CONTENTS

Metal Elements in Environment, Medicine and Biology

Tome VIII

ROUMANIAN ACADEMY
BRANCH TIMIŞOARA AND BRANCH CLUJ-NAPOCA

IOAN SILAGHI-DUMITRESCU, ZENO GÂRBAN, PETRU DRÃGAN

EDITORS OF SYMPOSIA SERIES

Metal Elements in Environment, Medicine and Biology

Tome VIII

EDITED IN COLLABORATION WITH
CORNELIU DAVIDESCU, GABRIELA GARBAN, IOSIF GERGEN,
SIMONA DRÃGAN, NICOLAE VASZILCSIN, ADINA AVACOVICI

Proceedings of the 8th International Symposium on
Metal Elements in Environment, Medicine and Biology
Timişoara, December 5-6, 2008

Publishing House “Eurobit” Timişoara
2008

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Second printing

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METAL ELEMENTS IN ENVIRONMENT, MEDICINE AND BIOLOGY 2008
Tome VIII (Eds. Symp. Series I. Silaghi-Dumitrescu, Z. Gârban, P. Drăgan

Proceedings of the 8th International Symposium on Metal Elements In Environment, Medicine and Biology, Timişoara, December 5-6, 2008
Publishing House “Eurobit” Timişoara, Roumania, 2008

ISSN 1583 – 4204

Ioan, Silaghi-Dumitrescu; Zeno, Gârban; Petru, Drăgan (Eds. Symp. Series)
Corneliu Davidescu, Gabriela Gârban, Iosif Gergen, Simona Drăgan, Nicolae Vaszilcsin, Adina Avacovici (Eds. Tome VIII)

ISSN 1583 – 4204

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FOREWORD

The 8th Symposium of the series „Metal Elements in Environment, Medicine and Biology” (M.E.E.M.B.) addresses to a distinctive domain of bioinorganic chemistry and life sciences: metallomics. It takes place in Timişoara under the auspices of Branch Timişoara and Branch Cluj-Napoca and of the Roumanian Academy,

Approached topics regard the complex problems of metallomics in different domains for interest in environment (geobiochemistry, ecology, contamination of habitual area etc.), medicine (applications in clinical chemistry, patobiochemistry, implantology, cardiology, nephrology, etc.), nutrition (distribution in processed food, obtainment of food supplements, methodology of foods fortification), pharmacy (the synthesis and testing of chemiotherapic drugs with metal content, obtainement of some vegetal extracts), biology (distribution of metals in the vegetal and animal reign etc.

First symposium had as honorary president Acad. Nicolae Cajal, vicepresident of the Roumanian Academy (1990-1994), who agreed the series of symposia to take place under the auspices of Branch Timişoara of the Roumanian Academy. Beginning with the 2nd symposium (1996) until now (2008), the symposia were under the patronage of the Roumanian Academy – Commission of Biochemistry, having as honorary president Acad. Ionel Haiduc.

The series of symposia „Metal Elements in Environment, Medicine and Biology” has been initiated in 1993 at the proposal of the „Working Group on Metal Research in Biological Systems” constituted of researchers from universities and some scientific institutions from Timişoara (1979).

Starting with 2008 the series of symposia take place under the auspices of Branch Cluj-Napoca of the Roumanian Academy and of Branch Timişoara of the Roumanian Academy with the perspective to extent the internal and foreign collaborations and the thematic area.

The aprovement of the Roumanian Academy given by President Acad. Ionel Haiduc and General secretary Acad. Ion Păun Otiman for the cooperation of Branch Cluj-Napoca and Branch Timişoara, can offer a benefit for the progresses in this domain.

In context of contemporary development focused on environmental, nutritional and public health problems, preoccupations in the domain of metallomics present a special interest.

It is well known the fundamental and applicative character of metallomics in this domain. It confers explanation for the continuity and even broadening of topics of this symposium series.

President of Branch Timişoara of Roumanian Academy,

Prof. Zeno Simon, PhD
corresponding member of Roumanian Academy

PREFACE

The on-going biennial series of Symposia „Metal Elements in Environment, Medicine and Biology” (M.E.E.M.B.) and the publishing of Tome I-VIII (1994-2008) confirm the existing interest for problems referring to the applications of Inorganic biochemistry, generally, and of Metallomics, especially, in fundamental and applicative domains.

The interest shown to the presence of metals in the living area and of their role in life sciences is proved by the continous development of metallomics.

The Series of M.E.E.M.B. symposia – taking place during one and a half decade – under the auspices of Branch Timişoara of the Roumanian Academy benefitted of the support offered by the president Prof. Zeno Simon, PhD – corresponding member of Roumanian Academy (editions 2006 and 2008) and former presidents Acad. Ion Păun Otiman (editions 2002 and 2004) and Acad. Toma Dordea (editions 1996, 1998 and 2000) to whom we express our thanks.

At each edition was remarkable the contribution of scientists from diverse universities and research institutes from inland and abroad.

As it is well known, implication of metals and their derivatives in environment, nutrition, medicine a.o. are of main interest nowadays. Between metal ions and bioactive compounds can occur complex interactions which have a beneficial or harmful action on the functioning of the organism. Also, metal ions can, in case of pollution, represent a danger for public health.

While traditional bioinorganic chemistry is focused on the role and interactions of a single metal in a protein or enzyme system, «metallomics studies global, multi-element interactions and relationships» (Koppendaal and Hieftje, 2007). One can appreciate the significant connexions between chemistry and life sciences and identify easily the important concepts of todays’ knowledge.

Actual meeting was supported by Prof. Nicolae Robu, Eng, PhD – rector of University „Politehnica” Timişoara and Prof. Alexandru Moisuc, Eng. PhD – rector of the University of Agricultural Sciences and Veterinary Medicine of Banat Timişoara. In the M.E.E.M.B. Symposia Series organisation and perpetuation a special contribution had Prof. Gheorghe Ciuhandu, Eng, PhD – mayor of Timişoara city.

As the series of M.E.E.M.B. Tomes have ISSN number, it is confirmed its perpetuation and the possibility of extend to an annual meeting (until now having a biennial character). In order to pursue the future evolution of the Symposium it is desirable to encourage young colleagues to continue their scientific involvement in the domain of metallomics.

Editors

METALLOMICS

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The chemical derivatives of metals were tested in medicine, with contradictory results, even from the middle ages. The toxicity of many metal compounds especially at uncontrolled concentrations, has made the possible medical use of metals to be regarded with reluctance by clinical doctors and significantly successful. The situation changed with the discovery [1] of the antitumoral activity of Cisplatin $[Pt(NH_3)_2)Cl_2]$, a very active compound against some forms of cancer. Cisplatin was clinically tested in 1972 [2], and in 1979 it was approved by U.S. Food and Drug Administration as a drug in the treatment of cancer [3]. In the years to come, several thousands platinum compounds were screened for antitumoral activity, and more efficient and less toxic derivatives were discovered, now known under several trade names, such as Iproplatin, Carboplatin, Oxaliplatin, Lobaplatin, Nedaplatin, etc. The extraordinary efficacy of platinum derivatives against various forms of cancer (especially testicular, ovarian, bladder, brain and throat tumors) awakened a renewed interest for the possible medical applications of metal combinations.

On the other hand, the understanding of the role of essential metals in biological process lead to a novel **interdisciplinary field**, the BIOINORGANIC CHEMISTRY [5], based mainly on **fundamental research,** but with applications constantly growing in importance. Lately, a new subject is emerging, studying the action of metals on living cells, their effect on the bio-control of cellular processes, through the influence on the metabolism, the cell biologic functions and biosynthesis and the medical implications occurring, called METALLOMICS [6], designation similar to that of new subjects known as genomics, proteomics, etc. METALLOMICS, a new journal from RSC Publishing, will be launched in January 2009. The announcement was made recently at the International Forum of Proteins meeting on Health, Food and Environment by Joseph A. Caruso, who will chair the Editorial Board.

The biological role of some metals (essential elements) is determined by complex interactions between metallic ions and bioactive organic compounds. The lack of essential metals (but also the excess) may cause specific illnesses. For instance, the iron as a component of hemoglobin plays an essential biological role, but the excess of iron in the organism leads to diseases. The copper is essential in the development of embryos, temperature control and the functioning of the nerve cell, but it is also involved in the Menkes disease. Chromium is essential in the carbohydrate and lipid metabolism, its deficiency can provoke diabetes, but the excess is toxic. Some metals are toxic in any concentrations (mercury, cadmium, lead, thallium) and their elimination from the organism represents a very important topic.

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 5-14, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

The implications of metals and their derivatives, including organometallic compounds, in medicine are numerous and varied [7, 8]. The following aspects can be mentioned: (a) toxicity and homeostasis; (b) therapeutic agents already incorporated in clinical practice (for instance the platinum derivatives displaying antitumoral activity, gold compounds with antiarthritis action, antibiotics with metal-mediated activity (bleomycin/iron, copper)); (c) radiopharmaceuticals for diagnosis and treatment (technetium 99, other radioactive isotopes); (d) contrast agents for Roentgen imagistic (barium sulphate) and magnetic resonance (gadolinium complexes); (e) antibacterial, antiviral, antiparasitic agents; (f) psychotherapeutic agents, antidepressants; (g) mineral food supplements; (h) redox mediators for biosensors with medical applications.

The recent interest in the biological role of metals and their implications in medicine is illustrated by several examples: a) research programs financed by grants in USA (National Institutes of Health) [9], Europe (COST programs) [10], Australia and other countries; b) conferences, congresses and theme-symposiums, like the prestigious Gordon Conferences in USA [11]; c) a large number of books published in the last years (from which a short selection is presented [12]).

As mentioned before, the metal-based drugs are intensely employed in the chemotherapeutical treatment of cancer and numerous aspects of their biological activities have already been studied. In clinical practice, the use of metal compounds is current, and in the anticancer therapy, platinum-based drugs have a very important impact on the disease remission and survival rate [13, 14]. A constant improving of these therapies is necessary though, because their clinical use is limited by unwanted secondary effects and the worldwide trend is to individualize the treatment for each patient. The therapeutic index of the cytotoxic drugs – the ratio between positive effects and their toxicity - is low, and its improvement is a major objective [15].

Next to platinum, other metallic and organometallic derivatives have been tested in an attempt to discover novel antimitotic compounds. A large number of publications showed the interdependency between the structure, biologic effect and biomedical applications of the novel metal compounds in cancer therapy. The study of cytotoxicity, that of the mutation-inducing capacity in the tumor cell and of the antimitotic effect leads to the identification of structure-effect dependency for numerous compounds. The incorporation of different metals in the structure of cell components constitutes the basis of their biological activity. Many aspects of their biological effects have been studied: apoptosis-inducement, genotoxicity, DNA lesions and the repair mechanisms, reactive oxygen species inducement, immunological implications, the molecular basis of the cell resistance and the synergy with irradiation effects. Several complexes of platinum and palladium that contain ligands with a high antitumoral activity [16] were identified and characterized.

Newer cytotoxic agents have a broader spectrum of anticancer activity, showing toxicity towards both platinum-sensitive and resistant lines. Organotin compounds were identified as novel antitumoral agents that act in the apoptosis of cervical and ovarian carcinomas in human cells [17, 18]. Vanadium [19] and ruthenium [20] also form complexes with selective antitumoral and antimetastatic effects, especially towards platinum-resistant tumors, and they display a low systemic toxicity in therapeutic levels. The activity [21] of rhenium and rhodium complexes is related to their structural and kinetic properties. Apoptosis is induced by the inhibiton of cellular tyrosin-phosphatases and the cleavage and fragmentation of cellular DNA. Gallium [22] displays tumor-growth inhibition properties, through mechanisms competing with cellular iron-acquiring. The arsines, arsenic trioxide and organoarsenic compounds are efficient against tumoral cells. Gold (III) is isoelectronic with Pt (II); its target is not the DNA, but the proteins, and the effects are protease-inhibition and apoptosis-inducement [23]. The efficiency of titan and molibdenum organometallic derivatives is correlated to the presence of cyclopentadienyl rings in their structure, and their biological activity is determined by the interaction with thiolic groups from the cell [24]. Lanthanum and gadolinium are also employed in cancer therapy as single agents or neoadjuvants in radiotherapy [25, 26].

The antitumoral effect of platin-based drugs in mammals is related to their binding to DNA components. A selective method of determining Cisplatin-DNA adducts is based on the use of element-selective mass spectrometry [27, 28]. The most important DNA adduct with Cisplatin is formed between adjacent guanine units of the same DNA strand (1, 2 intrastrand cross-links). The repair of the DNA lesions contribute to clinical acquired resistance and is an effect of the resistance to drugs.

Assays which measure DNA mutation frequency are appropriate for the assessment of genotoxicity [29, 30]. The single cell gel electrophoresis assay ("Comet assay") detects lesions and DNA repair, cellular mechanisms such as drug transport and detoxification and it is a method for measuring deoxyribonucleic acid strand breaks, and DNA-protein crosslink in eukaryotic cells. New platinum compounds that form more extensive lesions in DNA [31] have been identified. The preferred nucleotides for the intercalation of metal complexes in the double helix of DNA in cancer cells, which causes irreparable lesions in the macromolecule [32-34] have been evidenced. The interaction of metal complexes with other biologic molecules like proteins and peptides, through the terminal amine, the imidazolic group of histidine or the thiol group of methionine was reported [35].

Reactive oxygen species (ROS) play an important role in cell signaling, and when present at high concentrations, they can induce oxidative stress. Nitrogen oxides (NOx) react with oxygen radicals to generate highly damaging reactive nitrogen species that may inhibit DNA repair mechanisms [36]. This is recognized as playing an important role in the evolution of the cancer cell and he NOx generated by the metal-based drugs can influence the result of cancer treatment [37]. It has been proved that the addition of an NO-releasing moiety in chemotherapeutic drug molecule increases the anti-neoplastic activity of the drug, while it provides protection against apoptosis induced by oxidative stress and it reduces its cytotoxicity against normal, non-neoplastic cells by blocking the oxidative stress that would lead to their apoptosis [38-39]. Nitrogen monoxide-releasing agents currently used in biology and medicine are organic (NONO-ates [40-42], nitrosyl-glutathione) or inorganic (nitrite, sodium nitroprusside, or other coordination compounds with NO coordinated to transition metals [45, 46]); depending on their electronic structure, these donors can act as direct NO, NO^+ or NO^- donors, with different potentials that are not yet fully explored as far as the biomedical effect is concerned [43, 44].

Standard drugs like Cisplatin alter the nitric oxide synthetase (NOS) levels through regulation mechanisms and increase p53 protein levels inducing the apoptosis of tumor cells. The matrix metalloproteinases (MMPs) have been identified as the targets of the treatment in various pathological conditions, such as cancer, and their overexpression has been associated with the high potential of metastasis in human carcinomas. In order to optimize the therapeutic efficacy of any drug, it is useful to be able to monitor the MMPs *in vitro* as well as *in vivo*. Platinum complexes able to inhibit MMPs through a noncompetitive mechanism have been reported [28]. These results, along with the investigations of their mechanisms (DNA interaction and tumor cell growth inhibition), demonstrate that ligand modifications of platinum compounds can be exploited to target biological substrates other than DNA.

In this context, it is obvious that the understanding of the nitro-oxidative stress mechanism and the metabolism of nitrogen oxides/oxoanions is necessary – these aspects involve as primary targets the metalloenzimes [40-42, 48-53].

Metal-based antitumoral therapy has a significant impact on the immune system. Cell-mediated immunity parameters, such CD4+, CD8+, CD 28 which modulate T lymphocyte and the natural killer cells antitumoral response have been monitored in several studies [54, 55]. In the case of cervical cancer patients, neoadjuvant platinum-based therapy enhances the immune response against tumoral cells [56]. The key moment is the activation of the T lymphocyte through a signal from a co-stimulatory molecule, and the role of costimulatory receptors like CD28 and the glucocorticoid induced tumor necrosis factor (GITR-) has been established [57-59]. Platin-based antitumor drugs have radiosensitizing properties; they are considered as among the most active drugs in both neoadjuvant and salvage treatments for patients with cervical cancer [60].

The major impact of their association is a significant decrease in the loco-regional evolution, recurrence rates (50%) and metastases rates. Risk of death was decreased with 40% [61, 62]. Randomized clinical trials evidenced the superiority of platin-based radiochemotherapy versus radiation alone; the optimal chemotherapeutic regimens remain to be defined [63].

Metallomics is of interest for domains that are connex to inorganic biochemistry regarding lithiasic formations (renal, hepatic, sialic, thyroidic ones), problems of implantology (in dentistry, ortopedics), colloid-osmotic balances, dishomeostasis of hydroelectrolytic metabolism (with impact in nutrition, cardiology, nephrology etc.), biogenesis of DNA-biometal adducts (genetics, biotechnology), DNA-chimiotherapic drugs adducts (e.g. cisplatin) and DNA-metals with toxicogen potential adducts (xenobiochemistry, e.g. Pb, Cd, Sn, etc.) [64-66]

Our interest and contribution in this field is illustrated by the two-volume treatise on the applications of organometallic chemistry in cancer chemotherapy [8], a number of articles relevant for the proposed topic from which some of them are presented [67-69].

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EFFECTS OF FOLIAR SELENIUM APPLICATION ON PERFORMENCES IN PLANTS/ANIMALS/MAN

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ABSTRACT

Effects of foliar selenium (Se) application to plant food nutritional quality and the nutrient output of agriculture systems in selenium deficient areas and its influence to human needs for nutrients satisfaction are presented. Investigations results obtained during over 10 years of research showing that Se, when added in proper, for each crops specific, amount exerts beneficial effects through multiple mechanisms. Such produced crops have beside Se concentration in the optimal interval, improved quality and, due to higher tolerance to unfavorable external conditions, better yield. Researches conducted on animals that consumed mix feed diet prepared of such produced crops reflected beneficially to animal production and quality parameters of animal products. Examinations of the health benefits that offer consumption of mentioned plant and animal products showed that its contribution to daily Se intake was enough high that Se deficient population assure enough Se in natural form, as well as that, due to higher intake of many interrelated nutrients the benefit that foliar biofortification with Se offer to the Se deficient human population overcomes the benefit of any known Se supplement.

Key words: selenium, foliar supplementation, plants, animal, man.

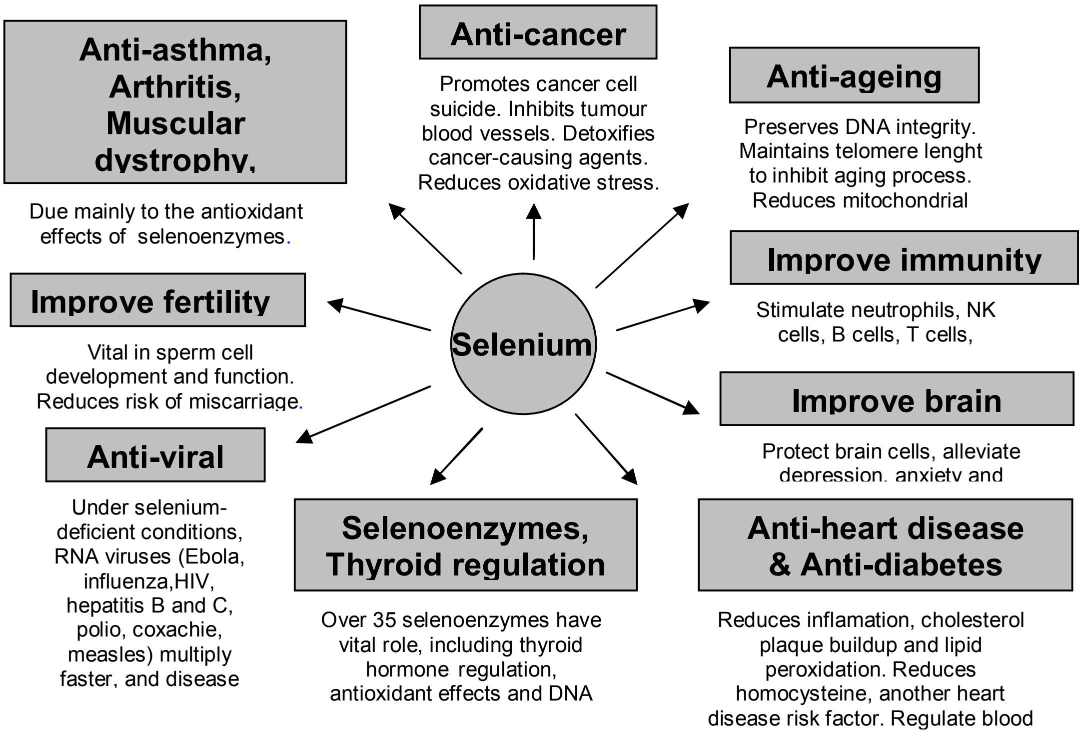
INTRODUCTION

Selenium (Se) is of fundamental importance to man and animal health. It is an essential component of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defence systems, and immune function. Its availability and content in food, due to irregularly distribution in the environment, may vary significantly depending on the region. Se protects against many disease problems in man (endemic cardiomyopathy - Keshan disease, bone and joint disease - Keshan-Beck-Syndrome) and animals (growth depression, white muscle disease, exudative diseases, liver necrosis, pancreatic degeneration, reproductive failure) (Tan and Huang, 1991; CAS, 1990; Oldfield, 2002).

Recent research indicates that Se is a versatile protecting agent against a variety of biologic and abiologic pathogenic agents, including certain viruses, toxic heavy metals, ozone, ionizing radiation, mycotoxins and industrial cancer causing compounds. It also positively affects the functions of the cellular or humoral immune system (Fig. 1) (Djujic, 2007)

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.15-26, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania



Anti-asthma, Arthritis, Muscular dystrophy,

Due mainly to the antioxidant effects of selenoenzymes.

Anti-cancer

Promotes cancer cell suicide. Inhibits tumour blood vessels. Detoxifies cancer-causing agents. Reduces oxidative stress.

Anti-ageing

Preserves DNA integrity. Maintains telomere lenght to inhibit aging process. Reduces mitochondrial

Improve fertility

Vital in sperm cell development and function. Reduces risk of miscarriage.

Selenium

Improve immunity

Stimulate neutrophils, NK cells, B cells, T cells,

Improve brain

Protect brain cells, alleviate depression, anxiety and

Anti-viral

Under selenium-deficient conditions, RNA viruses (Ebola, influenza,HIV, hepatitis B and C, polio, coxachie, measles) multiply faster, and disease

Selenoenzymes, Thyroid regulation

Over 35 selenoenzymes have vital role, including thyroid hormone regulation, antioxidant effects and DNA

Anti-heart disease & Anti-diabetes

Reduces inflamation, cholesterol plaque buildup and lipid peroxidation. Reduces homocysteine, another heart disease risk factor. Regulate blood

Fig. 1. The health benefits of Se

In order to prevent diseases resulting from Se deficiencies and assure safe and adequate Se intake, the production of high-quality food is essential. Equally important is the knowledge of maintaining food quality through the selection and the preservation of its quality components during food processing and preparation.

Production of food adjusted to consumer demands and environmental issues depends heavily on the availability of mineral and other nutrients to plants. This is why plants, animals and man have several essential nutrients in common.

According to existing knowledge Se is not essential plant nutrient. Its beneficial functions in plants is questonable. Additional nutrients to those required by plants, due to essentiality for man and animals, are Se, I, Cr and Co. Therefore they should be considered in food or feed production, and their deficiencies corrected by appropriate inputs.

Most plant nutrients are taken up from the soil solution. Leaves are able to take up only some forms of nutrient efficient. It is not easy to provide plants with exactly adequate amounts of all nutrients. Innumerable inter-related components that constantly interact causing negative or positive effects influence the extend and magnitude of Se and other nutrients flow through the soil – water – plants – animals – human chain in geographical area, its content and bioavailability in diet.

It is obvious that only multidisciplinary approach to the problem can offer a long-term solution for rapidly growing nutrients deficiency.

On the basis of field trials that we have conducted in Serbia the most feasible method to load enough Se in crops from Se deficient areas is foliar Se application. We invented procedure (*patent No, WO 99/31242*) and formulation (*patent No, WO 99/29639*) for foliar Se application that may assure in crops optimal level and distribution of Se in natural, bioavailable form, and improve negative impacts of Se deficiency to its yield, quality and tolerance to external and internal stressors.

The adopted procedure and formulations for foliar biofortification of food crops with Se is, in comparison with the biofortification through soil, more efficient and environmentally friendly, influences of soil and leaching factors are minimized, acceptability of added Se is fast (*over 50% of absorbed Se plant take in during the first 30-45 min of treatment*), quantity of Se needed for foliar treating is smaller.

Analyses of essential elements, proteins, amino and faty acids, vitamins, tolerance to oxidative damage and yield of crops showed that foliary nuriched plants with adequate Se level are source of high quality food. As such they may considerably improve Se and other essential nutrient intakes by man and animals. (Djujić, 2006).

The purpose of the present study was to presents improvements obtained in nutritional quality and agronomic properties of crops foliary treated with Se and impact of obtained food products use on performances of animals and man as and consumer in the food chain.

MATERIALS AND METHODS

Assays procedures and methods of investigations conducted in plants, animals and humans are described in our previous publications (Djujic et al, 2000a,2000b, 2002, 2003, 2004, 2005, 2006).

RESULTS AND DISCUSSIONS

Studies conducted on **crops** in period 1996-2008 showed that over 80% of total Se content is in L- (+) selenomethionine (SeMet) form, the favorable Se forms for man and animals. Obtained data indicates that proper Se range for each crop is different. For soybean it is 10 - 45 times higher than in corresponding control samples, while for wheat it is 10 - 15 times higher.

When Se concentrations in plants are under or above the proper ranges, antioxidant defense and seed quality decreases. Tolerance to higher Se concentrations in investigated plants, as a rule, was better than to lower Se concentrations. Data presented for TBARS, Se-dependent antioxidant enzyme, GSHPx activity and vitamin E contents in wheat and soybean seeds with different Se concentrations showes which Se interval was optimal for obtaining desired improvement in crop performences. (Fig. 2).

On the basis of repetitive data it was possible to obtain functional dependence between Se concentration and parameters important for antioxidative defence improvement and/or succeptibility to oxidative stress (Fig. 3 and Fig. 4).

Investigations showed that foliar Se application exerts beneficial effects on crops through multiple mechanisms. Such crops have increased contents of α - tocopherol (Fig. 5) and some of essential elements which optimal level is slightly lower (Zn, Cu, Mg) (Fig. 6), higher tolerance against detrimental effects of heat (Fig. 7), improved protein (about 10%), amino (Tab. 1) and fatty acids (Tab. 2) contents, yield, as well as tolerance to unfavorable external stressors (high temperature, UV-B radiation, drought).

Presented data, as well as over 15 years of the experience with the low Se soils in Serbia, shows that higher plants, similar as human and animals, require certain for each plant specific Se amounts for plant growth. It is evident that Se deficient crops that on average have 10 to 20 times less Se than are amounts that exert a beneficial effect on them have disrupted growth via several mechanisms.

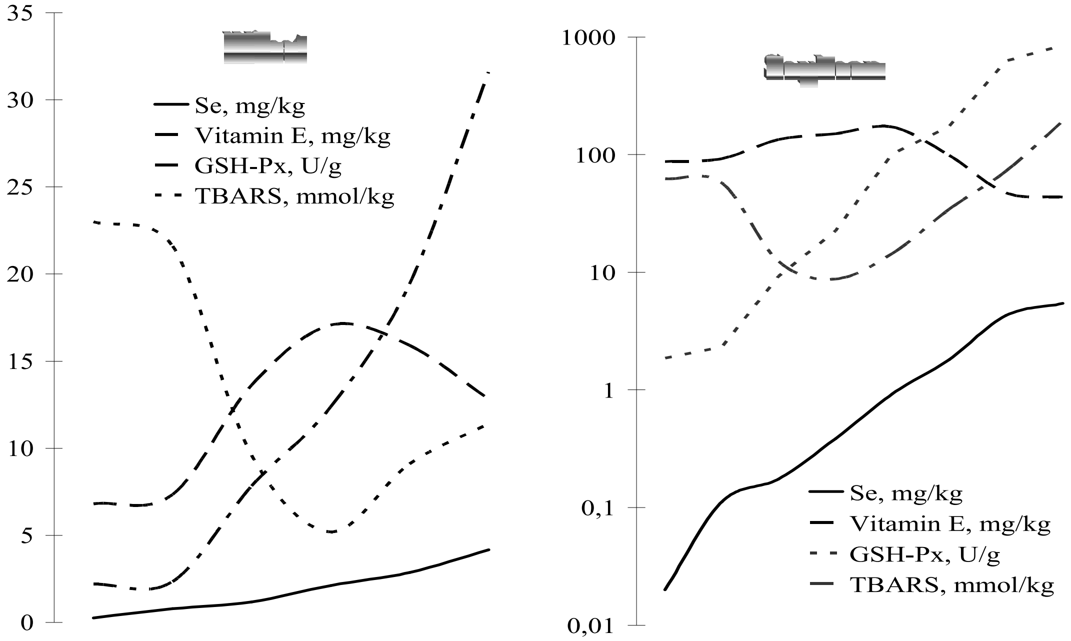
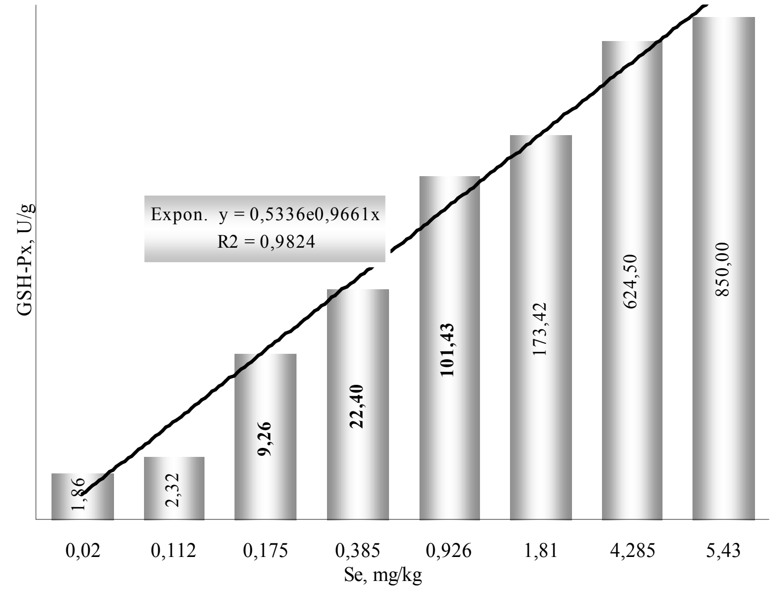
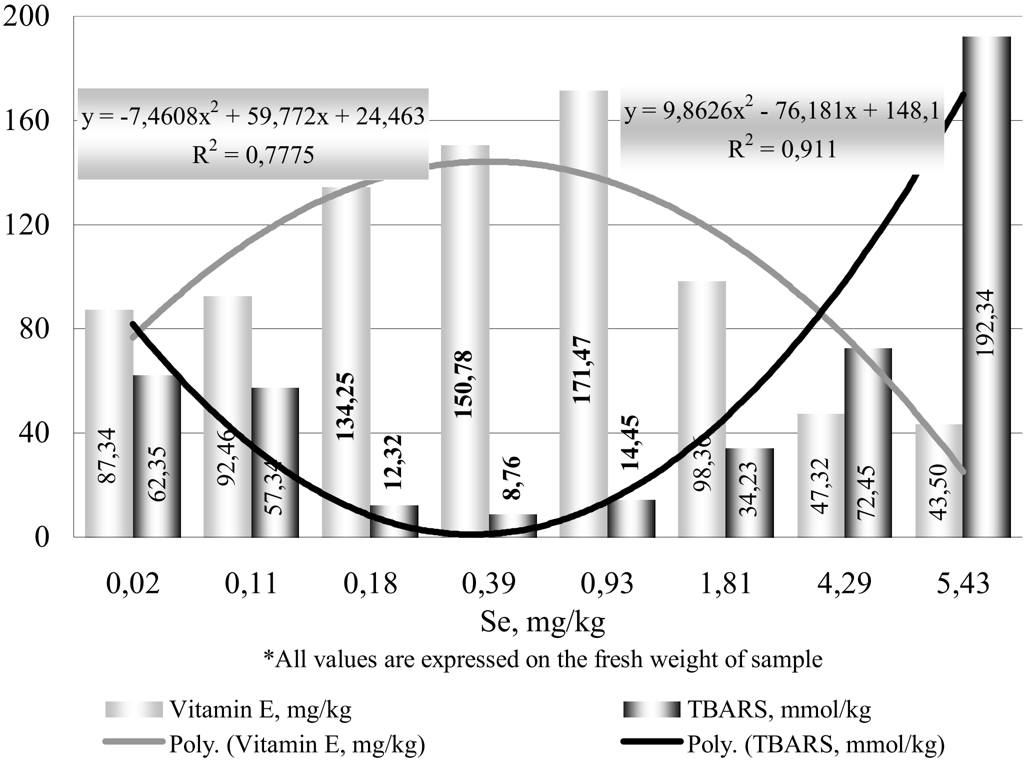


