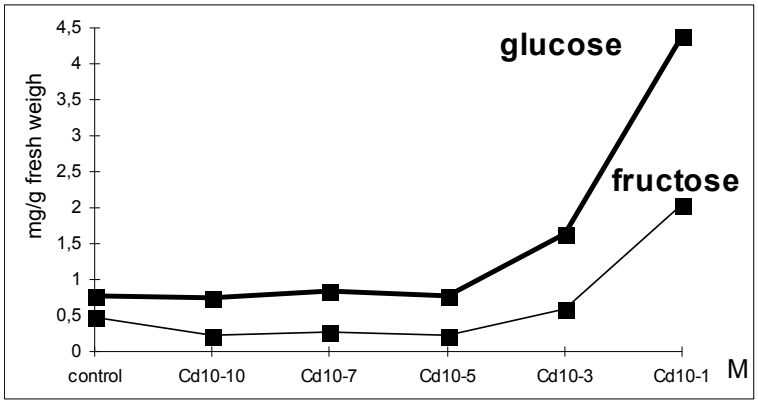

Fig. 2 Effect of cadmium stress on glucose and fructose contents 104 hours after the treatment

Differences shown earlier, at low cadmium concentrations, disappeared but at the higher cadmium concentrations caused increase in the carbohydrate contents after the 104 hours treatment.

Cadmium treatment completed with titanium ascorbate caused changes in the carbohydrate contents especially in the higher cadmium concentrations, 10^{-5} M and 10^{-3} M Cd^{2+}, results are shown in Table 1.

Table 1. Effect of titanium ascorbate on cadmium induced glucose and fructose contents

80 h	**Fructose μg/g fresh weigh**	**Glucose μg/g fresh weigh**	**Fructose μg/g fresh weigh +Ti-ascorbate (5μg/l)**	**Glucose μg/g fresh weigh +Ti-ascorbate (5μg/l)**
Control	387.93	1162.87	390.16	1221.23
Cd^{2+} 10^{-10} M	582.56	1770.29	578.34	1356.35
Cd^{2+} 10^{-7} M	684.48	1707.41	666.85	1689.32
Cd^{2+} 10^{-5} M	381.88	1183.09	345.23	1122.36
Cd^{2+} 10^{-3} M	1419.54	3641.89	1338.42	3365.29
Cd^{2+} 10^{-1} M	1606.17	4034.77	1890.71	5964.34
104 h				
Control	475.27	779.03	338.26	967.91
Cd^{2+} 10^{-10} M	218.42	755.91	299.68	969.34
Cd^{2+} 10^{-7} M	278.59	833.59	267.14	759.54
Cd^{2+} 10^{-5} M	231.34	768.25	212.21	713.25
Cd^{2+} 10^{-3} M	586.44	1645.52	498.98	1465.23
Cd^{2+} 10^{-1} M	2041.34	4387.42	2918.62	6141.34

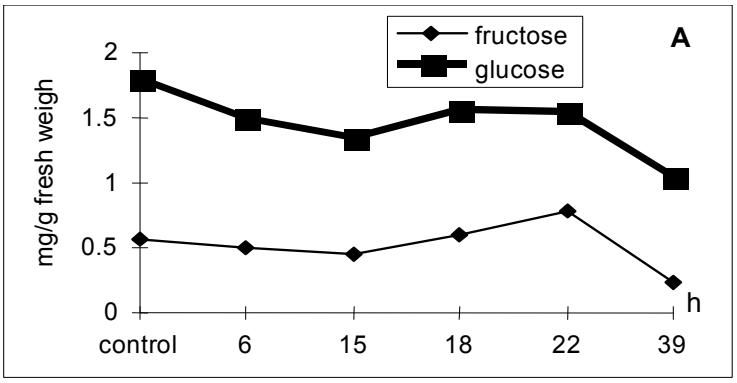

Fig. 3 Effect of 10^{-5} M (A) and 10^{-3} M (B) Cd^{2+} treatment on glucose and fructose content at the different sampling times

We have no found protective effect of titanium ascorbate at the 10^{-1} M Cd^{2+} concentration, where the water-soluble carbohydrate contents increased. These results are correspond to our erlier work.

Since the changes in the glucose and fructose content resulted at 10^{-5} M Cd^{2+} considerable increases therefore we took samples more frequently, to able to characterize the initial section of the stress induced mechanism (Fig.3).

In all cases glucose and fructose content changed in the nearly same way. In the early phase of stress.

Only the higher cadmium concentration, 10^{-3} M Cd^{2+} caused increases in the carbohydrate contents.

CONCLUSIONS

Water-soluble carbohydrates were measured by OPLC in the in wheat (*Triticum aestivum L.)* seedlings exposed to different concentration of cadmium (10^{-10}, 10^{-7}, 10^{-5}, 10^{-3}, 10^{-1} M Cd^{2+}) stresses for 80 and 104 hours. Our results suggest that the glucose and the fructose showed considerable response to Cd^{2+} treatments. In all cases glucose showed more sensitive stress effect than fructose. The increase of glucose and fructose contents at the low cadmium concentrations at 80 hours were higher than at 104 hours. The reason is presumably that after the cadmium stress with passing of time plants used to the stress and later only the higher cadmium concentracions cause stress effect. We have found stress effect at the early phase of cadmium treatment in the glucoce and fructose contents.

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OXIDATIVE STRESS AND ERYTHROCITE ANTIOXIDANT STATUS OF TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT COMPLICATIONS

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ABSTRACT

Oxidative stress plays a role in the pathogenesis of late complications of diabetes mellitus.The aim of the study was to evaluate some aspects lipid peroxidation of the antioxidative status in diabetics.We studied 79 type 2 diabetic patients and 30 healthy subjects.

Diabetic patients were divided according to carbohydrate, lipid status and existence of diabetic late complications. No significant differense could be observed between the controls and the diabetic group with good metabolic status. Compared to the controls poor metabolic status was associated with a decrease of the activity of two antioxidative enzymes: the superoxiddismutase (SOD) and the catalase (CAT), and a higher malondialdehyde (MDA) activity. Lower SOD and higher MDA activity were observed comparing diabetic patients with poor and good metabolic status. We found a significant decrease of the activity SOD and the CAT and higher MDA activity in diabetics v.s. control subjects and in diabetics wits complications v.s. those without complications. These results suggest that the inefficiency of the antioxidant system allows the oxidative stress to initiate the lesions vascular; this deficiensy could be improved by associating antioxidant therapy to the specific treatment.

Keywords: oxidative stress, diabetes mellitus, antioxidants.

INTRODUCTION

Leading pathology in endocrinology is diabetes mellitus, of which the large frequency of physical disability and high mortality of the population is characterised.

The researches of the last years testify the important role of nonenzymatic free radical oxidation in pathogenesis of diabetes mellitus and diabetic angiopathyas (Altomare, 1992;, Baynes, 1991; Baynes and Thorpe, 1999; Fonesca, 1997; Freitas, 1997; Wolf, 1993).

Among the patients diabetes mellitus shorten time of life of erythrocytes is marked, increase their agregation, modification of phospholipide's asymmetry of membranes of erythrocites, amplification of the tendency of their adhesion to endothelium of vascular wall, reduction deformability of erythrocytes (Cameron and Cotter; Hunt et al., 1988; Jain et al., 1989; Wali et al., 1988). The direct correlation between a level of hyperglicemia and degree of activation of lipid peroxide oxidation (LPO) in membranes of erythrocytes is marked.

The important role in prevention of consequences of peroxide stress belongs to a system of antioxidant defence and it's extra- and intracellular links, which functions in different levels and suppress processes of free radical oxidation and preventing redundant accumulation of free radicals or active peroxide forms.

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The ways of activation a LPO in case of diabetes mellitus and role of this universal mechanism of damage of cell's membranes in forming of vascular complications remain unclear.

AIM: Research of features of processes of lipid peroxide oxidation and the enzymatic's link of the antioxidant defence of erythrocytes among the patients with diabetes mellitus, and also revealing disbalance between the processes of lipid peroxide oxidation and antiperoxide enzyme activity.

MATERIALS AND METHODS

Tastes were caried out on 79 patients (47 women and 32 men) with non-insulun-dependent diabetes mellitus who were grouped in accordance with data of heir metabolic status and their present complications.

In control group were included 30 healthy persones (19 women and 11 men).

All these patients were examined and carried out a metabolic status, which included a presence in plasma of glucose's level, of lipide's spectrum: total cholesterol, HDL-cholesterol, triglycerides wiht the help of reagents sets "HUMAN" (Germany).

To all inspected persones the clinical inspection and metabolic monitoring included determination in plasma of glucose's level, of lipide's spectrum:; the researches were executed (GERMANY). The activity of processes of lipid peroxide oxidation in erythrocytes was evaluated on accumulation of malondialdehyde - final product of LPO. (Коробейников Э.Н., 1989). Superoxiddismutase's activity - specrtofotometric by sets "Oxis" (USA), catalase's activity in erythrocytes was determined specrtofotometric by kinetic method (Aebi H., 1970).

The statistical analysis of results was followed by parametrical t-criterion Student. $p<0.05$ was considered as statistically significant. For revealing correlation between investigated parameters the nonparametric correlation of Pirson is used.

RESULTS AND DISCUSSION

The data of clinical and laboratory examinatoins of the patients with diabetes mellitus and persons of control group are presented in table 1.

Table 1. Baseline characteristics of Type 2 diabetic patients

Specification	Control (n = 30)	Type 2 Diabetic (n = 79)
Sex (men/women)	11/19	32/47
Age (years)	54 ± 4.3	56 ± 3.4
Age at diagnosis (years)	-	5 ± 2.3
BMI (kg/m^2)	23.49 ± 0.21	30.28 ± 0.42 **
Systolic blood pressure (mmHg)	121.5 ± 3.31	141.23 ± 4.2 **
Diastolic blood pressure (mmHg)	78.83 ± 2.11	91.51 ± 3.41 **
Fasting plasma glucose (mmol/l)	4.74 ± 0.16	10.13 ± 0.26 **
Serum triglycerides (mmol/l)	1.35 ± 0.15	3.08 ± 0.21 **
Serum total cholesterol (mmol/l)	4.89 ± 0.18	6.61 ± 0.24 **
Serum HDL-cholesterol (mmol/l)	1.92 ± 0.2	1.05 ± 0.1 *
MDA (nmol/g Hgb)	0.181 ± 0.01	0.339 ± 0.02 **
SOD (U/g Hgb)	66.64 ± 3.1	32.06 ± 1.39 **
Catalase (μmol/s·g Hgb)	10.37 ± 0.63	6.29 ± 0.19 **

Data are expressed as means ± SEM. Significance of differences from the corresponding control value, * $p < 0,05$, ** $p< 0,01$

In comparison with control group the patients with diabetes mellitus was registered the significant increase of concentration malondialdehyde - 187,29%, and also decrease of superoxiddismutase's and catalase's activity up to 207,86% and 164,81% in relation to control group is marked (table 1).

In depending on parameters of metabolic status the superoxiddismutase's and catalase's activity is changed unequally. So among the patients with diabetes mellitus with normal parameters of the metabolic status (11 patients) - the glucose's concentration in blood was less than 6 mM/l and was marked the tendency of increase of level of malondialdehyde and significant decrease of superoxid-dismutase's activity in comparison with group of healthy persones accordingly up to 111,05% and 125,1%, and the catalase's activity in erythrocytes has no significant difference from parameters of control group.

Among the patients with diabetes mellitus with deviations of parameters of metabolic status, who have level of glicemia the more than 6,6 mM/l (68 patients) the significant increase of concentration malondialdehyde up to 200% and decrease of superoxiddismutase's and catalase's activity accordingly up to 232,76% and 178,3% in relation to control group is marked (table 2).

Table 2. The values of MDA and activity of SOD and catalase of patients Type 2 diabetic.

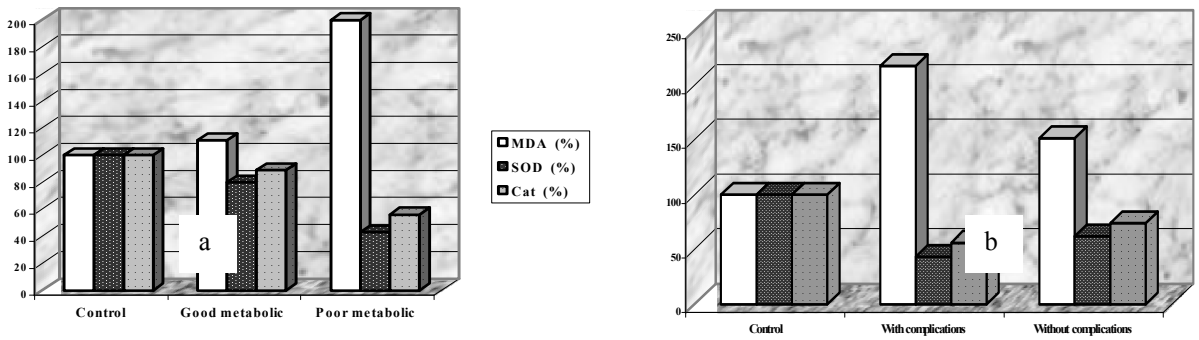
Variable	Control (n=30)	Diabetes mellitus			
		Good metabolic status (n=11)	Poor metabolic status (n=68)	Patients with complications (n=58)	Patients without complications (n=21)
MDA (nmol/g Hgb)	0.181 ± 0.01	0.201 ± 0.012	0.362 ± 0.003**	0.393 ± 0.01**	0.244 ± 0.02*
SOD (U/g Hgb)	66.64 ± 3.1	53.27 ± 2.18*	28.63 ± 1.12**	28.72 ± 1.06**	41.29 ± 2.01**
Catalase (μmol/s·g Hgb)	10.37 ± 0.63	9.239 ± 0.58	5.816 ± 0.01**	5.809 ± 0.18**	7.62 ± 0.2*

Data are expressed as means ± SEM. Significance of differences from the corresponding control value, * $p < 0,05$, ** $p < 0,01$

The significand differences of above mentioned parametres remained the same in comparison with patients diabetes with good metabolic status (fig. 1a.)

Fig. 1. Persent dependence of MDA, SOD, catalase of diabetic patients in comparison with control. The significant differences of examined parameters among the diabetic patients ith and without complications (table 2 fig. 1b). The given research states significant decrease of superoxiddismutase's, catalase's activity and of malondialdehyde's level .

The malondialdehyde's level at the patients with complicated diabetes



correlates with a level of basal glicemia (r = 0,71), duration of disease (r = 0,54). Between sex, age and blood pressure (both systolic, and diastolic) correlation is not

revealed. The significant inverse correlation between malondialdehyde and superoxiddismutase's (r = 0,81) and catalase's activity (r = 0,56) is marked.

Among the patients with not complicated diabetes mellitus the significant correlation (r = 0,71) between malondialdehyde's level and superoxiddismutase's activity is revealed.

The significant decrease of superoxiddismutase's and catalase's activity against a background of increased level of malondialdehyde, both the patients with diabetes mellitus, complicated anghiopatia, and at the patients with diabetes mellitus without anghiopatia, that coincides data obtained by other authors (Baynes, 1991;,Jiang et al., 1990; Jain et al., 1989; Wolf, 1993).

Among the patients with diabetes mellitus is observed relative hypozinkemia and hyperzinkuria, that can lead to reduction of synthesis Cu, Zn-dependent superoxiddismutase in erythrocyte's citosol, as Zn^{++} is a component which take part in stabilization of enzyme's molecule (Arai et al., 1987; Wolf and Dean, 1987).

It is possible to assume, that the more high level of LPO activity in erythrocyte's membranes of the patients with anghiopatias is caused by lowering of the antioxidant defence system.

The given research confirms unsufficient efficiency of antioxidant systems in non-insulindependent diabetes mellitus, that creates favorable conditions for a course of free radical processes and, as a corollary – development of the endothelium damages. In this context the necessity of introduction of antioxidant therapy for treatment of the patients diabetes mellitus is obvious.

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PREPARATION OF TOBACCO-CUT FOR ICP-MEASUREMENT IN ORDER TO DETERMINE MICROELEMENT CONTENT

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ABSTRACT

There has been increased interest in the concentrations of a broad range of chemical compounds in tobacco and cigarettes smoke over the past decades with particular reference to ones associated with health. Nicotiana tabacum is known to easily absorb heavy metals from soil and accumulate them in unusually high concentrations in leaves. These metals pass by the smoke into the human body, where accumulate, damage the organs (mainly the liver and the kidneys) and could act as promoters in connection with carcinogens. The objective of this study was to compare four destruction techniques: ashing, ashing with matrix modifier ($MgNO_3$-$NH_3H_2PO_4$), wet ashing and digestion with microwaves in order to determine trace element content – especially Cd, Pb, Zn and Fe - of tobacco-cut. The destruction-techniques were repeated with certified reference material (Lagarosiphon major) in order to select the most precise method. In case of Cd and Zn wet-ashing seems to be the most effective destruction technique. Considering Fe the highest value was detected after microwave-digestion.

Key words: tobacco-cut, trace elements content

INTRODUCTION

Tobacco-plant is known to easily absorb heavy metals, especially cadmium, from the soil and accumulate them in the leaves in unusually high concentration. Part of these metals is transferred by the smoke into the human body, where accumulate, damage the organs (mainly kidney and liver) and act as promoters in conjunction with carcinogens.

Inhalation of cadmium causes irritation and possibly an acute inflammatory reaction of the lungs. Long-term exposure produces chronic bronchitis and increased susceptibility to infections and emphysema. Cadmium causes excessive urinary loss of calcium. This is likely to decrease calcium absorption and bone mineralization and thus lead to osteoporosis and osteomalacia. Kidney damage has been observed in people who are exposed to excess cadmium. The effects of cadmium on the kidney take the form of renal tubular dysfunctions (proteinuria, aminoaciduria, glucosuria).

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Lead exposure has been shown to decrease intelligence scores (IQ) and cause hearing problems. Exposure to high levels of lead can cause bad damage in the brain and kidneys. Lead exposure may increase blood pressure. Also, a couple may have trouble with having children because high levels of lead may damage the male reproductive system.