Fig 2. Effects of the various Se concentrations in wheat and soybean seeds on TBARS and vitamin E level and GSH-Px activity



*All values are expressed on the fresh weight of sample

Fig 3. Effects of the various Se concentrations in soybean seeds on GSH-Px activity



*All values are expressed on the fresh weight of sample

Fig. 4. Effects of the various Se concentrations in soybean seeds on TBARS and vitamin E concentration

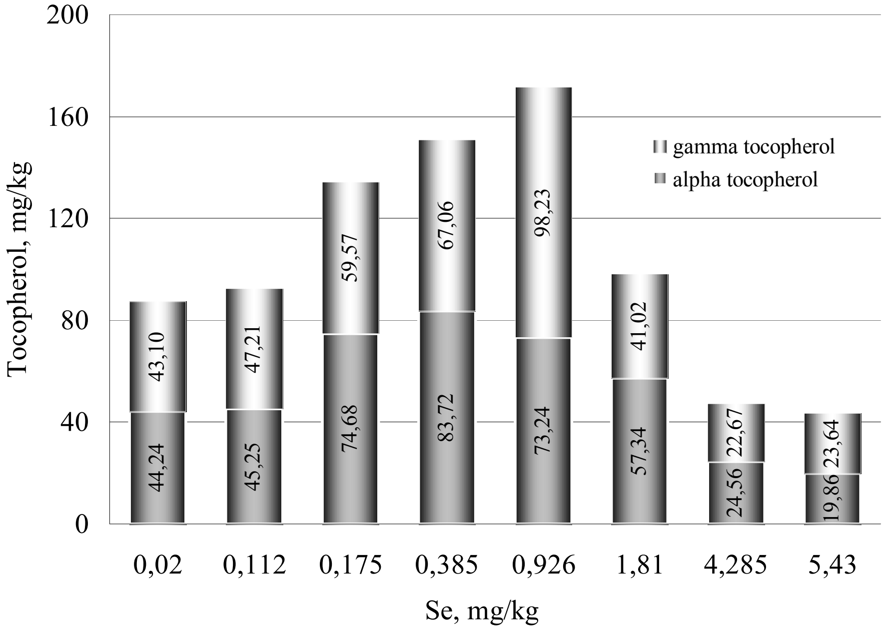


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Fig. 5. Influence of various Se concentrations in soybean seeds on α - and γ - tocopherol content

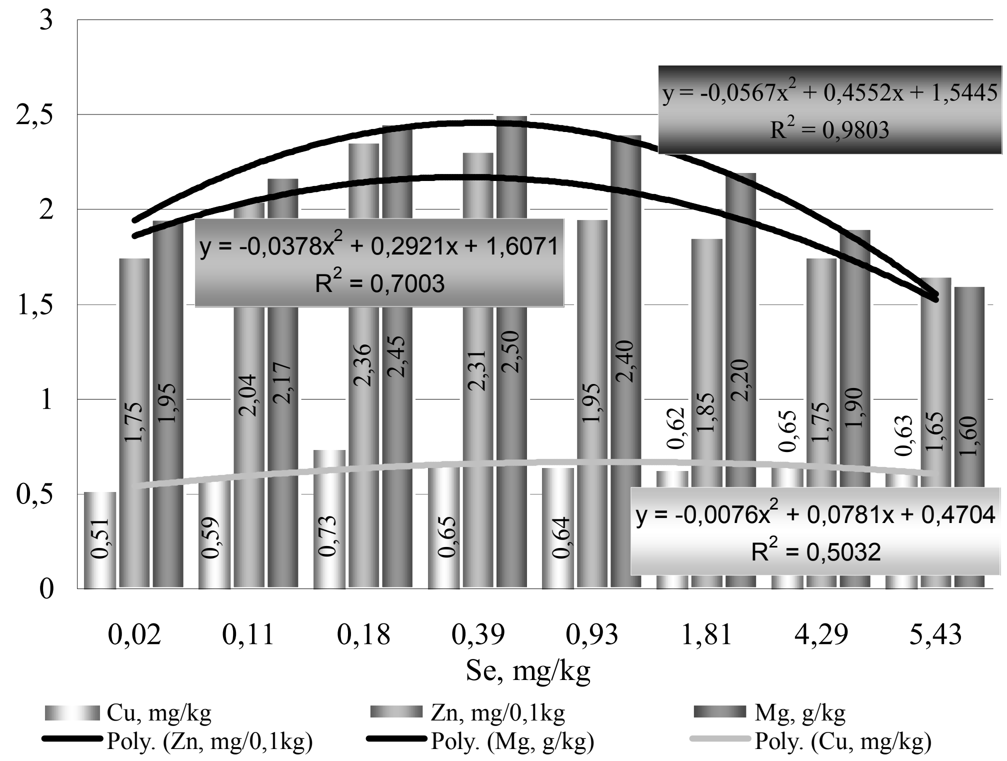

Fig 6. Effects of foliar Se supplementation on Se, Zn, Cu and Mg in soybean

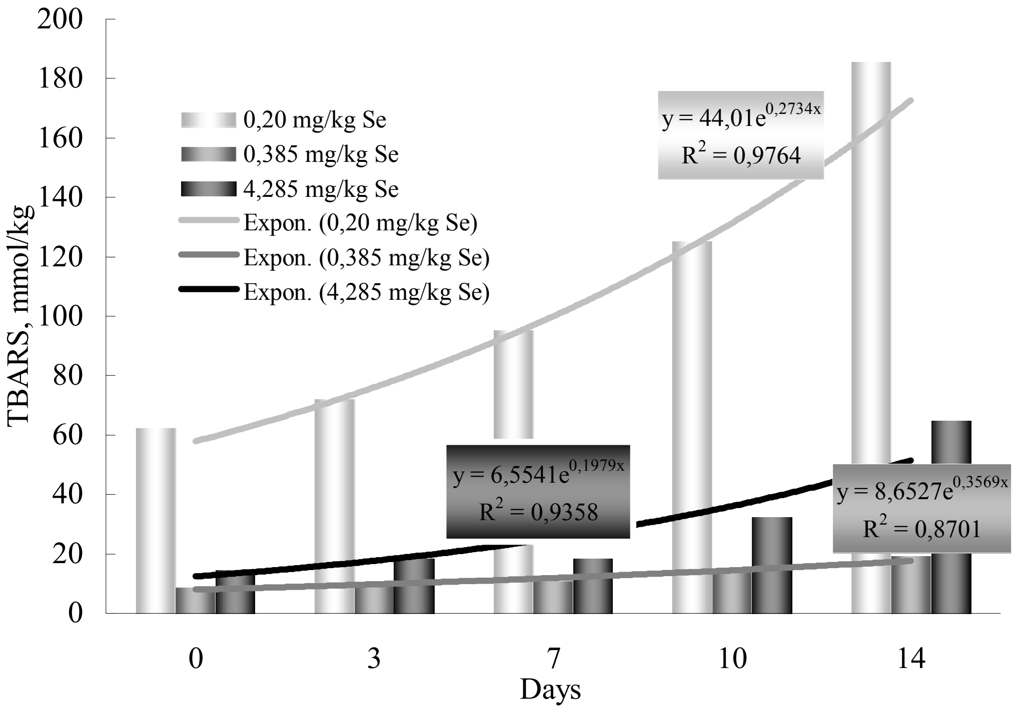

Fig. 7. Changes in TBARS concentration in soybean seeds foliary enriched with different Se concentrations obtained during the exposure to temperature of 45^{0}C

Table 1. Essential amino acids in Se-rich soybean seeds, Se rich wheat grain and seeds/grains from control groups

Amino acid concentration, mg/g protein	Soybean no added Se	Soybean added Se	Wheat no added Se	Wheat added Se
Histidine	30	24	21	23
Isoleucine	51	63	34	39
Leucine	82	78	69	72
Lysine	68	64	23	29
Met+Cys	33	32	36	39
Phe+Tyr	95	42	77	75
Threonine	41	41	28	30
Tryptophane	14	13	10	11
Valine	52	53	38	42
Total	466	410	336	360
Protein, %	35	38	13	14

Table 2. Essential fatty acids in Se-rich soybean seed, Se rich wheat grain and seeds/grains from control groups

of total lipids	Soybean no added Se	Soybean added Se	Wheat no added Se	Wheat added Se
$C_{14:0}$	-	-	3	3
$C_{16:0}$	11	9	14	12
$C_{18:0}$	2	3	5	4
$C_{18:1}$	20	19	25	20
$C_{18:2}$	64	74	49	56
$C_{18:3}$	3	4	4	5

Obviously, compensational mechanisms that each living organism has are insufficient to overcome stress caused by insufficient Se supplementation. Se deficient plants have significantly lower defense capacity to oxidative stress caused by oxygen radicals produced by internal metabolic and/or external factors. High Se additions are in turn toxic and induce pro-oxidative reactions that enhance lipid peroxidation, and disrupt metabolic harmony in plants. In Se deficiency as in excess oxidative capacity, growth, fertility, quality and nutritive value of obtained crops is reduced. Senescence of crops is faster, what reflects to the decreasing of its nutritive value.

By assuring proper Se supply nutritional value and tolerance to oxidative damage in plants may be significantly increased. This is of exceptional importance for the fast growing magnitude of damages induced by various complex and interrelated stressors in our living environment.

Investigations conducted on **domestic animals** (Japanese quails, chicken, pigs and caws) nourished with: mix feed diet fortified with inorganic Se (Na_2SeO_3), mix feed diet fortified with organic Se (brewery SeY) and mix feed diet composed of Se rich ingredients (crops biofortified with Se foliar) showed that the most significant improvement in animal health and nutritional quality of animal products were obtained at animal that received a mix feed diet composed of crops foliary biofortified with Se.

First investigations were conducted on Japanese quails and the benefits obtained with crops foliary biofortified with Se in mix feed diet surpassed our expectations (Djujic, 2004, 2006, 2007). Essays conducted on chicken showed that such nutrition also act beneficially to its resistance to oxidative stress and significantly improve meat and egg quality and mass.

Analysis of the Se content in laying quail and chicken eggs collected after 1, 3 and 6 months of receiving a feed mix composed of crops bioforified with Se showed that their eggs contained in average 6 times more Se than control groups. The protein analysis showed that Se rich nutrition improve more significant protein content in quail eggs (about 15%) than in chicken eggs (7%).

The n-3 fatty acids analysis in mixed eggs samples collected at 6th month of the experiments showed that, in comparison with the control group, eggs contains significantly more n-3 fatty acids (70% in quail eggs and 36% in chicken eggs).

Monitoring of the lipid peroxidation marker (TBARS), an important determinant of egg quality and nutritive value, the deterioration in fresh eggs and during storage, showed that eggs that originates from poulty nourished with feed mixture biofortified foliar with Se have much more delayed development of lipid peroxides and thus their nutritional value remains unchanged much longer (in chicken eggs 3 weeks and in quail eggs 12 weeks of storage) .

Furthermore, such produced poultry eggs have lower cholesterol content (32-50%), much less toxic elements in eggshell and inside part of egg and are richer in many in nutrition often deficient nutrient as are choline, vitamin A, riboflavin, vitamin B12, pantothenic acid, minerals - J, Cu, Cr, Si, Ca, Fe, Zn and essential amino acids than eggs obtained by ordinary farming procedure.

In our experiments on caws only mix feed diet composed of Se rich ingredients (crops biofortified with Se foliar) induced increasing in milk production and significant improvement in its nutritional value.

Analyses condacted on meat of animals thet were feed 6 months with mix feed diet composed of crops foliary biofortified with Se showed that such meat has improved Se and other essential elements concentration, as well as amino and fatty acid composition. Antioxidative enyzmes (GSHPx, SOD, CAT) and lipid peroxidation marker monitoring in them showed that tissues of animals nourished with feed mix composed of ingredients biofortified with Se have lower autoxidation of unsaturated fatty acids in which poultry meat is particularly rich and therefore longer shelf life.

Obtained data indicates that proper level of natural Se form in feed mix plays a key role in maintaining effective protection in various tissues against the damaging effects of free radicals and toxic products of their metabolism. Post-slaughter biochemical changes involved in the conversion of muscle to meat that are accompanied by a loss of cellular antioxidant defences and an increased propensity of meat lipids to undergo oxidation. Proper level and quantity of Se in nutrition of poultry contributes that undesirable changes in a number of quality parameters, including loss of water-holding capacity, texture and flavour became lower. As the lipids in poultry exhibit a higher degree of unsaturation than red meats due to a relatively high content of phospholipids, important factor for the oxidative stability of meats represent the degree of unsaturation of the phospholipids of the subcellular membrane. To improve oxidative stability, sensory quality, shelf life and acceptability of poultry now adding higher level of dietary antioxidants what, our experimental data shows, is not necessary when animal diet contains the proper level and form of Se.

On the basis of experimental data presented for animals, it can be concluded that foliar biofortification with Se offers following benefits to consumers and animals and its products producers:

- Eggs, milk and meat of such farmed animals represent an extremely rich source of natural Se forms and have significantly improved contents of many other, commonly deficient nutrients as are highly valuable proteins, omega-3 fatty acids, minerals, antioxidants).
- Content of undesirable toxic substances and its metabolic products was reduced.
- Body and egg mass, as well as eggs and milk production was higher in such feed animals.
- Animal products (eggs, milk and meat) have significantly longer shelf life and other quality parameters (loss of water binding capacity, texture, sensory quality).
- The consumption of products of such farmed animals may assure to man a daily needs for Se, highly nutritionally scored proteins, fat with the optimal omega-6/omega-3 ratio.

Comparison of data obtained on **population** groups that consumed supplements with 100 μg Se in the form of bakery SeY in the course of 8 weeks and products made from wheat foliar biofortified with Se over 6 weeks showed that efficiency of daily intake on average about 18 μg Se (12 - 35 μg) from Se enriched wheat influence in a shorter time effects comparable with the SeY supplement use. Increase of Se concentration in both groups were about 53% in plasma, 37% in erythrocytes, 44% in hair and 54% in urine (Djujić et al, 2000a). Data for oxidative stress reduction monitoring in blood plasma showed that although contribution of wheat biofortified with Se to daily selenium intake was over 5 times lower and in both cases the dominant Se-form was L – (+) Se-methionine, consumption of product obtained from Se enriched wheat reduced more efficiently oxidative stress (Djujić et al, 2000b).

In a Norwegian study it was demonstrated that high bioavailability of Se from wheat by feeding trial participants with Se-rich bread providing 100, 200 or 300 µg Se daily for 6 weeks. Serum selenium increased in a dose-response manner by 20, 37 and 53 µg/l, respectively, in the three groups ($p<0.001$) (Lyons et al, 2003).

Inclusion in diet soybean and other crops foliary biofortified with Se contributed that Se intake by Serbian residents increased by 170 %.. Investigations conducted on participants in this study showed that inclusion of crops biofortified with Se in diet did not only influence the increase of Se concentration and GSHPx activity in blood plasma and erythrocytes but optimized many other, for antioxidant defense and anti-inflammatory response important parameters (Djujić et al, 2002a, 2002b). Similar correlations between Se intake and Se and GSHPx levels in plasma and erythrocytes were found only in expressive Se deficient population groups from China and New Zealand (Xia et al, 1992; Whanger, 1998; Whanger and Butler, 1988; Schrauzer, 2000) confirming that the degree of deficiency influences Se absorption.

Monitoring of values obtained for lipid peroxidation in healthy subjects showed that good negative correlation exists between Se and TBARS (r ≥ - 0, 546). Investigations of lipid metabolism parameters in serum of investigated subjects showed that increased natural Se intake improves the level of these parameters.

Examinations of the health benefits that consumption of products prepared with crops foliary biofortified with Se and eggs obtained from quails that consumed feed mix composed of biofortified crops showed that its contribution to daily Se intake was high enough that Se deficient population of Serbia can assure even more Se in natural form than is RDA (Fig. 8). Investigations of Se, concentration and GSHPx activity in blood plasma before the beginning with Se rich food consumption and after 6 weeks of consuming Se rich food showed that increased Se intake reflected significantly to GSHPx activities, but that volunteers did not in the mentioned period reach GSHPx activities accepted as optimal for healthy individuals. Obviously, time of increased Se consumption was insufficient to fill up all Se pools in the body. Lipid peroxidation marker monitoring in plasma of the mentioned groups of volunteers showed that increase of Se intake acts beneficial to oxidative stress in Se deficient humans (TBARS values in plasma decrease significantly in all groups). The health-related quality of life (HRQoL) surveys obtained on the basis of the respondent's reports to five domains of function at start and the end of investigation indicate that in all investigated groups, food rich in natural Se improved more or less all five domains of HRQoL functions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) (Djujic at al., 2005).

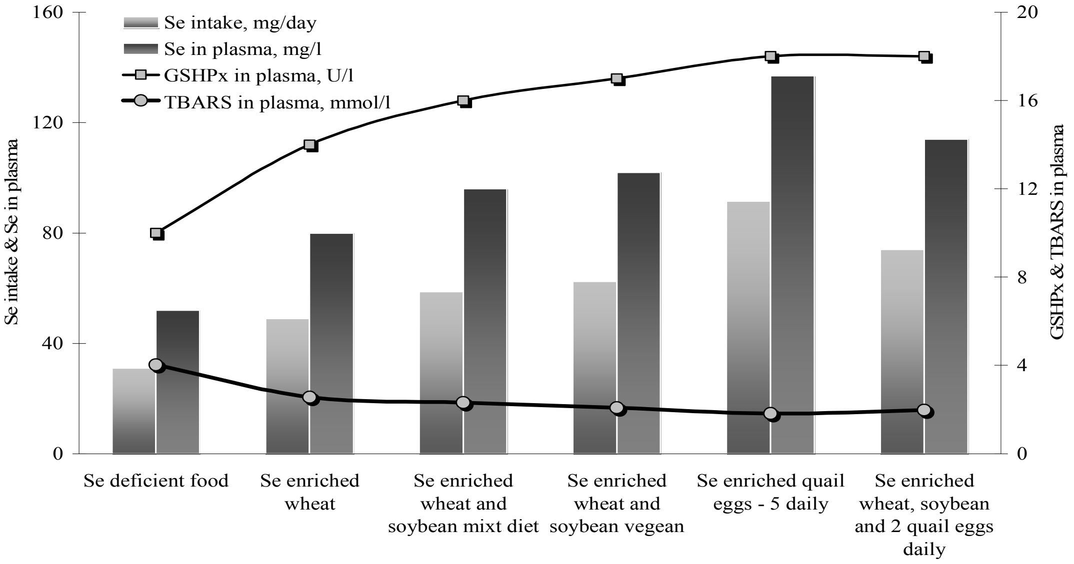

Fig. 8. Influence of food rich in natural Se consumption by Se deficient volunteers on its average daily intake, concentration in plasma and lipid peroxidation

Recent examinations, conducted on equipment that for diagnostic functioning of organism uses energy-information inter-exchange confirmed that consumption of food prepared from biofortified crops and/or animal products (eggs, meat, milk) obtained of animals that consumed crops biofortified with Se offer us possibility to prepare right combination for each in nutrient deficient individual and, at the same, time shows that none supplement can be so efficient as natural sources of essential and beneficial nutrients (Djujic and Lalic, 2008).

CONCLUSIONS

1. Monitoring the magnitude and nature of oxidative damage induced by various complex and interrelated environmental stressors, showed that Se deficiency is responsible for a higher susceptibility of plants, animals and man to oxidative damage.

2. Foliar Se supplementation may assure optimal Se interval in plants and optimize many for plants beneficial nutrient content what significantly increase tolerance to oxidative damage in plants.
3. Foliar Se supplementation effectively alleviate primary Se deficiency consequences in agriculture and at the same time offer solutions for the secondary problems in crops and animal breeding, assuring production of highly valuable nutritious food and formulation of nutritionally well balanced diets for increasingly malnourished consumers.
4. Health benefits that in synergistic action assure adequate Se intake and optimized intake of many essential and beneficial nutrients through the food chain were incomparable with effect that offer supplementation with chemical forms provided in artificial powders, supplements or pills as well as food fortification, and some of procedures for biofortification.
5. And results of such interventions were that living organisms in food chain have better developed antioxidative defence, and other compensational mechanisms of importance for defence against internal metabolic or external factors. This is of exceptional importance for the fast growing magnitude of oxidative damage induced by various complex and interrelated stressors in our living environment.
6. Examinations of the health benefits that offer consumption of mentioned plant and animal products showed that its contribution to daily Se intake was enough high that Se deficient population of Serbia can assure even more Se in natural form then is RDA, as well as that, due to higher intake of many interrelated nutrients, the benefit that foliar biofortification with Se can offer to the deficient human population overcomes the benefit of any known Se supplement.

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NORMAL AND PATHOLOGICAL INVOLVEMENT OF BIVALENT CATIONS IN ORO-MAXILO-FACIAL AREA

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ABSTRACT.

Bivalent cations have multiple actions in the oro-maxilar area. In case of parotide gland neoplasma, our data revealed significant increase in salivary magnesium concentration and a decrease in plasma zinc concentration. In maxilar sinusitis, there are no changes in calciu and magnesium concentration, whereas plasmatic zinc level is low. Zn, Cu, Ca, Mg and Mn also play important roles in the synthesis of dental enamel, and misbalances are involved in the pathogeny of tooth decay and other maxillary diseases.

Key words: Ca, Mg, Zn, parotide cancer, sinusitis, teeth.

Bivalent cations have multiple roles in normal functioning of all systems in the human body. They are involved in regulating the activity of lots of enzymes and influence cell functioning in complex ways.

Misbalances in the ionic concentrations of these cations (inside and outside the cell) deeply influence normal functioning of the human body. The oro-maxilo-facial area exposes a series of important particularities and complex functional roles. Some of the characteristics particular to this area are:

- the contact of the oral mucosa with blood and saliva
- the interaction between airways and digestive pathway
- phonation and deglutition headquaters
- teeth presence
- others.

Bivalent cations play important role is in the functioning of all the cells and salivary secretion.

Misbalances in intra and extra cell concentration of these cations are implicated in a series of pathogenic conditions.

Bivalent cations in saliva

Salivary concentrations of bivalent cations are a reflection of the saliva-secreting processes. This salivary secretion is realised from blood plasma.

Zn, Mg, Cu, Ca as well as other cations are found in the composition of sublingual calculi. Zinc and zinc-containing salts seem to be very important in this calculi formation ((Knuntilla M et al, 1981).

The are differences in the composition of bivalent cations in supra and subgingival calculi. Mn^{2+} concentration is significantly higher in supragingival calculus. Zn^{2+} concentration was 5.4 times higher subgingival calculus than in supragingival calculus (Knuntilla M et al, 1979 and 1981).

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.27-34, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

In case of acetyl-choline (Ach)-evoked amylase secretion of the parotid gland in a medium containing 1.1mM Mg^{2+}, Ach significantly increase the amylase secretion. Absence of magnesium or elevated magnesium concentration (5-10 mM) reduced Ach-evoked amylase secretion.

Ach stimulation of the parotid gland elevate the saliva Mg^{2+} concentration. The increase of is an active process, since Mg^{2+} has to move against the gradient.

Some medicines such as lidocaine (frequently used as local anaesthetic in dental practice), quinidine, amylorid, etc determine modification in the oral concentration of this cation.

In patients with chronic renal failure, changes in salivary ionic composition are frequently identified (Martins C. et al, 2006) Hemodyalisis also determine changes in saliva concentration and saliva flux.

The level of Ca^{2+} in saliva of diabetes mellitus patients was significantly elevated compared to healthy-matched controls. The concentration of Zn^{2+} and Mg^{2+} were significantly reduced (Mato AD, et al, 2004).

Una et al., 2004 showed that on chronic rheumatoid arthritis children the plama level of zinc and copper is significantly lower than in the control group, bu plasma magnesium level did not differ.

Bivalent cations in teeth pathology

Research performed by X-ray florescent techniques showed increased copper and zinc concentrations in canins regions of dentine compared with normal part of the teeth (Haris HH et al, 2008). Increased zinc concentration in the hydroxyl apatite was noticed in tooth decay regions.

We consider that the Cu^{2+}/Zn^{2+} ratio in tooth level and saliva might be correlated with the evolution and occurrence of tooth decay.

On the other hand, zinc citrate from tooth paste reduce by 17-49%the anerobic bacteria and streptococci at the leval of the oral cavity.

Additionally, in situ microbial biofilm formation was significantly inhibited in the zinc citrate group ($p<0.05$) (Srenivoisan et al., 2008).

The dental concentrations of some cations may suffer small changes with aging and they are not the same in enamel as well as in dentine.

Derise and Ritchey date (1974) show that manganese concentrations in the enamel is 7.41±0.13 ppm for 10-12 years and 6.67±0.12 ppm for 25 years. Zinc concentration in the same age groups was 190±4.1 ppm vs 180.3±3.8 ppm ($p<0.05$). Manganese concentration in dentine decreases from 6.20±0.16ppm in 10-12 years group of age to 5.52±0.15ppm after 25 years. The evaluation of zinc concentration differences between sexes indicated that zinc was significantly higher in both enamel and dentine in females ($p<0.01$).

Manganese concentration was significantly higher in the enamel than in dentine. Maximum manganese concentration both in enamel and dentine can be found in children under 12 years old. After this age, it decreases, and this fact can be linked to dentine evolution in puberty. Zinc enamel concentrations differences between sexes are highly significant (higher values for female). No significant differences were found in Fe concentrations in enamel and dentine between sexes (Knychalska-Karwan et al., 1985).

Copper concentration is important for the denthal osteoclasts activity stimulation.

On the contrary, increased zinc concentration in the osteoclasts enviorenment do not modify tooth resorbtion process (Li BB, Yu S.F., 2007). Mg ions have no effect on ostheoclasts apoptosis and active ring formation (Li X et al., 2008).

Hashemi et al., 2005 showed that the Ca/P ratio of was caries-afected dentine structures. After analysis of human molars extracted with coronal carians lesions was founded that CA/P ratio of caries was less than caries-free dentin surface. Milk carietic teeth contain less Mg, Mn and Cu and much more Zn as compared to normals (Niedzielska, 1990). Zinc concentration was higher both in the crown and the root of milk teeth than permanent teeth. Zinc level is higher in the teeth of the children than in the adult teeth (Gierat-Kucharzewska, 2003).

Cancers of oro-maxilar area

Cancer represents one of the most important medical problems. The malignant tumours of the oro-maxilar area represent 1-1.5% of human neoplasm. Salivary gland malignant tumours are a heterogenous group of cancers. The most important malignant parotide tumours anatopathological aspects are: adenocarcinoma, mucoepidermoid carcinoma and acinic cell carcinoma.

The frequency of parotide gland tumors is increasing now. The permanent contact of parotid gland tissue with saliva can be important for normal cell activity and some modifications of saliva composition can influence the malignant tumour evolution.

In our studies (Nechifor et al., 2005; Gradinaru et al., 2007), we found that plasma and saliva total-Mg concentrations was significantly higher in parotid malignant tumours patients vs. healthy controls. Plasma copper concentration of patients with parotid neoplasma was significantly higher compared to normal control and plasma zinc concentration was lower in parotid malignant tumour (0.017+/-0.01mmol/L vs. 0.024+/-0.01mmol/l in healthy controls, $p<0.01$).

Zinc plasma concentrations reduction can enhance the neoplasma development by reduction of the immunity (Fong et al., 2006). There are data regarding the role played by zinc in the reduction or prevention of oncogenesis. Bitar et al., 2007 showed that zinc might be an adjustment therapy for juvenile onset recurrent papilomatosis.

Changes in plasma and tissue concentration of some cations have been also identified in case of different forms of cancer (Tanaka et a., 2000).

In whole saliva collected from oral squamos carcinoma patients, magnesium concentration was significantly higher by 28%, compared to saliva people (Shpitzer T. et al., 2007). The albumin and K was lower in saliva collected from squamos in the most common head and neck malignant and is generally induced by tobacco exposure. Saliva annalisis is a reliable indicator for early diagnosis (Reznick, 2004).

Saliva play an important role in the pathogenesis of oro-pharingeal neoplasma. In patients with xerostomia induced by cancer radiotherapy the flow rate of stimulated whole saliva decreased 83% and this volume reduction was accompanied by a significant increase in Ca and Mg concentrations (Dreizen et al., 1976).

The taste

The exact mechanisms of gustative sensations development and influencing by concentrations of the bivalent cations are not yet very clear.

There are data that show calcium and magnesium air may be involved in taste perception. T1R3 receptor phenotype expression of TAs 1r3 taste receptor Tass1r3 taste receptor gene function as a gustatory calcium-magnesium receptor (Tordoff MG et al, 2008).

Oral calcium elicited less electrophysiological effect in the chorda tympani nerve in Tas1r knockout mice.

The oro maxillar infections

We consider that ratio between some bivalent cations concentration in plasma and saliva are very important for for the evolution of some infections in the oro-maxilar area.

We surveyed the plasmatic and salivary concentrations of different bivalent cations in patiens with oro-maxilo-facial area bacterial infections.

We noticed the Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+} plasmatic concentrations, the Ca^{2+}, Mg^{2+} salivary concentrations for 71 adults patients, both sex, hospitalized for soft tissues suppurations in the oro-maxilo-facial area (study group) and for 60 healthy volunteers, with a similar distribution upon age and sex (the control group). The suppurations were produced by different types of bacterial species: Gram-positive anaerobic cocci, facultative anaerobes (*Enterococcus spp*, *Streptococcus pneumoniae, etc*). The determinations were done by absorption spectrometry, before the beginning of the drug or surgical therapy, during the morning 12 hours after the last food ingestion. In this study we didn't include patients with other infections, pregnancy, liver cirrhosis, renal insufficiency, malabsorption syndrome, diarrhea, patients treated by diuretics/ cardiac glicozides/ antihypertensive drugs or other drugs containing cations.

In the oro-maxilo-facial bacterial infections the salivary magnesium concentration is statistically significant increased in comparison to the control group (4,76 ± 0,78 mg/1 vs 3,37 ± 0,65mg/1, $p<0,05$). The salivary Mg /Ca ratio in the study group is statistically significant increased in comparison to the control group. The zinc plasmatic concentration is statistically significant decreased in the study group in comparison to the control group (1,18 ± 0,51 mg/1 vs 1,53 ± 0,20 mg/1, $p<0,05$).

We consider that an increased Mg /Ca salivary ratio correlated to a decreased Zn^{2+} plasmatic concentration can be considered as a biological indicator of a suppurative process in the oro-maxilo-facial area and can favour the development of the bacterial infections in the mentioned area.

Our data (Nechifor et al., 2006) are in concordance other that show that Mg-dependent ATP-ase isinvolved in the invasion of salivary gland by some microorganisms like *Rhodnius prilixus* (Fonseca FV et al., 2006).

Periodontitis are bacterial infections of both tooth suppurating tissues. The essential elements of periodontitis are inflammation and teeth loss. This disease is frequent, being some of the most problems in dental medicine and some of the most frequent infections world-wide (Xu Y et al., 1999). Patients with periodontitis have decreased total saliva Ca and decreased serum Zn and Ca (Kuraner, 1991). Meisel et al., 2005 showed that magnesium deficiency is associated with periodontal disease. A cross-sectional investigation involving 4290 subjects aged 20-80yrs showed that increased serum Mg/Ca ratio was significantly associated with reduced probing death ($p<0.001$) and a higher number of remaining teeth ($p<0.005$) (Meisel et al., 2006). Higher serum Mg/total Ca ration can lower the periodontitis incidence (Meichant et al., 2006).

Our data show that increased calcium favours periodontitis.

Subjects with calcium antagonist treatment had significantly deeper gingival pocket and the probing depth was increased (Meisel, 2005).

Another aspect of the implication of the bivalent cations in salive and teeth in oral cavity pathology is constitute by the possibility that some of these cations possibly influencing dental enamel characteristics and biofilm formation. The biofilm is essential for the development of bacteria in the dental plaque. Mitrakul K et al., 2004 showed that copper can influence bacterial biofilm detachment in the *Streptococcus gordonii* imitation of dental placque development.

Situation seem to be different in case of fungal infections.

Calcium blocks fungicidal activity of salivary histatin 5 wich kills the fungal pathogen Candida albicans.

A high level of salivary calcium can favorize the development of fungicidal oral infections. Histatins constitue a group of cationic multifunctional proteins with antifungal activity. Their activity depends on cationic concentrations in the environment. They posses high fungicidal activity against azole-resistent fungal species (Tsai H, Bobek LA, 1998).