In the last two decades more and more details were found in international literature in connection with the element interactions (antagonism and synergism). The application of the essential elements can be effective in the prevention of toxicity or curative manner against the well-known negative physiological effects of some toxic heavy metals. For example, zinc can reduce any possible absorption of cadmium. Excess zinc by inhalation has been shown to reduce the carcinogenic potential of cadmium after exposure.

There are a lot of methods, which can be successfully used for single or multielement analysis. These methods today cover the whole range of trace elements in biological and environmental materials. However a proper sampling and preparation strategy has to be developed in order to achieve reliable and reproducible results. The following minimum requirements should be met:

- complete destruction of all organic material of the sample
- avoidance of losses
- avoidance of contamination

The classical destruction technique – *dry ashing* - is a very simple and popular method. At 600°C the organic compounds in the sample burn. But this method has some problems. First of all the vessels can be contaminated from former samples (Table 1) so influence the metal content of our sample.

Table 1. Contamination of some vessels by cadmium and zinc (ng/g) (Borszéki,1993)

Element	Glass	PTFE	Quartz
Cd	10-1000	-	10
Zn	300-3000	10	50-100
Fe	$2000/2x10^5$	10	200-800

Ashing has the risk of a loss of volatile elements (Table 2). The quantity of the loss depends on the temperature and the volatility characteristics of the metal compounds. The loss can be reduced using matrix-modifiers.

Table 2. The loss of cadmium and zinc during ashing (Borszéki, 1993)

Element	400°C	450°C	500°C
Cd	-	-	6-20%
Pb	-	2-5%	20- %
Zn	-	6-20%	20- %
Fe	6-20%/	2-5%	20- %

Acid digestion – *wet ashing* - can be carried out in a Teflon bomb under pressure for 20 minutes. Before the digestion the sample is treated with HNO_3 and H_2O_2 for 24 hours. Whereas the destruction is performed in a closed bomb, the volatility-loss can be avoided.

The first papers describing the application of *microwaves for digestion* organic and inorganic samples appeared in 1975. This method is very good to reduce digestion time from hours to minutes. The microwave generates approximately 5 billion molecular collisions every second. This enormous activity generates rapid solution heating. Solutions reach much higher temperatures than the boiling point at atmospheric pressure. Vessels are manufactured with special PTFE based materials with very high purity. So the risk of contamination is reduced to a minimum. The efficiency of this method can be increased if the microwave power is concentrated on the sample by special tools.

The *cool plasma asher* (CPA) consists of a high-frequency generator and a quartz vessel equipped with a cooling finger that prevents loss of volatile elements. The organic compounds in the sample will be oxidized in the oxygen plasma. This method achieves good decompositions of difficult sample matrixes without acids at a temperature slightly above 100°C.

The objective of this study was to compare four destruction techniques: ashing, ashing with matrix modifier ($Mg(NO_3)_2$-$NH_3H_2PO_4$), wet ashing and digestion with microwaves in order to determine trace element content – especially cadmium, lead, zinc and iron - of tobacco-cut.

MATERIALS AND METHODS

The sample, tobacco-cut mixture, was put at our disposal by a Hungarian tobacco factory. Before analytical measurements the sample was conditioned for 48 hours at 22°C and 60% humidity to achieve constant weigh and moisture (MSZ ISO 3402:1994).

The following destruction methods were used so as to compare the results:

- Tobacco-cut was ashed at 600°C for 90 minutes in opened system. The ashing was repeated using matrix modifier ($Mg(NO_3)_2$ and $NH_3H_2PO_4$) in order to reduce the loss of the volatile elements.
- The sample was digested by microwaves in closed Teflon bomb after the following programme:

0 W	1 minute
300 W	2 minutes
0 W	2 minutes
600 W	5 minutes

Before the destruction the cut was treated by nitric acid. Destruction was carried out in MLS-1200 MEGA microwave equipment from MILESTONE.

- Tobacco-cut was digested with concentrated nitric acid and hydrogen peroxide in Teflon bomb. Destruction was carried out at 100°C for 20 minutes under pressure.

The methods were repeated with certified reference material (Lagarosiphon major) in order to select the most precise method.

The metal content was measured by ICP technique.

Chemicals were purchased from SIGMA Chemical Company (Budapest, Hungary).

RESULTS AND DISCUSSIONS

The metal contents detected in our tobacco-samples were consistent with other foreign published data. Considering the certified reference material (CRM) in some cases our results were not equivalent with the data in the certified document.

The measured cadmium concentrations ranged from 0,88 up to 1,62 µg/g in the tobacco-cut (Table 3). The highest one was detected in the sample digested with microwave but considerable standard deviation was noticed. Practically similar values were shown in the wet-ashed sample, however, the standard deviation was very slight. The worst results were detected after dry-ashing, so it can be concluded, that the certain amount of the cadmium-compounds volatilise at 600°C in the opened system. The application of the matrix modifier did not prove the values.

In case of CRM the results were lower compared to the certified value by 13 to 18% excepting the values of dry-ashing.

Table 3. Cadmium-concentrations in tobacco-cut and Lagarosiphon major (µg/g)

	Tobacco-cut		**Certified reference material**	
Certified value			2,2±0,1µg/g	
	Concentration	SD	Concentration	SD
Dry-ashing	1,09	0,08	2,30	0,36
Digestion with microwave	1,62	0,20	1,80	0,03
Wet-ashing	1,51	0,08	1,90	0,04
Dry-ashing using matrix modifier	0,88	0,09	1,54	0,16

The lead concentrations in tobacco-cut at wet-ashing and digestion with microwave were below the detection limit (25µg/L) (Table 4). It can be explained, that the Teflon bombs were able to involve only 0,1-0,2 g sample, whereas dry-ashing required minimum 1 g material. On the other hand the extreme high standard deviation did not allow us to evaluate the results.

Considering the CRM the certified value for lead (63,8±3,2 µg/g) was in a good agreement with the measured concentrations after digestion with microwave (65,98) and wet-ashing (57,55). In the opened system a great loss was realised because of volatility. According to the results the closed system is recommended to destruction of tobacco in order to determination of lead concentration.

Table 4. Lead-concentrations in tobacco-cut and Lagarosiphon major (µg/g)

	Tobacco-cut		**Certified reference material**	
Certified value			63,8±3,2 µg/g	
	Concentration	SD	Concentration	SD
Dry-ashing	1,17	0,11	21,82	7,22
Digestion with microwave	< DL	-	65,98	9,79
Wet-ashing	< DL	-	57,55	4,46
Dry-ashing using matrix modifier	5,39	4,37	18,46	4,45

The measured zinc concentrations ranged from 32,27 up to 37,05 µg/g in the tobacco-cut and the standard deviations were slight (Table 5). Similar data can be found in the international literature.

The results of CRM were below the certified value (313±8 µg/g) actually it did not manage to approach the lowest one. So these methods did not seem to be too effective. The destruction methods for determination of zinc content demand further examinations.

Table 5. Zinc-concentrations in tobacco-cut and Lagarosiphon major (µg/g)

	Tobacco-cut		**Certified reference material**	
Certified value			313±8µg/g	
	Concentration	SD	Concentration	SD
Dry-ashing	33,04	1,62	221,65	20,81
Digestion with microwave	32,27	0,74	246,69	10,56
Wet-ashing	37,05	2,07	268,92	3,51
Dry-ashing using matrix modifier	33,75	1,39	187,55	10,88

The iron content of the tobacco cut was the highest (522,07 µg/g) after dry-ashing using matrix modifier and the standard deviation was slight (Table 6). However, similar value was detected after digestion with microwave but the standard deviation was considerable. The greatest loss was noticed after wet-ashing.

Considering iron content of CRM there was not certified value. The detected concentrations were ranging from 1344 up to 1770 µg/g and the standard deviations were very high. The lowest value was also measured after the wet-ashing similar to the tobacco sample (313±8 µg/g) (313±8 µg/g).

Table 6. Iron-concentrations in tobacco-cut and Lagarosiphon major (µg/g)

Certified value	**Tobacco-cut**		**Certified reference material**	
	Concentration	**SD**	**Concentration**	**SD**
Dry-ashing	447,33	11,50	1406,5	91,31
Digestion with microwave	521,84	31,35	1770,2	66,38
Wet-ashing	412,66	11,46	1344,3	64,30
Dry-ashing using matrix modifier	522,07	8,09	1377,8	48,17

CONCLUSIONS

In case of cadmium and zinc wet-ashing seems to be the most effective destruction technique.

Considering iron the highest value was detected after microwave-digestion.

Destruction techniques require further examinations in order to determine the lead concentration in tobacco-cut.

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THE VARIATION OF THE Cu, Zn SUPEROXIDE DISMUTASE BLOOD LEVEL IN IMMOBILIZED PATIENTS

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ABSTRACT

The aim of the study was to estimate the antioxidant defence of the immobilized patients. CuZn SOD and GSH determinations were performed in two groups: group I - 11 healthy subjects and group II - 15 patients from the Orthopaedic Hospital immobilized in bed and treated for osteitis. We also measured CuZn SOD concentration in group II, before and after an antioxidant treatment (vitamin C and E). The results revealed a significant decrease ($p<0.05$) of CuZn superoxide dismutase and glutathione blood concentration in immobilized patients. The CuZn superoxide dismutase determination in immobilized group, before and after the antioxidant administration, showed an increase of this parameter after 2 weeks of treatment. In patients maintained immobilized for a long time and with associated infections, the decrease of CuZn SOD and GSH can be caused by high consumption of antioxidant systems. In these patients it would be a benefit to associate scavenger therapy.

Key words: antioxidant defence, Cu, Zn superoxide

INTRODUCTION

Oxidative stress occurs in cells when the equilibrium between prooxidant and antioxidant species is broken in favor of the prooxidant state. It is due to reactive oxygen species generated either by the cellular metabolism such as phagocytosis, mitochondrial respiration, xenobiotic detoxification, or by exogenous factors such as ionizing radiation or chemical compounds performing redox reactions. Some reactive oxygen species (free radicals) are extremely reactive and interact with all the macromolecules including lipids, nucleic acids and proteins (Laval, 1996). The reactive oxygen species are hydroxyl radical, superoxide, nitric oxide, hydrogen peroxide.

1. Hydroxyl radical ($OH^{\cdot}$) is one of the most reactive. This radical, once generated, attacks whatever it is next to, as $OH^{\cdot}$ cannot migrate any significant distance within the cell. Its lifetime in vivo is small (Halliwell, 1994).

2. Superoxide radical ($O_2^{\cdot -}$) is the one-electron reduction product of oxygen. It is produced by phagocytic cells and helps them to inactivate viruses and bacteria. $O_2^{\cdot -}$ may be generated by "autoxidation" reactions, in which such compounds as catecholamines, tetrahydrofolates react directly with O_2 to form $O_2^{\cdot -}$. The $O_2^{\cdot -}$ then oxidizes more of the compound and sets up a free radical chain reaction (Halliwell, 1996).

3. Nitric oxide ($NO^{\cdot}$) is synthesized from the amino acid L-arginine by many cell types, including vascular endothelial cells, phagocytes and certain cells in the

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brain. $NO^{\cdot}$ is vasodilator agent, neurotransmitter and it may also be involved in the killing of parasites by macrophages.

Free radicals are constantly formed in the human body. Up a certain level, cells are protected from the detrimental effects of free radicals (Baeuerle, 1996). On line of defense are small molecules with antioxidative potential. These include an array of protective enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) as well as low molecular weight water and lipid soluble antioxidants such as ascorbate, glutathione, α-tocopherol and carotenoids (Esterbauer, 1996). A superoxide dismutase with cooper and zinc (CuZnSOD) at the active site is present largely in the cytosol (Halliwell, 1996), whereas manganese-containing superoxide dismutase (MnSOD) is localized to the matrix (Coyle and Puttfarcken 1993).

Antioxidant defences:

Superoxide dismutase (CuZnSOD) is a scavenger for the superoxide radical ($O_2^{\bullet -}$), accelerating its conversion to hydrogen peroxide (H_2O_2)·CuZnSOD works in collaboration with H_2O_2-removing enzymes, such as catalase and glutathione-peroxidases (Fulbert and Cals 1992; Halliwell et al*.,* 1992; Halliwell,1996).

$$2\ O_2^{\bullet -} + 2\ H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

Reduced glutathione (GSH) is a cysteine-containing tripeptide with reducing properties which play an important role in cellular protection against oxidative damage. During oxidative stress, GSH can scavenge various free radicals directly, as well as being a cofactor for enzymes, such as glutathione peroxidases (GPx). GSH presents synergistic interactions with other components of the antioxidant defense system, including superoxide dismutase (Mezzetti et al*.* 1990, Fulbert and Cals 1992, Halliwell et al*.* 1992, Halliwell 1996, Gerard-Monnier and Chaudiere 1996)

$$2\ GSH + 2\ R\bullet \rightarrow GSSG + 2\ RH$$

$$2\ GSH + H_2O_2 \xrightarrow{GOX} 2\ H_2O + GSSG$$

Catalases remove hydrogen peroxide generated by peroxisomal oxidase enzymes.

Ascorbate, α-tocopherol and carotenoids remove free radicals by reacting directly with them non-catalytically.

MATERIALS AND METHODS

The aim of this study was to estimate the antioxidant defence of the immobilized patients. The determination was performed in two groups: group I (control group): 11 healthy subjects - average = 28, and group II : 15 patients from the Orthopaedic Hospital immobilized in bed and treated for osteitis, mean age 47 = years.

The determination of CuZn SOD and GSH were performed in comparison with the control group. We also measured CuZn SOD concentration in group II, before and after the antioxidant administration. We used 0.5g/day vitamin C and vitamin E as antioxidant for a period of 2 weeks.

Total blood leukocytes count and subsets were determinate in both groups. All determination were made from peripheral blood.

Statistical analysis was performed using Student's T test.

Cu, Zn SOD determination (G. Szegli, Cantacuzino Institute, Bucharest, Romania - unpublished)

Principle:

The $O_2^{\bullet-}$ generating system is the redox couple formed by methylene blue and tetramethylenediamine (TMEA), as indicator being used the Nitro blue tetrazolium (NBT). When $O_2^{\bullet-}$ is produced, NBT is reduced to formazan, a colored compound with a maximum rate of absorption at 560 nm. The rate of absorption is directly proportional with $O_2^{\bullet-}$ concentration. CuZn SOD in the reaction mixture induces $O_2^{\bullet-}$ consumption, reducing the formation of formazan.

Reagents:

Phosphate buffer (KH_2PO_4 + $Na_2HPO_4 \bullet 12H_2O$) 0.05 M, pH = 7.8

Tetramethylenediamine (TMEA) 1.16 ml/10 ml distilled water

Methylene blue 0.003 g/50 ml distilled water

Nitro blue tetrazolium (NBT) 0.26 g/40 ml distilled water

Technique:

The reaction mixture for one sample contains:

Phosphate buffer	2.70 ml
Tetramethylenediamine (TMEA)	0.05 ml
Methylene blue	0.05 ml
Nitro blue tetrazolium (NBT)	0.20 ml

Heparinized blood was used. Before determination, CuZn SOD was extracted as following: 1ml blood + 0.25 ml chloroform + 0.5 ml ethyl alcohol; the mixture is centrifuged, just the supernatant being used. The sample contains 0.1 - 0.3 ml supernatant + 3 ml reaction mixture. The blank contains the same mixture, without the supernatant. The tubes were uniformly illuminated for 15 minutes with a neon lamp of 17 W, in a closed space, without other light sources. The extinction of both samples and blanks was determined spectrophotometrically at 560 nm, compared to the phosphate buffer.

One CuZn SOD unit is defined as the amount of enzyme which reduces with 50% the formazan in the blank. CuZn SOD is expressed in U/ml probe, its calculation depending on the dilution of the sample; if the extinction of the sample (Es) is less than ˝from that of the blank (Eb), the calculation formula is: [(Eb - Es)/Eb x 2 x 1]/ sample volume x sample dilution

The formula is used as long as the enzyme reduces the formazan of the blank to 40-60%.

GSH was determined using a modified form of Ellman's method (Albini, 1980).

Principle:

Dithiobisnitrobenzoic acid (DTNB) was added to plasma, at pH = 8; the reaction of DTNB with the -SH groups generates a colored compound with a maximum rate of absorption at 420 nm.

Reagents:

Phosphate buffer pH=8.0 (0.3683 g NaH_2PO_4 + 16.9749 g $Na_2HPO_4 \bullet 12\ H_2O$ + 4.675 g NaCl, in 1000 ml distilled water)

5,5' dithiobis-(2-nitrobenzoic acid) (DTNB) 40%, solved in the phosphate buffer

Technique

The sample contains: 0.15 ml plasma + 3.5 ml phosphate buffer + 0.5 ml DTNB

The blank contains: 0.15 ml plasma + 4 ml buffer

The optical density is measured spectrophotometrically after 15 minutes at 420 nm.

Calculation (μM/l): absorption x 2020

Normal values: 370-500± 65 μM/l

All chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA)

RESULTS AND DISSCUSION

Our results are presented in table 1.

Table 1. Statistical analysis

Statistical analysis	CuZnSOD UI/ml) group I	group II	GSH (μM/l) group I	group II
Average	10.872	5.686	465	251.133
SD	1.897	1.090	85.114	53.306
p value	p<0.0001		p<0.0001	

In the group of immobilized patients, the values of both antioxidant parameters were significantly reduced (p<0.0001) (fig.1 and fig. 2).

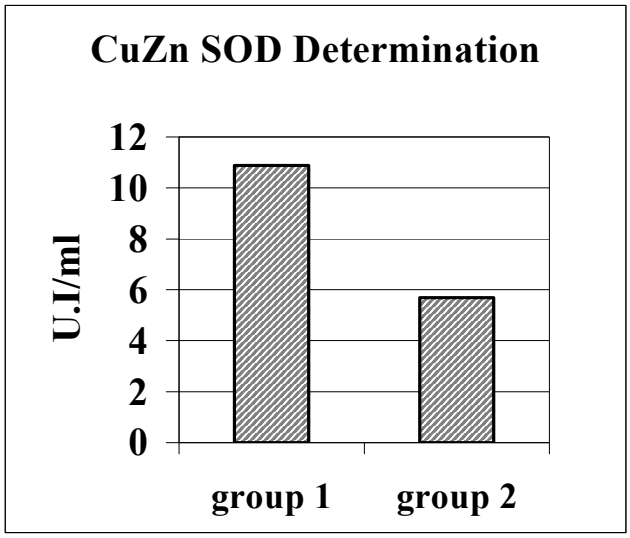

Fig. 1. CuZnSOD levels in controls and immobilized patients.