We studied Ca, Mg, Zn and Cu plasma concentrations and t-Ca and t-Mg levels in saliva in patients with chronic maxillary sinusitis without suppuration. Our results (fig. nr. 1 and fig. nr. 2) showed that in chronic maxillary sinusitis without suppuration no changes in t-Mg and t-Ca, but plasma zinc level is lower compared to normal control group (10.82mg/mL+/-0.24 vs. 1.46 mg/mL +/-0.27, p<0.01). The significiant decrease in serum zinc level a reduction of lymphocytes and macrophages immunity involvement. This decreased level of plasma zinc concentration can favourise the development of chronic sinusitis by reduction of defence of the human body against microbial infections.

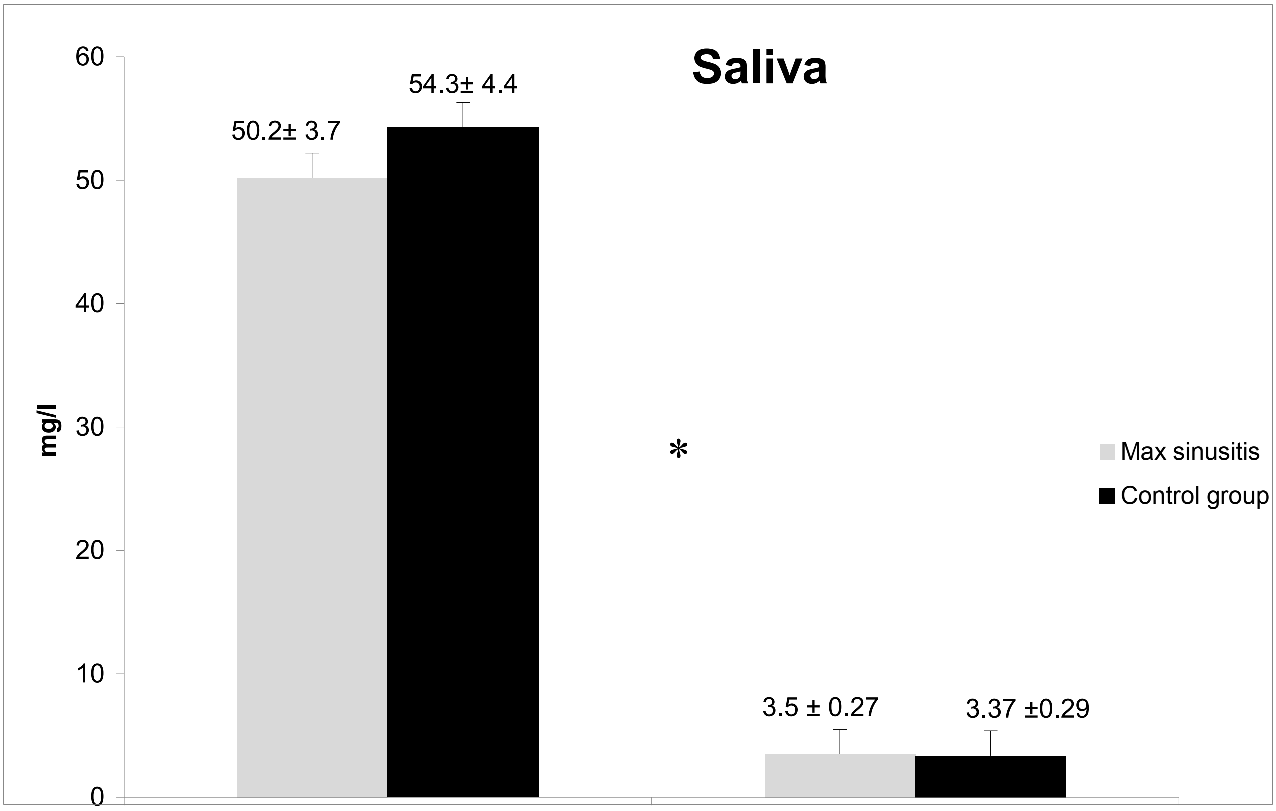

Fig.1 Salivary concentrations of calcium and magnesium in patients with maxilary sinusitis

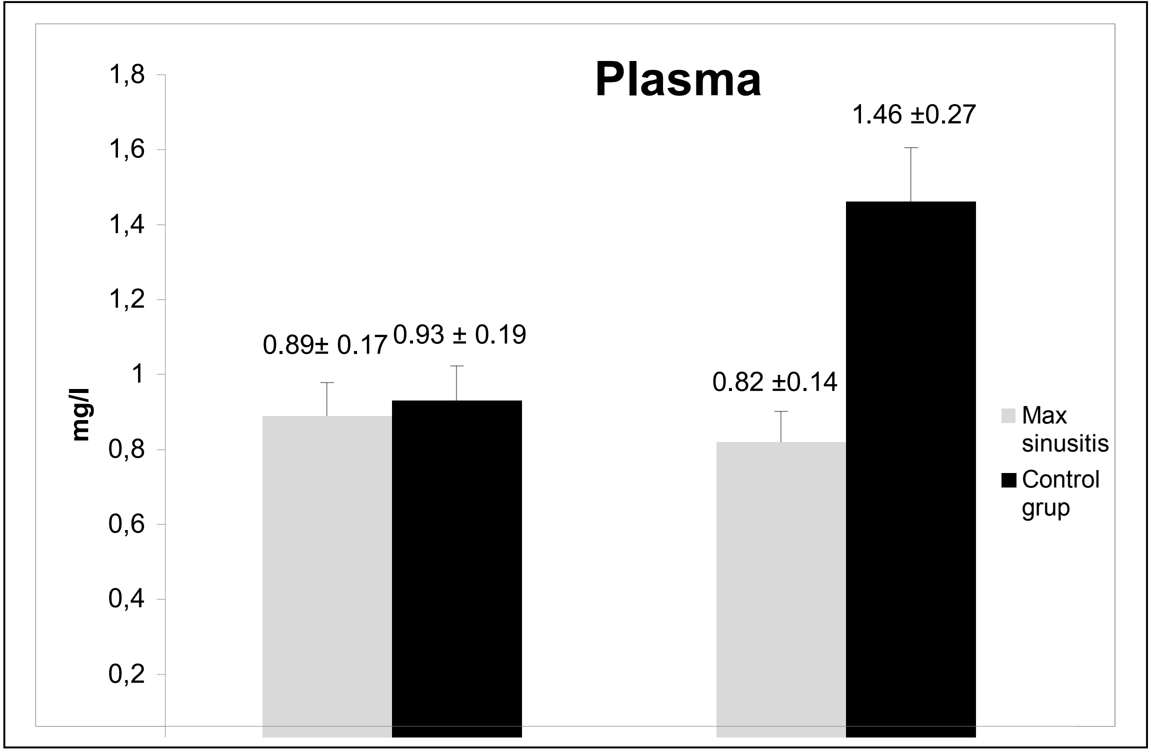

Fig. 2. Plasmatic concentrations of copper and zinc in patients with maxilary sinusitis

The influence on some locally administered drugs

Considering the use of the periodontal gel of some drugs (such as metronidazol), the bioadhesive properties of the gels was affected was affected by the variations of the Ca^{2+} concentrations in saliva (Varshosaz J et al., 2002).

Bivalent cations play important roles, unable to be disregarded, in the oro-maxilar area.

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POTENTIALLY METALLOENZYME INHIBITOR HYDROXAMIC ACIDS: EFFECTS OF AN IMIDAZOLE DONOR ON THE METAL BINDING CAPABILITY

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ABSTRACT

The role of R_C substituents, involving imidazole moiety, in complexation of various potentially metalloenzyme inhibitor hydroxamic acids, R_C-CO-N(R_N)-OH, (derivatives of amino acids and small peptides) with Cu(II), Ni(II) and Zn(II) ions has been investigated using pH-potentiometry and different spectroscopic methods. The main factors found to affect the competition of the imidazole with the hydroxamate moiety for the metal ions (e.g. the distance between these two functions, presence/absence of additional donor in the R_C chain, sort of metal ion) are planned to evaluate in the presentation.

Keywords: hydroxamic acid, imidazole, metal complexes, metalloenzyme inhibitors

INTRODUCTION

Although the coordination properties might be different and affected by numerous factors, but if an imidazole is present in a molecule, *e.g.* either in amino acids or in derivatives [1], in small peptides [2-6] or even in large protein molecules [7], very often this group is one of the most effective metal binding sites (often the most effective one) of that molecule. For example, in a large number of natural biomolecules, *e.g.* metalloproteines, this type of group plays a predominant role in the metal binding. Moreover, there is also a great affinity of an imidazole group for different non-covalent interactions (hydrogen bond, stacking interaction).

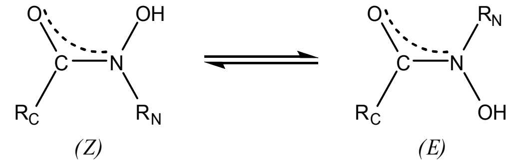
On the other hand, if a hydroxamic function is situating in a molecule, this can also play a determinant role in the metal binding (a five-membered chelate, via the two oxygen donors, is most frequently formed). The biological role of the hydroxamate based siderophores [8] and also the known metalloenzyme inhibitory effect of hydroxamic acids are highly based on the strong metal binding activity of the hydroxamate function [9,10]. Great effort has been made during the past years to develop selective inhibitors to various metalloenzymes, *e.g,* to Matrix Metallo-Proteinases (because large variety of serious diseases can be correlated to the disfunction of MMPs). One part of the substrate-based inhibitor molecules, developed up to present, consists of a hydroxamic metal-binding group and various R_C and R_N substituents. These substituents are planned for selective non-covalent interactions with the sub-sites of the enzyme [11, 12]. However, if any potential donor atom is situating in the substituents, this might modify (often significantly) the metal-binding ability of that hydroxamate-based compound [13, 14]. Evidently, if the mode of the

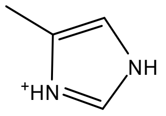
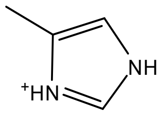
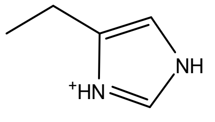
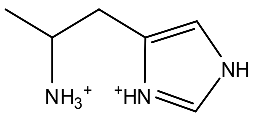
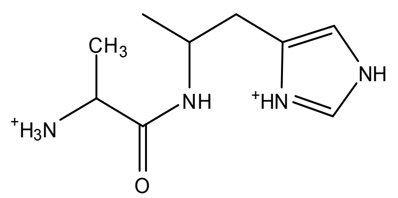
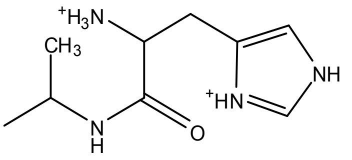
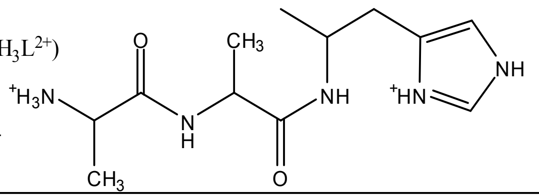
„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.35-40, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

metal binding is changing, this results in the change of the inhibitory effect too. That is the reason, why our main aim is to clarify the effects of the various donor atoms on the metal (first of all Zn(II), Ni(II) and Cu(II), because these metal ions are often included in the active centers of metalloenzymes) binding properties of hydroxamic derivatives. Since the imidazole is a common donor site in the biological systems, in the present study all molecules involve imidazole moiety at the R_c substituent. The effects of the distance between the imidazole and hydroxamic functions and also the effects of additional donor(s) situating in different positions compared to the above mentioned two functions on the metal complexation have been investigated by involving a series of imidazole-containing compounds (the formulae of them together with their dissociation constants, taken from ref 15, are listed in the Table 1).

Table 1: Formulae of the studied hydroxamic acids and their corresponding dissociation constants (pK_a)



Completely protonated forms of the ligands (L)	*R_C*	*R_N*
Imidazole-4-carbohydroxamic acid (H_2L^+) **(Im-4-Cha)** $pK_{a1} = 3.76$; $pK_{a2} = 8.82$		-H
N-Methyl-imidazole-4-carbohydroxamic acid (H_2L^+) **(N-Me-Im-4-Cha)** $pK_{a1} = 5.06$; $pK_{a2} = 8.57$		$-CH_3$
Imidazole-4-acetohydroxamic acid (H_2L^+) **(Im-4-Aha)** $pK_{a1} = 6.17$; $pK_{a2} = 9.05$		-H
Histidinehydroxamic acid (H_3L^{2+}) **(Hisha)** $pK_{a1} = 5.38$; $pK_{a2} = 7.11$; $pK_{a3} = 9.06$		-H
Alanyl-histidinehydroxamic acid (H_3L^{2+}) **(AlaHisha)** $pK_{a1} = 6.11$; $pK_{a2} = 7.72$; $pK_{a3} = 8.82$		-H
Histidyl-Alaninehydroxamic acid (H_3L^{2+}) **(HisAlaha)** $pK_{a1} = 5.56$; $pK_{a2} = 7.55$; $pK_{a3} = 9.02$		-H
Alanyl-alanyl-histidinehydroxamic acid (H_3L^{2+}) **(AlaAlaHisha)** $pK_{a1} = 6.31$; $pK_{a2} = 7.82$; $pK_{a3} = 8.84$		-H

MATERIALS AND METHODS

Reagents, metal ion and ligand stock solutions

The ligands are either commercially available (histidine hydroxamic acid, HisHa), or synthesized as previously published [15]. The purity of the ligands and the concentrations of the ligand stock solutions were determined by *Gran's* method. [16] The metal ion stock solutions were prepared from $CuCl_2 \cdot 2H_2O$ and $NiCl_2 \cdot 6H_2O$ (Reanal) dissolved in doubly distilled water. ZnO (Reanal) was dissolved in a known amount of HCl solution (0.1 M). The concentration of the metal ion stock solutions was determined gravimetrically via precipitation of quinolin-8-olates. The HCl concentration of the Zn(II) solution and the exact concentration of the carbonate-free KOH titrant were determined by pH-potentiometry.

Potentiometric and spectroscopic studies

The pH-potentiometric titrations were performed in the pH range 2.0-10.5 or until precipitation occurred on samples of 4.00 or 5.00 cm^3 at an ionic strength of 0.2 M (KCl) and at 25.0 ± 0.1 ^{o}C. During the titrations purified, strictly oxygen-free argon was continuously bubbled through the samples. Samples were always freshly prepared and titrations were completed within 3 hours. The ligand concentrations were varied within the range 0.001-0.0025 $mol \cdot dm^{-3}$ and the metal to ligand ratios were varied between the ranges of 1:4 - 1.5:1. The experimental data were collected by titrating minimum 4-5 different samples. The titrations were performed by using a Radiometer pHM84 instrument equipped with a Metrohm combined electrode (type 6.0234.100). Titrant KOH was added from a Metrohm 715 Dosimat auto burette. The electrode system was calibrated according to Irving et al. [17] so that the pH-meter readings could be directly converted into hydrogen ion concentration. The water ionization constant (pK_W) obtained was 13.76 ± 0.01. The pH-metric results were utilized to find the stoichiometry of species and to calculate the stability constants. The calculations were made with the aid of the computer program PSEQUAD [18].

UV-Vis measurements on systems containing Cu(II) and Ni(II) were performed. The ligand concentrations were varied within the range 0.0015-0.0025 $mol \cdot dm^{-3}$ and the metal to ligand ratios were 1:1, 1:2 and in the case of some Cu(II) containing systems were also 1.5:1. A Perkin Elmer Lambda 25 spectrophotometer was used to record the spectra in the region of 300-800 nm. Path length was 1 cm in all cases.

CD spectra for copper(II)-containing systems with all ligands at 1:1, 1:2 and 1.5:1 metal to ligand ratios were also obtained at 25 ^{o}C under a constant flow of nitrogen on a Jasco J-810 spectropolarimeter, which was calibrated with an aqueous solution of the ammonium salt of (1R)-(-)-10-camphorsulfonate. The concentrations of the ligands in the samples were 0.001 M in all cases. Measurements were carried out in water at different pH values, using 1 cm path length cuvettes in the 290-800 nm wavelength regions.

The ^{1}H NMR measurements were recorded on a Bruker Avance AM 360 by using D_2O as solvent and TSP ((trimethylsilyl)-propionic acid-D4, sodium salt) as standard under the following conditions: metal to ligand ratio was 1:1 in all samples and the analytical concentrations were 0.002 M. 0.1 M NaOD and DCl solutions were used to adjust the pD within the range of pD 2 – 11.

Anisotropic X-band EPR spectra of frozen solutions were recorded at 120 K, using a Bruker EMX spectrometer after addition of ethylene glycol to ensure good glass formation. Copper(II) stock solution for EPR measurements were prepared from $CuSO_4 \cdot 5H_2O$ enriched with ^{63}Cu to get better resolution of EPR spectra. Metallic copper (99.3% ^{63}Cu and 0.7% ^{65}Cu) was purchased from JV Isoflex, Moscow, Russia for this purpose and converted into the sulfate. The concentration of Cu(II) was 4 mM in all cases, and the metal to ligand ratios were 1:1 and 1.5:1.

On a few samples, electrospray ionization time-of-flight mass spectrometric (ESI-TOF MS) analysis was carried out using a Bruker BIOTOF II ESI-TOF instrument. The solutions were introduced directly into the ESI source by a syringe pump (Cole-Parmer Ins. Comp. type 74900) at a flow rate of 2 μL/min. The temperature of drying gas (N_2) was 100°C. The pressure of the nebulizating gas (N_2) was 30 psi. Voltages applied at the capillary entrance, capillary exit and the first and the second skimmers were -4500, 120, 40 and 30 V, respectively. The spectra were accumulated and recorded by a digitalizer at a sampling rate of 2 GHz. The spectrometer was operated at unit mass resolution and was calibrated to sodium trifluoroacetate.

RESULTS AND DISCUSSIONS

Imidazole analogues of α- and β-amino hydroxamic acids (Im-4-Cha, N-Me-Im-4-Cha, Im-4-Aha)

The relatively low basicity imidazole-N(3) was found to behave as anchor donor not only to Cu^{2+} and Ni^{2+}, but even to Zn^{2+}, although this latter metal ion prefers oxygen donors over nitrogen ones. By increasing the pH water insoluble polynuclear species have been found to form with Im-4-Cha. A pentanuclear metallacrown (Scheme 1) is exclusively formed above pH 4 between Cu(II) and Im-4-Aha. The stability of this species with Ni(II) and Zn(II) is significantly lower, and as a consequence, it appears together with various mononuclear bonding isomers in the Ni(II)-Im-4-Aha and Zn(II)-Im-4-Aha systems.

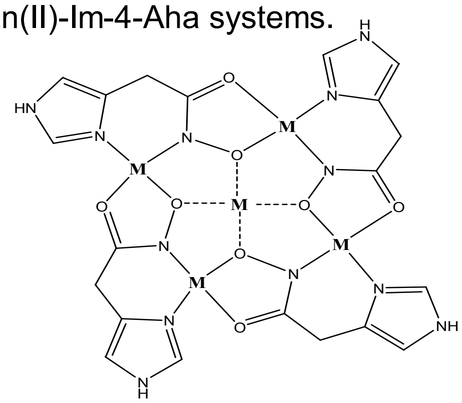


Fig. 1

Beside the mononuclear species a dinuclear complex, $[M_2L_3]$ (Scheme 2), was found in the Cu(II)- and Ni(II)-*N*-Me-Im-4-Cha. A possible bonding mode is that one of the three ligands bridges two metal ions, while each of the other two ligands binds to one metal. Stacking interaction between the imidazole rings of the latter two ligands might stabilize this species.

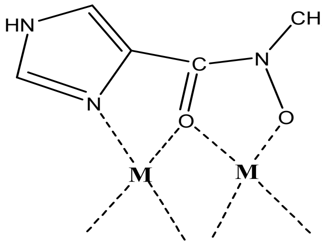


Fig. 2

Based on the results, this molecule, which is capable for the interaction with two metals, might be effective inhibitor of a metalloenzyme involving two divalent metal ions in the catalytic unit.

Hydroxamic derivative of histidine (Hisha)

In this molecule the R_C involves an imidazole in γ-position to the hydroxamic and to βüposition to the terminal amino group. As it is known from previous results [1], the imidazole takes part in the coordination first of all in the complexes with Cu(II), but also with Ni(II), where octahedral complexes with tridentate coordination of the ligand (N_{amino},$N_{imidazole}$,$N^{-}_{hydroxamate}$) dominate below pH 7. Above this pH, however, the hydroxamate-coordinated complexes become dominant. With Zn(II) ion, the coordination starts similarly to the simple α-amino hydroxamic acid derivatives, but NMR results indicate also imidazole coordination.

Hydroxamic derivatives of histidine-containing di- and tripeptides (AlaHisha, HisAlaha, AlaAlaHisha)

The lower basicity of the imidazole, compared to that of the hydroxamate gives a benefit to the former donor especially at lower pH in the studied peptide derivatives and the role of the hydroxamate generally increases upon increasing the pH. It is also a common feature that, upon increasing the pH, when the imidazole-N is displaced by the hydroxamate in the coordination sphere, the former moiety does not remain uncoordinated, but imidazole-bridged di- and polynuclear complexes are formed. In contrast with this, in the Cu(II)-AlaAlaHisha system, the hydroxamate can take part in the coordination only at lower pH, where the hydroxamate chelate can successfully compete with the amino – imidazole macrochelate, but can not compete with the very high stability 4N-coordinated (amino-, amide-, amide- and imidazole-N donors) bonding mode. As a consequence, in this system, the hydroxamate does not play measurable role in the coordination at high pH. With Ni(II), the formation of planar complexes is generally more favoured with hydroxamic derivatives of the histidine-containing di- and tripeptides compared to molecules containing carboxylic instead of hydroxamic moiety. This demonstrates the exceptional coordinating ability of the imidazole ring. Any indication for deprotonation and coordination of pyrrole-nitrogen was not found in the systems. With Zn(II), formation of various bonding isomers with rather low stability was found. The complex formation does not start below pH ca. 6 and precipitation occurs at pH. 7.5-9 depending on the ligand and Zn(II) to ligand ratio.

CONCLUSIONS

Increased selectivity of the hydroxamate-based metalloenzyme inhibitors can be expected, if, in addition to the interaction of the hydroxamate group with the catalytic metal, non-covalent interactions also occur between the substituents of the inhibitor molecule, R_C and R_N, and the enzyme. To achieve this, favoured coordination of the hydroxamate chelate over the imidazole to the metal ion is necessary. Because of the above mentioned facts, the role of R_C substituents, involving imidazole moiety, in Cu(II)-, Ni(II)- and Zn(II)-complexation of various hydroxamic acids (derivatives of amino acids and small peptides) has been investigated. A competition between the hydroxamate and imidazole functions was found in all studied systems, but the extent of that competition was found to differ from metal to metal, from ligand to ligand and to depend very much on the pH. The imidazole played the most determinant role in the Cu(II) complexes, somewhat less in the Ni(II)-containing ones, while the most negligible role occured in the Zn(II)-complexes.

Acknowledgements: This work was supported by the Hungarian Scientific Research Funds (OTKA T 046366 and T 049612).

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INTEGRATED MODELING OF GEOCHEMICAL AND BIOGEOCHEMICAL PROCESSES ACROSS SPACE-TIME SCALES

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ABSTRACT

In order to find out how the environment is organized one investigates structural elements and processes. Geochemical, soil and biogeochemical processes are amongst the most frequently used for characterizing the so called "hierarchical levels" of the environment's structure. The goal of the project we present in this paper is to test in five catchments hypotheses 1: the budgets of microelements assessed in large catchments don't close with the budgets of their sub-catchments and hypotheses 2: the bioaccumulation in mobile organisms (top consumers) cannot be predicted base on concentrations in organisms which are their trophic base, on their local abundances, and on the trophic preferences of the top consumers. The activities are organized under three objectives: one dedicate to the ecosystem level mechanisms supporting the processes at landscape level, one for testing the first hypotheses, and one for testing the second hypotheses. The activities are grouped in seven workpackages (wps): in objective 1 a wp for investigating the role of microorganisms (with a sub-wp for symbioses with plants), and wp for organo-mineral complexes, with a sub-wp dedicated to chemical speciation of metals, in objective 2 a wp for fluxes of metals by groundwater, and one for runoff and erosion (with a sub-wp for sediment transport in rivers), including math modeling, in objective 3: a wp for bioaccumulation and ecotoxicology in terrestrial systems (with sub-wps for plants, carabids, frogs and small mammals) and a wp for bioaccumulation and ecotoxicology in aquatic lotic systems. The seventh wp supports all objectives and is dedicated to math modeling with four sub wps: gis database, stochastic modeling, cellular automata modeling and neural networks modeling

Key words: biogeochemistry, soil science, ecology, geology, hydrology, metals, mathematical modeling

INTRODUCTION

In this paper we present a research project for complex exploratory research (acronym BIOGEOST) submitted under the call 2008, which passed the first selection phase and now is in the final evaluation phase. Whatever the result of this evaluation will be, we believe that the ideas and the approach specific to this project are valuable for discussion in the scientific community.

Consequently the structure of the paper will be informal, following the structure of the project. We have not presented the very technical part of the project, which is not of general interest. The paper can be seen also as a short review in the area of trace elements biogeochemistry.

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.41-54, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

1. IMPORTANCE AND RELEVANCE OF SCIENTIFIC CONTENT

After the failure of the heavily subsidized attempts to do produce holistic models of ecological systems in the 1970' in the frame of the International Biological Program, the financing of such research program went down to reasonable dimensions, despite the widespread recognition of the existence of a hierarchy of ecological systems, from local to global level, whose functional modelling is a desirable research objective (Loijen, 1998). The idea to do such costly modelling revived recently under the heading of "modelling the socio-ecological systems", but still the possibility of successful implementation of this research agenda is debated: the holistic approaches have failed in the past, and the reductionistic ones are said to not reflect the true characteristics of the systems. At this general level, the idea of this project is *to* implement a new strategy for modelling hierarchically organized ecological systems, coupling holistic and mechanistic approaches.

The complex systems such as the ecological ones are characterised by and intrinsic principle of incertitude (Jorgensen and Svirezhev, 2004; Stanciulescu, 2005). Because there are no precise solutions for complexly organized systems, there is no single method for their study. It isn't quite "anything goes", but we cannot tell what might work without trying it. Taking into consideration also the non-linearity of the complex systems and their real, non-stochastic, indeterminacy (due to biological evolution, for instance), one can say, from a philosophy of science standpoint, that there cannot be a final mathematical model of such systems: models of the functioning of ecosystems will evolve (in an evolutionary epistemology sense). Our strategy is to not look for a single comprehensive model, whose epistemic "mutations" and evolution are very costly, and consequently difficult to accept by the scientific community, but to produce a maximally coherent portfolio of models with a modular structure, most appropriate for a high rate of epistemic evolution occurring by piecemeal changes at the level of the modules and of the relations between them. By now the models belonging to the potential "modules" have evolved independently, disciplinary, without explicit concern about their relationships with other disciplinary developed models.

To give substance to this strategy, the project deals with modelling biogeochemical cycling of metals occurring in hierarchically organized ecological systems. That this is a basic research priority is proved by a comprehensive analyses of the bioaccumulation models coupled with ecotoxicological models done in the frame of a previous ESF project. In order to do describe the metals' circulation there is a need to develop food-web models (modifying and extending their structure), especially models describing natural, complete terrestrial ecosystems (www.esf.org/ecolmat, 2003). Starting from the conclusions of this ESF project we performed a review of the state of the art concerning the mathematical modelling of the cycling and effect of metals at landscape level (Iordache and Stelian, 2007). The general conclusions were:

1. Current models applicable at landscape scale describe mainly the abiotic side of the processes, the best example being reactive transport models. However, there are many opportunities for interdisciplinary transfer of ideas profitable for the field of mathematical modelling in biogeochemistry and ecotoxicology.
2. The limits related to modelling the involvement of the organisms cannot be surpassed at the current state of biological knowledge, despite the efforts in the systems biology field.
3. With respect to the development of the scientific knowledge in biogeochemistry and ecotoxicology the potential of modelling is underused, because the modelling efforts are focused mainly on the side rapidly relevant for socio-economic systems.
4. There is an important opportunity to use populations of similar contaminated and, eventually, bioremediated sites (seen as in situ experimental areas) for testing biogeochemistry hypotheses

with modelling tools (i.e. to build some kind of “long term *contaminated* ecological research” network).

The difficulty which blocked the refinement of this kind of models for *in situ* cases is related to the large heterogeneity of the control parameters, the distribution in space, as well as to the large number of relationships between the control parameters for each metal. In these conditions, to set up mathematical relations between the control parameters of metals mobility and their mobility proved to be not yet feasible *in situ*. It may be that the development of new mathematical tools, more adapted to biological phenomena, would contribute to changing the landscape of models in this area. For instance, Schryver et al. (2006) use non-linear analyses with artificial neural networks with good results to describe the relationships between groundwater contamination with metals and the structure of bacterial communities as measured by molecular markers. Beside neural networks, another new tool is the Multivariate Adaptative Regression Splines (MARS). It has been used for instance for predicting the denitrification rate based on data sets at European scale (Pinay et al, 2007).

These mathematical tools could be applied in order to describe relationships between parameters describing metals biogeochemistry at the *explanandum* (higher hierarchical) level, on the one side, and its control parameters at the *explanans* (lower hierarchical) level. Once the analyses are performed, one would have a holistic phenomenological description of the relationships in mathematical forms at site scale. One limitation of this approach is to uncover mathematical laws with 1 member of the reference class (the studied site). This limitation can be surpassed only by studying a population of sites across gradients. Another limitation of the holistic approach is that the discovered mathematical laws will give no indication about the mechanisms supporting them. This indication can be provided only by a mechanistic (bottom-up) approach. By now reductionism has created unwarranted biases in certain mathematical models and experimental designs. Mechanistic models may incorporate unnecessary lower level details that compromise verisimilitude and predictive success. However, without this approach one cannot have an image on the involved mechanisms, and thus on the possibilities of controlling the metals’ mobility. Therefore one can proceed this way, having in mind that the mechanistic frame has to be re-verified after each step of upgrading and prediction. Our idea is to develop (bottom-up) mechanistic models of metals’ behaviour under the (top-down) constraint of the results of the holistic modelling (figure 1).

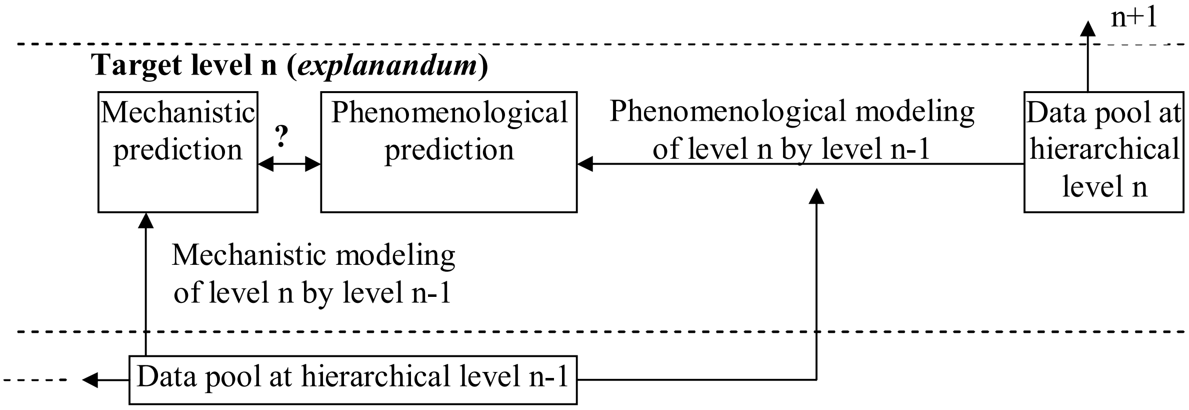


Fig 1. Overall structure of the modelling approach.

The main focus from the mechanistic perspective will be on the difficulty of allocating values to the parameters under high field heterogeneity. The heterogeneity of the real systems at a certain hierarchical level cannot be predicted based on principles (Dungan et al., 2002). The heterogeneity problem can be dealt with by empirical research for delineating in situ the elementary units of models application (systems identification) and using a portfolio of models covering the diversity of the elementary units. Different processes require different space-time scale of the elementary units and models' development. Programming in GIS can then be used for upscaling the models' results from the elementary units of application to the site and for linking the results of the models with different space-time scale (Iordache and Bodescu, 2005). The upscaling problem is linked to some extent to the reduction of the ontological levels problem, and can be solved partially by recognizing the irreducibility of ecological levels of organization, such as elementary ecosystems and landscapes (contaminated lands are usually at these levels). Then each level can be approached by specific model(s). The remaining part of the upscaling problem, within a certain level, directly reflects the heterogeneity problem. In this context, the general objectives of a research program in this area can be:

1. To uncover mathematical laws describing the mobility and effects of metals at contaminated landscape level.
2. To develop coupled top-down and bottom-up models of key processes of metals biogeochemistry and effects in a population of contaminated landscapes.