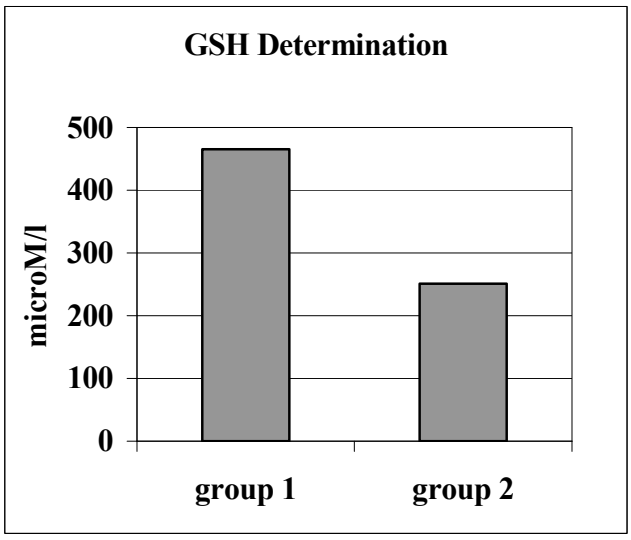

Fig.2. GSH levels in controls and immobilized patients

Total leukocytes count ranged between $8 \cdot 10^3/mm^3$ and $11 \cdot 10^3/mm^3$, with a mean value of $9.3 \cdot 10^3/mm^3$. The subsets of leukocytes revealed a slight increase in the percentage of neutrophils (78±5%).

Similar results, e.g. a significant decrease ($p<0.05$) of CuZn superoxide dismutase and glutathione blood concentration in immobilized patients were reported by Kedziora and Buczynski (1996).

Other investigators (Flagg et al. 1993, Bolzan et al. 1997, Siska *et al.* 1999) reported the existence of a large variability of CuZn SOD, GSH and GSH-related enzymes ascribed, at least in part, to the sex and age of the individuals. Our study also showed that CuZn SOD and GSH in the human blood exhibit a wide interindividual variability, but there were no statistically significant differences related to sex and age ($p>0.05$).

The CuZn superoxide dismutase determination in immobilized group, before and after the antioxidant administration, revealed an increase of this parameter after 2 weeks (fig 3).

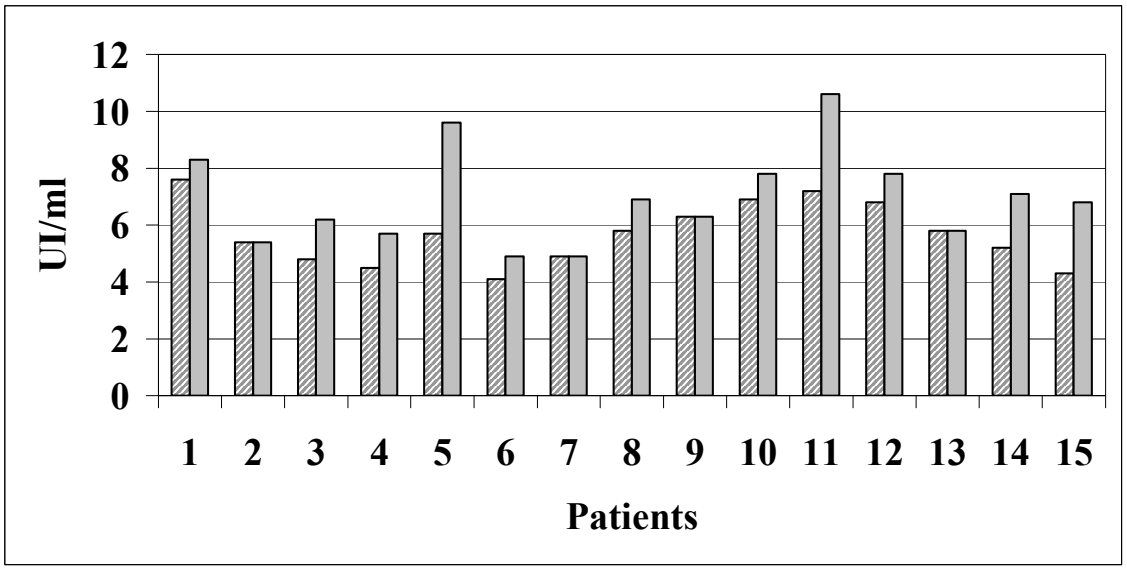

Fig. 3. SOD levels before and after antioxidant treatment

In patients maintained immobilized for a long time and with associated infections, the decrease of CuZn SOD and GSH can be caused by high consumption of antioxidant systems. The data suggested that the defense capacity in the immobilized patients is decreased, mainly due to a stress factor caused by their physical state. They have a high degree of susceptibility to infections. When the infectious process develops, the stress factor is amplified by increased production of free radicals in the cells, caused by leukocytes activation (neutrophils especially) and their response to pathogenic agents.

It would be a benefit to associate scavenger treatment like vitamin C and vitamin E in this type of patients. The antioxidant defence is improved if scavengers are administrated for at least 2 weeks.

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ALUMINIUM, RISK FACTOR FOR HUMAN AND ANIMAL HEALTH

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ABSTRACT

Based on bibliographical study, there are presented the sources of aluminium for animals and humans, the toxicity, the admitted intake from the diet, the local and systemic pathogenic effects, the clinical signs, and the therapy in aluminium intoxication. The local pathogenic effects consist in the limited absorption of phosphates, calcium, iron, from the digestive tract. The systemic pathogenic effects are: neurotoxicity due to the degenerative phenomena in the central nervous system, vitamin D resistant osteomalacia, anaemia, and probably immunosuppression. The clinical signs in animals are of neuro-muscular type in the acute intoxication, and osteomalacia, behavioural changes, contact dermatitis in the cumulative intoxication. In humans, the clinical signs are of neuro-muscular type (neurological and neuro-psychological signs within the dialysis associated encephalopathy, Alzheimer disease, Parkinson syndrome), and osteomalacia in the cumulative intoxication. The treatment may consist in the antidote desferroxamine or in the alternative chelation therapy with salicilhidroxamic acid and 3-hidroxipiridine.

***Key words*:** aluminium, sources, pathogenic effects, intoxication.

INTRODUCTION

Bibliographical references concerning the toxic effects of aluminium started to accumulate in the 1980's. Data about the essential role of aluminium are less numerous compared to data which demonstrate its toxicity for plants, animals and humans. As a consequence of the most different human activities (industrial, medical) the aluminium concentration in the environment (water, food, air) has changed and is changing continuously, reaching levels for which the organisms are not adapted and do not posses efficient homeostatic mechanisms and, consequently, may become harmful or even lethal (Ghergariu, 1996).

1. Aluminium sources for humans and animals

The aluminium sources, natural and human made, are numerous:

- **Telluric** (aluminium represents 8% from the total minerals existing in soil; is the 3rd element following the oxygen – 47.3% and silica – 27.7% - Ghergariu, 1996; Norseth, 1979): corundum – Al_2O_3, bauxite – Al_2O_3 and H_2O, cryolite – $AlF_3 \cdot 3NaF$, kaolin – $2SiO_2 \cdot Al_2O_3 \cdot H_2O$, zeolites – $4SiO_2 \cdot Al_2O_3 \cdot Me_2O \cdot 6H_2O$ or $4SiO_2 \cdot Al_2O_3 \cdot MeO \cdot 6H_2O$;
  - concentrations of 160 – 600 mg Al/kg were found in soil;
  - aluminium contained in the soil becomes bioavailable in the condition of acid pH;
- **Water**
  - hydrosphere: oceanic water (up to 1mg/l – Norseth, 1979), water from lakes, rivers (up to 10 mg/l - Norseth, 1979);
    - ⇒ the acidic medium and the acidic rains increase the aluminium concentration in water (Norseth, 1979);
    - ⇒ the water contaminated with emanated dust from primary or secondary aluminium industry, cement factories, effluents from the water stations, leather industry, textile industry, acidic drainage from mines;

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- drinking water: by the aluminium sulphate used for the coagulation of the colloidal and suspension particles, and by exceeded content of iron (Norseth, 1979);
  - ⇒ it contributes only by 0.4 – 2.1% from the total aluminium which may be accumulated by a person along his life;
  - ⇒ it is a risk factor for human consumer only in certain conditions (diet content, health status, genetic factors, age – children and aged persons are more susceptible);

- **Air**: the dust and/or smoke which contains in combination, or not, with fluor and other emissions from the primary or secondary aluminium industry, cement factories (aluminium silicate, aluminium oxide) (Guo et al., 1998);
- **Plants**:
  - by accumulation (only in some acid-resistant plants) from the soil reach in aluminium and with conditions of its bioavailability;
  - by direct contamination with atmospheric depots and water in the areas with risk of contamination;
  - feed treated with calcium and sodium aluminosilicate as detoxifying agent against aflatoxins and other mycotoxins (Ghergariu, 1996);
- **Row materials for food industry**:
  - vegetables originated from areas with aluminium emissions;
  - of animal origin: kidneys, liver, bones, brain, meat and milk from animals with chronic aluminium intake (Gergely et al., 1991).
    - ⇒ most row materials in uncontaminated areas contain below 10 mg/kg; the concentration of aluminium in milk varies within different references and different species: 3-79 µg/l in human milk and 4-33 µg/l in cow milk (Fernandez-Lorenzo et al., 1999); the eggs from Japanese quail contain 54% of aluminium in the eggshell and 38% in the egg-yolk.
- **Food products**: from work tools, foils, packages, additives for salting mixtures, backing additives, anti-agglomerating agents (powder milk, pudding, backing powder);
  - ⇒ daily intake by diet, in uncontaminated areas, varies depending on the diet structure: 20 mg/day (Gergely et al., 1991) – 80 mg/day (Sorensen et al., 1974, quoted by Norseth, 1979);
- **Therapeutic remedies**: antacids, buffered aspirin, drugs against haemorrhoids, injection with nutritive solution, fluids for dialysis and vaccines.
- **Cosmetics:** antiperspirants, tooth paste, packages for cosmetics products.

2. Aluminium impact on live organisms

2.1. Toxicity

Data on the toxic doses are meagre. Lorgue et al. (1987) give as toxic level in diet (ppm/dry matter): 1000 for ruminants, 200 for non ruminants. The aluminium toxicity for fish may be present in the acidic water at concentration below 50 µg/l (Poston, 1991); drinking water may contain maximum 50 ppb aluminium.

According to WHO, the tolerable aluminium intake for human adults is 7 mg/kg body weight/week, or 65mg/day, and 2 mg/day for children. Dairy products containing above 300 µg aluminium/l may lead to toxic effects (Hawkins et al., 1994).

2.2 Pathogenic effects

Aluminium is considered the main toxic element for invertebrates and fishes in acidic waters. The pathogenic effects consist in: inhibition of the development of oyster embryos, diminishing of the barrier properties of the branchial epithelium, and , as consequence, the asphyxia.

For mammals and birds, aluminium presents two types of potential toxic effects: local effects – in the **digestive tract** (the limitation of the absorption of some elements and compounds such as strontium, iron, fluor, calcium and, in consequence, metabolic disorders); in the **respiratory tract**, as a consequence of inhaling dust with aluminium (irritation, degenerative effects, sensitisation) and **systemic effects**: neurotoxicity, (formation of the senile amyloide tangles – Bitter et al. 1998), and neurofibrillae (Somova et al., 1997), paradoxical effects upon the bone metabolism: induction of the vitamin D resistant osteomalacia and the decrease of the remodelling capacity of bones but also the stimulation of "*de novo*" formation of bones in dog, the acceleration of the differentiation ratio of osteogenic cells, and the formation of bone nodules simultaneous with the inhibition of their mineralising (Bellowes et al., 1999), anaemia (Ganchev et al., 1998) and, possibly, immunosuppressive effect, toxic effects upon the foetuses: delayed ossification and development, behavioural disorders in new-borns, malformations.

2.3 Symptoms

2.3.1 Clinical signs in animals

There are not many data concerning the clinical signs occurring in accidentally intoxication. Lorgue et al., (1987) have described the following signs in accidentally poisoning in ruminants (bovine, ovine, caprine): diminishing of the appetite, low body weight increase in young animals, hypophosphataemia, tetanic seizures (resulted by interfering the magnesium metabolism), yield decreasing, and Ghergariu (1996) suggested that aluminium could be involved in the genesis of the enzootic osteomalacia and the grass tetany in ruminants. Other clinical signs are: medullar ataxia, increased lethality in sows, muscular contractions, convulsions, paresis and coma in dog, and in chronic poisoning: granulomatous enteritis in equine (Fogarty et al., 1998), behavioural disorders. Ocular contact lesions in rabbits: discoloration of iris , uveitis, opacity of crystalline were also described (Walmsley, 1999).

2.3.2 Clinical signs in humans

Acute poisoning is characterised by: muscular tremor, ataxia (staggering), convulsions, incapacity of correctly pronouncing the words (McLachlan et al., 1980, 1986, quoted by Walmsley, 1999), "fever of metallic smoke" (respiratory and digestive disorders), exaggerated mental activity followed by prostration (Walmsley, 1999).

Diseases resulted by the cumulative intake are: dialysis associated encephalopathy, spontaneous bone fractures (speaking disorders, directional disorientation, hallucinations, myoclonus, epileptic seizures, dementia and death within 6-8 months), Alzheimer disease (different opinions), encephalopathy of degenerative type, amyotrophyc sclerosis, dementia syndrome of Parkinson type (Bitter et al., 1998) (muscular tremor in standing and moving, hypokinesia, ataxia).

People who have been working for at least 10 years in aluminium contaminated environment presented: nervousness, nervous crisis in case of tiredness, decreasing of the neuromotor reaction and the accuracy of movements (Guo et al., 1998).

Dermal contact may cause cutaneous irritation by abrasion or, in case of chronic contact, dermatitis, allergy. Ocular contact produces after some time the discoloration of iris, partial atrophy of iris, uveitis, opacity of crystalline, pigmentation of eye-ground, capillary changes in the conjunctiva (Walmsley, 1999).

2.4 Treatment

Desferrioxamine is considered by some authors a very efficient chelating agent for aluminium, which helps its realising from tissues and its elimination. The therapy with

desferrioxamine implies some risks: allergic reactions with pruritus, anaphylaxis, disuria, diarrhoea, fever, muscular cramps, occasionally cataract and signs of neurotoxicity and does not take out completely and rapidely the aluminium from body.

The alternative to the therapy with desferrioxamine are the therapy with 3-hydroxypiridine, and salicilhidroxamidic acid.

CONCLUSIONS

Acute intoxications with aluminium are rare in human and animals. Severe pathogenic effects, especially the signs of neurotoxicity, are the consequence of the cumulative intake of small doses of aluminium.

The intensity of the pathogenic effects is increased by the health status deficiency, mainly by the morphological and functional integrity of the kidneys, age (high predisposition in children and aged people), but also in people with genetic predisposition.

Because the aluminium is a risk factor for animal and human health, the very important conclusion of this bibliographical study is the necessity of monitoring the aluminium sources and their impact upon the environment, by “environmental hygiene programmes and occupational hygiene programmes”.

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THE μ_3- OXOTRINUCLEAR IRON (III) COMPLEXES WITH AMINOACIDS – PHYSIOLOGIC ACTIVE SUBSTANCES IN CULTIVATION OF ALGAE AND CYANOBACTERIA

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ABSTRACT

Four new μ_3-oxotrinuclear iron high-spin (S=5/2) clusters with glycine, alanine, valine and treonine were synthesised and characterised by magnetochemistry, IR, Mossbauer spectroscopy. It was studied the influence of these compounds on productivity of different algae and cianobacteria. It was established the productivity of Dunaliella salina, Porphyridium cruentum, Spirulina platensis, increases up to ~25, 25, 37% respectively. It was observed that Fe(III)-Ala used in optimal doses for Spirulina platensis (5.0mg/l) stimulates rise of carotenoids up 1884.1%, C-phycocyanine – 167.8% and alophycocyanin – 199.0%, and Fe(III)-Gly: 141.6, 179.4, and 183.7% respectively. These two compounds are of great interest as chemical regulators in phicobilinogenethys and carotenogenethys.

Key words: iron, aminoacids, algae, cyanobacteria, stimulator, phycobiliproteids, carotenoids.

INTRODUCTION

One of the important directions in phycobiotechnology is to yield algae biomass with the content of synthesised bioactive substances. Phycobiliproteides, carotenoids and other pigments gain importance because they are applied in different branches of industry, e.g. pharmaceutics, food industry, cosmetics, etc. That is why, problems referring to elaboration of new original methods for obtaining above-mentioned substances is of great interest.

Further development of cultivation technology is necessary. And at the same time, physiological active compounds used as regulators of growth and productivity play an important part (Semenenko, 1982; Daskaliuk and Shalari, 1999). Coordination metal compounds (Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Fe^{3+} and others) call our attention. These compounds proved to be metabolic stimulators in algae cells. They contribute accumulation of different valuable bioactive substances such as proteins, vitamins, glucids, lipids, etc. (Rudic, 1995; Rudic and Cepoi,1995).

The main goal of this work is to study the influence of new coordination compounds of Fe^{3+} on productivity and biosynthesis of chlorophyll, carotenoids and phycobiliproteids by alga *Dunaliella salina* Teod., *Porphyridium cruentum* Näg. and cyanobacterium *Spirulina platensis* (Nordst.) Geitl as biotechnological model organisms.

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MATERIALS AND METHODS

Synthesise. The coordination compounds I-IV were synthesised by the substitution reaction of acetate anions from the iron(III) cluster $[Fe_3O(CH_3COO)_6(H_2O)_3]NO_3 \cdot 3H_2O$ with racemic aminoacid respectively. The acetate cluster was obtained according (Earnshaw et al., 1966).