This project should be seen as one step in such a long-term research program.

2. RESEARCH METHODOLOGY
(AIM, OBJECTIVES, AND WAYS OF REACHING THEME)

The goal of the project is to test the following hypotheses:

- Hypotheses 1: The budgets of microelements assessed in large catchments don't close with the budgets of their sub-catchments. Rationale: the boundary conditions of the sub-catchments have their own dynamic, specific to the functioning of the system of larger scale (for instance the change in the landscape structure can be unpredictable at small scale, but predictable at larger scale) 2) buffering and remobilization areas can occur on the flow path of underground and surface waters (so called hot spots and hot moments). Testing method: coupling models for the budgets in small catchments and comparing the results with the results of models developed for the integrating catchment. Math modeling of the microelements budget will be performed as integrated modeling (separate sub-models for the involved mechanisms coupled in between).
- Hypotheses 2: The bioaccumulation in mobile organisms (top consumers) cannot be predicted base on concentrations in organisms which are their trophic base, on their local abundances, and on the trophic preferences of the top consumers. Rationale: The emergent properties at large scale would interfere with the simple accumulation in trophic chains by the way it controls the availability of trophic resources at large scale and indirectly the feeding behaviour of mobile organisms. Testing method: the performances of deterministic models of bioaccumulation (cellular automata type) will be compared with neural networks based models to predict the accumulation in plants, carabids, rodents and birds. We expect that the differences in predictions will become larger as the mobility of the organisms increase, and that the neural networks based models will be more accurate in the validation phase.

These two hyptheses have an atractor role for the whole project.

Complementarily, in each WP the scientific groups involved will test specific, local hypotheses. In this way the data sets obtained will be useful both for the disciplines involved in the project, and for the systemic biogeochemical approach providing the identity of the project.

The experimental and theoretical activities, having as final end to produce new elements of knowledge and developing principles and concepts, will be organized, from a management perspective under three separate objectives dedicated to 1) Biogeochemical mechanisms at ecosystem level 2) Testing the hypotheses concerning the budget of trace elements at landscape scale, 3) Testing the hypotheses about trophic-dynamic modules emergent at landscape scale.

The activities are grouped in seven workpackages (wps): a wp for investigating the role of microorganisms (with a sub-wp for symbioses with plants), and wp for organo-mineral complexes, with a sub-wp dedicated to chemical speciation of metals, a wp for fluxes of metals by groundwater, and one for runoff and erosion (with a sub-wp for sediment transport in rivers), including math modeling, a wp for bioaccumulation and ecotoxicology in terrestrial systems (with sub-wps for plants, carabids, frogs and small mammals) and a wp for bioaccumulation and ecotoxicology in aquatic lotic systems; the seventh wp supports all objectives and is dedicated to math modeling with four sub wps: gis database, stochastic modeling, cellular automata modeling and neural networks modeling.

Figure 2 presents the Pert diagram of the project and the relations between WPs and objectives. Table 1 presents the Gant diagram of the project.

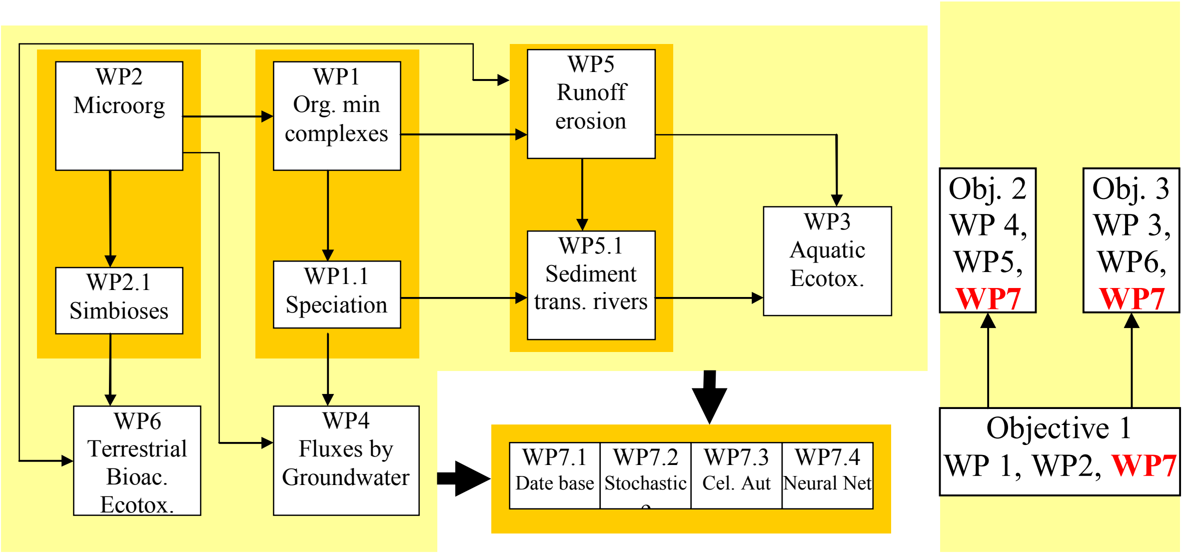
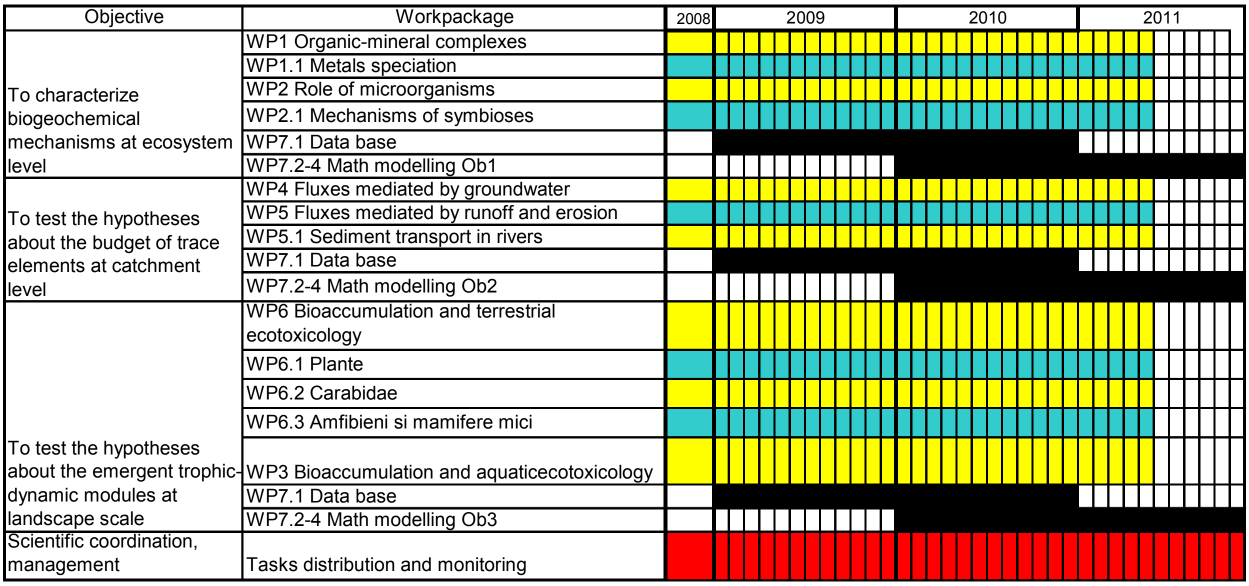


Fig 2. Pert diagram of the project and the relationships between workpackages and objectives.

Table 1. Gant diagram of the project.



Objective	Workpackage	2008	2009	2010	2011
To characterize biogeochemical mechanisms at ecosystem level	WP1 Organic-mineral complexes	🟨	🟨	🟨	🟨
	WP1.1 Metals speciation	🟦	🟦	🟦	🟦
	WP2 Role of microorganisms	🟨	🟨	🟨	🟨
	WP2.1 Mechanisms of symbioses	🟦	🟦	🟦	🟦
	WP7.1 Data base		⬛	⬛	
	WP7.2-4 Math modelling Ob1			⬛	⬛
To test the hypotheses about the budget of trace elements at catchment level	WP4 Fluxes mediated by groundwater	🟨	🟨	🟨	🟨
	WP5 Fluxes mediated by runoff and erosion	🟦	🟦	🟦	🟦
	WP5.1 Sediment transport in rivers	🟨	🟨	🟨	🟨
	WP7.1 Data base		⬛	⬛	
	WP7.2-4 Math modelling Ob2			⬛	⬛
To test the hypotheses about the emergent trophic-dynamic modules at landscape scale	WP6 Bioaccumulation and terrestrial ecotoxicology	🟨	🟨	🟨	🟨
	WP6.1 Plante	🟦	🟦	🟦	🟦
	WP6.2 Carabidae	🟨	🟨	🟨	🟨
	WP6.3 Amfibieni si mamifere mici	🟦	🟦	🟦	🟦
	WP3 Bioaccumulation and aquaticecotoxicology	🟨	🟨	🟨	🟨
	WP7.1 Data base		⬛	⬛	
	WP7.2-4 Math modelling Ob3			⬛	⬛
Scientific coordination, management	Tasks distribution and monitoring	🟥	🟥	🟥	🟥

The field sites include five catchments with contaminated lands, spread over the climatic gradients of Romania (E-W, N-S, table 2).

Table 2. Catchments studied by the partners. Acronyms explained in figure 5.

Studied catchments	UAIC-Ggrf	UAIC-Geol	UBB-Geo	UBB-Bio	UB-Eco	UB-Ghydro	UB-Gch	ICPA	USAM VB	UBM
Săsar	x	x	x	x	x	x	x	x	x	x
Abrud-InferiorArieş	x	x	x	x	x	x	x	x	x	
Ampoi-Geoagiu	x		x	x	x	x	x	x	x	
LeşuUrsului	x	x		x	x	x	x	x	x	
Pantelimon	x			x	x	x	x	x	x	

Table 3. Types of approaches in the studied systems.

Type of system	Type of approach			
	Field study		Experimental	Math simulation
	extensive	intensive		
Low order catchement	x			x
Highly contaminated lands		x	micro, mezo, field scale	x

Each catchment will be approached by several research methods at three scales of analyses (table 3): contaminated ecosystem level, small order catchment, and medium order catchment.

For the symbiotic part in WP2 we will use ectocmycorriza (EM) as study model (figure 2), hypothesizing a peculiar organization of the trophic-dynamic modules of this group. From this perspective we are able to identify the following components of EM structural diversity in a tree island: number of TDMs, species richness inside each TDM (α), at tree level (β) and forest level (γ), evenness inside each TDM.

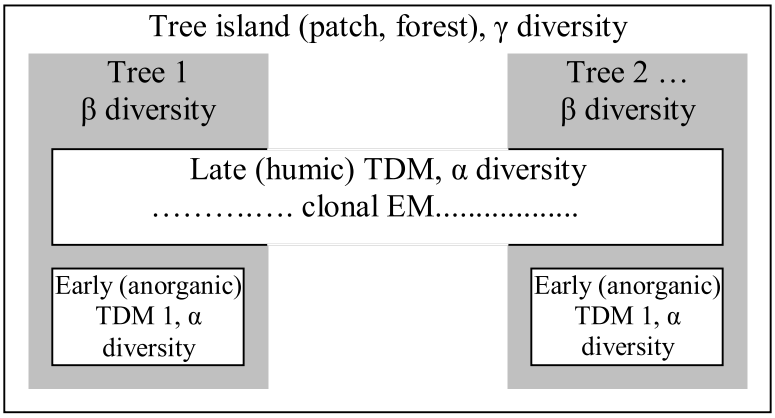


Fig. 3. Model of EMs community structure in a forest.

This way of conceptualizing the structural diversity allows a direct connection with functional interpretation. For instance, changes in microbial diversity reported in the literature did not always correspond to changes in functional redundancy. The reason for this is that diversity is usually characterized unstructured, at tree island level, which mixes the diversity of different TDMs. As functional redundancy of species occurs only at infra-TDM level, an increase of overall diversity (across pooled TDMs) does not necessarily imply an overall increase in functional redundancy. One can have for instance decrease of redundancy in a TDM coupled with an increase in another TDM, or simply the appearance of new TDMs in the system's structure. The functional diversity, excepting for the redundancy component, will change only with the change in the number of TDMs. The research done will allow us to test this concept model.

The main objective of the groundwater workpackage is a transient coupled model at different scale of space and time for geochemical and biological processes as. Five experimental sites (Sasar, Abrud – Inf. Aries, Ampoi- Geoagiu, Leşul Ursului, Pantelimon) have to be characterized with respect to flow paths, and in subsequent years the waters sampled from soil, surface and ground water wells. The result of monitoring for formation of groundwater, chemistry of soil and runoff water will be used in fitting operation of the transient coupled model. The support of coupled models will be the spatial distribution of metals (WP1) and an important factor of stability of the environmental conditions is the sediment transport in the rivers (WP5) and the elements of aquatic ecotoxicology (WP3). In the final stage the fitted transient coupled model will be connected with sthocastic models for bioaccumulation and terrestrial ecotoxicology (WP6).

In workpackage 6 we will provide the population level data needed for developing bioaccumulation models for four model organisms characterized by different space-time scale of activity. A comprehensive analyses of the bioaccumulation models coupled with ecotoxicological models was done in the frame of an ESF project (www.esf.org/ecolmat, 2003). They state that ecosystem modelling and, more specifically, modeling of food-web processes, provides a proper vehicle to study the ecotoxicological impacts and to make the risk assessment. They conclude that an important obstacle to the use of food-web models in ecotoxicological risk assessment is the lack of hard data, or more precisely a database containing these data. This includes ecophysiological and life history parameters (data on consumption, mortality and the like, as well as data on concentration-effect relationships for those parameters) for the various functional groups in an ecosystem. Supplementary research in order to use food-web models for ecotoxicological risk assessment involves the further development of food-web models (modifying and extending their structure), especially models describing natural, complete terrestrial ecosystems. It also involves collecting the required input data and parameter values either in the laboratory or in the field. Furthermore, a comprehensive set of field data is required for validation purposes(www.esf.org/ecolmat, 2003). Andretta et al. (2006) discuss the most important theoretical aspects of polluted soil Risk Assessment Methodologies, which have been developed in order to evaluate the risk, for the exposed people, connected with the residual contaminant concentration in polluted soil, and make a short presentation of the major different kinds of risk assessment methodologies. They also underline the relevant role played, in this kind of analysis, by the pollutant transport models and describe a new and innovative model, based on the general framework of the so-called Cellular Automata (CA), initially developed in the UE-Esprit Project COLOMBO for the simulation of bioremediation processes. This line of research will be followed in the last protocol, modeling.

The data used for modeling (WP7) will have three sources: comprehensive critical analyses of the (experimental and field) literature, field investigations and experimental activity.

Figure 4 shows the relationships between the models developed based on experimental data and those developed based on field data. As suggested in figure 4, the dataset produced as a result of the experiments will be characterized by a higher number of parameters, but a lower variability of parameters values, while the dataset produced from the field study will consist in a lower number of parameters but with much higher variability of values due to the natural space-time heterogeneity. These data sets with different characteristics will be used for the production of mathematical models following the scheme pictured in figure 4. In order to set up the nonlinear correlation model we will use the software MATLAB with the needed modules, and the software MARS.

The models developed based on the experimental data will be extrapolated at field scale by a soft programmed in GIS and using maps of the basic control parameters distribution and eventually modular submodels of derived control parameters such as the redox potential in soil. The results obtained after the extrapolation will allow us to check the nonlinear correlation obtained from field data, and, to the extent that the fit between the two approaches will be good, to point out some of the mechanisms underlying the field correlations.

A variety of other inter-comparisons between the models will be performed, as described in figure 4. The overall result will be a coherentised portfolio of models used finally for the simulation of metals mobility in different ecosystems types and degrees of metals contamination, at different scales.

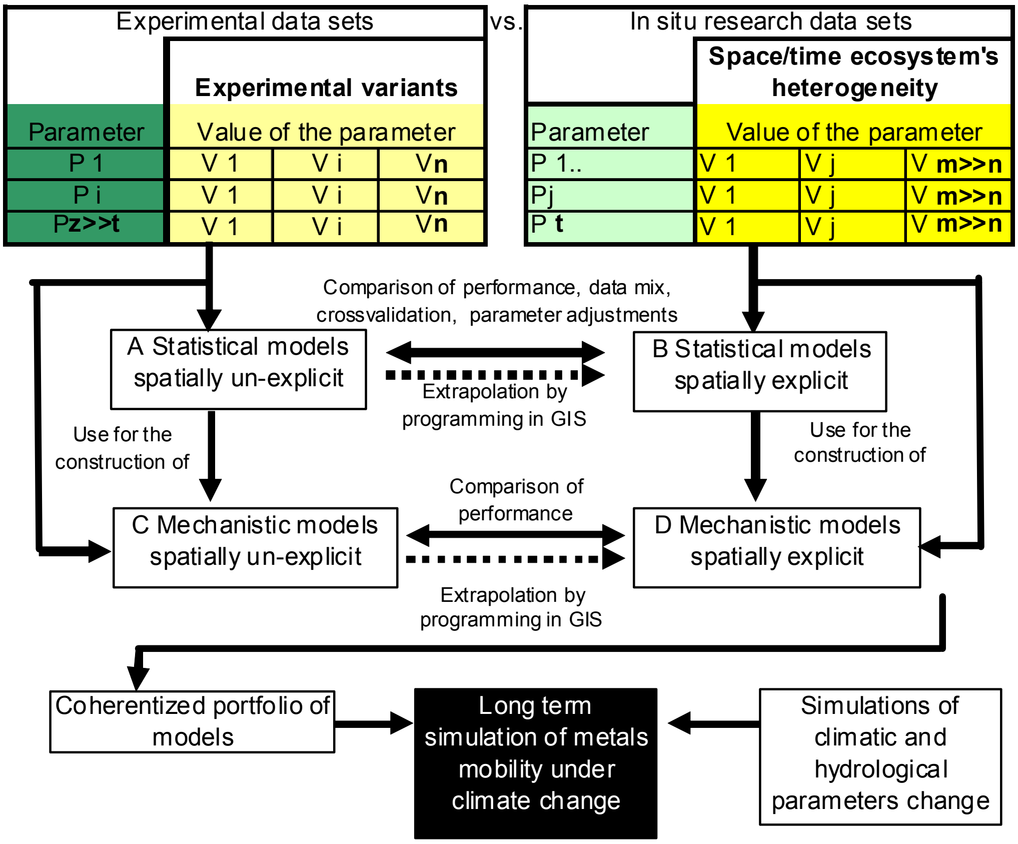


Fig. 4. Relationship between spatially explicit and un explicit models in this project.

3. NOVELTY AND ORIGINALITY DEGREE

After the accumulation of knowledge concerning local and global biogeochemical processes, in the last 10-15 year the international interest moved towards the study of systems of intermediary scale, between local and global (as demonstrated by the increased number of article in ISI journals). The applicative stake is huge, being linked to understanding how local actions are connected with global effects. The researches lead to the proposal of concepts like hot spots (HS) and hot moments (HM), meaning zones in space and periods of time in which the rates of the geochemical and biogeochemical processes are much larger than in other zones and areas. A question not yet answered is at what scale should these concepts be applied. From an applied perspective the scale is imposed by the management problem to be solved, and then the question vanishes (actually this is the answer of the socio-ecological approache). From a naturalistic perspective, adopted in this project, the question can be reformulated as follows: are there objectively certain space-time scales of analyses at which HS and HM can be identified, or rather HS and HM can be identified at any scale of analyses ? O positive answer to the first question would support the theory that the hierarchical theory of the environment (according to which there is a number of hierarchical level and each level is characterized by certain emergent properties which can be identified; the presence of HS and HM could be such emergent properties). If the answer to the second question would be positive, then the notion of hierarchical level would become inappropriate and we should re-conceptualize the relation between the scale of analyses and the occurrence of emergent properties (eventually by using and quantitative indicator for the appearance of emergent properties as the space-time scale of analyses increases). In this context, the project contributes to testing the general hypotheses that there is emergency of biogeochemical properties at certain space-time scales, not reducible to the functioning of the systems identifiable at smaller scale. In particular, the hypothes is that HS and HM organizes relatively independently at certain space-time scales. In systems analyses terms, the hypotheses implies that large scale systems are not fully decomposable in subsystems, that there is not a homomorphic model able to describe all its relevant properties. From the perspective of math modeling the hypothes implies that one cannot build models fully reducing the functioning of large scale systems to that of small scale ones, and then one should develop independent models at least in what concerns the some properties of the system. These consequences would be imposed by the nature of the environment, and not by pragmatic constraints (which currently lead to a *de facto* independent approach of the geochemical and biogeochemical modeling of systems characterized by different scales). One could then ask: which are those ST scales at which the properties we are interested in emerge? An next: do these scales vary in space and time ? The project, beyond its direct contribution to geochemistry and biogeochemistry, contributes to the international efforts to answer such theoretical questions. Applicative importance: Soil Directive, WFD, and ecological risk assessment, by objectives.

4. INTERDISCIPLINARITY AND/OR TRANSDISCIPLINARITY

We will provide arguments for the fundamental character of the project, and next we point out its inter- and trans-disciplinarity. Then the epistemic legitimacy of the approach proposed in this project as compared with other approaches in the research field is discussed.

4.1. The fundamental character

We provide two kinds of arguments for the fundamental character of the research proposed: by the results of the critical analyses of the existing scientific knowledge, and by referring external authorities (National Science Foundation, European Research Council, European Science Foundation, and Directorate General XII – FP7).

4.1.1. Arguments by the critical analyses of the knowledge

In order to find out how the environment is organized one investigates structural elements and processes. Geochemical, soil and biogeochemical processes are amongst the most frequently used for characterizing the so called “hierarchical levels” of the environment’s structure. Implicitly, geochemical and biogeochemical parameters are conceived to emerge only at certain levels of environment’s organization. On the other hand, obtaining general as general as possible knowledge (eventually applicable in all systems of a certain hierarchical level) requires the study of a statistical population of similar sites, which is a difficult task in the case of large systems like catchments. Such research projects are relatively rare (for instance those from the Hubbard Brook experimental area, http://www.hubbardbrook.org/), and have concentrated mostly on macroelements. The idea to intentionally contaminate ecosystem was considered un-ethical (despite the fact that the method was applied in the Experimental Lakes Area, http://www.dfo-mpo.gc.ca/regions/central/science/enviro/ela-rle_e.htm). Alternatively, recently arroused and consolidated the idea to use sites already contaminated for fundamental research of geochemical and biogeochemical processes (Kozlov and

Zvereva, 2007). Their advantage for fundamental research is the high number and the low socio-economic interest in using these sites for other purposes. As Romania’s potential in this respect is, unfortunately, large, the institutions of our consortium oriented their research in this direction and several projects of smaller dimension. Two members of the project team performed a comprehensive critical analyses of literature about mathematical modelling of the biogeochemical cycles of microelements (Iordache and Stelian, 2008), and its results together with the experience accumulated in previous projects will be used in this proposal.

4.1.2. Argument by the authority of prestigious institutions

In the last decade the National Science Foundation had an integrated program dedicated to geochemical and biogeochemical research (http://www.nsf.gov/pubs/1999/nsf999/nsf999.htm), which is currentluy continued by the U. S. Department of Energy Office of Science in direct correlation with contaminated areas (http://www.lbl.gov/ERSP/generalinfo/geochem_biogeo.html). At the level of European Science Foundation the biogeochemistry of trace elements was promoted in the context of polluted systems (http://www.esf.org/fileadmin/be_user/research_areas/LESC/Documents/GPollGoteborg. pdf), of the use of lanthanides for inferring some characteristics of the biogeochemical cycles, of the limiting factors for the development of organisms, and of the modeling of bioaccumulation in food chains (www.esf.org/ecolmat). The European Research Council recently funded (by FP7) 8 Ideas starting grants in area PE8 (Earth system sciences), and 13 are on the reserve list. The FP6 program financed in the area of our proposal an ERA-NET project (SNOW-MAN, http://www.snowman-era.net/index.php). Members of our consortium are in course of involvement in this ERA-NET’s research project with a la carte funding, and they are also contributing to the scientifical aspects of the extention of group of funding agencies with a Romanian one. A priority of FP7 is “Soil processes modeling”.

4.2. Interdisciplinarity and transdisciplinarity of the proposed fundamental research

The interdisciplinary character is obviously underlined by the name of the scientific field (geo-chemistry, bio-geo-chemistry). As for the transdisciplinarity, one can envisage by this term either the fact that the project supports the creation of a new discipline, or that the project supports the creation of a new way of science transcending the disciplinary approach. In the first interpretation, one can argue that biogeochemistry is a new emerging discipline, as suggested by the increasing number of journals dedicated to the field. If one envisage a transdisciplinary character in the second sense, one could argue (from the perspective of the philosophy of science – Staicu and Iordache, 2007) that transdisciplinary approaches by intention function as cultural mutations, and will be subject to selective pressure. Consequently one need as many such mutations as possible, in order to ensure the selection of the most effective one. The integration of all scientific efforts in a single intentionally transdisciplinary approache (e.g. of the „socio-ecological systems” type is ineffective from an evolutionary perspective, despite its efficiency on the short term by centralizing the resources.

5. CONSORTIUM FUNCTIONING SCHEME

The places of the partners of this project in the Consortium for the biogeochemistry of trace elements and the complementarities with major infrastructure projects are presented in figure 5. The management structure of the project is depicted in figure 6. The steering committee of each WP (SCWP) will include on representative from each research unit with expertise in the WP area. Each SCWP will have on WP leader and will send two delegates to the Coordination committee (CC) of the project in such a way to ensure equilibrium of organizations in its structure.

6. MEASURES FOR FULFILLING THE DEONTOLOGICAL NORMS/STANDARDS OF RESEARCH

The large number of feedback loops and the external connection in the structure of the project management (figure 6) will allow 1) the internal control of the quality of results, 2) the external control of the quality of results 3) the early mitigation of any disputes between the institutions or member, including those dealing with ethical aspects. On could remark also the democratic organization, but with clear responsibilities, of the management structure.

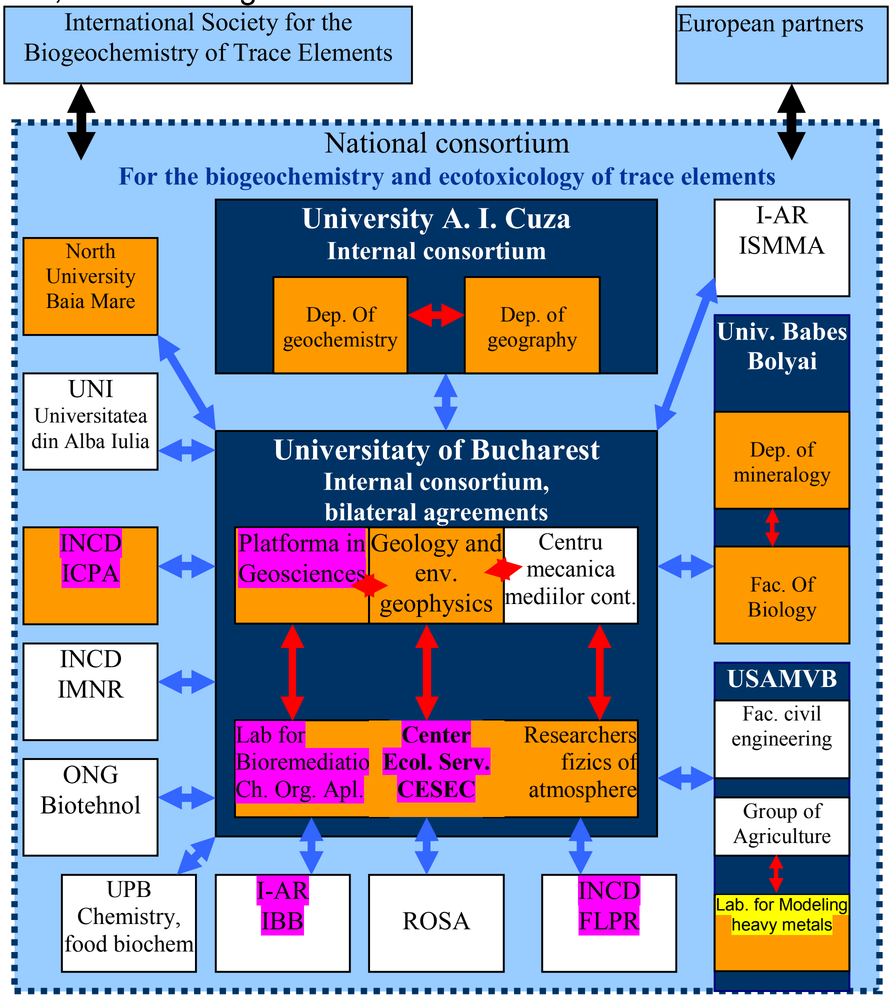


Fig.5. Current structure (open to other potential partners) of the national consortium for the biogeochemistry and ecotoxiciology of trace elements.

With orrage are indicated the partners involved in the BIOGEOST project. The institutions on pink are holders of the infrastructure for trace elements biogeochemistry purchased in the running INFRABIM Capacities project. The institution on yellow is holder of the infrastructure for metals in food chain purchased in the running HEVMETFOOD project. *Legend:* Biotehnol = research center externalized from USAMV Bucuresti, FLPR= National Institute for the Physics of lasers, plasma and radiations, IBB=Biology Institute of Bucharest, Romanian Academy, ICPA = National Institute for Pedology and Agrochemistry, IMNR= National Institute for rare and non-ferrous metals, ISMMA = National Institute for statistics and applied mathematics, Romanian Academy, ROSA = Romanian Space Agency, UPB= University Politehnica of Bucharest.

CONCLUSIONS

We developed a large integrated project in order to catalyze the cooperation between soil scientist, geologists, hydrologist, biologists and ecologists in the frame of the interdisciplinary field of trace elements biogeochemistry. We believe that the above elements and approach can function as an attractor also for other Romanian institutions interested in interdisciplinary environmental research. In particular, for operational reasons we have not included research directions dealing with human and animal health in the project, but this is a necessity and could be done in complementary projects. The value of this approach is confirmed at European level by the success of similarly organized project including Romanian partners (www.umbrella.uni-jena.de).

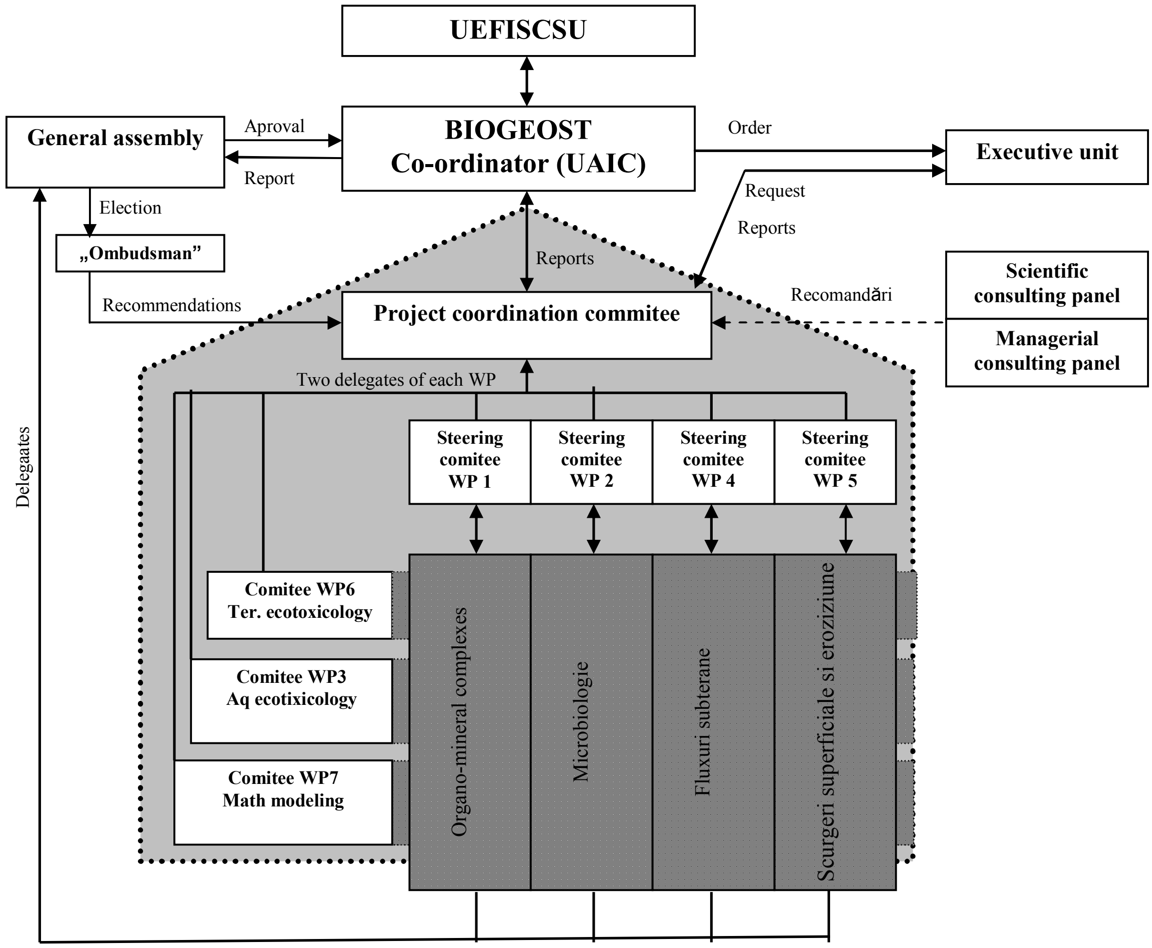


Fig. 6. Structure of BIOGEOST project
(adapted from FP6 IP ALARM, Settele, J., et al., 2005, GAIA, 14, 69-72).