0.71 g (0.001mol) of μ_3-oxotrinuclear acetate of iron(III) and 0.45 g (0.006mol) of glycine were dissolved in 25 ml of water. The purpure solution was filtered and was evaporated on water bath at 40-50^oC up to gel obtained. The gel was treated by isopropilic alcohol and the fine powder was obtained. The precipitate was filtered and washed by isopropylic alcohol. The rest complexes II-IV were obtained using the analogue procedure.

IR spectra of compounds were measured on a specord M-80 spectrophotometer in the region 250-4000cm^{-1} in nuloy and fluoride vaseline.

Mossbauer spectra of complexes at RT and 77 K were measured on the electrodynamics equipment. ^{57}Co in Cr-matrice was used as source. Fe metallic was used as calibrate.

Magnetic susceptibilities of compounds were determined by the Gouy method over the range from liquid nitrogen to room temperature and correcting for diamagnetism with the appropriate Pascal constants. Molar conductivity was measured in water solution (~10^{-3}mol) at 25^oC.

The green alga *Dunaliella salina* Teod. CALU-834 (Rudic et al.,1994) was grown and periodically agitate in 0.25 l Erlenmeyer flasks at the temperature of 28-32 ^0C, illumination 18-24 thous.erg/cm^2·s., at pH of the medium 8,5-8,8 in the presence of coordination compounds in concentrations of 1.0; 5.0 and 10.0 mg/l.

The red alga *Porphyridium cruentum* (Näg.) CNM-AR-01(Rudic and Cepoi,1995) was grown on the nutrient medium of the following composition, g/l: NaCl-7.0, KCl-7.5, $MgSO_4 \cdot 7H_2O$-1.8, $NaNO_3$-5.0, K_2HPO_4-0.2, KBr-0.04, KI-0.05, microelements, mg/l: $FeCl_3 \cdot 6H_2O$-27.0, $ZnSO_4 \cdot 5H_2O$-0.02, $MnSO_4 \cdot 5H_2O$-0.3, $CuSO_4 \cdot 5H_2O$-0.05, H_3BO_3-0.6, MoO_3-0.02, $NaWO_3$-0.05, at pH of the medium 6,8-7,2. Alga was grown on the above mentioned medium in the presence of coordination compounds in concentrations of 1.0; 5.0 and 10.0 mg/l in the 0.25 l Erlenmeyer flasks at the temperature of 24-26 ^oC, illumination during the first days 8-10 those. erg/cm^2·s. and the next days 12-15 those. erg/cm^2·s. , pH was kept constant at 6,8-7,2. The culture was periodically agitated.

Cyanobacterium *Spirulina platensis* (Nordst.)CALU-835(Rudic et al.,1995) was grown on nutrient medium Nr.16 (Gromov and Titova,1983) in the presence of 5.0; 10.0;15.0 and 20.0 mg/l concentration of coordination compounds in the 0.25 l Erlenmeyer flasks at the temperature of 34-36 ^oC, constant illumination 12-15 those. erg/cm^2·s. during the first days and 18-24 those. erg/cm^2·s during the next days, periodically agitated.

Alga productivity was determined photometrycally with the following re-count to dry biomass (Rudic,1993; Rudic and Cepoi,1996). Chlorophyll were determined according to Okuntsev method (Okuntsev et al., 1966), B-phycoerythrin by Gant and Lipschultz method (Gant and Lipschultz, 1972) carotenoids - by Neamtu method (Neamtu and Tamas, 1986), but phycocyanins by the methods adopted to the given objects (Rudic,1993).

RESULTS AND DISCUSSIONS

Using the traditional method of coordination chemistry it was synthesised 4 new monoanionic trinuclear complexes of iron(III) with different aminoacids with the general composition $[Fe_3O(A\text{-}H)_6(H_2O)_3]NO_3 \cdot nH_2O$, were A-H: Gly(I), Ala(II), Val(III), Tre(IV).The complexes are soluble in H_2O, DMF, DMSO and take part from the binary electrolytes (μ= 157-164 $Om^{-1}cm^2\ mol^{-1}$ in water).

The IR spectra of I-IV confirm the formation of some new compounds in which aminoacid anion is in deprotonated state. In the range 3250-3130 cm^{-1} two bands of absorption which are characteristic for oscillation of uncoordinated NH_2 groups but which are incorporated in system of hydrogen bond. For uncoordinated aminoacids this oscillation are placed in the range of 3450-3300cm^{-1}, and for coordinated amino groups for zwitter-ion state it is situated in the range of 3100-2900cm^{-1} (Kogan, 1986; Acbarov, 1990) .

The coordinated carboxylic groups are presented in IR spectra by two bands at 1600cm^{-1} (ν_{as}(COO)) and 1410cm^{-1} ((ν_s(COO)). The difference $\Delta\nu$(COO) indicates the bridge mode of coordination of carboxylic groups (Nakamoto, 1991).

The middle intensive band at ~ 600 cm^{-1} ($\nu_{as}(Fe_3O)$ and ~380 cm^{-1} (ν_d(Fe-O) are characteristic for μ_3-oxo trinuclear cluster.

Mossbauer spectra of I-IV represent one doublet (fig.1) with parameters δ_{Na}^{+} = 0.71- 0.74 (80K), 0.63-0.64 (300K); ΔE_Q = 0.73 ÷ 0.80 (80K),0.65 ÷.76 (300K) which are characteristic to iron(III) in high spin state (S=5/2) (Herber and Goldanskii, 1969).

The values of effective magnetic moment of I-IV at room temperature are 3.45 ÷ 2.80 MB and are lower than for pure spin state of iron (III) (S=5/2) 5.90 ÷ 6.05 MB. With decreasing of temperature up to 100K these values are diminished up to 2.75 ÷ 2.10 M.B., respectively. The temperature dependence of magnetic moments of investigated complexes indicates the presence of antiferromagnetic interaction between paramagnetic ions (Kalinnikov, 1980; Tsukerblat, 1983). Using the GDVV model (Van Vleck, 1932; Tsukerblat, 1983) for C_{2v} symmetry of complexes the values of exchange parameters J_1, J_2 were determined. They are: J_1= -37÷-11cm^{-1} and J_2 = -50÷-28cm^{-1} and are characteristic for μ_3-oxotrinuclear Fe_3O compounds (Zelentsov 1975).

The synthesised complexes were tested as biological active compounds in growth of some algae and cyanobacteria like Dunaliella Salina, Porphyridium cruentum, Spirulina Platensis. The results of productivity and content of pigments in biomass of the green alga *Dunaliella salina* Teod.CALU-834 during its cultivation in the presence of coordination compounds of Fe(III) with aminoacids are presented in Table 1. According to the data, productivity of *D. Salina* increases if nutrient medium contains thrinuclear clusters of Fe (III) with aminoacids, optimal dose is 5.0 mg/l. Stimulation in synthesis of chlorophyll *a* by using Fe (III)-Gly and less using of Fe (III)-Ala is noticed too. At the same time Fe(III)-Tre inhibits biosynthesis of pigments almost in all cases when doses 5.0 and 10.0 mg/l were used.

Presence of trinuclear clusters of Fe (III) with aminoacids in the nutrient medium during cultivation of marine red alga *Porphyridium cruentum* (Näg.)CNM-AR-01 shows different effects (tab.2).

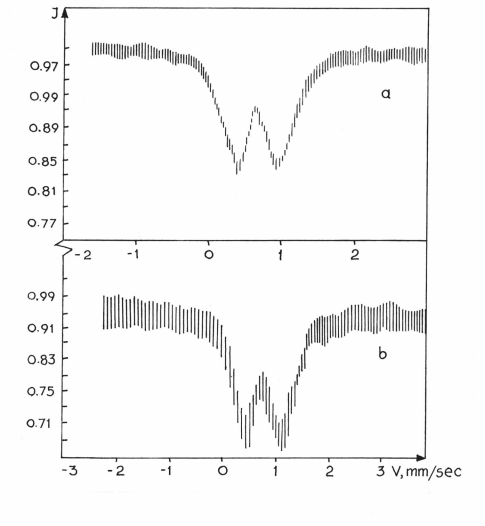

Fig. 1. Mossbauer spectra of investigated iron complexes $[Fe_3O(A\text{-}H)_6(H_2O)_3]NO_3 \cdot nH_2O$, a- 300K, b- 80K.

For instance, Fe (III)-Gly inhibited productivity of *P.cruentum* in all concentrations, meantime the other three complexes stimulated a little biomass accumulation without any regularities. Only Fe (III)-Ala increased considerably ($P<0.05$) content of pigment B-phycoerythrin in biomass from 13.96% (10.0 mg/l) up to 35.71% (1.0 mg/l). Phycobilinogenethis had a positive effect on obtaining of C-phycocyanin and alophycocyanin during utilisation of all coordination compounds with the exception of Fe(III)-Tre.

The results of productivity and content of phycobiliproteids and carotenoids in biomass of cyanobacterium *Spirulina platensis* (Nordst.) CALU-835 is presented in Table 3.

All coordination compounds of Fe(III) with aminoacids stimulate productivity of *S. platensis* under certain concentrations. Thus, optimal dose for Fe(III)-Gly, Fe(III)-Ala and Fe(III)-Val was 5.0 mg/l and these doses increased biomass by 32.85; 37.14 and 20.71% respectively. But Fe(III)-Tre only in doses of 10.0 and 15.0 mg/l showed the same effect. Content of chlorophyll ***a*** in biomass of *S. platensis* was determined. It was proved that used compounds inhibited its synthesis to a different extent with the exception of Fe(III)-Ala, dose – 5.0 mg/l. Content of chlorophyll ***a*** reaches only 35.77 – 40.78% in comparison with control.

An obvious stimulation of phycobilinogenethys had been found in all the experiments when *S.platensis* cultivated in the presence of thinuclei clusters of iron with aminoacids. Only Fe(III)-Gly in concentration 15.0 mg/l did not show the above mentioned effect in case of C-phycocyanin. When spirulina was cultivated in the presence of 5.0 mg/l of Fe(III)-Ala the highest results of phycocyanin content were obtained. They are 179.36% for C-phycocyanin and 199.05% when Fe(III)-Gly with concentration 10.0 mg/l was used.

Results referring to total content of carotenoids in biomass of *S.platensis* vary widely in dependence on the used coordination compounds. Thus, Fe(III)-Val in all used concentrations inhibits synthesis of carotenoids by 43.93%; 43.74 and 24.36%, the rest compounds stimulate carotenogenethys in dependence on used concentrations by 35.14 – 41.56% (Fe(III)-Gly), 57.28 – 84.13% (Fe(III)-Ala) and 24.87 – 87.80% (Fe(III)-Tre).

Comparative analysis of this study (Table 1-3) shows lack of any essential regularities. Thus, productivity increase of *D.salina* and *P.cruentum* in optimal coordination compounds concentrations of Fe(III) does not exceed 25.51 – 26.81%. In case of *S.platensis* cultivation this index is 33.57 – 37.14%. Pigments of photosynthesis in studied algae and cyanobacterium *S.platensis* react in a different way. Content of both chlorophyll and carotenoids in biomass of *D.salina* and B-phycoerythrin at *P.cruentum*, with some exceptions, does not react significantly to the presence of trinuclear clusters of Fe(III) with aminoacids in culture medium. At the same time synthesis of C-phycocyanin and alophycocyanin both in *P.cruentum* and *S.platensis* increases strongly, in comparison with control 2-3 times as much. The obtained results confirm prof. V.Isac's opinion (1993) that coordination compounds of iron stimulates phycobilinogenethys in *S.platensis*. These results

with good prospects can have practical use in phycobiotechnology of industrial produce of C-phycocyanin and alophycocyanin.

It should be mentioned stimulation of carotenogenethys in *S.platensis* during cultivation in the presence of 5.0 mg/l of Fe(III)-Ala – 184.13% which took place tighter with hypersynthesis of C-phycocyanin (167.82%) and alophycocyanin (199.05%).Fe(III)-Gly which is used in optimal doses stimulate rise of above mentioned indices: carotenoids – 141.56%, C-phycocyanin – 179.36% and alophycocyanin – 183.71%. Thus, these two compounds are of great interest as chemical regulators in phycobilinogenethys and carotenogenethys and their use future will have a valuable effect in phycobiotechnology.

As a result of our investigations it was established that coordination compound of Fe(III) with aminoacids stimulate synthesis of phycobiliproteids in the red marine alga *Porphyridium cruentum* (Näg.) CNM-AR-01 and cyanobacterium *Spirulina platensis* (Nordst.) Geitl. CALU-835.

Studied coordination metal compounds are of great interest for phycobiotechnology in biomass production with high content of phycobiliproteids and carotenoids.

Table 1. Productivity and content of pigments in biomass of alga green *Dunaliella salina* Teod. CALU 834 in cultivation in presence of Fe(III) compounds with aminoacids.

Nr	Coordination compound	Concentration, Mg/l	Biomass		Chlorophyll a		Chlorophyll b		Carotenoids	
			g/l	% C*	%	% C	%	% C	%	% C
1.	Fe(III) – Gly	1.0 5.0 10.0	1.12±0.06 1.16±0.09 1.14±0.07	114.28 118.36 116.32	2.56±0.10 2.55±0.08 2.40±0.12	123.02 122.71 115.52	1.63±0.09 1.54±0.07 1.50±0.08	112.96 106.79 104.01	5.62±0.10 6.14±0.08 5.30±0.06	111.29 121.68 104.95
2.	Fe(III) – Ala	1.0 5.0 10.0	1.15±0.10 1.18±0.05 1.23±0.09	117.34 120.41 125.51	2.41±0.06 2.39±0.05 2.32±0.08	116.14 115.11 111.72	1.68±0.04 1.49±0.10 1.48±0.12	116.98 103.70 102.78	5.36±0.04 6.30±0.05 5.91±0.06	106.14 124.75 116.98
3.	Fe(III) – Val	1.0 5.0 10.0	1.02±0.06 1.20±0.02 1.10±0.02	104.08 122.45 112.25	2.18±0.12 2.09±0.16 2.07±0.08	104.83 100.72 99.69	1.52±0.14 1.47±0.10 1.39±0.08	105.56 101.23 96.91	5.09±0.12 5.02±0.14 4.75±0.03	100.39 99.41 94.01
4.	Fe(III) – Tre	1.0 5.0 10.0	1.08±0.14 1.22±0.12 1.04±0.15	110.20 124.49 106.12	2.19±0.09 2.05±0.06 1.97±0.12	105.14 98.66 94.66	1.43±0.06 1.41±0.03 1.35±0.09	99.07 98.15 93.52	5.10±0.08 4.90±0.12 4.83±0.07	100.99 97.03 95.64
5.	Martor(C*)	-	0.98±0.04	100.00	2.08±0.06	100.00	1.44±0.08	100.00	5.05±0.10	100.00

Table 2. Productivity and content of pigments in biomass of alga red *Porphiridium cruentum* (Näg.) CNM-AR-01 in cultivation in presence of Fe(III) compounds with aminoacids.

Nr.	Coordination compound	Concentration, mg/l	Biomass		B-phicoerythrin		R-phycocyanin		Alophicocyanine	
			g/l	% C*	%	% C*	%	% C*	%	% C*
1.	Fe(III) – Gly	1.0 5.0 10.0	5.92±0.61 5.62±0.74 5.44±0.65	93.37 88.64 85.80	3.09±0.04 3.15±0.06 3.18±0.02	100.32 102.27 103.24	0.89±0.05 0.84±0.05 0.80±0.06	247.22 233.33 222.22	0.38±0.02 0.32±0.02 0.36±0.04	271.43 228.57 257.14
2.	Fe(III) – Ala	1.0 5.0 10.0	6.44±0.62 7.94±0.32 6.51±0.24	107.57 125.23 102.68	4.18±0.02 3.68±0.04 3..51±0.07	135.71 119.48 113.96	0.42±0.08 0.49±0.02 0.56±0.06	116.66 136.11 155.55	0.29±0.01 0.32±0.04 0.38±0.06	207.14 228.57 271.42
3.	Fe(III) – Val	1.0 5.0 10.0	8.04±0.58 6.52±0.61 6.24±0.74	126.81 102.84 98.43	3.19± 0.06 3.28±0.05 3.62±0.07	103.57 106.49 117.53	0.69±0.08 0.75±0.06 0.74±0.05	191.66 208.33 205.55	0.18±0.01 0.22±0.02 0.44±0.01	128.57 157.14 314.28
4.	Fe(III) – Tre	1.0 5.0 10.0	6.42±0.51 7.12±0.48 7.51±0.39	101.26 112.30 118.45	3.15±0.04 3.29±0.05 3.18±0.02	102.27 106.81 103.25	0.41±0.01 0.39±0.04 0.26±0.01	113.88 108.33 72.22	0.12±0.02 0.18±0.02 0.24±0.04	85.71 128.57 171.42
5.	Control (C*)	-	6.34±0.40	100.0	3.08± 0.09	100.00	0.36±0.02	100.00	0.14±0.01	100.0

Table 3. Productivity and content of pigments in biomass of cianobacteria *Spirulina platensis* (Nordst.) Geitl. CALU – 835 in cultivation in presence of Fe(III) compounds with aminoacids.