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MINERAL ELEMENT CONTENT OF LEAVES OF TABLE BEET VARIETIES AND THEIR ROLE IN NUTRITION

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ABSTRACT

In our experiment the production of baby beetroot, grown by a method different from the traditional one, was examined with shorter maturation time (70 days) and different genotypes on alluvial meadow soil. In this growing phase the tender leaves are edible so the examination of mineral element content of lamina and petiole was considered important. It was found that the Mg, Ca, Mn, Fe, Cu and Zn content of the lamina was the multiple of the values measured in the petiole. The nutrition-physiologically important ion ratio – $(Ca^{2+}+Na^{+})/(Mg^{2+}+K^{+})$, the optimal value of which in the human organism is ~1.0 – showed better, i.e. lower values in the petiole in all varieties. So, it is worth consuming not only the lamina of beetroot leaves but also their petioles because their mineral element content and composition are very favourable. There is difference between the varieties especially in the Fe content. In this regard the best genotype was the Boro F_1.

Key words: table beet, mineral element, lamina, petiole, ion ratio, genotypes

INTRODUCTION

The mineral element content of root of tablebeet and its nutrition physiological effects have been demonstrated in several studies (Stefanovits Bányai et al., 2002, Blázovits et al, 2007). It is well-known from the technical literature that the leaves of tablebeet were used by the people of the ancient times mainly for medicinal purposes. As a result several scientific papers were published on the bioactive materials of tablebeet leaves. The folic acid content (vitamin B derivative) is very high, thus the consumption of leaves of tablebeet is very useful from point of view of nutrition physiology (Maison, 1994). Based on the above mentioned arguments not only the root of the table beet but also its foliage can enrich our vegetable selection. Thus the leaves of 6-8 cm size gained at thinning can be used for making salads.

The modern alimentation prefers the consuming of more vegetables preferably in fresh form. The special salads have become very popular. In gastronomy they are called mesclun. The word comes from the Latin word misculare that means mixing. Nowadays this word is used for naming the multicoloured mixed salads. For making

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these salads the very young, tender leafy parts of all vegetables are used. They should be very fresh and crunchy and possibly different in colour (claret, yellow, as well as light and dark green). If we prepare a mesclun salad ourselves the leafy parts of the following vegetables should be mixed into it: green lettuce, leaves of baby spinach, tender spinach beet and table beet leaves as well as purslane, lamb's lettuce, dandelion, chervil and parsley.

The favourable nutrition physiological effects of tablebeet are proven by its mineral element content, which is shown in Table 1 compared to the root of tablebeet.

Table1. Mineral element content in different parts of beetroot

Elements		Element content (mg/100g raw material)		
		in leaves	in the flesh	in the rind
Macro-elements	Ca	156.0	10.04	21.19
	Mg	170.3	22.23	44.33
	K	687.7	296.4	375.70
	Na	540,8	121,4	158,60
	P	37,38	49,4	65,65
Micro-elements	Zn	0,88	0,40	0,54
	Fe	2,91	0,85	2,09
	Cu	0,20	0,11	0,13
	Mn	1,15	0,22	0,28
	Al	5,68	0,50	0,58

Based on our measurements (Takácsné Hájos et al., 2000) it is visible that the Ca, Mg and K content of rinds of tablebeet leaves is several times higher than that the values measured in the root.

In growing of this plant the thinning of tablebeet individuals at the 2-4 leaves stage is a part of the production technology when the surplus individuals are removed. These plants are suggested to be used for making fresh vegetable salads as at this stage the leaves can be used together with the petioles. The folic acid content of tablebeet is also very important, however its amount is highly dependent on the varieties.In the varieties characterized by intensively green leaves this compound has been found in higher proportion (Wang - Goldman, 1996).

Taking into consideration the above mentioned reasons and the measurement data, the leaves of tablebeet could be utilized for human consumption offering a bigger choice of vegetables.

MATERIALS AND METHODS

The experiments were conducted in the Horticultural Experimental Farm of Tessedik Sámuel College, Faculty of Agricultural Water and Environmental Management, on alluvial meadow soil. Six beetroot varieties (Beta vulgaris L. ssp. esculenta var. rubra) were examined. Among them there were five Dutch varieties (Taunus, Boro F_1, Pablo F_1, Bikores, Little Ball) and one Hungarian variety (Rubin). The sowing was carried out on 26 April, 2006 where the row space was 15 cm and the size of the plot was 2 m x 0,3 m. Harvesting was carried out on 6 July, 2006 and after it the following measurements were taken: Mineral element values were determined by an atomic absorption method using ICP OES IRIS Thermo Jarrel ASH device. It was conducted in the Institute of Field and Vegetable Crops, Novi Sad. The characterization of soil of our experimental field is shown in Table 2.

Table 2 Soil composition

pH	EC	NO_3-N	P	K	Ca	Mg	Na	Cl	HCO_3
	mScm^1	mgkg^{-1}	mgkg^{-1}	mgkg^{-1}	mgkg^{-1}	Mgkg^{-1}	mgkg^{-1}	mgkg^{-1}	mgkg^{-1}
6.9	0.15	8.23	1.26	21.13	12.33	6.96	16.86	11.43	32.10

pH and EC values include distilled water extract in a ratio of 1:2.

RESULTS AND DISCUSSIONS

It can be concluded that the Mg, Ca, content of the lamina is multiple of the values measured in the petiole (Tables 3-4, and Fig.1), whereas considering the differences in K and Na supply the differences can be neglected in most varieties. The nutrition-physiologically important ion ratio – $(Ca^{2+}+Na^{+})/(Mg^{2+}+K^{+})$, the optimal value of which in the human organism is ~1.0 – showed better, i.e. lower values in the petiole in all varieties. So, it is worth consuming not only the lamina of beetroot leaves but also their petioles because their mineral element content and composition are very favourable.

Table 3 Element content (g kg^{-1}) and ion ratio in the petiole of table beet varieties

varieties / elements	K	Mg	Na	Ca	$(Ca^{2+}+Na^{+})/(Mg^{2+}+K^{+})$
Taunus	61,74	2,75	39,63	4,31	0,681
Boro F1	54,15	3,41	26,73	4,14	0,536
Pablo F1	54,36	3,53	31,09	4,30	0,611
Bikores	48,72	2,33	26,46	3,55	0,588
Rubin	54,47	2,69	34,75	4,83	0,692
Little Ball	53,62	2,83	30,29	5,15	0,628
mean	54,51	2,92	31,49	4,38	0,62
St. dev.	4,16	0,46	5,03	0,56	0,06

Table 4 Element content (g kg^{-1}) and ion ratio in the lamina of table beet varieties

varieties / elements	K	Mg	Na	Ca	$(Ca^{2+}+Na^{+})/(Mg^{2+}+K^{+})$
Taunus	50,81	17,31	36,97	15,14	0,765
Boro F1	43,15	20,00	35,66	15,97	0,818
Pablo F1	41,67	19,98	33,39	17,60	0,827
Bikores	45,69	20,22	37,26	16,35	0,813
Rubin	44,73	15,66	33,08	16,45	0,820
Little Ball	46,46	19,26	39,71	18,75	0,889
mean	45,42	18,74	36,01	16,71	0,82
St. dev.	3,16	1,85	2,52	1,28	0,04

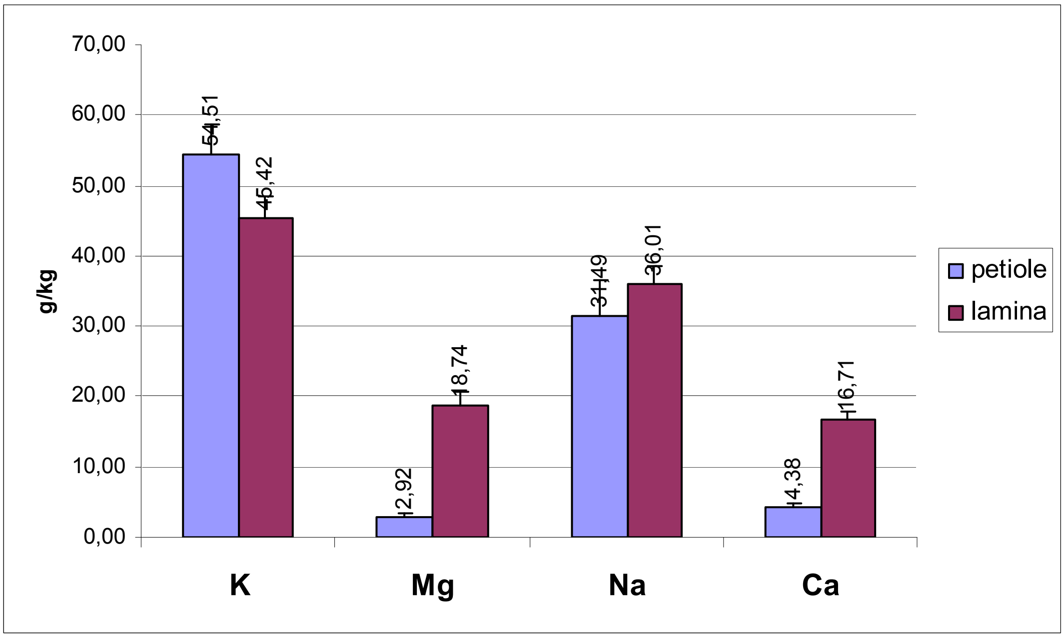

Fig. 1. Mezo-element content in the different part of table beet leaves

Evaluating the mezzo-elements (Table 5-6, Fig.2) we considered, that higher amount of them is the Fe in the table beet leaves. The most important statement, that the Mn and Fe content of lamina is multiple as those in petiole.

Table 5 Mezo-element content (mg kg^{-1}) in the petiole of table beet varieties

varieties / elements	**Cu**	**Zn**	**Mn**	**Fe**	**Al**
Taunus	13,05	15,43	54,86	209,53	305,43
Boro F1	18,99	20,97	51,51	523,53	513,65
Pablo F1	25,31	20,75	52,31	456,40	588,10
Bikores	15,79	20,94	39,82	445,63	582,73
Rubin	17,60	17,46	60,84	369,70	414,55
Little Ball	11,09	20,10	45,10	384,40	388,90
mean	16,97	19,28	50,74	398,20	465,56
St. dev.	5,01	2,31	7,39	107,65	114,17

Table 6 Mezo-element content (mg kg^{-1}) in the lamina of table beet varieties

varieties / elements	**Cu**	**Zn**	**Mn**	**Fe**	**Al**
Taunus	42,60	27,93	357,83	743,43	689,15
Boro F1	52,55	50,93	238,55	1144,08	1189,90
Pablo F1	61,83	49,28	334,47	876,18	1075,70
Bikores	65,09	49,41	238,02	1035,00	903,78
Rubin	66,02	39,55	354,42	983,00	809,75
Little Ball	47,71	37,10	333,70	1102,88	1195,48
mean	55,97	42,37	309,50	980,76	977,29
St. dev.	9,77	9,11	56,05	149,53	209,28

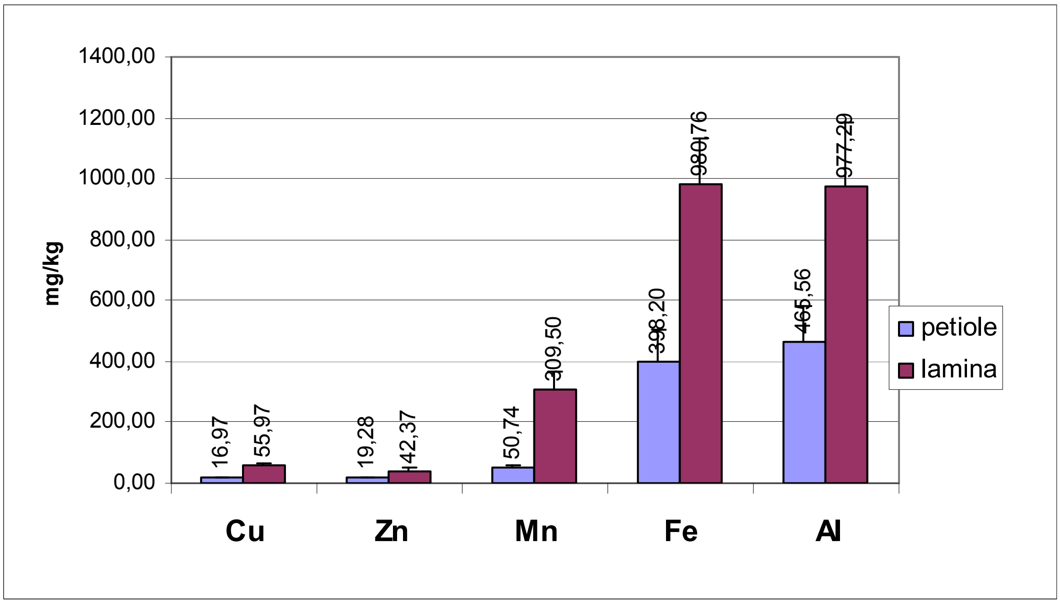

Fig. 2. Mezo-element content in the different part of table beet leaves

We measured great difference in Fe content between the varieties (Fig.3) which is may be connected with the assimilation ability of lamina. The Boro F1 exceeded in several important mineral element content. It is affirmed, that choice of varieties is very important in this roil too.

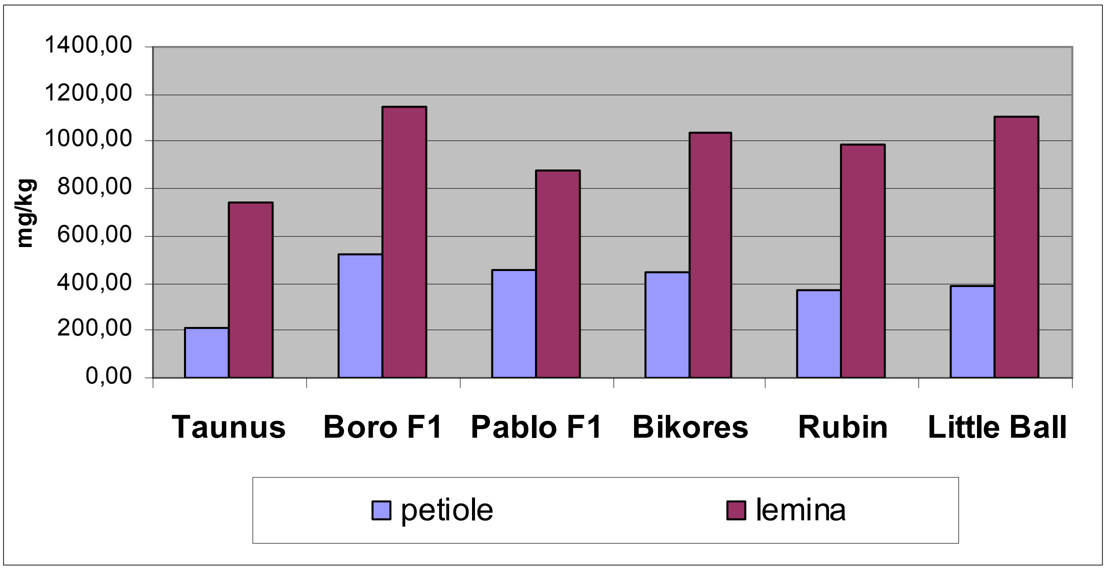

Fig. 3. Fe-content in the table beet varieties

CONCLUSIONS

It can be concluded that the Mg, Ca, Mn, Fe, Cu and Zn content of the lamina is multiple of the values measured in the petiole, whereas considering the differences in K and Na supply, the differences can be neglected in most varieties. The nutrition-physiologically important ion ratio – $(Ca^{2+}+Na^{+})/(Mg^{2+}+K^{+})$, the optimal value of which in the human organism is ~1.0 – showed better, i.e. lower values in the petiole in all varieties. So, it is worth consuming not only the lamina of beetroot leaves but also their petioles because their mineral element content and composition are very favourable. By the way it seems very important the choice of varieties too, because there is great difference in mineral element content.

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Dedicated to the memory of
Professor Petru Dragan (1932-2007)

CHARACTERISTICS OF THE METALLOGRAME IN CYSTINE UROLITHIASIS – INVESTIGATION MEANS

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ABSTRACT

Urinary stone disease reveals a multifactorial ethiogenesis. All types of renal stones originate on excessive supersaturation of the urine with a poorly soluble material. This depends not only on solute concentration but also on pH, ionic strength and the existence of soluble complexes.

Cystine crystals are found in urine of almost exclusively pacients with a genetic disease giving an impairment in renal and intestinal cystine transport with decreased proximal tubular reabsorption resulting an increased cystine excretion.

Our study used infrared spectroscopy (IRS) for the determination of urolithiasis' types and atomic absorption spectroscopy (AAS) to draw the metallograms.

Keywords: cystine urolithiasis , metallograms

INTRODUCTION

Cystine is an amino acid dimmer formed from two molecules of cysteine joined by a disulphidic bond. Cystinuria is an autosomal recessive disorder of the amino acid transport in thekidney leading to an abundance of cystine in the urine. This is a poorly soluble molecule that crystallizes at concentration of above 300 mg/L. This value may be regarded as the "clinical saturation point" for cysteine. Over this concentration, cystine calculi may form or enlarge. There have been described more types of cystinuria: due to two mutations of the SLC3A1 gene encoding a subunit of the transport mechanism; two mutations of the SLC7A9 gene encoding the transport channel itself. There also appear mixed types from the combination of recessive and dominant alleles (Matouschek, 1981; Heininger, 1986; Goodyer, 1998). Cystinuria is the most common defect in the transport of an amino acid.

Although the exact molecular basis is not yet elucidated, future investigations are based on molecular and gene therapy.

MATERIALS AND METHODS

Cystine crystals form hexagonal-shaped crystals which can be viewed upon microscopic analysis of the urine. Cystine stones are visible on standard abdominal

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.61-66, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

radiographs because of the relative density of the sulphur constituent of cystine (Robertson and Peacock, 1984; Watts, 1984; Coe and Parks, 1988; Martins et al., 2002; Ahmed et al., 2006; Evan et al., 2006).

Sample prelevation. Investigations have been performed on 92 calculi surgically removed or spontaneously eliminated by pacients from the University Clinic of Urology Timişoara.

Analytical investigations. In previous investigations we obtained by IRS the standard spectra of the pure compounds frequently found in kidney stones. In the first step we recorded the spectra of each calculus and established, by comparison with the data base, the composition of uroconcrements. Subsequently, by AAS, was determined the metal composition, i.e. the content of alkaline, alkaline-earth and transitional metals.

Statistical evaluation. By the use of a computerized method, the mean value (X) and standard deviation (SD) were obtained.

RESULTS AND DISCUSSIONS

Although cystine is not the only overly excreted amino acid in cystinuria, it is the least soluble of all naturally occurring amino acids. The key process in the development of kidney stones is supersaturation. As to frequency among other types of urolithiasis, cystinic calculi are about 1% in adults and up to 8% in children. The stones are usually multiple, they tend to recur after surgical removal, and bacterial infections often complicate the clinical signs (Williams and Chisholm, 1976; Ala-Opas et al., 1990; Galka et al., 1990; Sakhaee, 1994)

The IRS identification of unknown compounds makes use of standard spectra that offer data on characteristic absorption domains and vibrational states. Fig. 1 presents the standard spectra of cystine.

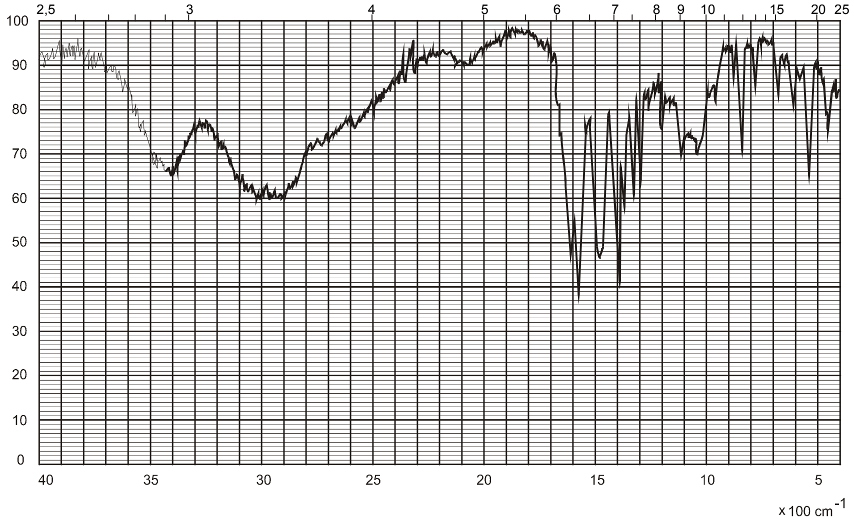

Fig. 1. Standard spectra of cystine

The cystine transporter also promotes the reabsorption of the other dibasic amino acids such as: cystine, lysine, ornithine, arginine, homoarginine. The structural formula of the amino acids excreted in high amounts are presented in figure 2.

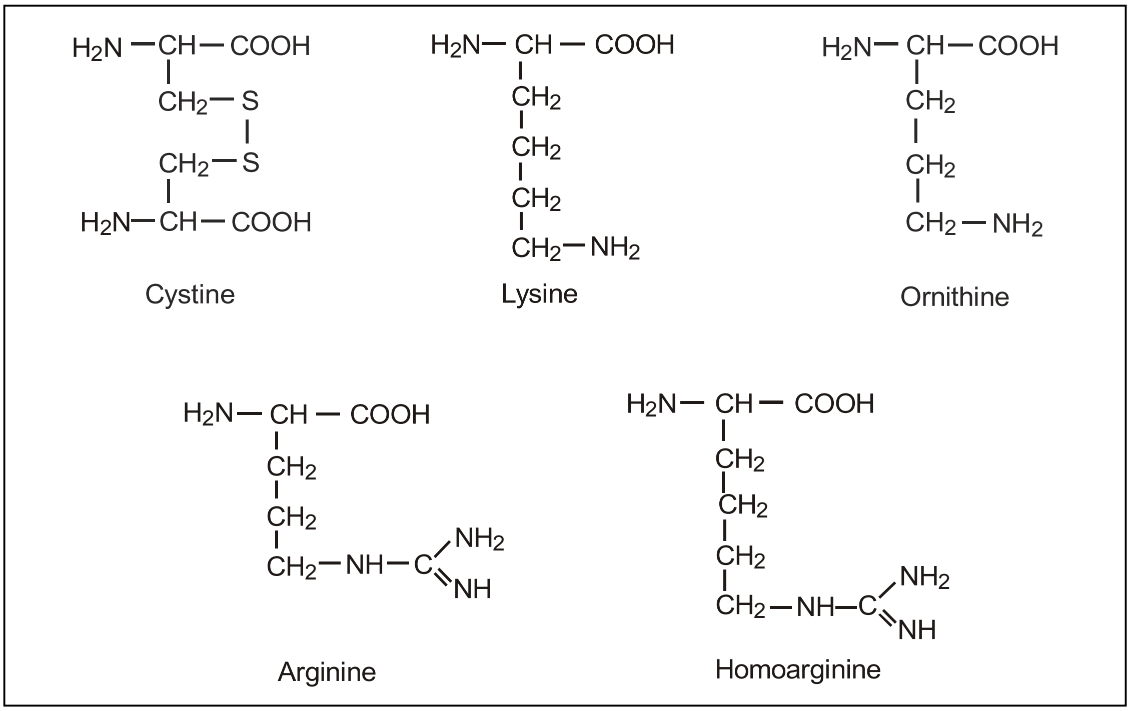

Fig. 2. Amino acids in excess decelated in cystinuria

Metal ions present in the blood and urine play an important role in the organism. Some of these metals can prevent the concrements formation at the level of urinary tract, gall bladder or salivary gland while the others inhibit the crystallization.

Metal ions compete in the process of coprecipitation of the calculi' ingredients by the bioinorganic mechanism of lithogenesis.

A synopsis of the types of studied lithiasis is given in table 1 and table 2. One can observe that out of all 90 calculi only 3 contained cystine.

Table 1. Simple urolithiasis revealed by IRS

Type of urolithiasis	Composition		Number of cases		
			Total	Men	Women
SIMPLE	Oxalates (O)		21	12	9
	Purinic derivatives	Urates (U)	15	7	8
		Xanthine (X)	--	--	--
		2,8-dihydroxyadenine (2,8-DHA)	--	--	--
	Phosphates (F)		14	5	9
	Cystine (C)		3	1	2
Total – simple urolithiasis			53	25	28

Table 2. Mixed urolithiasis revealed by IRS

Type of urolithiasis	Composition		Number of cases		
			Total	Men	Women
MIXTE	Binary	Oxalates- Phosphates (O-P)	13	6	7
		Oxalates - Urates (O-U)	5	1	4
		Oxalates - Cholesterol (O-COL)	3	2	1
		Urates -Xanthine (U-X)	3	1	2
		Phosphates - Oxalates (P-O)	6	2	4
		Phosphates - Carbonates (P-CARB)	3	1	2
	Ternary	Phosphates – Oxalates - Carbonates (P-O-CARB)	1	1	--
		Oxalates - Urates - Phosphates (O-U-P)	3	1	2
Total –mixte urolithiasis			37	15	22

The presence of metals in the organisms, their homeostasis, their involvement in metabolic processes as well as the perturbation of these processes, e.g. urolithiasis, represent problems of special interest (Daranyi et al., 1994; Garban et al., 1996; Dragan et al., 1998; Worcester et al., 2006). Determination of the metals present in uroconcrements can contribute, indirectly, to the explanation of the mechanism of heterogenous nucleation to which organic and inorganic metabolites compete.

Determination of concentration of the main alkaline, alkaline-earth is presented in table 3.

Table 3. Metallogram of cystinic uroconcrements (alkaline and alkaline-earth metals)

Metals		**UM**	**No. of cases**	Concentration of metals **X ± SD**
Alkaline	Na	µg/g calculus	11	242.53 ± 58.10
	K		11	601.77 ± 183.15
Alkaline-earth	Ca		11	3050.86 ± 1416.78
	Mg		11	320.18 ± 121.37

Determinations regarding transitional metals, that exist in more reduced quantities than the alkaline and alkaline-earth ones, are presented in table 4. There are given results for a greater number of calculi, i.e. there were added 8 more from a previous period of analytical determinations.

Table 4. Metallogram of cystinic uroconcremets (microlements)

Metals	**UM**	**No. of cases**	**Concentration of metals X ± SD**
Zn	µg/g calculus	11	41.20 ± 14.45
Fe		11	14.51 ± 4.05
Cu		11	15.06 ± 4.16
Mn		11	4.36 ± 1.83

Researches performed at the University Clinic of Urology Timişoara revealed the role of metals inthe ethiopathogeny of lithiasis and led to the hypothese of bioinorganic mechanism of urolithogenesis (Garban and Daranyi, 1994; Dragan et al., 1998). Obviously, this mechanism does not facilitate the formation of starters and thereafter, of uroconcrements (Williams şi Chisholm, 1976).

CONCLUSIONS

1. The appearance of metals in urolithiasis is the consequence of their presence in urine;
2. By means of infrared spectroscopy the types of lithiasis (simple and mixte ones) have been established;
3. Metallograms were drawn using atomic absorption spectroscopy, revealing: alkaline metals: Na < K; alkaline earth metals : Mg < Ca; microelements: Mn < Fe< Cu< Zn
4. Metals play a predictive role on the bioinorganic composition of cystinic uroconcrements.

Acknowledgements. The authors express their thanks to the doctors from the Clinic of Urology Timişoara who cooperated at the collection of calculi from surgical interventions. It is mentioned that the University Clinic of Urology, University of Medicine and Pharmacy "Victor Babeş" Timişoara, was led by Prof. dr. Petru Dragan (1932-2007).

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CONSEQUENCES OF SUBACUTE INTRATRACHEAL EXPOSURE OF RATS TO CADMIUM OXIDE NANOPARTICLES: ELECTROPHYSIOLOGICAL AND TOXICOLOGICAL EFFECTS

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ABSTRACT

Cadmium is a metal used in various industrial applications, whereby emission of Cd-containing fumes is likely. The submicron sized particles in the fumes represent an extra risk due to their high mobility within the organism and high surface area. Toxicity of Cd on the liver, kidney and bones is well known, but there are less data on its neurotoxicity. Here, male Wistar rats were treated for 3 and 6 weeks by intratracheal instillation of CdO_2 nanosuspension. The treated rats' body weigh gain was significantly decreased, and in the high dose (0.4 mg/kg Cd daily) rats the weight of lungs and thymus was significantly increased. In this group, the spectrum of spontaneous cortical electrical activity was shifted to higher frequencies, the latency of the somatosensory evoked potential was lengthened and its frequency following ability, impaired. The visual evoked potential also had increased latency. Cadmium had access from the airways to the brain and exerted neurotoxic effects.

Key words: cadmium, nanoparticles, lungs, body weight, organ weight, electrophysiology

INTRODUCTION

Cadmium has various industrial applications in steel and other alloys, pigments, in batteries and in electroplating. Occupational exposure is mainly due to inhalation of metal fumes (WHO, 1992), and the exposure of smokers due to Cd content of tobacco leaves is similar (Elinder et al., 1983). Cd is a heavy metal with considerable general toxicity. Its main target organs include the liver, kidneys and bones, and the respiratory and cardiovascular systems (ATSDR, 1999), but Cd exposure can also cause behavioral and neurological disorders. In occupational Cd exposure, reduced visuomotor performance and difficulties of concentration and postural balance were observed (Viaene et al., 2000). In children, a straight relationship between hair Cd and visual or auditory evoked potential parameters was found (Thatcher et al., 1984), and school behavioural problems were reported (Marlowe et al., 1985). Similar effects were observed in rats (Agar et al., 1990; Yargicoglu et al., 1996). Our previous works on Cd neurotoxicity revealed altered electrocorticogram (ECoG) power spectrum, and effects on cortical evoked potentials and peripheral nerve action potentials (Papp et al., 2003; Institóris et al., 2002).

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.67-72, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

Cd containing metal fumes (from smelting, casting, welding etc.) contain both microscopic and submicroscopic particles, and the two show dissimilar behavior after inhalation (Elder et al., 2006). The health importance of submicron particles has been recognized newly, and attributed to their high number and large surface area (Oberdörster et al., 2005). Nanoparticles (NPs) easily cross barriers within the organism, such as the respiratory or olfactory epithel and the blood-brain barrier, not penetrable for larger (microscopic) particles. Transport from the olfactory epithel directly into the CNS via then olfactory nerve has also been described.

Within the framework of the *Regional University Knowledge Centre for Environmental and Nanotechnology, Szeged, Hungary* a series of animal experiments has been carried out to see the nervous system effects of toxicologically relevant nanoparticles. In the present work, CdO_2 nanoparticles were given to adult rats, and general and nervous system effects were observed.

MATERIALS AND METHODS

Adult male Wistar rats of 320-350 g body weight were housed under standard conditions (22 - 24°C, 12-hour light/dark cycle with light on at 06:00) with free access to tap water and standard pellet. The rats were divided into 4 groups of 20 animals each at start. Nanoparticles of CdO_2 (20±5.7 nm diameter) were synthesized at the Department of Applied Chemistry, University of Szeged, and were suspended in distilled water. The suspension was instilled into the rats' trachea, in the daily doses shown in Table 1., 5 days a week (Monday to Friday). The volume instilled was 1 ml/kg b.w. Treatment was continued for 3 and 6 weeks, so that 10 rats from each group were sacrificed after 3 weeks treatment, and the remaining 10 after 6 weeks. There was an untreated control group (Con), and a vehicle control group (W), as shown in Table 1.

Table 1. Treatment groups and doses.