Nr.	Coordi-nation compound	Concen tration, mg/l	Biomass		Chlorophyll a		C-phicocyanin		Alophicocyanin		Carotenoids (sum)	
			%	% C*	mg%	% C*	%	% C*	%	% C*	mg/%	%C*
1.	Fe(III) – Gly	5.0 10.0 15.0	1.86±0.07 1.51±0.04 1.46±0.04	132.85 107.85 104.28	883.2 ± 18.99 887.7 ± 6.18 838.6 ± 9.89	92.55 88.22 85.33	11.98±0.57 12.45±0.12 6.49±0.16	167.79 134.37 90.90	9.70±0.59 6.15±0.20 6.10±0.22	183.71 116.47 115.53	1238.45 ± 32.45 1151.92 ± 42.01 1040.56 ± 26.59	141.56 135.14 119.94
2.	Fe(III) – Ala	5.0 10.0 15.0	1.92±0.04 1.67±0.04 1.59±0.04	137.14 119.28 113.57	1116.2 ± 18.6 867.3 ± 3.21 812.10 ± 15.73	110.54 90.24 81.48	10.55±0.14 11.39±0.26 8.14±0.27	147.76 159.32 119.00	10.51±0.04 8.58±0.41 8.36±0.30	199.05 162.50 158.33	1599.43 ± 51.99 1365.48 ± 8.09 903.06 ± 2.14	184.13 157.28 94.65
3.	Fe(III) – Val	5.0 10.0 15.0	1.69±0.01 1.61±0.01 1.58±0.01	120.71 115.00 112.87	765.6 ± 5.82 819.5 ± 4.79 919.5 ± 6.51	76.09 81.67 92.83	10.10±0.11 10.46±0.11 9.81±0.05	141.96 146.50 137.40	9.85±0.93 7.79±0.16 6.78±0.05	186.55 147.55 128.40	785.18 ± 27.04 847.11± 24.71 1137.40± 3 2.91	56.02 56.02 75.64
4.	Fe(III) – Tre	5.0 10.0 15.0	1.47±0.04 1.87±0.04 1.79±0.03	105.00 133.57 127.85	373.9 ± 19.34 373.3 ± 7.09 361.8 ± 8.02	40.78 36.35 35.77	9.90±0.26 9.40±0.29 9.45±0.35	134.73 131.65 132.35	8.31±0.57 9.61±0.12 7.82±0.46	157.38 182.00 148.10	823.44 ± 35.68 909.84 ± 26.53 1199.64 ± 18.43	106.85 124.87 187.80
5.	Martor (C*)	-	1.40±0.05	100.00	994.0 ± 8.39	100.0	7.14±0.26	100.00	5.28±0.22	100.0	989.65 ± 6.53	100

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INVESTIGATIONS CONCERNING THE METALLIC COMPOSITION OF THE SURGICALLY REMOVED SIALOCONCREMENTS

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ABSTRACT

The study of the biogenesis of lithiasis concrements presents interest under three aspects: anatomic - by localization; physiopatologic - by the homeostatic disturbances; pathobiochemical - by composition and by the mechanism of formation, of precipitation and/or co-precipitation.

From anatomic point of view, the lithiasis presents numerous localisations, the most important ones being the cholelithiasis and the sialolithiasis.

Investigations presented in this paper, performed on a smal number of cases (14 pacients), followed the metallogram of salivary calculi known also as "sialoconcrements". On that purpose there were made determinations by atomic absorbtion spectroscopy (AAS) on some alkaline metals (Na, K), alkaline-earth metals (Ca, Mg) and on some metallic trace elements (Zn, Cu, Mn, Pb , Cd, Al , Cr).

Obtained data reveal that the greatest quantities of metals have been decelated in the case of alkaline-earth metals (in the relation Ca>Mg) and alkaline/earth metals (in the relation Na>K) – values expressed in mg/g; as to metallic trace elements, a reduced quantum was decelated, a decrease of the main constituents in the series Al>Pb>Cu>Cr – values expressed in µg/g.

Key words: metallograms of sialoconcrements

INTRODUCTION

The saliva is a mixture of the secretion from three pairs of glands. About 750 ml are produced per day by man, of which the submandibular glands contribute with 70%, the parotid glands with 25% and the sublingual glands with 5% (Bell et al., 1976).

Physically, the saliva is a viscous, colourless and opalescent liquid. The average specific gravity is 1,003 (ranging between 1,002 - 1,010). The pH varies between 6.2 - 7.4 and it may become more acid in the presence of air (Ganong, 1995). Under microscope, the salive shows nucleated squamous cells from the buccal lining, disitegrating leucocytes and gland cells, as well as a large variety of microorganisms.

It contains organic constituents of the group of glycoproteins, that confer viscosity and lubricating properties. Among enzymes it contains an α-amilase (ptyalin) at an optimum pH 6.8 which is activated by chlorides and catalyzes the breakdown of starch to maltose. There were also decelated other proteins, including numerous enzymes, such as carbonic anhydrase and in small amounts, amino acids, urea acid, citrates etc. The saliva also diverse cationic (Na, K, Ca, Mg, Cu etc) and anionic (P, Cl, I, F etc.) compounds.

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In the presence of carbon dioxide or by bacterial action, salivary constituents can precipitate and their deposition takes place on the teeth as tartar or as calculi in the salivary ducts.

At the level of salivary glands, especially of salivary ducts, crytals with the specificity of lithiasis and sometimes even concrements can be produced. These are known as salivary lithiasis or sialolithiasis.

MATERIALS AND METHODS

Analytical investigations were made on salivary calculi surgically prelevated. The sialoconcrements were extracted at the level of submaxilar glands and their ducts. The size of concrements was of 3-11 mm diameter. After prelevation the calculi were washed repeateadly with distilled water, desicated and thereafter powdered.

Investigations followed the determination of main alkaline (Na, K), alkaline-earth (Ca, Mg) and trace metal elements (Zn, Cu, Mn, Pb, Cd, Al, Cr) concentrations.

For analytical determinations were used atomic absorbtion spectroscopy (AAS) methods. It was used an apparatus “Analyst-100” produced by Perkin Elmer. Obtained data were statistically evaluated determining the mean value (X) and the standard deviation (SD).

RESULTS AND DISCUSSIONS

The most frequent depositions of salivary calculi (i.e. sialoconcrements) appear at the level of submandibular glands and mostly on their excreting ducts (Teodorescu-Exarcu, 1981; Garban, 2000).

Forming of sialoconcrements and their localisation is sometimes a consequence of repeated infections that facilitate the spatial expansion and localisation mainly at the level of the submandibular gland.

Data referring to the composition of saliva are compiled from literature after Altman and Dittmer (1968, 1974) – see Table 1.

Tabel 1. Chemical composition of saliva - anorganic substances
(after Altman and Dittmer, 1968, 1974)

Component		UM	Saliva (probe)	Average	Range
Cations	Ca total	mg / dL	Mixte Submaxillary Parotid	5.80 8.80 3.50	5.2 – 9.7 4.4 – 13.1 2.1 – 6.7
	Co	μg / dL	Mixte	2.44	0 – 12.53
	Cu	μg / dL	Mixte	31.70	5.0 – 76.0
	Mg	mg / dL	Mixte	0.50	0.15 – 0.93
	K	mg / dL	Mixte	80.3	56 - 148
	Na	mg / dL	Mixte	23.2	8 – 56
Anions	Cl	mEq / L	Mixte	15.5	8.4 – 17.7
	F	mEq / L	Mixte		0 – 0.005
	I	μg / dL	Parotid Submaxillary	6.46 3.65	
	P total	mg / dL	Mixte	20.40	
	P anorg	mg / dL	Mixte	14.90	7.4 – 21.1
	P org	mg / dL	Mixte	5.50	
	Bicarbonate	mEq / L	Mixte	6.44	3.48 – 10.70

Performed investigations did not concern organic compounds 8e.g. cholesterol) or anorganic ones (e.g. oxalates, phosphates) which can appear in diverse types of lithiasis. Metal determination in sialoconcrements offers important data for the physiopathology of lithogenesis process and be used as a clinical guideline in stomatology (Yamamoto et al., 1984; Vasiluță, 2000)

The study focused on the determination of alkaline (Na, K), alkaline-earth (Ca, Mg) concentrations (Tabel 2).

Tabel 2. Concentration of main metallic alkaline and alkaline-earth macrobioelements

Specification	UM	n	Concentration X + SD	Range of concentrations
Na	mg/g	14	5.83 ± 2.46	3.64 – 9.11
K		14	0.54 ± 0.19	0.36 - 0.72
Ca		14	234.61 ± 79.20	178.80 – 288.40
Mg		14	1.68 ± 0.72	1.14 – 2.69

There were also determined some biogene trace- and ultra trace elements, mentioning Zn, Cu, Mn (Tabel 3).

Tabel 3. Concentration of some metallic tracebioelements

Specification	UM	n	Concentration X + SD	Range of concentrations
Zn	μg/g	14	33.62 ± 14.11	17.23 – 53.62
Cu		14	4.54 ± 2.09	2.72 - 6.87
Mn		9	1.30 ± 0.61	0.76 – 2.19

As to potential toxic elements, there were decelated Cd, Pb, Al, Cr in a very small amount (Table 4).

Tabel 4. Concentration of some trace elements with toxicogen potential

Specification	UM	n	Concentration X + SD	Range of concentrations
Pb	μg/g	6	9.55 ± 4.08	5.07 – 13.07
Cd		7	0.72 ± 0.33	0.41 – 1.69
Al		14	167.87 ± 73.12	91.20 – 324.11
Cr		5	3.18 ± 1.47	1.21 – 5.85

Usually treatment of salivary lithiasis means surgical intervention and, in the last decade, lithotripsie procedures based on treatment with ultrasounds.

Shock wave lithotripsy has been the first choice treatment of salivary gland stones, the lithotripter having an ultrasound scanner (10 MHz) that enables focusing the stone and allows monitoring of stone disintegration with fragment size under 2 mm. It is now considered that shock wave lithotripsy is a non-operative treatment, an alternative to surgery, a successful treatment with low risk (Kater et al., 1994). Surgical interventions present risks during the general anesthesia due to its consecutive effects.

CONCLUSIONS

1. Study of metal composition in sialoconcrements evidenced the decrease of concentration in the succesive series:
   a) for alkaline and alkaline-earth metal bioelements:

   Ca > Na > Mg > K

   b) for trace metal bioelements the decrease series is:

   Cu > Zn > Mn

   c) for metal elements with toxic potential the quantum presents the following decrease:

   Al > Pb > Cr > Cd

2. As a general remark, for all metallic elements the variation domaine varies from case to case very much. In some cases metals as Pb, Cd, Cr and Mn did not show.
3. Data refering to metal composition of sialoconcrements present importance for physiopathology and is a veritable guideline for the dentistry clinic.

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EXPERIMENTAL STUDIES ON THE METABOLIC EFFECTS INDUCED BY ZINC IN LABORATORY ANIMALS

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ABSTRACT

Metal elements are biocomponents of living matter, or biochemical effectors (inhibitors and / or activators) of various metabolic processes. These compounds are of interest for different domains of science, e.g.: ecology, nutrition, pharmacology, toxicology etc.

Studies on the metallic elements are often made on experimental animal models – and in this work the study was also performed on Wistar strain rats, pursuing some metabolic effects induced on some metabolites, on their distribution in organism a.o. The study presents the results obtained after "per oral" administration (gavage) of zinc administered as chlorines, in two experimental groups (E_1 and E_2). Data were reported to a control group (C).

Researches followed the modifications induced on the quantum of some trace metal elements in kidneys. It was determined the concentration of some metallic bioelements (Zn, Cu, Mn) and of some elements with toxicogene potential (Al, Cr, Pb). Different homeostatic variations, depending on the administered metallic chlorines, were observed.

Key words: zinc effects laboratory animals

INTRODUCTION

Metal elements and their various compounds (organic or inorganic) are involved in different biochemical processes with consequences on morpho-physiological status and even with pathological manifestations (Mills et al., 1985).

In this context biominerals have essential role in morphogenesic processes being components of some bioconstituents, they intervine as biochemical effectors (activators/inhibitors) in metabolic interactions, maintain the biochemical homeostasis of fluid compartments in organism, contribute to the maintenance of osmotic pression, of acid-base balance a.o. (Mertz, 1987; Garban, 1999).

Experiments on animals offer concludente data on the biochemical role of metallic elements and evidence some dyshomeostazic effects, too. The latest effects have various explanations, starting from the competitive interactions till to their fixation in different tissues (Ciudin and Marinescu, 1997; Cotrău et al., 1991).

The present paper deals with the effects of zinc on the homeostasis of various metals in rats.

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MATERIALS AND METHODS

Experiments were performed on laboratory animals – Wistar strain rats – divided in three groups: one control (C) and two experimental (E_1, E_2). Each group comprised 10 animals (male and female) with an average weight of 100 ± 10 g. By gavage a total amount of 1mL solution per 100 g body weight (b.w.) was administered on day 4th and 7th of the experiment. To animals from C group tap water was administered and to those from E_1 and E_2 groups zinc chlorine solution.

The quantum of zinc in solution was calculated according to the Recommended Daily Intake (RDI) for Zn, i.e. 0.214 mg / kg body weight (Devlin, 1992; Derek, 1995). Animals from group E_1 received a $ZnCl_2$ solution in which Zn concentration was twofold of the RDI and E_2 group $ZnCl_2$ solution in which the Zn concentration was fourfold the RDI. In the calculation of the concentrations was made reporting to the metallic elements Zn (from the chlorine). In the volume of the administered solution of 1 mL / 100 g b.w. Zn concentration was twofold the RDI for E_1 group and for E_2 – fourfold the RDI (values was calculated starting from 0.214 mg / kg b.w.).

The animals were killed on day 15 of the experiment. The concentration of Zn, Mn, Cu (biometals) and of Al, Cr, Pb (potentially toxicogene metals) in renal tissue was determined. For this purpose we used a Perkin Elmer type Analyst 100 atomic absorption spectrophotometer (AAS).

Data were processed statistically being determined mean values and standard deviation.

RESULTS AND DISCUSSIONS

Zinc - considered as an essential microelement for living organisms - was found in different tissues, especially in various compounds with biocatalytic role. In this context one can mention the following metalloenzymes: carboxypeptidases, alkaline phosphatase, lactic dehydrogenase, glutamicdehydrogenase, DNA-polymerase, RNA-polymerase, Zn-Cu-superoxyde-dismutase a.o. These enzymes participate in different phases of the material and energetic metabolisms (Reinhold, 1976; Mertz, 1987).

Zinc intervine also in the action of some hormones, e.g.: insuline, hypophisar gonadotrope hormones.

In the animal and human organism zinc was found in higher concentration in liver, kidneys, muscle, brain, blood etc. (O'Dell and Savage, 1960; Hooper, 1980; Mills, 1989; Garban et al., 1999)

Having in view the biochemical and morphophysiological importance of zinc we proposed the study of its action on some metallic bioelements (Zn, Cu, Mn) and on some metallic elements with toxicogene potential (Al, Cr, Pb).

In Table 1 there are presented the obtained concentrations of biometals in renal tissue.

Table 1. Concentration of biometals in renal tissue after $ZnCl_2$ administration

Bio-metals	Groups				
	Control (n = 10)	E_1 (n = 10)		E_2 (n = 10)	
	X ± SD	X ± SD	ΔX	X ± SD	ΔX
Zn	19.64 ± 3,49	16.15 ± 2.38	- 3.48	15.63 ± 2.83	- 4.01
Mn	5.01 ± 0.78	3.27 ± 0.74	- 1.74	3.08 ± 0.81	- 1.93
Cu	0.68 ± 0.24	1.22 ± 0.83	+ 0.54	1.41 ± 0.79	+ 0.73

Note: n – number of cases

The results show that the administered zinc acts on its own homeostasis and on the homeostasis of Cu and Mn. In case of Zn-Zn relationship it was found a direct proportionally decrease of zinc concentration in the renal tissue with the increase of the administered Zn concentration.

A similar situation was revealed in the case of Zn-Cu relationship. As to Zn-Mn relationship a direct proportionally increase of Mn concentration was observed with the increased Zn administration.

In Table 2 there are presented the concentrations of Al, Cr and Pb in renal tissue after zinc administration

Table 2. Concentration of metals with toxicogene potential in renal tissue after $ZnCl_2$ administration in two different doses

Metals with toxicogene potential	Groups				
	Control (n = 10)	E_1 (n = 10)		E_2 (n = 10)	
	X ± SD	X ± SD	ΔX	X ± SD	ΔX
Al	5.13 ± 1.61	5.24 ± 1.53	+ 0.11	6.22 ± 2.79	+ 1.09
Cr	0.41 ± 0.09	0.45 ± 0.15	+ 0.04	0.59 ± 0.19	+ 0.18
Pb	0.16 ± 0.07	0.17 ± 0.03	+ 0.01	0.74 ± 0.28	+ 0.58

It was observed only a slight increase in the concentration of metals with toxicogene potential after $ZnCl_2$ administration. In case of Al the increase is higher than for Cr and Pb.

In the renal tissue of animals from group E_2 (the administered Zn with higher concentration) the values of Cr and Pb were more increased. This situation might be explained by the existence of some competitive interactions which determine a higher elimination of Cr and Pb.

Studies revealing modifications in the concentration of some metallic elements in renal tissue are of importance because they can offer details on the storage of these metallic elements and pleads for the activation of the eliminative process (Mills, 1985; Mertz, 1987; Garban, 1996). Further studies in this domain could offer information with toxicological utility, too.

CONCLUSIONS

1. Excedentary zinc administration - higher doses than RDI - modify the concentration of Zn, Cu and Mn (biometals) as well as the concentration of Al, Cr and Pb (metallic elements with toxicogene potential) in the renal tissue.

2. The concentration of renal Zn and Cu content decrease while the Mn concentration increase in E_1 and E_2 groups.

3. Metals with toxicogene potential as Al, Cr and Pb show a decrease in the renal tissue of the E_1 and E_2 groups. These dyshomeostasic effects induced by zinc suggest the necessity of further researches related to the enhancement of toxicogene effects.

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THE CONCENTRATION OF Ca, Mg, Fe, Cu, Zn, Li, Mn AND Cr IN SOME FOODS IN TRANSYLVANIA AREA

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ABSTRACT

The intake of minerals varies widely both qualitatively and quantitatively in different geographical areas. This paper aims to determine the concentrations of Ca, Mg, Fe, Zn, Li, Mn and Cr in different plant and animal products from diverse areas of Transylvania.These elements were determined by atomic absorption spectro-photometry.

The obtained results are similar to those published by other authors, but Ca, Mg and Fe vales were much lower. The content of trace elements in plants is inflenced by a number of factors. Primarily, they are dependent on the parent rock and the soil- forming process affected by the aspect of availability of the elements toplants in dependence on the pH value, within the complex of action of further elements in soil (e.g. Na, Ca, P) which indicate the formation of linkages of soluble or disoluble forms, futher on the growing season, the factors of climate and the like. The level of macro and trace elements is also specific of the plant speies, cultivar, individual, and it may become affected by anthropogenic lods in direct dependence on the plant's phenophase.

Vegetation time (spring, summer, autumn) the parts of plants and the technical treatment of plant material (chopping, silage, drying) can also change the macro and microelements concentrations.