Group	Code	Treatment and dose	Duration
Untreated control	Con	---	3 and 6 weeks
Vehicle control	W	Distilled water 1 ml/kg b.w.	
Low dose	LD	CdO_2 nanosuspension, 0.04 mg Cd/kg b.w.; 1 ml/kg b.w.	
High dose	HD	CdO_2 nanosuspension, 0.4 mg Cd/kg b.w.; 1 ml/kg b.w	

Intratracheal instillation was done in diethyl ether anesthesia. The rat was suspended on a 60° inclined board so that its upper incisors were held by a wire loop to keep the animal's mouth open. The trachea was illuminated transdermally. The tongue was pulled forward with a pair of non-traumatic forceps, and a custom-made laryngoscope was used to visualize the glottis. The nanosuspension (or distilled water for group W) was instilled into the trachea by means of a syringe and 1.2 mm OD plastic tubing, inserted between the vocal chords. The untreated control group (Con) had neither ether anesthesia nor instillation, while the water control (W) group was anesthetized and instilled with distilled water. The nanosuspension was vigorously sonicated before, and repeatedly during, administration to prevent agglomeration.

Body and organ weights were the endpoints for general toxic effect of the nano-CdO_2. The rats' body weight was measured each Monday during treatment, and once more on the day of sacrifice, and the mean body weight of the groups was plotted against time to see the course of weight gain. Following electrophysiology (see below), the rats were sacrificed by an overdose of urethane, dissected, and the organ weight of the brain, liver, lungs, heart, kidneys, spleen, thymus and adrenals was measured. Relative weights were calculated by relating organ weights to 100 g body weight.

Electrophysiological recording was done 3-4 days after the last instillation. In urethane anesthesia the animal's head was fixed and the sensory areas of left hemisphere were exposed. The wounds were sprayed with 10% lidocaine, and a thin layer of petroleum jelly was applied on the dura to prevent drying. After 30 minutes recovery, silver electrodes were placed on the primary somatosensory (SS), visual (VIS) and auditory (AUD) areas. Electrocorticogram (ECoG) was recorded from these areas for 6 minutes, and the relative spectral power of the frequency bands (delta, theta, alpha, beta1, beta2, gamma; standard human EEG bands) was determined.

Then, sensory evoked potentials (EPs) were recorded by the same electrodes. For somatosensory stimulation, 2 needles were inserted into the contralateral whiskery skin to deliver square electric pulses (3-4 V, 0.05 ms, 1-10 Hz). Visual stimulation was produced by a high-luminance white LED aimed directly at the rat's right eye, driven by 0.2 ms pulses at 1 Hz. The acoustic stimuli were clicks (1 Hz, 40 dB) guided from a miniature earphone into the animal's right ear via the hollow ear bar. Fifty stimuli of each modality per rat were applied and the evoked activity was recorded. After averaging, latency and duration of the evoked responses was measured manually (for details, see Lukács and Szabó, 2007).

The change of latency of the somatosensory EP with increasing stimulation frequency was also investigated as a possible indicator of the action of the treatment on the state of the cortex. All electrophysiological recording and analysis was done by means of the Neurosys 1.11 software (Experimetria Ltd, Budapest, Hungary). From the general toxicological, behavioral and electrophysiological data, group means (±SD) were calculated. The results were tested for significance with one-way ANOVA and the post hoc analysis was done by Scheffe's test.

RESULTS AND DISCUSSSIONS

Intratracheal exposure by the nanoparticulate CdO_2 had marked effect on the rats' body weight gain. As shown by Table 2, the untreated controls' (Con) weight

Table 2. Body weight gain (grams) of the rats during the 6 weeks of treatment

Groups Weeks	Con	W	LD	HD
0	360±18	360±18	353±26	353±24
1	382±20	375±21	364±27	351±31
2	416±24	400±24	388±32*	359±31**$^{\#\#}$
3	434±23	409±24*	400±31**	378±35***$^{\#\#\#\circ\circ}$
4	462±49	426±28*	409±38***	411±37***$^{\#\#}$
5	495±27	445±26	430±37**	438±36**
6	510±27	444±32	439±34***	450±33***

*,**,***: $p<0.05$, 0.01, 0.001 vs. Con; $^{\#\#,\#\#\#}$: $p<0.01$, 0.001 vs. W; $^{\circ\circ}$: <0.01 vs. LD.

gain was undisturbed. In the vehicle control (W) group (anesthesia and instillation but no CdO_2) the weight gain was lower, and with the advance of time became more and more similar to that seen in the treated rats. In the HD group, there was hardly any weight increase in the first weeks.

Then, some compensation seemed to take effect and the weight gain was similar to that seen in the LD group and approached that of the vehicle control (W).

The relative weight of the lungs was significantly higher in the HD group vs. Con after 6 weeks exposure (Fig. 1). In the W and LD groups, there was no noteworthy increase. There was also significant increase of the relative thymus weight in the HD group, and decrease of the relative spleen weight in all treated groups. After only 3 weeks treatment, the trends were similar but less expressed.

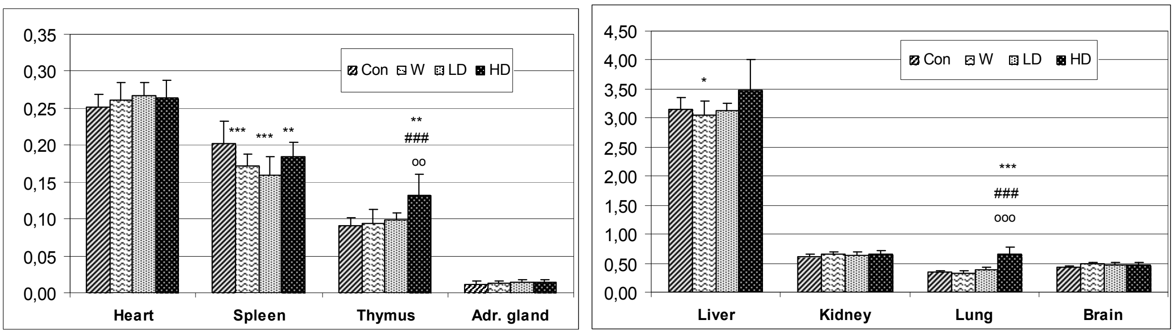

Fig. 1. Relative organ weights (ordinate, organ weight/[0.01 x body weight]) of the rats. For group codes, see Table 1. *, **, ***: $p<0.05$, 0.01, 0.001 vs. Con; $^{\#\#\#}$: $p<0.001$ vs. W ; $^{\circ\circ}$,$^{\circ\circ\circ}$**:** <0.01, 0.001 vs. LD.

Electrophysiological effects: The most typical alterations were observed in the somatosensory cortex. In the ECoG, there was a dose-and time-dependent shift from slower to faster waves which became significant in the HD group after 6 weeks exposure (Fig. 2). A similar trend, without significance, was seen in the other cortical areas.

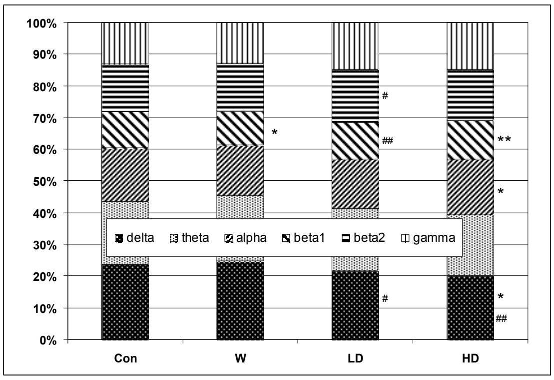

Fig. 2. Band spectrum of the somatosensory electrocorticogram. Group codes and significance marks as in Fig. 1.

The somatosensory EP showed significant latency increase in the HD group vs. Con (Fig. 3). The slight dependence of the latency on the inter-stimulus interval (inverse frequency) seen in Con was more expressed in the treated groups, up to the significant difference between the latencies obtained with 1000 and 100 ms interval in the HD group. The changes of the EP duration were less clear-cut but the increase in HD vs. Con was significant. The latency of the visual EP changed similarly to the somatosensory EP in the treated animals (Fig. 4). The changes of the auditory EP were inconclusive.

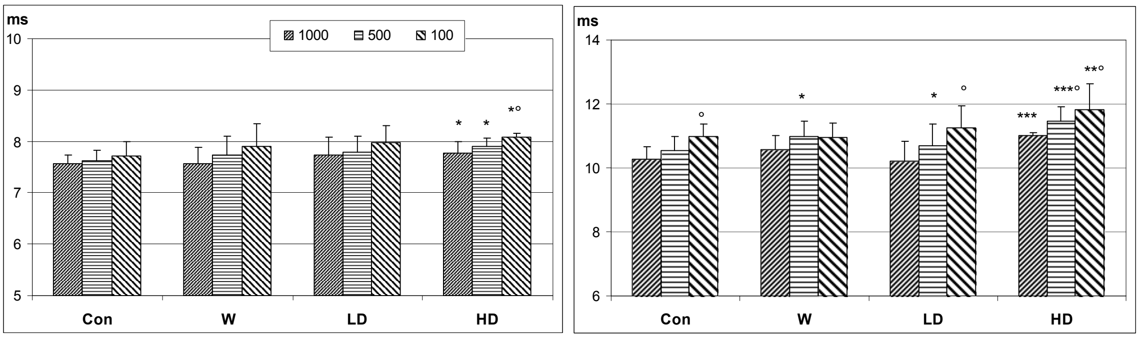

Fig. 3. Latency (left) and duration (right) of the somatosensory evoked potentials in the control and treated rats at varied inter-stimulus intervals (in ms, see insert).
*,**,***: $p<0.05$, 0.01, 0.001 vs. control; ° : $p<0.05$ vs. lowest frequency stimulation in the same group.

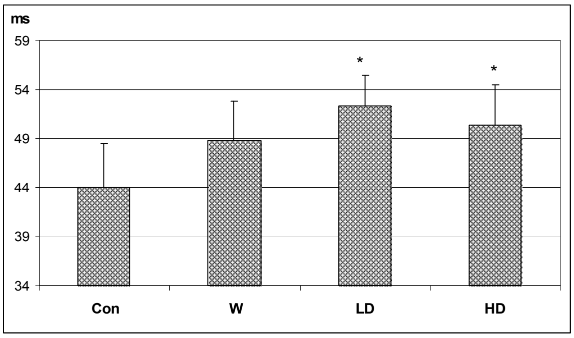

Fig. 4. Latency of the visual evoked potential in the control and treated rats. 1 s inter-stimulus interval only. Displayed as in Fig. 3.

The general and electrophysiological changes indicated that cadmium instilled into the rats' trachea in form of CdO_2 nanoparticles was in fact absorbed and unfolded its toxic effects. The ECoG shift was similar to that found with oral application of dissolved Cd for 12 weeks, alone (Papp et al., 2003) and combined with the insecticide propoxur (Institóris et al., 2002). The body and organ weight effects were also in agreement with our previous results. The time trend of body weights and the increase of alterations after 6 vs. 3 weeks indicated a gradual build-up of Cd and its effects in the treated rats' body. Absorption of Cd following inhalation (or similar exposure) has been described repeatedly. Takenaga et al. (2004) found Cd in the blood, liver and kidney of rats after inhalation of a much smaller amount than our dose. In exposed workers, elevated urine Cd level was associated with with reduced visuomotor performance and difficulties of concentration and stance (Viaene et al., 2000). First of all the latter effect indicated that Cd had access from the lungs to the brain, despite its low permeability across the blood-brain barrier (ATSDR, 1999). In rats exposed by Cd orally for two months, deposition in the cortex, hippocampus and other brain regions was seen (Clark et al., 1985). EEG alterations were seen in rats after only 9 days of Cd exposure (Vataev et al., 1994).

Literature data and our own results sufficiently prove that Cd exposure bears a considerable risk of nervous system damage and that Cd, entering the organism via the airways, has access to the CNS. This means that workplace airborne Cd has to be strictly regulated, Cd containing waste be treated accordingly, and Cd replaced with less hazardous substances where technically possible.

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BIOACCUMULATION OF METALS IN FOOD CHAINS OF WETLANDS

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ABSTRACT

We studied the accumulation of metals in food chains of Danube floodplain, from soil and detritus towards vegetation, Carabidae and frogs. We found no simple pattern of the bioaccumulation of metals in the food chain, but most of the bio concentration occurred at the level of Carabidae, and not at the level of Rana sp. Mobile groups seem to not be a good indicator of pollution with metals at local level in large flood plains.

Key words: heavy metals; flood plains; bioaccumulation;

INTRODUCTION

Floodplains are involved in the retention of the metals transported by rivers (Finlayson, 1994; Wallach et al., 2001) and are a usual study model for the biogeochemistry of metals (Hobbelen et al., 2004; Griethuysen et al., 2005; Boivin et al., 2007). The flood plain of the lower Danube River has a length of hundreds of km and consists of riparian and insular micro landscapes (Vădineanu et al., 2001), 80% of which are diked. However, the lower Danube flood plain preserves several natural areas under natural flooding regime which are actively involved in the retention of nutrients and metals from the Danube basin (Iordache and Ruță,2006).

The issue of the retention time of metals in flood plains is an important and still unresolved one. Remobilization processes and transfer through food chain are possible, with subsequent ecotoxicological risks. In particular, the metals can affect planktonic systems (Baca and Threlkeld, 2000), birds (Levengood and Skowron, 2001), amphibians (Bank et al., 2005), and detritus consuming invertebrates (Weis et al., 2002).

Estimating bioaccumulation of metals along the food chains is subject to the challenge of how to integrate the space-time heterogeneity of the distribution of organisms. This is an important issue especially where the fluxes between ecosystems are intense, as in flood plains. Mobile species would presumably intercept metals from larger areas than sessile species, but biogeochemical data to support this statement are scarce. Ground beetles (Carabidae) and frogs (Rana sp.) are mobile species commonly occurring in flood plains, usable as overall indicators of wetlands functionality.

Carabidae have been subject of previous studies in areas contaminated with metals, with focus on the distribution (Read et al., 1987, Andrews et al., 1989,

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.73-80, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

Rabitsch, 1995; Heikens et al., 2001) or the effects of metals (Lock et al., 2001; Stone et al., 2001; Lindqvist et al., 2005). Frogs have also been subject of such studies (Birdsall et al., 1986; Roe et al., 2005; Loumbourdis et al., 2007). By their diet, frogs integrate fluxes from various sources, ranging from phytofagous, to detritivorous and to raptor invertebrates (Cogălniceanu, 1997; Cogălniceanu et al., 1999). Carabidae species are an appropriate model for the diet of frogs because they belong to all these trophic groups (Ciubuc, 1997). However, the study of metals accumulation in Carabidae and frogs has seldom been put in the larger context of a food network.

In this context, our purpose in this article is to characterize the accumulation of metals in food chains of Danube floodplain, from soil and detritus towards vegetation, Carabidae and frogs. The results will show to what extent these compartments are useful as direct indicators of the local contamination with metals.

MATERIALS AND METHODS

Site description

We studied three micro landscapes located in the Small Island of Brăila, Danube floodplain (Figure 1). Two landscapes are located in islands (H and I), and one in the riparian area (G). “H” landscape is a late succesional one, with mature forests and marshes in its structure, and with low sediment dynamic during floods. “I” landscape is an early succesional one, with young forests and former channels in its structure, and with high sediment dynamic during floods. While H and I are in a natural state, “G” landscape is impacted by human activities: part of it is disconnected from floods by a dike, it includes a planted forest and it is grazed non-intensively by livestock.

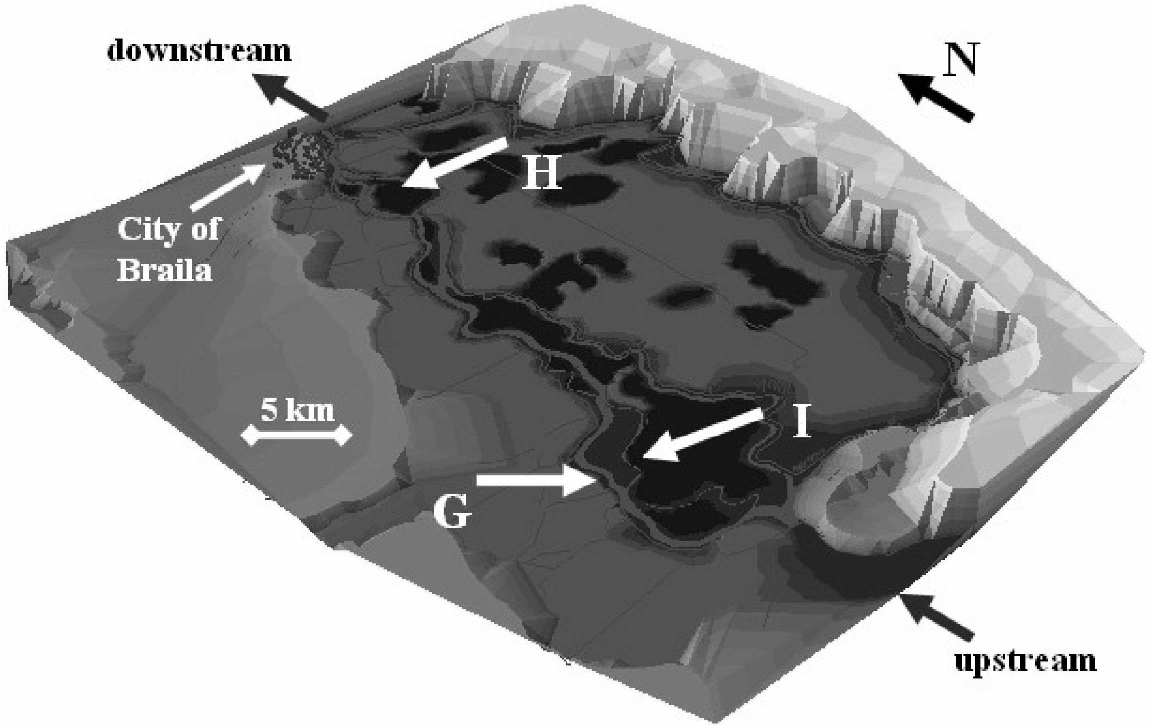


Fig. 1 The location of transects in the Danube floodplain. The vertical dimension is exaggerated in order to point out the floodplain

In each micro landscape we had four stations, from the Danube to the internal part of the landscape. Transect H started from the shore (H1), crossed a mature forest located on a high levee (H2), a natural forest from the nearby depression (H3), and a marsh (H4). Transect I crossed the shore (I1), a low forested levee (I2), a silted former Danube channel (I3), and a high forested levee (I4). Transect G included a shore (G1), a planted forest on high levee (G2), a depression (G3), and a diked depression (G4).

Sampling program

Top soil was sampled monthly during one year in five replicates. Detritus was sampled in five replicates using a 0.25 x 0.25m quadrate at several moments, depending on the state of the ecosystem (flooded or not). We had detritus of terrestrial origin (decomposed litter from previous years), and detritus of aquatic origin (snails and mussels at I1 and I3, snails at G1, snails and aquatic macrophytes at H3 and H4; details and species in Iordache and Ignat in preparation). In some ecosystems both types of detritus were present (H3, H4). We sampled previously known dominant herbaceous plant species (Cristofor et al., 1997) at three moments during the growing season in nine replicates. The dominant plant species differed from one ecosystem to another (details and species in Iordache in preparation). Terrestrial invertebrates were sampled fortnightly with nine traps in each ecosystems (method described in Ciubuc, 1999), in the frame of a project dealing with biodiversity. Dominant species of Carabidae were provided for analyses of metals concentrations, as follows: Pseudophonus rufipes (phytofagous), Carabus cancellatus (raptor), Chlaenius festivus (raptor), Chlaenius spoliatus (mostly detritivorous, sometimes raptor) and Poecillus cupreus (detritivorous). Frogs (Rana sp.,) were sampled on a transect base during several campaigns (using the methods described in Cogalniceanu, 1997), in the frame of a project dealing with the amphibians community. From transects G and I we analyzed adults (15 replicates), and from transect H adults, juveniles and tadpoles (10-15 replicates).

Samples preparation and chemical analyses

The soil samples were sieved at < 0.5 mm. The detritus of aquatic origin was separated in organic material and shells, in order to analyze only the organic material, accessible to detritivorous Carabidae. After harvesting, the plants were weighed (fresh weight) and partitioned for analysis into aboveground and underground part of plants. In this article we use the data characterizing the aboveground parts. Because Carabidae had been stored in ethanol before analyses (a method used also by Rabbitsch in 1995) we checked the effect of the preservation method by analyzing Pseudophonus rufipes individuals (the most frequent species) captured alive and stored by freezing or in ethanol (for 3 months). The t test using ten sample units of each type indicated no statistical differences between the concentrations of the analyzed metals. Adult frogs had been sub sampled for tissues: muscle, liver, gonad (in case o females) and “rest of body”.

Soil, terrestrial detritus, plants and amphibians were dried in an oven at 105^{o}, aquatic detritus and carabids were dried at 60^{o}, then ground in an uncontaminated mill and stored at -20 ^{0}C until processing. All samples were analysed for Fe, Mn, Zn, Cu, Cr, Pb and Cd. For soil samples it was used the pseudo-total extraction with aqua regia (Hoffmann, 1991); for detritus, plant samples, Carabidae and amphibians the digestion was performed using nitric acid. The analyses were performed by F-AAS and GF-AAS (Perkin Elmer). The quality of the analyses was checked using BCR reference materials (river sediment, plant, and mussel tissue).

Data processing

A weighted average of the concentration of metals in adult frogs was computed using the concentrations in each sub sample (tissue), and the weight of each sub sample. Such an average concentration in whole body is of interest not only from bioaccumulation point of view, but also when computing the stock of metals in the population of frogs.

Bioaccumulation in plants was assessed for each species and ecosystem based on the average concentration of metals in the soil of the ecosystem. Bioaccumulation in detritivorous Carabidae (including *Chlaenius spoliatus*) was assessed at landscape level based on a weighted average of metals in the detritus of the landscape. The concentration of detritus at each station was multiplied with the number of months in which the detritus was available to Carabidae (i.e. not flooded during the growing season, 2-6 months depending of the ecosystem), and the sum of concentration-months was divided by the total number of months-ecosystems. A similar approach was used for the bioaccumulation in phytofagous Carabidae. Bioaccumulation in raptor invertebrates (excluding *Chlaenius spoliatus*) was assessed based on the annual average of metals concentrations in the source compartments (detritivors or phytofags). Bioaccumulation in frogs was assessed based on the whole body concentration of adults and the annual average of metals concentrations in the source compartments.

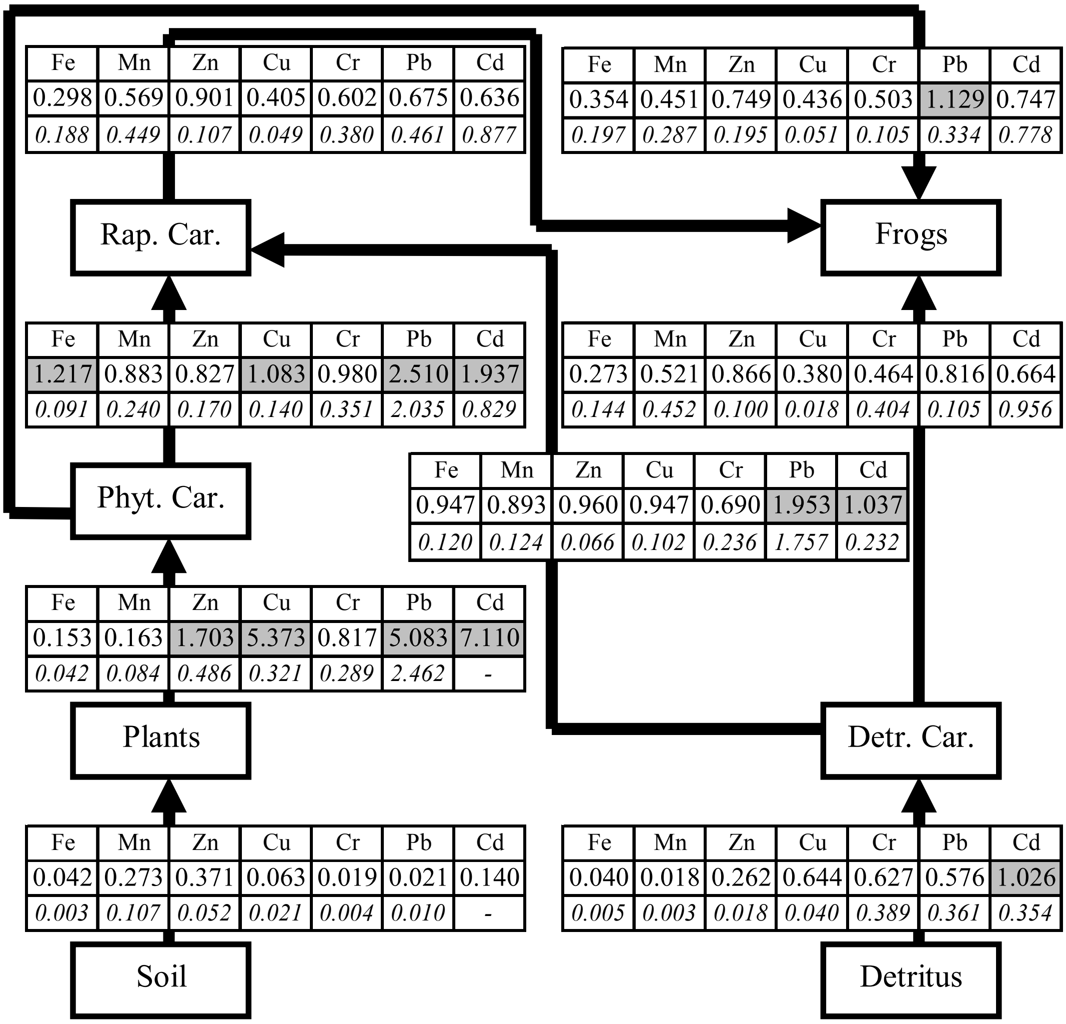
RESULTS AND DISCUSSIONS

The average bioaccumulation factors in all three micro landscapes are presented in Figure 2. Factors larger than 1 (bioconcentration) occurred most often for Pb and Cd, and in some cases for Fe, Zn, Cu. The metals were concentrated especially from plants to phytofagous Carabidae, and from phytofagous Carabidae to raptor Carabidae. The plants did not concentrate the metals from soil (although there are exceptions in case of some species which concentrate Mn in frequently flooded areas). Detritivorous Carabidae did not concentrate the metals from detritus, excepting in case of Cd. Frogs did not concentrate the metals from Carabidae, excepting for Pb from phytofagous species.

In Table 1 are presented differences of the concentration of metals average values between the stations of each transect and between transects (Mann-Whitney test). We do not present here the underlying data for space reason.

The mechanisms of metals retention in very large rivers are comparable to those in constructed wetlands), but the scale is different (Iordache et al., 2000; Iordache, 2003a, b). The percent of metals retained in the lower Danube floodplain from the upstream fluxes varied between 2.2% in case of Cd and 20.79% in case of Fe, but there are important differences in the pattern of metals' retention between the micro landscapes and ecosystems, depending on their succession stage and human impact (Iordache and Ruța, 2006). In this context one might expect to find consequent differences in the distribution of metals in the biotic compartments of the ecosystems.

Our results (further detailed in Iordache and Neagoe, in preparation) show that the number of significant differences between the ecosystems of a micro landscape decreases as one goes up in the food chain (from soil to detritus, plants, and Carabidae). Also the usefulness of the biological compartments for comparing the micro landscapes tends to decrease as we go to more mobile species. This is due probably to the fact that more mobile species can integrate fluxes from large areas (by having access to food in many ecosystems). A final answer to this issue can be provided only by population ecology studies. It is also due to the heterogeneous pattern of metals transfer from one trophic level to another, impinging on the existence of a simple correlation between the metals distribution at the bottom and at the top of the food chain.



Fe	Mn	Zn	Cu	Cr	Pb	Cd
0.298	0.569	0.901	0.405	0.602	0.675	0.636
0.188	*0.449*	*0.107*	*0.049*	*0.380*	*0.461*	*0.877*

Fe	Mn	Zn	Cu	Cr	Pb	Cd
0.354	0.451	0.749	0.436	0.503	1.129	0.747
0.197	*0.287*	*0.195*	*0.051*	*0.105*	*0.334*	*0.778*

Fe	Mn	Zn	Cu	Cr	Pb	Cd
1.217	0.883	0.827	1.083	0.980	2.510	1.937
0.091	*0.240*	*0.170*	*0.140*	*0.351*	*2.035*	*0.829*

Fe	Mn	Zn	Cu	Cr	Pb	Cd
0.273	0.521	0.866	0.380	0.464	0.816	0.664
0.144	*0.452*	*0.100*	*0.018*	*0.404*	*0.105*	*0.956*

Fe	Mn	Zn	Cu	Cr	Pb	Cd
0.947	0.893	0.960	0.947	0.690	1.953	1.037
0.120	*0.124*	*0.066*	*0.102*	*0.236*	*1.757*	*0.232*

Fe	Mn	Zn	Cu	Cr	Pb	Cd
0.153	0.163	1.703	5.373	0.817	5.083	7.110
0.042	*0.084*	*0.486*	*0.321*	*0.289*	*2.462*	-

Fe	Mn	Zn	Cu	Cr	Pb	Cd
0.042	0.273	0.371	0.063	0.019	0.021	0.140
0.003	*0.107*	*0.052*	*0.021*	*0.004*	*0.010*	-

Fe	Mn	Zn	Cu	Cr	Pb	Cd
0.040	0.018	0.262	0.644	0.627	0.576	1.026
0.005	*0.003*	*0.018*	*0.040*	*0.389*	*0.361*	*0.354*

Grey cells indicate values of the bioaccumulation factor bigger than 1. Legend: Phyt. Car. = phytofagous Carabidea, Det. Car. = detritivorous Carabidae, Rap. Car. = raptor Carabidae.

Fig. 2 Bioaccumulation of metals in the food chains of the investigated transects (average and *SD*).

Studying the metals biogeochemistry of flood plains is relevant for the design of decision support tools (Iordache et al., 2001), in particular for the monitoring system (Iordache and Bodescu, 2005). Floodplains management by restoration would lead to an important increase in the retention of metals and to a reduction of the pressure on the current wetlands (Iordache, 2002).

Table 1 Differences of the concentration of metals average values between the stations of each transect and between transects (Mann-Whitney test).

Transect		Fe	Mn	Zn	Cu	Cr	Pb	Cd
G	S	14*, 24*, 34**	12**, 13**, 14***, 23**, 24**, 34***	12**, 14**, 23**	12*, 13*	NS	NS	NS
	D	12*, 13*, 14*	12*, 13*, 14*	12*, 13*, 14*	12*, 13*, 14*, 23*	12*, 13*, 14*	12*, 13*, 14*, 23*	12*, 13*, 14*
	P	NS	NS	NS	NS	NS	NS	NS
	C	NS	NS	NS	NS	NS	NS	NS
H	S	12*, 13***, 14***, 23**, 24**	12***, 14*, 23***, 24***, 34**	12***, 13***, 14***, 24***, 34***	12**, 24*	NS	NS	12*, 13***, 24**, 34*
	D	NS	23*	NS	NS	NS	NS	NS
	P	34*	23*	NS	NS	NS	NS	NS
	C	NS	NS	NS	NS	NS	NS	NS
I	S	12**, 13*, 14**	12**, 13**, 14**, 23*, 34*	12**, 13*, 14**, 34*, 24*	12*	NS	NS	NS
	D	12*, 14*, 23*, 34*	12*, 14*, 23*, 34*	12*, 13*, 14*, 23*, 34*	12*, 14*, 23*, 34*	12*, 13*, 23*	12*	12*, 14*, 23*, 34*
	P	NS	12*, 13*, 23*, 34*	NS	NS	NS	12**	NS
	C	NS	14*(pr)	14*(cc)	12*(cc), 14*(cc)	NS	NS	NS
All	S	GH**, GI**, HI**	GH***, GI**	GH***, GI***, HI***,	GH*, GI***	GH*	HI*	HI*
	D	NS	GH*	GH*, HI*	GH*, GI*	GH*	GH*, GI*	NS
	P	NS	NS	GI*	NS	NS	NS	NS
	C	NS	HI*(cc)	GH*(c sp.), HI*(c sp.)	GH*(pr)	NS	NS	GI*(pr), GI***(c sp.), HI***(c sp.)
	F	NS	NS	NS	NS	NS	NS	HI*

Legend: S = soil, D = detritus, P = plants, C = Carabidae, F = frogs, cc = Carabus cancellatus, c sp. = Chlaenius sp., pr = Pseudophonus rufipes.G, H, I = transect name, 1-4 = station, NS not significant, *, **, *** significant at p<0.05, <0.01, and<0.001

Our results suggest that the monitoring of the pollution state of large floodplains slightly contaminated with metals, current or restored in the future, would find no much use of mobile groups of organisms as indicators of local, ecosystem or micro landscape level, pollution. This may not be the case in the flood plains of smaller rivers the lower Danube.