Key words: metals, foods

INTRODUCTION

The intake of minerals from the environment, through foods, water, air, varies widely both qualitatively and quantitatively in the different geographical areas of the world, and is not always adequate for the needs of the organism. Some possible causes are different nutrition habits, changes in the mineral contents in foods. Metals and other elements can be present in food for a number of reasons – for example, due to their natural presence in the environment, as a consequence of agricultural practice (e.g. application of fertilizers) and human activities or as a result of industrial activities (Anke and Groppel, 1987; Anke et al., 1984; 1986; MAFF, 1988). This paper aimes to determine the concentrations of Ca, Mg, Fe, Cu, Zn, Li, Mn and Cr in plant and animal products from different areas of Transylvania in order to ensure that these concentrations do not pose a risk to the consumers' health.

MATERIAL AND METHODS

Edible plant samples (54 onion samples, 54 carrot samples, 60 dried wite beans samples, 50 spinach samples, 56 tomato samples, 46 radish samples, 45 cabbage samples, 50 potato samples); cereal-based products (48 white lour samples, 52 maize lour samples and 50 bread samples); and animal products (42 cow milk samples, 50 meat samples) were submitted to laboratory

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determinations. The vegetable and animal samples were taken from individual households, markets and other commercial establishments.

Edible vegetables were harvested depending on the season in which the mature plant appeared.

In the case of vegetable samples, mineral dosage was performed using washed and dried vegetable tissue in oder to evaluate the degree of retention of macro- and microelements in the plant.

Food samples, dried in the oven at 105^0 C for 24 hours were submitted to wet digestin by HNO_3/ $HClO_3$ mixture.

Ca, Mg, Fe, Cu, Yn, Li, Mn, and Cr determination was performed by atomic absorption spectrophotometry using a Perkin Elmer spectrophotometer, with atomization in the oven. The results were expressed in ppm fresh weight.

RESULTS AND DISCUSSIONS

The results obtained (arithmetic media, median) have been shown in for Ca, Mg and Fe in table 1.

Table 1. Median and arithmetic mean for Ca, Mg and Fe

Food	No.	Ca		Mg		Fe	
		Median	Arthmetic mean	Median	Arthmetic mean	Median	Arthmetic mean
Lettuce	47	496.10	612.75	234.67	258.82	12.03	14.45
Spinach	50	734.25	821.53	203.68	281.42	6.96	8.43
Garlic	32	285.10	322.75	157.30	191.26	14.86	16.20
Green Onion	54	398.44	487.40	352.47	360.70	15.26	16.40
Radish	46	102.34	117.50	38.05	42.30	1.85	2.34
Potatoes	50	358.71	436.04	202.89	266.84	24.45	26.84
Carrots	54	398.40	431.79	157.62	169.01	11.06	12.44
Dried White Beans	60	872.47	1050.24	650.90	728.01	82.78	91.10
Maize Flour	52	1190.23	13361.12	497.81	577.19	410.07	436.50
White flour	48	317.02	353.55	750.26	769.04	112.23	119.33
Bread	50	684.89	815.10	402.66	494.69	202.80	219.90
Tomatoes	56	229.70	270.44	177.02	185.72	11.06	12.30
Cabbage	45	598.20	624.12	290.11	312.41	7.52	8.70

In oder to compare the results obtained by us, we present below Ca, Mg and Fe content of vegetables according to the Food Composition Tables Published by I. Gontea (1971) and Geigy Scientific Tables 1981 (Table 2).

Table 2. The mean Ca, Mg and Fe contents
(according to Gontea, Geigy Scientific Tables 1981 and M. Anke)

Vegetables	Ca (ppm)		Mg (ppm)		Fe (ppm)		
	1.	2.	1.	2.	1.	2.	3.
Potatoes	150	400	350	560	2	18	77
Green onion	400	270	300	80	10	5	40
Carrots	400	370	150	210	7	7	150
Radish	400	300	200	150	15	10	
Lettuce	250	350	400	110	10	20	
Spinach	810	1060	570	620	30	31	
Dried beans		770		1590		63	
Cabbage		460		230		5	
Tomatoes		130		110		6	36
Garlic		380		360		14	

For the majority of vegetables, our results are similar to those published by Gontea and Geigy. In the of same vegetables, differences may be found, for example we recorded much lower mean Ca, Mg and Fe values than those published by the above authors.

Also, there are differences in Ca, Mg and Fe concentrations in other vegetables as well, differences which may be partly due to cultivar, culture practice, soil,m maturity, storage, area of growth, location factors and handling effects. Mean Cu and Zn concentration values in the vegetable and animal products investigated by us are shown in Table 3. For comparison, we present in Table 4 the data provided by Food Composition Tables according to Geigy Scientific Tables (1981) and with T. Zawadzka (1989).

Table 3. Copper and zinc contents in vegetable and in animal products (ppm)

Food	No of samples	Cu		Zn	
		Median	Arthmetic mean	Median	Arthmetic mean
Lettuce	47	0,62	080	6,87	7,00
Spinach	50	1,96	2,30	4,53	5,10
Garlic	32	0,75	0,87	4,21	4,80
Green Onion	54	0,72	0,80	0,73	0,78
Radish	46	1,47	1,60	4,67	5,80
Potatoes	50	1,86	2,10	9,82	10,20
Carrots	54	1,19	1,20	15,78	17,30
Dried White Beans	60	11,78	12,40	20,26	22,70
Maize Flour	52	0,66	0,69	5,69	6,50
White flour	48	0,41	0,50	7,46	8,40
Bread	50	1,32	1,44	5,07	5,80
Tomatoes	56	1,37	1,50	14,63	16,80
Cabbage	45	0,64	0,66	2,55	3,70
Cow milk	42	0,53	0,55	3,62	4,2
Pork meat	50	1,04	1,20	20,1	21,4

Table 4. The mean Cu and Zn contens in ppm
(according to Geigy Scientific Tables, Krause and Zawadzka)

Vegetables	Cu (ppm)			Zn (ppm)	
	1.	2.	3.	1.	2.
Potatoes	1,6	3,8	0,73	8,7	3,33
Green onion	1,3			0,9	
Carrots	0,8	6,8	0,45	5,2	2,92
Radish	1,3		0,24	1,6	2,23,319
Lettuce	0,7	10,9	0,46	1,6	
Spinach	0,7			2,0	
Dried beans	8,4	8,8		28,0	2,24
Cabbage	0,6	2,3	0,25	1,8	1,49
Tomatoes	1,0	6,8	0,45	0,6	
Garlic	2,6			5,9	
Maize flour	1,7			77,0	
White flour	2,0			25,0	
Bread	1,7	2,0		8,0	
Cow milk	0,1	1,8		3,8	
Pork meat	1,3	2,3		19,0	

The lowest mean Cu concentrations were foud in white flour and milk samples, and the highest mean values in drid white beans.

We found the lowest mean Zn values in green onion samples and the highest ones in dried white beans, this being a good source of dietary Zn.

The necessary daily amount established by US Food Nutritional Board and OMS is 2-4 mg Cu for adults and 0,05 mg/kg body weight for children (OMS, 1973).

It is known that a unilateral diet with cereal-based products and milk can lead to the appearance of anemia due to a low Cu Intake (Gonțea, 1971; Mărgineanu et al., 1984). Human dietary intake of Zn has been reported to be 10-15 mg/day (OMS, 1973).

A diet based on red meat, chlorophyll-rich plants (spinach, lettuce), beans, cereal flour, potatoes, carrots represent according to our results important Zn sources.

The mean Mn, Li and Cr contents expressed in ppm wet weight in the studied vegetables and animal products are listed in table 5.

The highest mean Mn concentration was found in dried beans samples, and the lowest mean values in green onion.

If we accept that the necessary Mn intake is for adults 3-5 mg/day (Tomas JW. 1970) or 6-10 mg/day (1), it follows that the majority of foods that are crrently consumed by man and were analyzed by us are than sufficient to meet these requirements.

Human dietary intake of chromium has been reported to be 24-47 □g/day (Bunker et. al, 1984). The lowest mean Cr values were found in white flour and maize flour samples and the highest contents in dried beans and meat samples. Meat, potatoes, bread and flour apparently provide appreciable dietary amounts, with 100 g (fresh weight) covering the safe and adequate allowance recommended by the National Academy of Sciences (1980).

Table 5: The Mn, Cr and Li content of several vegetables and animal products in ppm wet weight

Food	No.	Mn		Cr		Li	
		Median	Arthmetic mean	Median	Arthmetic mean	Median	Arthmetic mean
Lettuce	47	4,94	6,77	1,42	1,50	0,20	0,285
Spinach	50	5,98	6,33	0,31	0,37	0,78	0,863
Garlic	32	1,72	1,80	0,18	0,27	0,04	0,039
Green Onion	54	0,41	0,46	0,73	0,75	0,007	0,009
Radish	46	0,70	0,78	0,86	0,90	0,007	0,008
Potatoes	50	1,22	1,52	1,19	1,20	0,004	0,005
Carrots	54	1,02	1,09	0,18	0,29	0,009	0,010
Dried White Beans	60	19,03	20,43	1,58	1,70	0,021	0,020
Maize Flour	52	4,90	5,54	0,11	0,14	0,020	0,022
White Flour	48	5,67	6,87	0,09	0,18	0,020	0,024
Bread	50	6,71	7,62	0,76	0,81	0,42	0,050
Tomatoes	56	5,02	5,46	0,20	0,22	0,028	0,030
Cabbage	45	5,70	6,91	0,53	0,55	0,040	0,050
Cow milk	42	0,39	0,40	0,86	0,95	0,012	0,015
Pork meat	50	1,00	1,29	1,50	1,72	0,043	0,050

We recorded the lowest mean Li concentrations in potatoes and the highest ones in spinach. Li content is content is strongly influenced by geological origin of the surface on which plants develop as well as by the plant species. Li content of foods in Germany, Belgium, Hungary, Bulgaria and Czech Republic varies between 1-40 mg /kg dry weight, depending on the species and soil. According to Hullien and Li contents in potatoes are 0.07- 0.28 ppm.

Our results are comparable to those of other investigators.

CONCLUSIONS

The area of the sources and requirements of macro- and microelements remains open to research, as there are differences between the values published by different authors.

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EXPERIMENTAL STUDY CONCERNING THE CHRONIC EFFECTS OF ALUMINIUM FROM DRINKING WATER ON WHITE RATS

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ABSTRACT

Biochemical, enzymatical, histological, electrophysiological effects of the cronic oral aluminium administration have been studied in rats. The experiment was made on 45 male Wistar rats divided in 3 equal groups: a central group and 2 other groups that received doses of aluminium 0,125 mg/Kg body and 1,25 mg/Kg body respectively orally, enclosed in food daily, for 90 days. The maximal values of the aluminium medium concentration in different surface sources from est Romania territory treated with aluminium sulphate was determinated: alteration of the general toxicity indicators, of some biochimical indicators, histopathological alterations of the brain, liver, kidney. We obtained data that evidence intraneuronal lipofuscins in brain cells of the both doses of aluminum sulphate administrated and individual variability of lipofuscins accumulation. Our results confirm the accumulation of malonic dialdehyde and the implication of the free radicals. The data revealed neuromuscular and thermoalgezic sensibility alteration and also of the vassels permeability in rats in experimental conditions.

Keywords: water, aluminium, toxicity.

INTRODUCTION

Although aluminium is not an essential element for mammals, it is present naturally in most foods and is widely used in various non prescription drugs, especially some antacids, buffered aspirins, antidiarrhoeal products, as well as Al-containing phosphate binders to prevent hyperphosphatemia in pacients with predialysis chronic renal failure (Domingo 1993; Greger 1993). On the other hand, Al sulfate is extensively added as a coagulant agent during the drinking water – purification processes in order to flocculate the organic matter and so clarify the water (Domingo 1993; Greger 1993).

It has become generally accepted that Al^{3+} may cause intoxication and disease in some rather artificial situations, e.g., chronic hemodialysis and havy occupational exposure to Al-fumes (welding) or dust of soluble Al^{3+} compounds.

Previous research regarding the concentration of Al in drinking water of different surface sources from lassy territory where there are put in evidence frequently, medium maximal concentration of 0,35 mg/l (C.M.A.= 0,05 mg/l and exceptional concentration of 0,2 mg/l).

This chronic experiment made on Wistar rats, to which was orally given (in the water together with the food) aluminum sulfate, had a purpose the research of the effects resulted from the average maximal concentration of remaining

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aluminum in the analyzed drinking water (3,5 mg/dm^3).

We have considered that this chronic experiment, with doses of aluminium sulphate calculated in relation with animal body weight or normal exposure frequency (human-animal) at the drinking water, was imposed.

MATERIALS AND METHODS

The experiment was made on 45 male Wistar rats divided in 3 equal groups: a central group and 2 other groups that received doses of aluminium 0,125 mg/Kg body and 1,2 mg/Kg body respectively orally, enclosed in food, daily for 90 days.

This study hasn't had as an objective to establish the lethal toxic dose of the aluminum sulfate orally administered (in the water) to the rat. By reporting the doses administered to the lots exposed for three months to DL_{50}=730 mg Al/kg (established by Llobet, 1987), dose 1 = 1/584, dose 2 = 1/58.4) thus the administered doses were relatively small. (WHO 1996)

The histopathological study was made on brain, kidney, spleen and liver. The specimens obtained were fixed in buffer formalin 10%, enclosed in paraffine, sectioned at 4 μ thickness and stained with PAS and Schmorl.(Shin, 1995)

GSH (reduced glutation) were determined in blood, retina and crystallin lens; SH – groups and TBARS (thiobarbituric acid-reagible substances) were determined in serum and homogenates (liver, retina, crystalline lens); SOD in erytrocytes; glutation peroxidases in serum. (Evens, 1990; Palmer, 1997)

EPI- INFO program was used for statistic analysis. (Jaba, 1995)

RESULTS AND DISCUSSIONS

By studying the indicators for general toxicity (mortality, body weight, organ coefficient) we have found ascertained: zero mortality for the animals in the control, 6,60% for those from the lot no.1 and 13,33% for those from the lot no.2; animals weight and organ coefficient had a statistic significant modification in the lots 1 and 2 compared to the control. The changes in the weight of the studied organs: brain, liver and kidneys draws the attention on their sensibility at aluminum.

Aluminum chronic administered in the water as sulfate has determined the appearance of an oxidative stress in the blood, liver, retina and crystalline lens, biochemically supported by the increase of MDA (marker of lipidic peroxidation) while the livel of SH groups in the liver, retina and crystalline lens (markers of oxidative affectation of the proteins) and the concentration of GHS in the blood, retina and crystalline lens (a major antioxidant) are low. (Evens, 1990)

At a cerebral level (retina and crystalline lens), the oxidative stress was biochemical emphasized by histopathological changes and by the presence of lipofuscin (corresponding to lysosomal accumulation of MDA) and has increased at the same time with the dose of administered aluminum. Specific coloration with eriocromcyanin R emphasizes the presence of *Al* at an encephalic level.

At a hepatic level, lipidic peroxidation (biochemical emphasized by MDA increase) and proteins oxidation (biochemical emphasized by a decrease in SH groups) have determined to appear lesions of lipidic and protidic degeneration pointed out at the histochemical examination and they have increased at the same time as the administered dose of *Al*.

In the blood, lipidic peroxidation (emphasized by MDA) had a significant statistic increase when the administered dose of *Al* was also increased, white proteins oxidation, emphasized by an GSH statistic significant decrease in lots 1 and 2 compared to control, hasn't recorded any differences depending on the administered dose of Al.

When some enzymatic systems of antioxidant defense were followed, during the oral administration of *Al* as a sulfate trough the drinking water, they have revealed the complexity of the processes through while this xenobiotic is manifesting its toxicity on the organism. We have ascertained different reactions regarding body's effort to counteract the oxidative reactions provoked by the aluminum: after three months of administering a dose of 0,125 mg Al/body kg/day, the activation of protection mechanisms is only supported by the increase of serum glutation peroxidase (inductable enzyme) while GSH in the blood and erythrocyte's SOD have recorded concentrations below those determined for the control; after three month of administering a dose of 1,25 mg Al/body kg/day, the investigated systems that are taking part in the antioxidant defense are found at lower levels compared to those of the control animals, this affectation being a kind of "green signal" for *Al* toxic reaction. The indicators determined from the antioxidant defense system had a lower concentration in the lot 2 compared to the lot 1.

Oxidative stress (lipidic proxidation and protidic oxidation) is also important for a smaller dose (0,125 mg Al/body kg/day – 10 times maximum concentration recorded for the investigated surface waters). This affirmation is supported by the fact that lipofuscins was emphasized in the brain sections of the rats in the lot 1 at a level comparable to that emphasized in the brain sections of rats in lot 2.

Affectation of proteic metabolism is also sustained by the changes in the following indicators: lowering in Hb concentration for lots 1 and 2 compared to control, a significant decrease depending also on the dose; a statistic significant decrease in the serumal proteins concentration, depending on the administered dose of *Al*; the increase in seric GGT (biomarker of hepatic disfuntionality); its concentration in the serum had a statistic significant increase in the lot 2 compared to control.

As a result of hepatic affectation, TGP (an enzyme of exclusivity hepatic origin) had a statistic significant decrease in lots 1 and 2 compared to control, without being recorded any differences between the two administered doses of *Al*.

Affectation of lipidic metabolism was also reflected by the statistic significant modification in the concentration of triglycerides in lots 1 and 2 compared to the control (but not between lots 1 and 2) and in cholesterol concentration for the lot 2 compared to the control.

Histopathological aspects that were emphasized come to complete and support the biochemical data that were obtained.

Because some of the morphopathological aspects were also noticed on the specimen of control animals, the changes reported on the sections obtained in this experiment with aluminum sulfate, are loosing specificity. Further more, doses are to small to emphasize the toxicity of Al ion or its effects.

Despite all this, we can underline the existence of some parenchymal and mesenchymal lesions in the vital organs that were studied (kidneis, liver, brain) with individual variations, lesions that are alike for both doses, but with a more striking note for the dose of 1,25 mg/body kg of aluminum sulfate.