CONCLUSIONS

1. The number of significant differences between the concentration of metals in ecosystems of a micro landscape decreases as one goes with the analyses up in the food chain (from soil to detritus, plants, and Carabidae).
2. There is not a simple pattern of the bioaccumulation of metals in the food chain, but most of the bioconcentration occurred at the level of Carabidae, and not at the level of Rana specie.
3. Mobile groups seem to not be a good indicator of pollution with metals at local level in large flood plains.

Acknowledgements. This research was supported by several research projects of the National University Research Council (Romania). We thank to Dr. Dan Cogalniceanu and Dr. Constantin Ciubuc for providing the samples of frogs and beetles.

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ALUMINIUM NEUROTOXICITY

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ABSTRACT

In this paper is described the role and consequences of aluminium toxic effects at the nerve cells. Aluminium was investigated as an environmental factor that could trigger inflammatory events in the brain and could enhance existing age-related inflammation. The model of aluminium toxicity was induced by chronic treatment of Wistar rats with aluminum chloride, 1.5% solution instead drinking water for 6 months. Al-treated animals was hypoactive to a catatonic behavior and disrupted exploratory movements. Histological analyze revealed most degenerated neurons, especialy in the cerebral cortical V layer and Purkinje cells.

Key words: aluminium, neurotoxicity, rats, models of Alzheimer's disease

INTRODUCTION

Aluminium is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere. It is the third most prevalent element and the most abundant metal in the earth's crust. Dietary aluminium is ubiquitous, but in such small quantities that it is not a significant source of concern in persons with normal elimination capacity. Aluminium produces toxic effects at the cell membrane by altering the physical properties, interfering with the function of voltage-activated ionic channels and altering the secretion of transmitters (Becaria, 2002). Within the cell, aluminum can affect many key processes in the nucleus, cytoplasm and mitochondria, including glucose metabolism, signal transduction, neurotransmitter synthesis, phosphorylation and dephosphorylation of cytoskeletal proteins, slow axonal transport of neurofilament proteins and inhibition of nucleotide activity (Walton, 1998).

It is estimated that the normal person takes in between 3 and 10 milligrams of aluminum per day. Aluminum is the most abundant metallic element in the earth. It can be absorbed into the body through the digestive tract, the lungs and the skin, and is also absorbed by and accumulates in the body tissues. Aluminum is found naturally in air, water and soil. It is also used in the process of making cooking pots and pans, utensils and foil. Other items such as over the counter pain killers, anti-inflammatory products, and douche preparations can also contain aluminum. Aluminum is also an additive in most baking powders, is used in food processing, and is present in antiperspirants, toothpaste, dental amalgams, bleached flour,

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.81-86, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

grated cheese, table salt, and beer (especially when the beer is in aluminum cans). The biggest source of aluminum, however, comes from our municipal water supplies.

No known physiologic need exists for aluminum; however, because of its atomic size and electric charge (0.051nm and 3+, respectively), it is sometimes a competitive inhibitor of several essential elements of similar characteristics, such as magnesium (0.066 nm, 2+), calcium (0.099nm, 2+), and iron (0.064nm, 3+). Approximately 95% of an aluminum load becomes bound to transferrin and albumin intravascularly and is then eliminated renally. Aluminum was investigated as an environmental factor that could trigger inflammatory events in the brain and could enhance existing age-related inflammation (Campbell, 2004).

The contribution of aluminum to neurodegenerative diseases such as Alzheimer's diseases (AD), based on the relation of aluminum concentracion in drinking water, was indicated in the epidemiological study (Rondeau et al., 2000). Urban water supplies may contain a greater concentration because water is usually treated with the element before becoming part of the supply. Autopsies on many people who have died of Alzheimer's disease showed accumulations of up to four times the normal amount of aluminum in the nerve cells in the brain, especially in the hippocampus which plays a central role in memory. Aluminum causes an oxidative stress within brain tissue. Aluminum excess has a direct effect on hematopoiesis and has been shown to induce anemia. Findings on peripheral smears in patients with aluminum toxicity include *microcytic anemia* (*hypochromic, normochromic*), *anisocytosis*, *poikilocytosis*, *chromophilic* cells, and basophilic stippling. Note that these are the same findings observed in patients with lead poisoning.

Aluminum can also be found in bone marrow of *macrophages*. Since the elimination half life of aluminum from the human brain is 7 years, this can result in cummulative damage via the element's interference with neurofilament axonal transport and neurofilament assembly. Some experts believe it plays a role in leading to the formation of Alzheimer like *neurofibrillary* tangles. Aluminum is absorbed from the GI tract in the form of oral phosphate-binding agents (aluminum hydroxide), parenterally via immunizations, via dialysate or total parenteral nutrition (TPN) contamination, via the urinary mucose through bladder irrigation, and transdermally in antiperspirants. Lactate, citrate, and ascorbate all facilitate GI absorption. If a significant load exceeds the body's excretory capacity, the excess is deposited in various tissues, including bone, brain, liver, heart, spleen, and muscle. This accumulation causes morbidity and mortality through various mechanisms.

Toxic effects are dependent upon the amount of metal ingested, entry rate, tissue distribution, concentration achieved, and excretion rate. Mechanisms of toxicity include inhibition of enzyme activity and protein synthesis, alterations in nucleic acid function, and changes in cell membrane permeability. Aluminum toxicity is usually found in patients with renal impairment. Acute intoxication is extremely rare; however, in persons in whom aluminum clearance is impaired, it can be a source of significant toxicity.

Pathophysiology of Aluminium toxicity

Aluminum toxicity was originally described in the mid-to-late 1970's in a series of patients in Newcastle, England, through an associated *osteomalacic* dialysis *osteodystrophy* that appeared to reverse itself upon changing of the dialysate water to deionized water (ie, aluminum-depleted water). Previously, the only known dialysis-associated bone disease was *osteitis fibrosa cystica*, which was the result of abnormalities in vitamin D production that resulted in a secondary hyperparathyroidism, increased bone turnover, and subsequent peritrabecular fibrosis. In aluminum-related bone disease, the predominant features are defective mineralization and osteomalacia that result from excessive deposits at the site of osteoid mineralization, where calcium would normally be placed.

Aluminum also has a direct effect on *hematopoiesis*. Excess aluminum has been shown to induce *microcytic anemia*. Daily injections of aluminum into rabbits produced severe anemia within 2-3 weeks. The findings were very similar to those found in patients suffering from lead poisoning. Aluminum may cause anemia through decreased heme synthesis, decreased globulin synthesis, and increased *hemolysis*. Aluminum may also have a direct effect on iron metabolism: it influences absorption of iron via the intestine, it hinders iron's transport in the serum, and it displaces iron's binding to transferrin. Patients with anemia from aluminum toxicity often have increased reticulocyte counts, decreased mean corpuscular volume, and mean corpuscular hemoglobin. Other organic manifestations of aluminum intoxication have been proposed, such as a slightly poorer immunologic response to infection, but the mechanism by which it exerts its effect is complex and multifactorial.

When there is a high level of absorption of aluminum and silicon, the combination can result in an accumulation of certain compounds in the cerebral cortex and can prevent nerve impulses being carried to and from the brain properly. Long term calcium deficiency can further aggravate the condition. Workers in aluminum smelting plants on a long term basis, have been know to experience dizziness, poor coordination, balance problems and tiredness. It has been claimed that the accumulation of aluminum in the brain could be a possible cause for these issues.

MATERIALS AND METHODS

The model of aluminum toxicity (Culic et al., 2007) was induced by chronic treatment of *Wistar* rats with $AlCl_3$ for four weeks (2mg Al/kg, i. p. daily). The treated animals were compared to the controls. For histological examination the brain tissue was fixed in 10% formaline immediately after authanasia of animals. Tissue was rutinely included in parafin wax, cutting and staining by Walton procedure. Specific neurodegeneration was noted under UV light at fluorescent microscope Leica.

RESULTS AND DISCUSSIONS

In aluminium-treated animals by parenteral administration of aluminum chloride (2mg/kg for 28 days) we noted focal neurodegeneration like dark-stained neurons, particulary in the cerebral cortex. On the Walton staining those neurons was intensive red and under UV light showed brightly fluorescence (figure 1).

Mechanisms underlying aluminum neurotoxicity are not completely resolved. For both aluminum treated rats (intracerebroventricular $AlCl_3$ injections for 5 days) and saline-infused controls, no major signs of great histological changes were evident in cresyl violet-stained sections (Platt et al., 2001). Platt and her colleaques found by fluorescent Morin staining that Al was found in white matter of striatum, corpus callosum and cingulated bundle. Immunoreactivity of astrocytes and phagocytic microglia based on glial fibrillary acidic protein and ED1 markers, revealed a greater inflammatory responce in Al-injected animals compared to controls, while damage of the cingulated bundle in Al-treated animals led to a severe anterograde degeneration of cholinergic terminals in cortex and hippocampus, as indicating by acetylcholinesterase labeling.

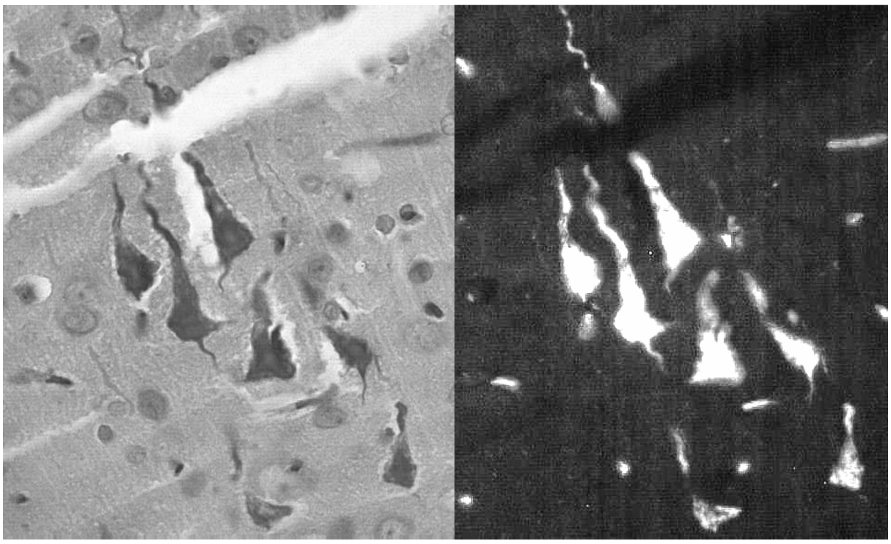

Fig 1. Dark neurons in the lamina V of rat cerebral cortex, Al-treated rat (Walton stain, white and UV light, magnified 1000)

Al-maltolate treated rabbits developed progressive behaviral symptoms consisting of forward head tilting tremor following touch or passive movement of the extremities, hemiplegic gait and splaying of the extremities (*Yokel*, 1989). Al-gluconat treated rats (0.85 mg/kg i.p., three times a week, 6 mounths) also developed impaired spatial memory deficits, hypoactive to a catatonic behavior and disrupted exploatory movemenrs (Miu and *Andreescu*, 2003). Aluminum induced learning and memory impairement model (Sun et al., 2005) was established by gavage of $AlCl_3$ (600mg/kg for 90 days) and in the hidden platform trials, escape latencies of the naloxone-treated rats (naloxone 0.8mg/kg, i.p. daily for 7 days) and the number of entries in the target area were significantly shorter than that of the non-treated model rats. We did not observe any changes in motor control, like paresis or tremor, in the Al treated animals. There has been a certain progress in investigation of animal models of AD (Price and Sisodia, 1994) as AD is a most common dementing disorder of late life comprasing complex neurobiochemical and neuropathological events characterized by extracellular deposition of β-Amyloid proteins, formation of intraneuronal neurofibrilary tangles and selective neuronal loss.

Aluminum is neurotoxic in both experimental animals and certain human conditions but whether aluminum presents a health hazard to humans as a contributing factor to Alzheimer's disease is still for debate (Savory et al., 1996). There are special heterozygous APP23 mice (Van Dam et al., 2003) and single, double and triple transgenic mice (Bloom et al., 2005) as models for human neurodegenerative disorders like AD. While Al still remains a mistery, Al-maltolate treated aged rabbits are suggested as a reliable and sensitive animal model for understanding AD neuropathalogy (Bharathi et al., 2006). Besides a great number of animal models, the tecniques of computational simulation have begun to be applied to modeling neurodegenerative disease (including the model of Alzheimer dementia), focusing on attractor-based network dynamics and on linking sequences of attractor states and their role in cognition (Frinkel, 2000).

Treatment of aluminum toxicity includes elimination of aluminum from the diet, TPN, dialysate, medications, antiperspirants, and an attempt at the elimination and chelation of the element from the body's stores. Avoidance of aluminum is easily achieved once the need to do so is recognized. Educate pregnant and breastfeeding females, and any patient with compromised renal function, about the use of aluminum-containing antacids and the potential dangers of their use and overuse. A safe alternative includes calcium carbonate, such as found in Tums. Educate patients to refrain from driving or operating hazardous machinery if they develop dizziness or impaired vision or hearing during treatment. Eat a diet that is high in fiber and includes apple pectin.

Use stainless steel, glass, or iron cookware. Stainless steel is the best choice. Beware of any product containing aluminum or dihydroxyaluminum. A hair analysis can be used to determine levels of aluminum in the body. Research has shown that the longer you cook food in aluminum pots, the more they corrode, and the more aluminum is absorbed into the food and hence into the body. Aluminum is more readily dissolved by acid forming foods, such as coffee, cheese, meat, black and green tea, cabbage, cucumbers, tomatoes, turnips, spinach and radishes. Acid rain leeches aluminum out of the soil and into drinking water.

Depending upon the degree of dementia and overall medical frailty of the patient, most improve in with deferoxamine therapy. Some patients, however, succumb to their underlying disease processes before any noticeable improvement in mental status or anemia occurs. Whether aluminum toxicity itself is fatal is unknown. Typically, patients' underlying diseases and medical frailty lead to early morbidity and mortality. Treatment of aluminum toxicity includes elimination of aluminum from the diet, TPN, dialysate, medications, antiperspirants, and an attempt at the elimination and chelation of the element from the body's stores. Avoidance of aluminum is easily achieved once the need to do so is recognized. Elimination is accomplished through the administration of deferoxamine through any of several routes. Deferoxamine, the metal-free ligand of the iron-chelate isolated from the bacterium Streptomyces pilosus, is used for acute and chronic iron toxicity and aluminum toxicity. It has a high affinity for ferric iron and does not affect iron in hemoglobin or cytochromes. Educate pregnant and breastfeeding females, and any patient with compromised renal function, about the use of aluminum-containing antacids and the potential dangers of their use and overuse. A safe alternative includes calcium carbonate, such as found in Tums. Educate patients to refrain from driving or operating hazardous machinery if they develop dizziness or impaired vision or hearing during treatment. Eat a diet that is high in fiber and includes apple pectin.

CONCLUSIONS

None of the models available mimics exactly cognitive, behavioural, electrophysiological, biochemical and histopathological abnormalities observed in neurological disorders characterised by cognitive impairment. Partial reproduction of neuropathology and/or cognitive deficits of Alzheimer’s disease, vascular dementia and dementia occurring in Huntington’s and Parkinson’s diseases, or in other neurodegenerative disorders may represent a basis for understanding pathphysiological traits of thesedisease and for contributing to their treatments (Tayebati, 2006). Hopeffully, different methodological approaches in investigation of various model will contribute to delineate pathogenic mechanisams, early diagnostics and test new therapies for treating AD in humans (Culic et al., 2007)

Acknowledgement. The authors wish to thank Ministry Science of the Republic of Serbia for financial support this work with Project: European Dimension of Change of the Education System in Serbia, No. 149009 (2006-2010).

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CORRECTION OF IODINE DEFICIENCY BY AN IODIZED VEGETABLE OIL – FLORIOL IODINE PRODUCT – (PARTIAL RESULTS)

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ABSTRACT

An iodine deficit in a population may delay the development of the brain of fetus and children with irreversible mental retardation. It was reported that still 33% of world population is affected by iodine deficiency. The aim of the study: to estimate if oral iodized oil is a major alternative or complement to iodized salt for correcting iodine deficiency in a population; to follow-up the effect of low doses of iodized vegetable oil (IVO) on urinary excretion of iodine (UIC) in studied population of 2 kind of IVO: 1st enriched with Lipiodol (IVOL); 2nd enriched with Brassiodol (IVOB). 3 population groups were randomly selected in Bucharest, an area where the median UIC is 128 µg/l: 1st (n=34) received 60 µg iodine/day in 1 ml IVOL in dairy; 2nd (n=40) received 60 µg iodine in 1 ml of IVOB in dairy; 3rd group (n=34) received 50 µg iodine per day IVOB in cooking meals. Anthropometric measurements were made. Serial UIC level for each subject were done 1 month before IVO adm -12 samples and 1 month during IVO adm -12 samples. The administration of daily low doses of IVO is followed by an increase of UIC (p < 0.001). There were no significant statistical differences between UIC of the 2 groups that received IVO enriched with 2 different vehicles of iodination (p= 0.24) and also between the 2 forms of administration (dairy products and cooking meals; p= 0.55). IVO constitutes an additional measure to iodized salt for iodine deficit prophylaxis in the future.

Key words: iodine deficiency, iodized vegetable oil, iodine deficit prophylaxis

INTRODUCTION

An iodine deficiency in the diet of a population may entail not only a change of the thyroid function in all age groups but also a delay in the development of the brain of fetus and children, whose clinical demonstration will be an irreversible mental retardation. This complication can only be avoided if a correction of the iodine deficiency is made very early during the intellectual development, which means during pregnancy and childhood (Delange, 1994).

In 2003, 2 billion inhabitants of 136 countries of the world were affected by iodine deficiency that is 33 % of the world population. It seemed consequently that iodine deficiency was the main cause of potentially avoidable mental retardation (Dr. de Benoist, personal communication).

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.87-94, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

At the general assembly of the World Health Organization (WHO) and the World Summit for Children in 1990, it was agreed that the most effective strategy aiming at the long-term elimination of iodine deficiency in the world was the establishment of programs of universal salt iodization, that is the enrichment in iodine of salt intended for food, feed and for the food industry (WHO, UNICEF and ICCIDD, 1994; Mannar and Dunn, 1995).

The salt iodization campaigns had a remarkable effect on the progressive elimination of iodine deficiency in the world and constitute an unprecedented success of public health in the field of the micronutrient deficiencies. Indeed, the household consumption of iodized salt in deficient countries had increased from less than 10 % in 1990 to 67 % in 1999, with a remarkable figure of 90 % in Latin America (WHO, UNICEF and ICCIDD, 1999; Delange et al, 2001).

However, a reassessment of the iodine nutrition in the world in 2005 showed that 54 countries were always affected by iodine deficiency, in particular in Central and Western Europe (Andersson et al, 2005). It was thus suggested strengthening the establishment of the salt iodization programs in these countries while organizing in parallel a systematic iodine supplementation for pregnant and lactating women and young children. An additional possibility is the introduction in these countries of iodine fortified food others than salt (de Benoist and Delange).

Romania is one of the countries of Central Europe which has suffered a lot due to iodine deficiency (Simescu et al, 2002). Until 2002, a national voluntary salt iodization program brought a partial correction to this deficiency but collided with a series of difficulties of logistic order (Dunn,1987). In 2002, the Romanian government adopted a Decision on compulsory addition of salt in iodine (Decision 568 of 5 June 2002). This resulted in 2005-2006 in a correction of iodine deficiency in almost all the districts where the iodine status was estimated (Dr. Simescu, personal communication), but monitoring the iodine uptake in pregnant women obtained by determination of urinary iodine concentration in a representative group showed a persistence of low values (median UIC 55 µg/l in 2002, 55 µg/l in 2005 and 80 µg/l in 2006-2007). Iodine deficiency in pregnant women represents an emergent health problem.

The aim of the study: to estimate additional contribution to the control of iodine deficiency resulting from the iodine fortification of another basic food of the population namely sunflower oil; to follow-up the effect of low doses of iodized vegetable oil (IVO) on urinary excretion of iodine (UIC) of 2 kind of IVO: first enriched with Lipiodol (poppy seed oil as vehicle of iodination); second enriched with Brassiodol (utilizes rapeseed oil as vehicle of iodination).

MATERIALS AND METHODS

The iodized sunflower oil is produced from sunflower (*Heliantus annuus* L.) seed. It is free from any contaminants or toxic compounds and fulfill the requirements and regulations of National Food Law (HG 150/2004, HG 412/2004, HG 924/2005, HG 1198/2002)) and National Oil Standards (Ordin 454/917/2001-22/2002, Ordin 495/997/2002-10/2003). The product contains iodized poppy seed oil in form of fatty acid ethylesters in order to provide the requested iodine content.

In this study, low daily intakes of iodized oil (organic iodine) were administered to studied population with a recent correction of their iodine deficiency.

Two groups of schoolchildren aged 6-14 y.o. and 1 group of young adults 24-40 y.o. were randomly selected in Bucharest, an area where the median UIC is 128 µg/l: first group of schoolchildren(n=34) received a complement of 60 µg iodine/day in 1 ml IVO (enriched with Lipiodol) mixed in dairy dessert; second group of young adult (n=40) received a complement of 60 µg iodine in 1 ml of IVO enriched with Brassiodol, mixed in dairy dessert; third group of schoolchildren (n=32) received 50 µg iodine per day from IVO enriched with Brassiodol used in cooking meals. At the beginning of the study anthropometric measurement were registered. Serial determinations of the UIC level for each child, as an index of the effect of iodine supplementation and an estimation of the iodine stored in the body, were done 3 times/week, for 4 weeks (12 samples), before IVO administration and during 4 weeks (12 samples) under administration of vegetable oil. Urine samples were also taken twice a month during 6 months after administration of iodized oil in 2 groups of schoolchildren (the study is ongoing).

The samples of urine were taken in the morning at getting up time with the aim to reduce the variability of the urinary iodine due to the volume and were kept at -20°C without preservative. For determination of UIC we used Sandell and Kolthoff method.

Statistical analysis of the results was made using SPSS program.

RESULTS AND DISCUSSIONS

The data of the first group of schoolchildren which received IVO enriched with Lipiodol showed an increase of UIC on the second day after administration and the persistence of significant increased values during the month of administration ($p< 0,001$). Elimination of UIC after a month of IVO administration showed still a significant high median value (median UIC = 177.5 µg/l, $p=0,14$). During the 3 to 6 months after IVO administration median UIC came back to values registered before IVO administration (table 4 and fig. 1).

The median UIC before IVO administration was 140 µg/l (table 2) and during IVO administration 192.5 µg/l (table 3). Median UIC of serial determination in first group of children before IVO administration are showed in table 1.

Table 1: Serial determination of urinary iodine concentration in first group of children in first month before iodized vegetable oil administration

Date	Mean UIC (µg/l)	Median UIC (µg/l)
23.04.2007	166	150
26.04.2007	130.3	122.5
28.04.2007	139	127.5
30.04.2007	132.8	120
02.05.2007	128.4	115
04.05.2007	121.3	113.8
07.05.2007	126.9	116.3
09.05.2007	171.8	152.5
11.05.2007	159.7	150
14.05.2007	131.3	130
16.05.2007	159.6	150
18.05.2007	164.3	167.5

Table 2: UIC value before IVO administration in first group children

No of samples	Mean UIC (µg/l)	Median UIC (µg/l)	SD (µg/l)
347	144	140	39.8

Table 3: UIC value during IVO administration in first group children

No of samples	Mean UIC (µg/l)	Median UIC (µg/l)	SD (µg/l)
345	196.1	192.5	38.2

Table 4: UIC value 6 month after IVO administration in first group children

Month after IVO administration	No of samples	Mean UIC (µg/l)	Median UIC (µg/l)	SD (µg/l)
1st month	42	169	177.5	44.6
2nd month	56	147.3	140	29.3
3rd month	57	136.8	127.5	24.8
4th month	54	14.2	135	26
5th month	54	134.7	125	24.5
6th month	26	139.3	135	25.7

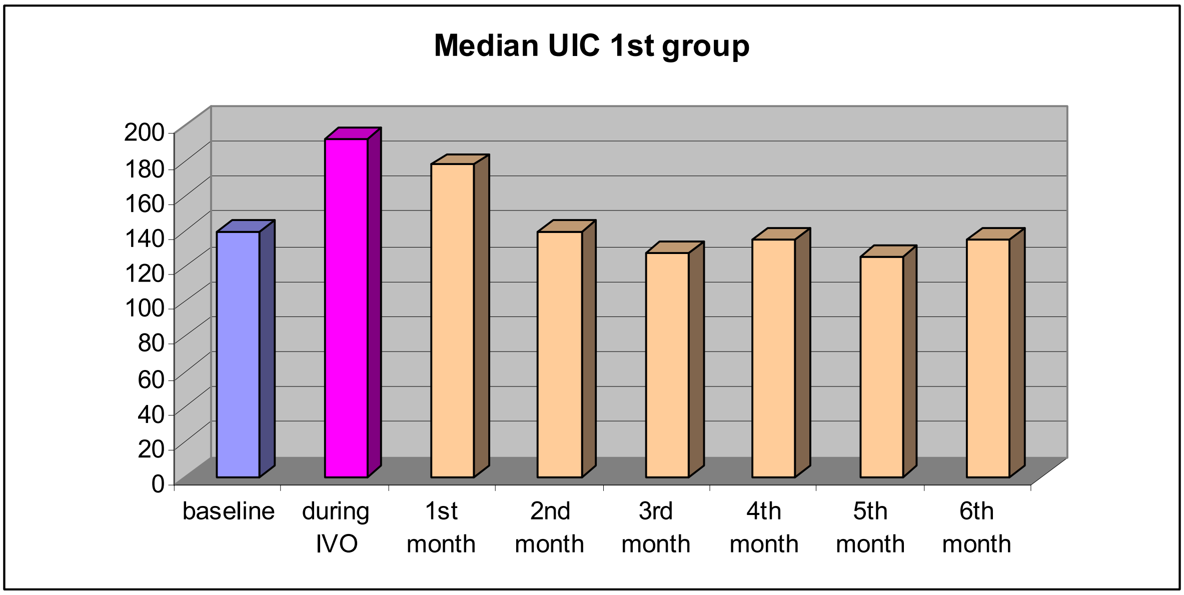

Fig.1: Evolution of UIC in first studied group

In second group of young adults who received IVO enriched with Brassiodol in amount of 60 µg iodine mixed in dairy products, median UIC before IVO administration was 135 µg/l (table 5) and during IVO administration 192.5 µg/l (table 6).

Table 5: UIC value before IVO administration in second group of young adults

No of samples	Mean UIC (µg/l)	Median UIC (µg/l)	SD (µg/l)
107	134.50	135.00	12.7

Table 6: UIC value during IVO administration in second group of young adults

No of samples	Mean UIC (µg/l)	Median UIC (µg/l)	SD (µg/l)
220	190.30	192.50	7.30

In third group of schoolchildren who received IVO enriched with Brassiodol in amount of 50 µg iodine per day mixed in cooking meals, median UIC before IVO administration was 131.25 µg/l (table 7) and during IVO administration 195 µg/l (table 8). The median UIC during IVO administration is significantly higher than basal values ($p < 0,001$). The follow-up 5 months after IVO administration revealed the persistence of high median UIC at 4 months ($p=0,03$ at 4 months). The values of UIC 5 months after IVO administration are showed in table 9 and fig. 2.

Table 7: UIC value before IVO administration in third group of schoolchildren

No of samples	Mean UIC (µg/l)	Median UIC (µg/l)	SD (µg/l)
68	136.65	131.25	25.17

Table 8: UIC value during IVO administration in third group of schoolchildren

No of samples	Mean UIC (µg/l)	Median UIC (µg/l)	SD (µg/l)
377	193.04	195.00	18.24

Table 9: UIC value 5 months after IVO administration in third group of schoolchildren

No of samples	Mean UIC (µg/l)	Median UIC (µg/l)	SD (µg/l)
41	193.11	195.00	8.33
47	186.76	187.50	6.73
54	175.42	175.00	6.57
50	167.20	167.50	6.86
52	143.1	142.5	10.3

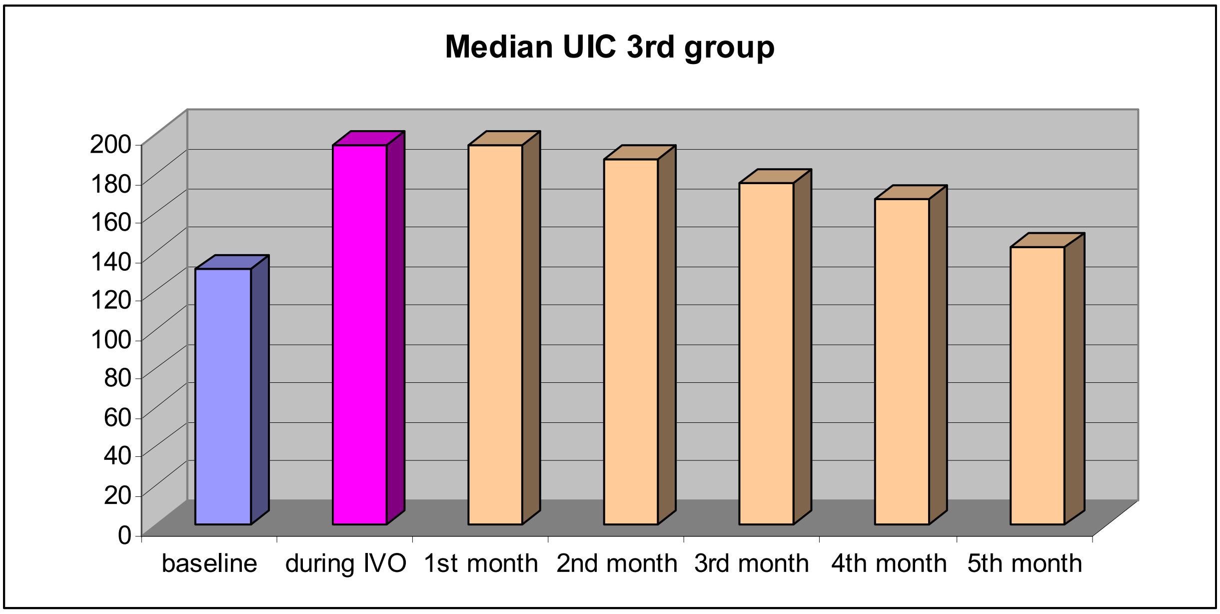

Fig.2. Evolution of UIC in 3rd studied group

Table 10: Median urinary iodine concentration and SD in 3 studied groups

No. of subjects	Iodine source	Iodine dosage	Baseline 4 weeks		During daily oral FIP 4 weeks	
			Median UIC(µg/l)	SD	Median UIC(µg/l)	SD
34	I. FIP Lipiodol	60 µg/day	140	39.8	192.5	38.2
40	II. FIP Brassiodol	60 µg/day	135	12.7	192.5	7.3
34	III. FIP Brassiodol	50 µg/day	131.25	25.17	195	18.24

The samples of UIC during the month preceding the administration of IVO reflect environmental availability of iodine. The results showed in table1, 2, 5 and 7 are concordant with previous reported data regarding the iodine uptake availability in Bucharest population.

UIC measured during the month of IVOL and IVOB administration revealed early increased values – first day after onset of administration – and the persistence of I127 high output during the month of administration.

After IVOL administration we observed the persistence of significant values of iodine concentration in urine only in the first month. We didn't observe significant high values in the next 5 months.

After IVOB administration we observed a prolonged elimination of iodine in urine. Median UIC in 1st, 2nd and 3rd and 4th month after IVOB administration showed significant high values.

The administration of daily low doses of IVO is followed by an increase of UIC ($p < 0.001$). There were no significant statistical differences between UIC of the 2 groups that received IVO enriched with 2 different vehicles of iodination ($p= 0.24$) and also between the 2 forms of administration (dairy products and cooking meals; $p= 0.55$).

IVO constitutes an important additional measure for iodine deficit prophylaxis in the future with beneficial effects especially in population groups which need iodine supplementation (e.g. pregnant women) and in population groups with salt intake restriction.

CONCLUSIONS

1. The administration of daily low doses of IVO is followed by an increase of UIC ($p < 0.001$).
2. There were no significant statistical differences between the 2 groups that received IVO enriched with 2 different vehicles of iodination ($p= 0.24$) and also between the 2 forms of administration (dairy products and cooking meals; $p= 0.55$).