Aluminum disturbs proteic metabolism (degenerative lesions), influences vascular-circulatory processes (congestive lesions in liver and kidney), inhibits proliferative processes (the absence of reactional processes in the spleen), and the regenerative processes are rare (few binuclear hepatocyte).

Aluminum sulfate administered to the white rat under experimental conditions, induced the appearance of lipofuscins in the neurons for both exposed lots (at the two administered doses) without being able to strictly differentiate according to the dose, individual variations being pointed out.

Due to existing relations between lipofuscins and lipidic peroxidation (trough its marker MDA) (Tokutakes 1995) it is supported the implication of free radicals in the conditions of the experiment. The increase in MDA concentration for the lot 1 (which has received 0,125 mg Al/body kg/day, meaning 0,031 mg Al sulfate/body kg/day) statistic significant, and for the lot 2 (it has received 1,2 mg/body kg/day, meaning 0,31 mg Al sulfate /body kg/day) is thus reflected through the positive and very intense coloration for lipofuscins of some layers from the cortex and cerebellum.

Qualitative determination of aluminum in the target organs for aluminum according to the data in the literature (liver, kidneys, brain, cord) (WHO 1996) revealed: in the control, which wasn't given aluminum orally through drinking water, we have found no positive section at the coloration with solocrom. We can say that histochemically wasn't evidentiated any impregnation with Al for this lot; this fact has allowed us to compare lots 1 and 2 with control.

Our study reveals a special affinity of Al for the brain and endothelial cell.

Brain presented for the lots 1 and 2 individual variations and depending on each section at the coloration with solocrom. It was histochemically evidentiated the presence of *Al* at the level of cerebellum (in the nuclei of Purkinje cells) directly proportional with the administered dose of *Al* also in concordance with the presence of lipofuscins at this level; in the cortex the impregnation with *Al* was better marked at the lot 2, in some sections being obvious the impregnation with *Al* of the endothelium.

Heart and liver presented *Al* impregnation histochemically evidentiated through coloration with solocrom (more than 8μg/tissue g) only at the level of the erythrocite, both in lot 1 and lot 2, but more intense in lot 2.

Kidney presented *Al* impregnation histochemically evidentiated through coloration with solocrom (more than 8μg/tissue g) at the level of erythrocite, both in lot 1 and in lot 2 (generally more intense in lot 2) and in the endothelium.

The study has revealed individual variations of impregnation of the renal tubular epithelium.

Aluminium administrated in thouse conditions encreses endothelium vasoconstrictory response to the oxidative stress. Biphasic complex responses result from the potentiation of the oxidative stress on endothelial cell in the second group of rats. This finding is correlated with the histopathologycal aspect of endothelial and perivascular oedema and the presence of aluminium inside vessels wall (evidentiated with Eriocromcyanin R).

The causes of this "functional ageing" consecutive to aluminium impregnation of endothelial cell are to be revealed by enzymatic and morphofunctional studies.

CONCLUSIONS

Aluminium is known today as a toxic element (WHO 1996), fact that requires to establish only CMA in the standard of drinking water, toxic substances do not beneficiate from the exceptionally admitted concentration.

Maximal concentration admitted (0,05 mg/dm^3 for *Al*) stipulated by the drinking water standard in force seems to be very difficult to follow if we consider the way surface waters are treated (aluminum sulfate is being used as a coagulant) with methods similar to those from the analyzed plants.

Maximal Average Concentrations for Al determined in the water from surface sources treated with aluminum sulfate as a coagulant have produced changes in the parameters for general toxicity, in some biochemical and enzymatic indicators, histopatological lesions, changes in neuromuscular sensibility, thermoalgezics and vascular permeability at the white rat under experimental conditions.

The qualitative determination of the impregnation with aluminum in the target organs, emphasized a particular affinity of the aluminum for the brain cell and endothelial cell.

We consider as being useful to improve the methodologies researching the toxicity of some chemical substances whitin experimental or demographical studies by considering the bioxicologic parameters that characterize the antioxidant defense. Through this approach can be emphasized in a more realistic manner the toxic effects of the environmental polluants; in the case of exposure to small or moderate doses, toxic effects do not always show, but the body makes an effort to adapt, effort characterized by the activity of this defense system.

There are necessary further studies to determine toxic-kinetic effects under the conditions of reducing the renal function.

These studies will have to mention the retention of aluminum end its toxicity in the incipient phases of kidney function decrease.

There are also necessary further studies to determine wheaten the aluminum in the drinking water has a greater biodispponibility then the aluminum coming from other sources (food), a special attention being required to evidentiate the factors that change its absorption by the body.

Further studies with radionuclid ^{79}Al can bring important news.

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ESTIMATED AVERAGE FOOD INTAKE OF ALUMINIUM

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ABSTRACT

Considerable evidence supports the idea that aluminium is an essential neurotoxic cofactor in the development of the neurodegenerative processes. Since aluminium is a major component of the earth's crust, it is naturally present in varying amounts in most foodstuffs consumed. The actual concentration in food from various territory will vary widely depending upon the food product, the type of processing used and, in particular, the level of the geographical area in which food crops are grown. In general, the foods highest in aluminium are those than contain aluminium additives. The aluminium content of a variety of vegetals (salad, onion, garlic, radish, carrot, parsley) and animals (milk, meat) food was measured using eriochromcyanine R spectroscopy method. Aluminium concentration ranged from 0,009 mg% in sausagees samples to 0,19 mg% in salad samples. The medium aluminium concentration was higher in vegetables food samples (in leafs-0,14 mg%). For animals food samples (mean aluminium concentration 0,24mg%) was higher in milk.

Keywords: method, aluminium, concentration, and food.

INTRODUCTION

Considerable evidence supports the idea that aluminium is an essential neurotoxic cofactor in the development of the neurodegenerative processes (Evans et al. 1990; Flaten et al.1996; Golub et al. 1992). Since aluminium is a major component of the earth`s crust, it is naturally present in varying amounts in most foodstuffs consumed.

The actual concentration in food from various territory will vary widely depending upon the food product, the type of processing used and, in particular, the level of the geographical area in which food crops are grown. In general, the foods highest in aluminium are those than contain aluminium additives. (e.g., grain products (flour) processed dairy products, infant formulae, etc.) (Duggan, 1992; Gargominz and Astier, 1995).

We recognized today aluminum as a toxic element, the aluminum concentration in the medium factor also in the aliments (foods) it is very important parameter. His toxicity is a certain characteristic, and it is also recognized in unanimity as a bad factor for heath especially in the conditions of a urology dysfunction.

It is a major component of the ground; aluminum is present in a natural way, in many aliments, in function of the research area, so we proposed to determinate the aluminum concentration from the aliments of vegetal and animal origin.

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MATERIALS AND METHODS

We determinate the content of aluminum in 30 samples, from animal and vegetal alimentary products. For the determination we realized on aliment samples, a digestion in wet medium, and than we us UV-VIS spectroscopy determination, based on the reactivity between aluminum and the eriochromcyanin R. We used an adapted form of the STAS 6326-90.

The Principle of the method

The aluminum ions made with the eriochromcyanin R, only on a pH = 6.0 and complex colored pink- red, at which we measured the absorption at a wavelength λ =535 nm.

We used:

Devices and materials:

- UV-VIS Spectroscopy Jasco V550l
- Reagents: Nitric acid concentrate; Hydrochloric acid d = 1.19; Sulfuric acid 98%; Sulfuric acid 1N; Sulfuric acid 0.01 M; Sodium hydroxide solution 2N; phenolphthalein indicator solution; Ascorbic acid solution 0.1 %.; Acetate blotting solution; Eriochromcyanin R, solution

We doing an etalon scale in 8 tubes and after 15' we read the extinctions at the UV – VIS spectroscopy face of a mark tube, and we obtain an etalon curve which help us to read the concentration in the samples.

The samples are digested in a water bath in the presence of nitric acid 70 %, 10 ml and 5 ml sulfuric acid 98%, using 10 g analyzing product. From the analyzing solution we take 10 ml sample, and we working on it like in the method indicate. We drawing the gauge curve, and after we read the extinctions at the UV-VIS spectroscopy we calculated the concentration in [mg Al^{3+}/ mg products].

RESULTS AND DISCUSSION

The vegetal foods that we analyzed we have 8 sortiments of vegetables: lettuce, onion, and cabbage, green garlic, radish, carrots, parsley's leaves and roots.

- The medium concentration of the aluminum in the vegetal samples was 0.1364 mg %
- The biggest concentration was determinate in the green lettuce 0.1968 mg %, and in green onion 0.1952 mg %, and the smallest in carrot's roots 0.0882 mg %, and in radish 0.898 mg %.
- In the salad samples the aluminum concentration is between 0.1572 mg% – 0.1968 mg % with and medium of 0.1754 mg %, and in onion samples the variation is between 0.1263 mg% – 0.1952 mg%, in medium 0.1633 mg%.

In the animal foods at which we determineted the aluminum concentration was: milk, and meat products, 5 assortments of sausage,

- The medium concentration of aluminum in the meat samples was 0.0241 mg %
- The biggest concentrations was determinate in milk samples, 0.080 mg%, and the smallest are in the Italian sausages 0.0094 mg %
- In the milk samples the aluminum concentration are between 0.032 mg% – 0.08 mg % being in medium 0.203 mg%, and in the sausages samples are 0.0094 mg% – 0.033 mg%, in medium 0.0275 mg %

The small concentration of aluminum determinate in mineral water, cow milk ($< 2 \div 3.5$ μg/kg), and big natural concentration exist in soybean (12 mg/Kg), tea (623 mg/Kg), baked potatoes (skin on), spinach, plum juice (Pennington, 1995).

In general, big concentration of aluminum in foods exist after using admixture with aluminum (aluminum-silicate, antidry, aluminum-phosphate acid as fermenting agent, etc.), in flour, cakes, and their decorations (1,5 g/Kg), different milk assortments for childrens, etc.

The preparation and the storing of foods in several recipients made from aluminum, determinate a big concentration in their content, and specialy when that content is acid (Duggan 1992, Voroniuc 1996).

The preparing of the tomato juice in the aluminum recipient's goes to real increase of the aluminum concentration: to 0.5 mg/Kg in natural juice till to 3.3 mg/Kg in the make one. In that time, the rice prepared, or the potatoes, in the same conditions didn't go to a bigger concentration (Gargominy, 1995).

The aliment's aluminum, which comes from the baking recipients it's a small part from the daily ingestion of aluminum (EHC – WHO 1997) (9).

The total ingestion from foods and beverage (excluding drinking water) in different countries it is showed in table number2, being estimated between 1–20 mg/day, 1 – 13 mg/day (WHO, 1997).

The expert's comity in admixture for foods and beverage FAO/OMS fixed the limit dose in aliments at 0 – 7 mg/Kg weight/week (the equivalent of 60 mg/day for an adult) (ATSDR, 1992).

Crapper Mc Lachlan (1991) recommends a decrease of total ingestion of aluminum, under 3 mg/day.

CONCLUSIONS

- The medium concentration in aluminum was bigger in the vegetal samples: in that one was around 0.1364 mg%, and in animal samples was 0.0275 mg%)
- The aluminum concentration was in general bigger in leaves than in the roots: the biggest concentration was determinate in green lettuce 0.1968 and in green onion 0.1952, and the smallest are in carrot's roots, and radish 0.898 mg%.
- For the animal products, the biggest concentration was in milk 0.080 and the smallest in Italian sausages, 0.0094 mg%.
- For the milk samples we have great difference in function of the producing unit, the concentration of it was bigger in the samples from S.C. Agroind. Bucium comparing with S.C. P.C.B. Dancu and S.C. Agrocom Strunga.
- For the meat products we don't registered any important difference which cosigned the aluminum concentration function of the producing unit.
- The normal daily aluminum intake among Western Europeans is less than 10 mg/day (370 umol/day) but Crapper Mc Lachlan and colleagues recommended 3mg or less for daily) intake (about 1/6 of the adult average daily intake and Al concentration in processed water should be less than 50 μg/l) (7,9)

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SCIENTIFIC EVENTS

AWARD OF DOCTOR HONORIS CAUSA TITLE

On the day of April 18th 2000 at the University of Agricultural Sciences and Veterinary Medicine of Banat Timişoara was awarded the title Dr. Honoris Causa to

Prof. Dr. Manfred ANKE
Faculty of Biology and Pharmacy
University "Friedrich Schiller" Jena - Germany.

The title was granted by the decision of the Senate of University of Agricultural Sciences and Veterinary Medicine of Banat Timişoara from March 14th , 2000 with the following mention:

"For the prestigious scientific activity and for outstanding merits in promoting and developing Roman-German interuniversity relations of co-operation."

At this ceremony, Prof. Dr. Manfred Anke presented a dissertation on the topics: "The Nickel Intake of Humans in Europe – Risks and Problems."

Prof. Dr.h.c. Manfred Anke -dissertation

The award of the Dr. h.c. title of the University of Agricultural Sciences and Veterinary Medicine of Banat Timişoara was preceeded by a "Laudatio" in the honour of Prof. Dr. Manfred Anke.

The Commission constituted for this occasion was made of:

President: *Acad. Păun Ion Otiman* – Rector of University of Agricultural Sciences and Veterinary Medicine of Banat Timişoara

Members: *Prof. Dr. Petru Drăgan* – University Clinic of Urology, Faculty of Medicine, University of Medicine and Pharmacy Timişoara

Prof. Dr. Zeno Gârban – Department of Biochemistry and Molecular Biology, Faculty of Food Products Technology, University of Agricultural Sciences and Veterinary Medicine of Banat Timişoara

Prof. Dr. Ing. Ilie Julean – Department of Analytical Chemistry, Faculty of Industrial Chemistry and Environmental Engineering, University Politehnica Timişoara

Prof. Dr. Ing. Aurel Lăzurean – Department of Agrotechnical Sciences, Faculty of Agronomy, University of Agricultural Sciences and Veterinary Medicine of Banat Timişoara

At the festivity took part members of the Roumanian Academy, of the Academy of Agricultural Sciences and Forestry as well as members of the Academy of Medical Sciences. A large university staff and students took part. The invitation at Doctor Honoris Causa award ceremony is given on page 347.

A comprehensive presentation of the personality of the distinguished Prof. Dr. Manfred Anke is given below.

Prof. Dr. Manfred Anke was born on September 26, 1931 in Altenheim, Saxony – Germany. He graduated highschool in Chemnitz.

Studies continued at "Friedrich – Schiller" University Jena, Faculty of Agriculture and subsequently at the Faculty of Chemistry.

In 1959 doctorate in Agricultural Chemistry with the theme: "Untersuchungen über den Spurenelementgehalt (Fe, Mn, Cu, Mo, Co) der Grünland- und Ackerfutterpflanzen verschiedener Bodenarten sowie Maβnahmen zue Erkennung und Verhütung von Mangelerscheinungen bei Milchkühen".

In 1965 qualification as a University lecturer with the thesis: „Der Mengen- und Spurenelementgehalt des Rinderhaares als Indikator der Calcium-, Magnesium-, Phosphor-, Kalium-, Eisen-, Zink-, Mnagan-, Kupfer-, Molybdän- und Kobaltversorgung".

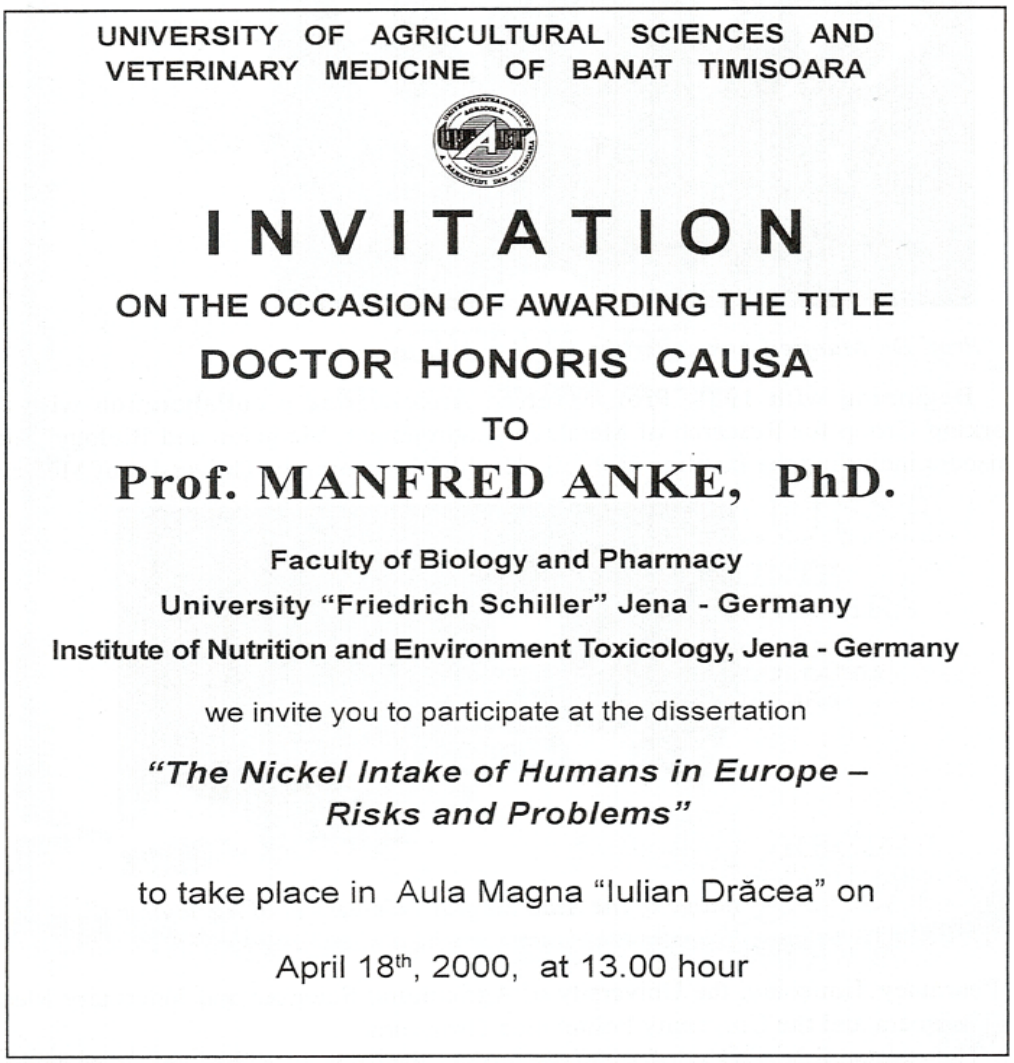
He passes all university degrees, becoming in 1990 professor at the Faculty of Biology and Pharmacy at Friedrich Schiller University Jena.

Prof. Manfred Anke published numerous articles and chapters in over 20 volumes with the topics metals in animal and man food, trace element supply, the role of metals in metabolism, toxic effects, implications of metal deficiencies in pathological states in man and animal, ecotoxicology of macro- and trace elements.

He collaborated with prestigious publishing houses such as: Rawn Press – New York; Academic Press; Springer Verlag – Berlin, Heidelberg; Marcel Dekker Ink. New York etc.

All the scientific activity of Prof. Dr. Manfred Anke comprises over 850 papers published in various periodicals, proceedings volumes of congresses, symposia, conferences and international meetings.

Some referent points of the activity: 1976 member of the Parent Committee "Trace Elements in Man and Animal"; in 1980 nominated honorary professor and starts the Series



UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE OF BANAT TIMISOARA

INVITATION

ON THE OCCASION OF AWARDING THE TITLE

DOCTOR HONORIS CAUSA

TO

Prof. MANFRED ANKE, PhD.

Faculty of Biology and Pharmacy

University "Friedrich Schiller" Jena - Germany

Institute of Nutrition and Environment Toxicology, Jena - Germany

we invite you to participate at the dissertation

"The Nickel Intake of Humans in Europe – Risks and Problems"

to take place in Aula Magna "Iulian Drăcea" on

April 18th, 2000, at 13.00 hour

Invitation at Doctor honoris causq award ceremony

of International Symposia "Mengen- und Spurenelemente" in Leipzig and Jena (organized annually in the period 1981-1999); 1983 awarded with "Klaus Schwartz" price at the University of California San Diego, USA; since 1990 member of the Iodine Study Group

Bonn; 1990 establishment of the Institute for Nutrition und Environment in Jena; 1993 organization of the World Congress on Trace Elements TEMA-8 in Dresda; 1995 Dr. h.c. of the University of Brno; year 2000 means for the Series of Symposia “Mengen- und Spurenelemente” coordinated by Prof. Dr. Manfred Anke, twenty years of continuity.

Prof. Dr. Manfred Anke after the Dr. H.C. title award

Beginning with 1988, Prof. Manfred Anke started a collaboration with the “Working Group for Research of Metals in Environment, Medicine and Biology” from Timişoara including the Institute of Public Health Timişoara, the University of Medicine

Image of the Aula Magna – University of Agricultural Sciences and Veterinary Medicine Timişoara

and Pharmacy Timişoara, the University of Agricultural Sciences and Veterinary Medicine Timişoara and the University Politehnica Timişoara.

The outstanding activity of the distinguished Prof. Dr. Manfred Anke was accomplished by opening new horizons in the investigation of metal and non-metal compounds in inorganic biochemistry, nutrition (plants, animals and men), pharmacology, toxicology, biochemical pathology a.o.

SENATUL
UNIVERSITATII DE STIINTE AGRICOLE A BANATULUI - TIMISOARA

IN SEDINTA DIN 14 martie 2000
CONFERA DOMNULUI

Prof. Dr. MANFRED ANKE

TITLUL DE
DOCTOR HONORIS CAUSA
AL UNIVERSITATII DE STIINTE AGRICOLE A BANATULUI - TIMISOARA

PENTRU PRESTIGIOASA ACTIVITATE ŞTIINŢIFICĂ ŞI MERITE REMARCABILE ÎN PROMOVAREA ŞI DEZVOLTAREA RELAŢIILOR DE COLABORARE INTERUNIVERSITARĂ GERMANO-ROMÂNE.

TIMISOARA
Nr. 24 / 18 aprilie 2000

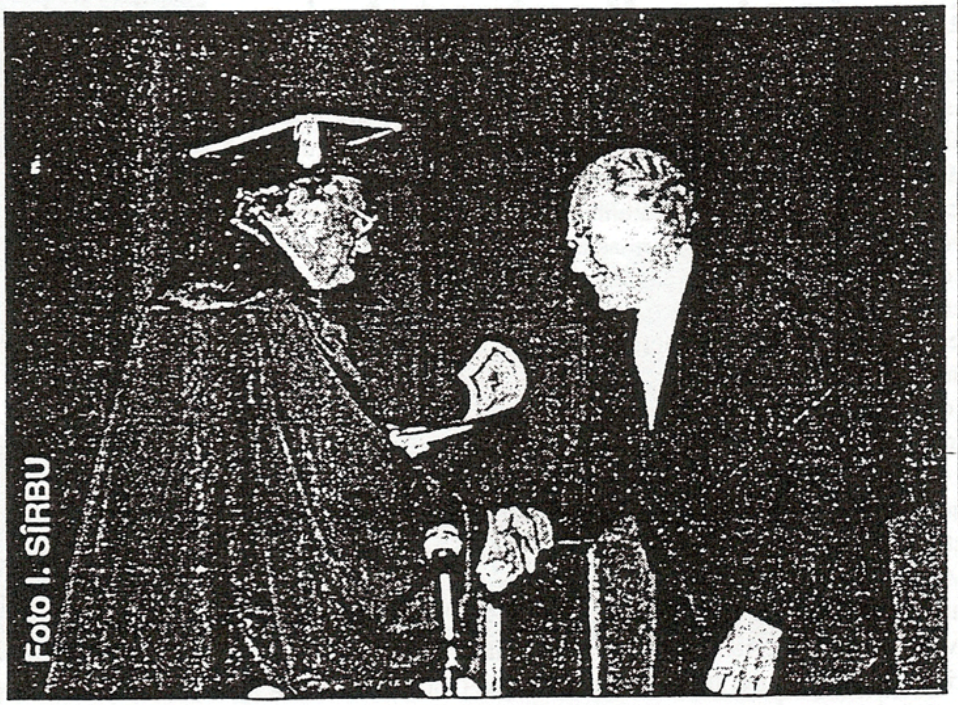
RECTOR
Acad. Păun Ion Otiman

The event presented in local newspaper

Renașterea
BĂNĂȚEANĂ

Profesorul Manfred Anke a fost primit în comunitatea academică a Universității de Științe Agricole a Banatului

În cadrul unei ceremonii care a reunit numeroși studenți și cadre didactice ale Universității de Științe Agricole și Medicină Veterinară a Banatului din Timișoara, domnul prof. univ. dr. Manfred Anke, de



la Facultatea de Biologie și Farmacie a Universității "Friedrich Schiller" Jena — Germania, a fost distins cu titlul de Doctor Honoris Causa al USAMVBT. După ce domnul prof. univ. dr. Gavril Stanciu, prorectorul instituției, i-a înmânat diploma, oaspetele german a susținut disertația cu tema "Rolul nichelului în organismul uman în Europa — riscuri și probleme". Decizia senatului universitar de a-l primi în comunitatea sa academică pe domnul Manfred Anke se datorează, în primul rând, anvergurii științifice europene pe care o probează, dar, mai ales, rolului Domniei sale în promovarea și dezvoltarea relațiilor interuniversitare româno-germane.

LĂCRĂMIOARA URSA

The event presented in local newspapers

Prima oră 3 Miercuri, 19 aprilie 2000

Un doctor honoris causa pentru întreaga comunitate universitară timişoreană

Profesor doctor Manfred Anke

Prezenţa profesorului doctor Manfred Anke în mediul universitar timişorean datează încă din 1988, când a fost integrat "Grupului de lucru pentru cercetarea metalelor în mediu, medicină şi biologie", din care mai făceau parte cadre didactice, medici şi cercetători de la Clinica universitară de urologie a Universităţii de Medicină şi Farmacie şi Filiala Timişoara a Academiei de Ştiinţe Medicale. Ulterior, colaborarea lui cu cercetătorii timişoreni s-a extins, incluzând Universitatea de Ştiinţe Agricole şi Medicină Veterinară a Banatului şi Universitatea "Politehnica". Onoarea de a-i acorda titlul de doctor honoris causa i-a revenit conducerii USAMVB, care, în cursul zilei de ieri, în prezenţa reprezentanţilor din cele trei universităţi, a numeroase cadre didactice şi studenţi, i-a înmânat diploma şi însemnele prestigioasei distincţii.

Profesorul Manfred Anke s-a născut la 26 septembrie 1931 în localitatea Alteheim din Saxonia (Germania). A absolvit succesiv facultăţile de agronomie şi de chimie ale Universităţii "Friedrich Schiller" din Jena, unde şi-a început apoi cariera didactică şi de cercetător la Institutul de Nutriţie Animală din acelaşi oraş. Primul doctorat şi l-a susţinut cu teza "Conţinutul în microelemente (Fe, Mn, Cu, Mo, Co) al divereselor plante de pe păşuni şi din culturi, dependent de originea geologică a terenului", iar pe cel de-al doilea, în Filosofia Ştiinţei – domeniul chimiei, cu o teză având ca temă "Conţinutul în macro şi microelemente al părului, ca un indicator al suplimentării cu aceste elemente". Ambele lucrări sunt rodul unor preocupări cărora li s-a consacrat încă din primii ani ai activităţii sale ştiinţifice, şi anume studierii relaţiilor referitoare la macro-, micro- şi ultramicroelemente, interesând, deopotrivă, ştiinţele agricole şi alte domenii interdisciplinare: nutriţia, toxicologia, biochimia, farmacologia, patologia biochimică, fiziopatologia etc. De-a lungul anilor, cercetările prof. dr. Manfred Anke s-au concretizat prin publicarea a peste 950 de lucrări în diverse reviste sau în volumele publicate în urma participării la numeroasele manifestări ştiinţifice internaţionale. A participat, de asemenea, la scrierea a peste 20 de monografii dedicate diferitelor aspecte privind metalele în hrana animalelor şi omului; rolului lor în metabolismul uman şi al altor animale. Prestigiul dobândit de aceste lucrări în lumea ştiinţifică internaţională a fost recunoscut prin includerea lor în volumul "Trace Elements Metabolism in Animals", publicat în 1970 la Londra şi Edinburgh, avându-i ca editori pe reputaţii Mills şi Livingstone. Din 1976, dr. Manfred Anke este membru al Comitetului Internaţional de Studiu al Microelementelor la om şi animale, iar în 1995 i s-a decernat titlul de doctor honoris causa al Universităţii din Brno. Prin acordarea aceluiaşi titlu de către USAMVB prestigioasei sale personalităţi, întreaga comunitate ştiinţifică timişoreană a demonstrat încă o dată că ştie să preţuiască valorile şi, în acest fel, să dovedească că este ea însăşi o şcoală de prestigiu internaţional.

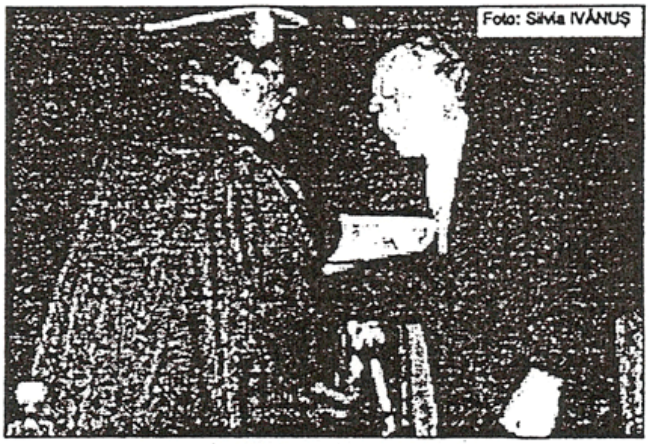
ION DANCEA

The event presented in local newspapers

Cultură TIMIȘOARA

Un profesor german - Doctor Honoris Causa al USABT

Ieri, în Aula Magna a Universității de Științe Agricole și Medicină Veterinară a Banatului a avut loc decernarea titlului de Doctor Honoris Causa domnului Manfred Anke, profesor doctor la Facultatea de Biologie și Farmacie din cadrul Universității "Friedrich Schiller" Jena - Germania și reprezentant al Institutului de Nutriție și Toxicologia Mediului, din Jena - Germania.



Născut la 26 septembrie 1931 în Saxonia, Manfred Anke a devenit lector universitar în 1965, dovedindu-se a fi un pasionat al cercetărilor legate de macro, micro și ultramicro elemente și rolul lor metabolismul uman și animal. Având la activ peste 950 de lucrări de specialitate publicate în mai multe țări, profesorul Manfred Anke a început colaborarea cu universitățile timișorene în 1988, mai întâi cu Universitatea de Medicină și Farmacie, iar ulterior și cu Universitatea Politehnica și USABT. După decernarea titlului de Doctor Honoris Causa, Manfred Anke a susținut o dizertație pe tema: "The Nickel Intake of Humans in Europe - Risks and Problems".

Smaranda PUIU

RETROSPECTIVES

SERIES OF INTERNATIONAL SYMPOSIA UNDER THE AUSPICES OF ROUMANIAN ACADEMY – BRANCH TIMIŞOARA WITH THE THEME "METAL ELEMENTS IN ENVIROMENT, MEDICINE AND BIOLOGY" [M.E.E.M.B.]

1. **"Metal Elements in Environment, Medicine and Biology" – Tome I**
   (Editors Drăgan Petru, Gârban Zeno)

   Foreword by Prof. Mihai Şerban, PhD – corresponding member of Roumanian Academy, president of Comission of Biochemistry of Roumanian Academy Bucureşti

   Proceedings of 1st International Symposium, October 21-23, 1993
   Organized under the auspices of Roumanian Academy – Branch Timişoara with collaboration of University of Medicine and Pharmacy Timişoara, University of Agricultural Sciences and Veterinary Medicine Timişoara

   Publishing House "Mirton" Timişoara (First Edition – 1994, Second Edition – 1995)

2. **"Metal Elements in Environment, Medicine and Biology" – Tome II**
   (Editors Gârban Zeno, Drăgan Petru)

   Foreword by Prof. Ionel Haiduc, PhD – vicepresident of Roumanian Academy Bucureşti

   Proceedings of 2nd International Symposium, October 27-29, 1996
   Organized under the auspicies of Roumanian Academy – Branch Timişoara and Comission of Biochemistry of Roumanian Academy Bucureşti

   Publishing House "Eurobit" Timişoara (First Edition – 1996, Second Edition – 1997)

3. **"Metal Elements in Environment, Medicine and Biology" – Tome III**
   (Editors Gârban Zeno, Drăgan Petru)

   Foreword by Prof. Toma Dordea, PhD, Eng. – president of Branch Timişoara – Roumanian Academy

   Proceedings of 3rd International Symposium, October 26-28, 1998
   Organized under the auspicies of Roumanian Academy – Branch Timişoara and Comission of Biochemistry of Roumanian Academy Bucureşti
   Publishing House "Eurobit" Timişoara (1998)

I N M E M O R I A M

Prof. Dr. GAVRIL NEAMȚU

(1933 - 1998)

On October 28th, 1998 passed away Prof. Dr. Gavril Neamțu. His death means a regretable loss for the biochemical and biological sciences.

Born in Mintiul Gherlei – Cluj county (Romania) on January 1st, 1933 he graduated the Pedagogical Highschool of Gherla – Cluj county and thereafter the Faculty of Natural Sciences – Chemistry from "University Babes-Bolyai" Cluj-Napoca (1957).

Initially he worked as a highschool teacher (1957-1962). Beginning with 1962 he was assistant professor at the Department of Medical Veterinary Biochemistry from the Faculty of Veterinary Medicine Cluj-Napoca. Obtained the degree of doctor in science in 1968 and became university professor, in Chemistry and Biochemistry at the Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca in 1990.

The scientific activity comprises over 160 papers published inland and abroad. Regarding the didactic work he published as single author or with collaborators 9 volumes (courses and handbooks for students). Prestigious publishing houses edited diverse treatises of biochemistry, such as: "Biochimie vegetală" – Publ. House Ceres, București (1981); "Biochimie ecologică" – Publ. House Dacia, Cluj-Napoca (1993); "Substanțe biologic active – vitamine" – Publ. House Ceres, București (1996) and "Biochimie alimentară" - Publ. House Ceres, București (1997).

Prof. Dr. Gavril Neamțu took part at numerous national and international scientific events. One has to mention the papers regarding the research in the domain of carotenoids. For example: Trondheim – Norway (1966); New Mexico – USA (1969); Varna – Bulgaria (1970); Madison – USA (1978); Liverpool – England (1981); München – Germany (1984); Boston – USA (1987); Kyoto - Japan (1990); Leiden – Holland (1996) etc. His papers have been cited in numerous periodicals and treatises, by other authors, inland and abroad.

In 1981 he was awarded the Price "Gheorghe Spacu" of the Romanian Academy for outstanding results in the research of carotenoidic pigments.

During 1974-1986 he was Secretary and in the period 1986-1998 Vicepresident of the Commission of Biochemistry of Romanian Academy – Branch Cluj-Napoca. In this quality he organized various scientific meetings on Biochemistry. In 1990 he was elected in the National Council of the Romanian Society of Biochemistry affiliated at the Federation of European Biochemistry Societies (FEBS).

Prof. Dr. Gavril Neamțu had also a contribution in the series of symposia "Metal Elements in Environment, Medicine and Biology", together with his candidates for the doctorates degree.

The scientific and didactic activity of Prof. Dr. Gavril Neamțu was appreciated inland and abroad for the contribution to the discovery of 5 new natural carotenoidic compounds and 7 carotenoidic compounds obtained through semisynthesis.

His knowledge on carotenoids has been also applied in research concerning vegetal carotenotaxonomy. Some of his work has been accomplished in research stations with agricultural profile, Institute of Biological Research Bucharest, Institute of Chemical Research Bucharest. He took part in an international cooperation programme between the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca and the University of Rhode Island – USA.

The remarkable activity of Prof. Dr. Gavril Neamțu brought him a national and international recognition Numerous coworkers are going to continue the prestigious activity developed by the school of biochemistry in the field of carotenoids.

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