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MINERAL IMBALANCE – REFLECTION TO BLOOD VESSELS

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ABSTRACT

The purpose of this study was to research reflection of disturbances in chemical elements content to health status of blood vessels. In this study, we used two non invasive diagnostic methods particularly efficient in early diagnostic of different pre-pathological disturbances and pre clinical stage of disease development, when disease symptoms are not expressed or absent: Bio-resonance method and elemental hair analyze. Examination were conducted on 103 subjects (67 females and 36 males) aged 12-86 years.

In the largest number of subjects, early forms of abnormalities were discovered in functioning of myocardium (58%); abdominal and thoracic aorta (63%); arteries coronaries (66%); brain arterial circulation (69%); venous circulation (75%); arterial blood pressure (50% hypertension, 15% hypotension, 45% variable), pancreas (69%). These subjects were without symptoms, detectable disorders in total plasma Mg concentration and dysfunction of tested organs by classical diagnostic methods. Only Mg concentration in their hair was higher than normal. With these very comfortable methods for patients, we can very early detect pre pathological disturbances and pre clinical stage in health status. Also we can design nutrition program for specific person and follow results.

Key words: hair, elements, imbalance, blood vessel, bio-resonance,

INTRODUCTION

It is now clear that disturbances in trace elements are associated with vascular diseases risk. Conducted studies on vascular diseases showes thet they are the first cause of morbidity and mortality in our and many Vestern countries.

The main risk factors for vascular diseases, hypertension, dyslipidemia, diabetes and obeisity related to diet. Conducted stydies showes that the great variation in vascular disease death rates among various nations exists. Other studies showes that between death rates of venous diseases, cholesterol and other risk factors on one side and the dietary intake of 34 elements exist relationships (Yacowitz et al., 1965; Klevay, 1977b). Among the conducted study most related to imbalances in Ca, Mg, K, Na, Zn, Se, Cu, Li, Mo, V, Cr, Si, Cd, Pb and Sn.

The mechanisms by which particular elements or their compounds may affect vascular disease risk are not clear, but it is likely that they involve effects on enzymes, hormones, and messenger molecules. Observations of known enzymes

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.95-104, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

showed that about 30% enzymes contain mineral elements and/or require minerals for activity.

The vascular diseases are the first cause of morbidity and mortality in Serbia and Western populations. Researches conducted in Serbia showed that: 95% of the population takes in about 40% of the RDA for selenium (Se); 60% females take in less iron (Fe) than is RDA; 85% of the population take in from 75-85% of the RDA for zinc (Zn);15% have a lower intake of copper (Cu) and chromium (Cr) than is the RDA (Djujić et al., 2000). Hair analyses of toxic elements, on the other side indicates that contamination with some of toxic elements as is As riches in males the maximal clinical reference level for biomonitoring of 0,1 mg/kg. Comparison of smokers with non-smokers showed that they have higher concentrations of Pb, Cd and Al in hair (Jeremic et al., 2004).

The relationship between hair element concentrations and human health is a complex process related to exposure, absorption, and tissue distribution of essential and toxic elements. Studies correlate elements in hair with exposure, disease and physiologic or pathologic effects of nutritional excesses or deficiencies. A long-term record that provides hair analysis reflects normal and abnormal metabolism, assimilation and exposure (Suzuki, 1982; Airey, 1983).

Hair analysis is useful for biological monitoring of most of the toxic metals and as well as a prognostic tool to ascertain whether an individual has a specific biochemical uniqueness, which can then be addressed in a therapeutic or prophylactic program. Levels of elements in hair are often even up to 300 times higher than those in serum or urine. As sauch hair is sensitive barometer of early imbalances and often reflects them before symptoms appear.

The BIO-RESONANCA is one of the most promising non-invasive diagnostic and therapeutic method in the field of energy-information, medicine. It is based on the conception of integrity of human organism and as such offers possibilities for sophisticated assessments.

For diagnostic and activation natural mechanisms, directed onto restoring and maintenance of healthy conditions it uses processes of energy – information inter-exchange that are a base of the functioning organism. Examinations conducted by the practitioners that use Bio-resonant diagnostic showed that such one screening test provides very useful information's that help in identifying the underlying root of patient's complaint (Gotovski et al., 2000; Djujic and Lalic., 2006; Lalic and Djujic, 2008).

The aim of the present study was to evaluate interrelations between blood vessels health status markers obtained by Bio-resonance diagnostic and concentrations of essential and toxic elements in hair of subjects from Serbia.

MATERIALS AND METHODS

The studied population were 103 subjects (66 females and 37 males) aged 12-86 years from various urban residential regions of Serbia, aged 8-78 years. Volunteers were, according to their ages, devided in 5 groups (<20; 21-35; 36-50; 51-65;>65 years).

The material for analysis was hair samples 3-5 cm in length taken from at least six spots of the occipitial scalp. About 0,5 g of sample was collected from each subject. The samples were thoroughly washed according to the method advised by the IAEA Advisory Group (1985) (acetone, 3X water, acetone), dried at 105^0 C to constant weight, and mineralised by using wet aching procedure. In the hair were analysed fallowing elements: Al, As, Ca, |Cd, Co, Cr, Cu, Fe, Hg, I, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Si, Sn, Ti, V and Zn. For analytical determination of all elements except iodine was used atomic emission spectrometry with inductively coupled argon plasma (ICP-AES) (spectrometer Optima 2000 DV, Perkin Elmer, USA),while I was determined by ion-selective electrodes. The accuracy of the method was confirmed by simultaneous analysis of the certified reference material (Chinese human hair GBW09101 powder). Hair element concentrations were expressed as µg/g dry weight.

For noninvasive diagnostic of functional abnormalities in cardiovascular sistem functioning and biochemical parameters in tissues Device Sensitive Imago was used. electromagnetic oscillations that living body radiate in wide frequency spectrum, that govern practically all biochemical and morphological processes in organism were measured by interface block "AUR-uM", model 3x'30s (Producer – Center of New Technology. Omsk, Russia), equipped with programs that energoinformational data transform to diagnostic and corrective therapy procedures (RUS Patents No.: 2001610661 and 2001110-691, 2001).

As such it assures quick diagnostic of organs functional status and internal interconnections between diseases of organs and systems of organism. In this study we examined functional disturbances in vascular tissues and fallowed coeficients of distribution of other, for vascular status validation, relevant parameters (myocard; aorta; coronary artery; average artery; brain blood vessels; average vene; liver; thymus; thiroidea; chromosomes 1,2,3 and 19; cholesterol in myocard, aorta, brain blood vessels and liver; LDH in average artery; c-reactive protein in myocard, average artery and liver; alkaly phosphatase in average artery and liver; creatinin phospho-kinase in myocard and average artery; potassium in myocard and average artery; Ca and Mg in average artery). Beside mentioned parameters in examined population group were checked to parasites, bacteria and viruses.

RESULTS AND DISCUSSIONS

Investigations of data obtained for functional disturbances in vascular sistem by bio-resonance diagnostic for different age groups (1/ <20; 2/ 21-35; 3/36-50; 4/ 51-65; 5/ >65) are presented In Tables 1 – 5, while data obtained for biochemical indices of significance for assesment risk for vascular sistem functioning are presented in Tables 6 - 10.

Correlation analysis of data obtained by bioreyonance diagnostic showed that between maz parameters exist correlations (Table 11).

Disturbances from "normal range" obtained in hair elements in different age groups were, in dependence of grade of deficiency or sufficiency, divided in 4 groups (1 – appears only changes in the metabolism of the element and may be transient; 2 – the impairment of the most sensitive biochemical functions and the most labile sources of elements are affected, but this may be compensated by other systems; 3 - appears consequential defects important for health - metabolic, immunological, cognitive, or development processes that do not express overt symptoms; 4 - appears identifiable symptoms that increase with the severity of deficiency - clinical phase) are presented in Table 12.

Table 1. Functional disturbances in vascular system functioning for group 1*, expressed in %

	Coefitients**				
Parameter	**0**	**1**	**2**	**3**	**4**
Aorta	28,57	28,57	42,86	0	0
Coronry arteries	42,86	28,57	28,57	0	0
Brain arteries	28,57	42,86	28,57	0	0
Averige arteria	28,57	42,86	28,57	0	0
Averige veins	28,57	57,14	14,29	0	0
Chromosome 1	14,28	85,71	0	0	0
Chromosome2	14,28	85,71	0	0	0
Chromosome 3	42,86	57,14	0	0	0
Chromosome19	42,86	57,14	0	0	0

*12-20 ages; n=7

**0 - physiological functional activity; 1 - initial compensatory disturbances of function without symptoms; 2- functional compensatory disturbances with symptoms; 3 - pathological disturbances of homeostasis – compensatory mechanisms disturbed; 4 - compensatory adaptable processes ended – irreversible changes incompatible with live

Table 2. Functional disturbances in vascular system functioning for group 2*, expressed in %

	Coefitient**				
Parameter	**0**	**1**	**2**	**3**	**4**
Aorta	25	50	18,75	6,25	0
Coronry arteries	31,25	46,88	18,75	3,13	0
Brain arteries	18,75	50	28,13	3,13	0
Averige arteries	18,75	43,75	37,5	0	0
Averige veins	12,5	62,5	18,75	6,25	0
Chromosome 1	18,75	81,25	0	0	0
Chromosome2	18,75	81,25	0	0	0
Chromosome 3	31,25	65,63	3,13	0	0
Chromosome19	36,32	63,68	0	0	0

*21-35 ages; n=32

**0 - physiological functional activity; 1 - initial compensatory disturbances of function without symptoms; 2- functional compensatory disturbances with symptoms; 3 - pathological disturbances of homeostasis – compensatory mechanisms disturbed; 4 - compensatory adaptable processes ended – irreversible changes incompatible with live

Table 3. Functional disturbances in vascular system functioning for group 3*, expressed in %

	Coefitient**				
Parameter.	**0**	**1**	**2**	**3**	**4**
Aorta	22,22	44,44	22,22	11,11	0
Coronry arteries	22,22	27,78	38,89	11,11	0
Brain arteries	22,22	38,89	33,33	5,56	0
Averige arteries	0	50	33,33	16,67	0
Averige veins	11,11	33,33	55,55	0	0
Chromosome 1	22,22	77,78	0	0	0
Chromosome2	22,22	7,78	0	0	0
Chromosome 3	5,56	94,44	0	0	0
Chromosome19	26,46	73,54	0	0	0

*36-50 ages; n=18

**0 - physiological functional activity; 1 - initial compensatory disturbances of function without symptoms; 2- functional compensatory disturbances with symptoms; 3 - pathological disturbances of homeostasis – compensatory mechanisms disturbed; 4 - compensatory adaptable processes ended – irreversible changes incompatible with live

Table 4. Functional disturbances in vascular system functioning for group 4*, expressed in %

	Coefitient**				
Parameter	**0**	**1**	**2**	**3**	**4**
Aorta	2,86	22,86	31,43	31,43	2,86
Coronry arteries	8,57	40	28,57	17,14	5,71
Brain arteries	5,71	28,57	48,57	11,43	5,71
Averige arteries	0	42,86	37,4	20	0
Averige veins	5,71	25,71	45,71	17,14	5,71
Chromosome 1	0	100	0	0	0
Chromosome2	5,71	94,29	0	0	0
Chromosome 3	2,86	97,14	0	0	0
Chromosome19	14,29	85,71	0	0	0

*51-65 ages; n=35

**0 - physiological functional activity; 1 - initial compensatory disturbances of function without symptoms; 2- functional compensatory disturbances with symptoms; 3 - pathological disturbances of homeostasis – compensatory mechanisms disturbed; 4 - compensatory adaptable processes ended – irreversible changes incompatible with live

Table 5. Functional disturbances in vascular system functioning for group 5*, expressed in %

	Coefitient**				
Parameter	**0**	**1**	**2**	**3**	**4**
Aorta	0	9,09	36,36	17,14	0
Coronry arteries	0	9,09	81,82	9,09	0
Brain arteries	0	0	45,46	45,46	9,09
Averige arteries	0	18,18	72,73	9,09	0
Averige veins	0	18,18	45,46	27,27	9,09
Chromosome 1	0	100	0	0	0
Chromosome2	0	100	0	0	0
Chromosome 3	0	100	0	0	0
Chromosome19	9,09	90,9	0	0	0

*66-86 ages; n=11

**0 - physiological functional activity; 1 - initial compensatory disturbances of function without symptoms; 2- functional compensatory disturbances with symptoms; 3 - pathological disturbances of homeostasis – compensatory mechanisms disturbed; 4 - compensatory adaptable processes ended – irreversible changes incompatible with live

Data presented for the youngist group of examiners (\20 years) (tables 1 and 6) comparison with data for older group of examiners (20-35 years) (tables 2 and 7) showed that vascular sistem functions in youngist group are more disturbed than in older group. In order to find explanation for this we compared incidence of inflamations with viruses and bacteria in both groups and discovered that 42% of young participants have infection with adenovirus, often together with streptococus, while in older group 25% has chronic inflamation with adenovirus and 15,6% with candida.

Disturbances in elements present in hair analysis (Table 12) showed that youngist group of examiners suffer from stronger defficiency of many essential elements (I, Co, Se, Mn, Zn, Si, Ca) except Cu that is find in excess. At the some time they have higher concentrations of some of potentialy toxic elements than older group (Li, As, Al). Obviousli life styel of compared groups differ, as well as their habits. Younger generation was less physicaly active, eat more fast food and various artificiel drinks, has more direct contacts with peoples vith various inflamatory processes what obviously reflects negatively to vascular sistem functioning and corresponding biochemical parameters.

Table 6. Disturbances in biochemical parameters in group 1*, expressed in %

	Coefitient*		
Parameter	**<0,8**	**0,8-1,0**	**>1**
LDH average arteria	0	28,75	71,43
Alk. Phos. average arteria	57,14	28,57	14,29
Creat.Phos.Kin. average arteria	28,57	57,14	14,29
Cholesterol average arteria	14,29	85,71	0
C-react. prot. average arteria	42,86	57,14	0
Ca average arteria	28,57	57,14	14,29
K average arteria	0	85,71	14,29
Mg average arteria	14,29	71,43	14,29
Cholesterol aorta	28,57	57,14	14,29
Cholesterol brain arteries	0	14,29	85,72

*0,4-0,8 normal; 0,8-1 physiological tendency to higher value; <1 pathological changes

Table 7. Disturbances in biochemical parameters in group 2*, expressed in %

	Coefitient*		
Parameter	**<0,8**	**0,8-1,0**	**>1**
LDH average arteria	15,63	50,00	34,38
Alk. Phos. average arteria	68,75	31,25	0
Creat.Phos.Kin. average arteria	43,75	40,63	15,63
Cholesterol average arteria	59,38	34,38	6,25
C-react. prot. average arteria	65,63	31,25	3,13
Ca average arteria	46,88	37,50	15,63
K average arteria	68,75	21,88	9,38
Mg average arteria	62,50	28,13	9,38
Cholesterol aorta	62,50	25,00	12,50
Cholesterol brain arteries	6,25	34,38	59,38

*0,4-0,8 normal; 0,8-1 physiological tendency to higher value; <1 pathological changes

Table 8. Disturbances in biochemical parameters in group 3*, expressed in %

	Coefitient*		
Parameter	**<0,8**	**0,8-1,0**	**>1**
LDH average arteria	5,55	44,44	50,00
Alk. Phos. average arteria	50,00	33,33	16,67
Creat.Phos.Kin. average arteria	33,33	38,89	27,78
Cholesterol average arteria	44,44	27,78	27,78
C-react. prot. average arteria	44,44	33,33	22,22
Ca average arteria	61,11	22,22	16,67
K average arteria	66,67	22,22	11,11
Mg average arteria	50,00	27,78	22,22
Cholesterol aorta	55,56	33,33	11,11
Cholesterol brain arteries	0	22,22	77,78

*0,4-0,8 normal; 0,8-1 physiological tendency to higher value; <1 pathological changes

Intercomparison of data for groups 3 (36-50 years), 4 (51-65) and 5 (>65 years) shoved that vith ages I deficiency became more serious, as well as taht many other elements have decreasing or increasing treands. Changes in macroelements clierly indicates that acidity of the organism increases with ages, while disturbances in microelement indicates that with aging antioxidative and imunological defence become weeker.

Table 9. Disturbances in biochemical parameters in group 4*, expressed in %

	Coefitient*		
Parameter	**<0,8**	**0,8-1,0**	**>1**
LDH average arteria	14,29	31,43	54,29
Alk. Phos. average arteria	71,43	22,86	5,71
Creat.Phos.Kin. average arteria	28,57	51,43	20,00
Cholesterol average arteria	48,57	37,14	14,29

Table 9. Disturbances in biochemical parameters in group 4*, expressed in % (cont.)

Parameter	**Coefitient****		
C-react. prot. average arteria	51,43	40,00	8,57
Ca average arteria	48,57	42,86	8,57
K average arteria	45,71	37,14	17,14
Mg average arteria	54,29	25,71	20,00
Cholesterol aorta	51,43	34,29	14,29
Cholesterol brain arteries	8,57	14,29	77,14

*0,4-0,8 normal; 0,8-1 physiological tendency to higher value; <1 pathological changes

Table 10. Disturbances in biochemical parameters in group 5*, expressed in %

	Coefitient*		
Parameter	**<0,8**	**0,8-1,0**	**>1**
LDH average arteria	0	54,55	45,46
Alk. Phos. average arteria	72,73	18,18	9,09
Creat.Phos.Kin. average arteria	36,36	36,36	27,27
Cholesterol average arteria	54,55	36,36	9,09
C-react. prot. average arteria	63,64	27,27	9,09
Ca average arteria	72,73	27,27	0
K average arteria	45,45	27,27	27,27
Mg average arteria	63,64	9,09	27,27
Cholesterol aorta	27,27	45,46	27,27
Cholesterol brain arteries	0	0	100

*0,4-0,8 normal; 0,8-1 physiological tendency to higher value; <1 pathological changes

Table 11. Correlations obtained between parameters determined by Bio-resonance

Average arteria cholesterol/ Average arteria Ca	0,79
Average arteria cholesterol/ Average arteria K	0,75
Average arteria cholesterol/ Average arteria Mg	0,46
Average arteria c-react.prot/ Average arteria K	0,63
Average arteria c-react.prot/ Average arteria Ca	0,56
Average arteria c-react.prot/c-react prot mocard	0,86
Average arteria cholesterol/Average arteria alk. phos	0,59
Liver c-react.prot/Liver cholesterol	0,50
Ages/aorta function	0,46
Ages/Average arteria	0,37
Ages/Miocard function	0,49

Correlation analysis data presented in table 11 showing that disturbances in vascular system functioning highly correlates with Ca, K and Mg and cholesterol in arteries, moderate with c-reactive protein and alkaline phosphatase

Table 12. Average disturbances from normal range in hair elements concentrations in investigated groups of residents.

Ages	Average disturbance grades in hair elements
<20	I (-1,8); Co (-1,2); Cu(+1,0); Se (-2,6); Zn(-1,4);Mn(-1,0); Cr (-0,6); Si(-1,1); Fe (+0,8); Mg (-0,9); Ca (-1,1); P(+0,6); Na(+0,8); K(-0,9)
21-35	I (-2,5); Co (-1,1); Cu(+0,6); Se (-2,1); Zn(-1,0); Mn(-0.6); Cr (-1,1); Si(-1,6); Fe (+0,5); Mg (+1,4); Ca (+2,2); P(+1,1); Na(+0,3); K(-0,6)
36-50	I (-3,0); Co (-1,0); Cu(+0,3); Se (-1,9); Zn(-1,6); Mn(-0,7); Cr (-0,7);Si(+0,3); Fe (+0,8); Mg (+2,2); Ca (+1,8); P(+0,9); Na(+0,9); K(+0,4)
51-65	I (-3,2); Co (-1,1); Cu(-1,2); Se (-2,3); Zn(-1,8);Mn(-1,2); Cr (-1,3); Si(+0,9); Fe (+1,6); Mg (+2,8); Ca (+3,0); P(+2,1); Na(-0,5); K(+0,9)
>66	I (-2,2); Co (-1,0); Cu(-1,5); Se (-2,6); Zn(-1,2); Mn(-1,6); Cr (-1,0); Si(+0,8); Fe (+2,4); Mg (+3,0); Ca (+3,6); P(+2,6); Na(+0,3); K(+1,3)

Monitoring of data obtained for inflamations influenced by viruses and/or bacteria in older age groups showed that with aging decrease no of infections with adenovirus, but increases infection iduced by bacteria (in age group 36-50 27,8% with Candida; 51-65 14,3% Candida often with Echerichia; >65 27% Streptococcus and/or Staphylococcus).

CONCLUSIONS

In the largest number of subjects, early forms of abnormalities were discovered in functioning of abdominal and thoracic aorta (63%); arteries coronaries (66%); brain arterial circulation (69%); venous circulation (75%); arterial blood pressure (50% hypertension, 15% hypotension, 45% variable). These subjects were without symptoms, detectable disorders and dysfunction of tested organs by classical diagnostic methods. Only hair concentrations in elements important for acid[base balance, antioxidative defense and immunological status regulation in their hair were disrupted.

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FEW WORDS ABOUT METAL COMPOUNDS IN CANCER TREATMENT

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ABSTRACT

Presently, there is large interest in the research of metal complexes as possible anticancer agents. In our investigations we have evaluated the effect of > 50 complexes of Zn(II), Cu(I, II), Co(II), Ni(II) and Fe(II, III) with different ligands (Mannich bases, aminoacids, cholic acids, mixed ligands) on viability and proliferation of cultured human and animal tumour cells. The most promising cytotoxic and antiproliferative agents were found among mixed ligand metal complexes.

Key words: malignant neoplasms, anticancer chemotherapy, metal compounds, cytotoxic and antiproliferative properties, tumour cell lines

INTRODUCTION

Cancer, a group of more than 100 different diseases, manifests itself as uncontrolled cellular reproduction, local tissue invasion and distant metastases. Cancer therapy employs surgery, radiation, chemotherapy and immunotherapy. Treatment becomes complicated because malignant cells are not pathogens that have specific cure, but are the body's own cells that must be killed or physically removed (Levi et al., 2001). While therapeutical results obtained by surgery and radiotherapy - which are locoregional interventions - are close to their maximum accomplishment, success of drug therapy - the only systemic approach - is far from being satisfactory (Kostova, 2006).

Cancer chemotherapy meets problems

Despite decisive progress in understanding the molecular biology of cancer development, cytotoxic anticancer drugs continue to be the cornerstone of modern antitumour therapies (Gonzalez-Nicolini, Fussenegger, 2005). Several obstacles can prevent successful chemotherapy of neoplastic diseases including: 1) Tumour heterogeneity phenomenon (Alexandrova, 2001); 2) Multidgug resistance (Alexandrova, 1998; Sarkadi et al., 2004; Leslie et al., 2005); 3) Undesirable side

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 105-112, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

effects - the occurrence of myelosuppression, mucositis, cardiodepression, peripheral neuropathy, and central neurotoxicity can complicate treatment especially in older patients (65 years of age and older) (Repetto, 2003; Ocean, Vohdat, 2004). Consequently, the need to find a safe and highly effective cure for neoplastic diseases remains a major challenge for modern science (Levi et al., 2001; Gonzales-Nicolini, Fussenegger, 2005; Kostova, 2006).

Metal compounds as antitumour agents

The earliest reports on the therapeutic use of metals or metal-containing compounds in cancer and leukemia date from the sixteenth and nineteenth centuries (Kopf-Maier, 1994; Desoize, 2004). They were forgotten until the 1960s, when the anti-tumour activity of the inorganic complex cis-diammine-dichlorplatinum (II) (cisplatin) was discovered.

Table 1. Metal complexes with Mannich bases

Code	Formula	Molecular weight (g/mol)
TS-1	$Cu_2(BAMP)(NCS)_4$	846.00
TS-2	$Cu_2(BAMP)I_3$	1007.00
TS-3	$Co_2(BAMP)Cl_4$	746.00
TS-4	$Co(BAMP)(NCS)_2$	661.00
TS-5	$Fe_2(BAMP)Cl_4$	740.00
TS-6	$Fe_2(BAMP)Cl_6$	811.00
TS-7	$Cu(TAMEN)(NO_3)_2$	1046.00
TS-8	$Co_2(TAMEN)Cl_4$	1121.00
TS-9	$Co_2(TAMEN)(NCS)_4$	1211.00
TS-10	$Fe_2(TAMEN)Cl_6$	1186.00
TS-11	$Ni(TAMEN)(ClO_4)_2$	1119.69
TS-12	$Ni(TAMEN)(NCS)_2$	1036.69
TS-13	$Ni_2(BAMP)(CH_3COO)_4$	839.38
TS-14	$Ni_2(BAMP)(Cl)_4$	745.38
TS-15	$Fe(TAMEN)(NO_3)_3$	1103.85
TS-16	$Fe(BAMP)(NO_3)_3$	727.85
TS-17	$Cu(TAMEN)(ClO_4)_2$	1124.55
TS-18	$Cu(TAMEN)(CH_3COO)_2$	1043.55
TS-19	$Cu_2(BAMP)(ClO_4)_4$	1011.10
TS-20	$Co(TAMEN)(ClO_4)_2$	119.93
TS-21	$Fe_2BAMPpy_2Cl_4$	898.33
TS-22	$Fe_2BAMPpy_2Cl_6$	969.23
TS-23	$Co_2BAMPpy_2Cl_4$	904.50
TS-26	$Cu_2BAMPdipyCl_4$	911.71
TS-27	$Fe_2TAMENdipyCl_4$	1270.75
TS-28	$Fe_2TAMENdipyCl_6$	1341.66

The antitumour power of platinum compounds

The square plannar platinum (II) complex, cis-$[PtCl_2(NH_3)_2]$ (cis-DDP, cisplatin), was first synthesized in 1844 by Michael Peyrone in Turin and is historically known as Peyron's chloride. Fifty years later in his theory of coordination chemistry Alfred Werner correctly assigned to this compound a cis-geometry, which was confirmed in an X-ray crystal-structure determination reported in 1966. The biological activity of cis-platin was accidentally discovered in 1965 by Barnet Rosenberg and his coworkers who reported first the inhibitory activity of this compound on E. coli division. In the seventies efficacy in human cancer patients was established. A little bit later these scientists performed experiments with sarcoma 180 and leukemia L1210 bearing mice and the results obtained led to cisplatin entering phase I clinical trials in 1971. Approval to cisplatin for treatment of testicular and ovarian cancer was given in 1978 (Rosenberg et al., 1965, Lippert, 1999; Desoize, 2004; Abu-Surrah and Kettunen, 2006).

Evidence for antitumour activity in humans was first reported against testicular and ovarian cancer, in terms of both objective response and of prolonged survival. Because of its marked renal toxicity, cisplatin was almost withdrawn from clinical trials. Interest was rekindled when hyperhydration with isotonic saline circumvented this problem. One of the attractions of cisplatin, besides its efficacy, is that its toxicity is different from that of other anticancer drugs, thus making it attractive for combination regimens. Today cis-platin is the world's leading anti-tumour drug for the chemotherapy of human cancer. This platinum compound continues to be used in 50-70% of all cancer patients especially against testicular and ovarian cancer, and it is also used in case of head and neck cancer, cervical and bladder carcinomas, bronchogenic carcinoma, small-cell and non-small cell lung cancers, lymphoma, osteosarcoma, melanoma and neuroblastoma (Wong and Giandomenico, 1999; Galanski et al., 2003; Abu-Surrah and Kettunen, 2006).

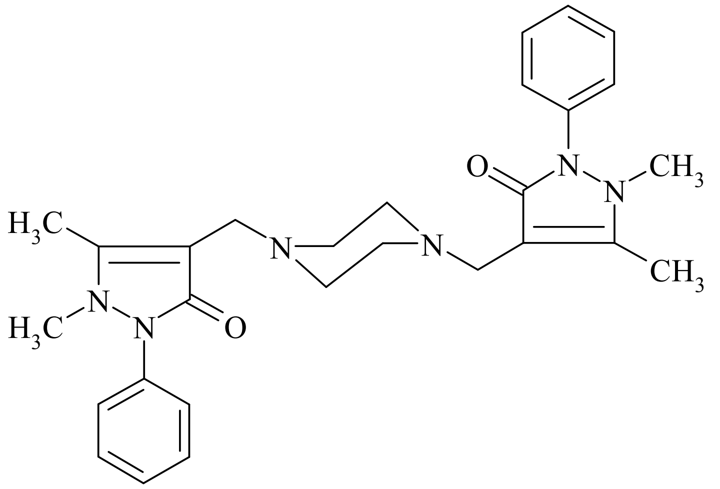

Fig. 1. N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP)

Although cis-platin is highly effective anticancer agent, it is not ideal because of: 1) Low or no activity against some of the most common and socially important malignant neoplasms such as breast, gastrointestinal and renal cancers, leukemia); 2) The development of drug resistance during treatment is not rare; 3) It has major toxicity limitations of which nephrotoxicity is the most notable, although nausea and vomiting, peripheral neuropathy, and myelotoxucuty can also raise major concerns (Galanski et al., 2003; Abu-Surrah and Kettunen, 2006).

The landmark discovery of the antitumour activity of cisplatin was an extremely fortunate one for a number of reasons: 1) Cis-platin gave and still gives patients suffering from various types of cancer hope for a prolonged and a better quality of life, and the chance of a cure; 2) It had an impact on inorganic and coordination chemistry that cannot be overestimated; 3) It is a wonderful example of that basic research is capable of achieving and a pledge for the support of basic science (Lippert, 1999).

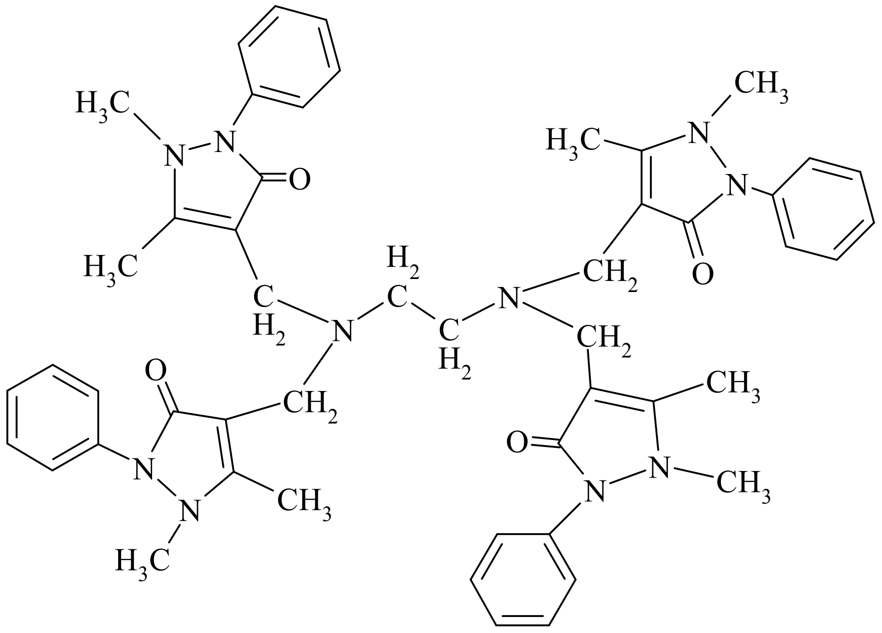

Fig. 2. N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN)

Since the introduction of cisplatin around three thousand platinum derivatives have been synthesized and tested against cancer cells; but, at most, only thirty compounds have reached clinical trials and more than half of those have already been rejected. The main aim of these intensive investigations was to obtain drugs with at least an equal activity but reduced toxicity compared to cisplatin. Today, six are used clinically: three of them – cisplatin, carboplatin and oxaliplatin, are used world-wide, whereas the other three are applied in a few countries - nedaplatin in Japan, lobaplatin in China, and heptaplatin – in south Korea. Some platinum complexes are still under clinical investigation, including those developed for oral administration (Desoize and Madoulet, 2002; Abu-Surrah and Kettunen, 2006; Choy, 2008).

The antitumour properties of non-platinum compounds

The unresolved problems (narrow spectrum of cancers that can be treated; side effects; resistance phenomena).in platinum-based anticancer therapy have stimulated increased research efforts in the development of novel non platinum-containing metal species as cytostatic agents. As a result, a wide variety of ruthenium (Alessio et al., 2004; Kostova, 2006b), gallium (Collery, 2002; Jacupec, Keppler, 2004), titanium (Desoize, 2004), gold (Messori, Marcon, 2004; Kostova, 2006a), vanadium (Chatterjee, Bishayee, 1998; Evangelou, 2002), lanthanides (Kostova, 2005), zinc (Alexandrova et al., 2002), copper (Alexandrova et al., 2003), cobalt (Alexandrova et al., 2004), nickel (Alexandrova et al., 2006),iron (Alexandrova et al., 2005) compounds have been reported to express promising antitumour properties in preclinical studies. Some of them (titanium, ruthenium, gallium compounds) have already been evaluated in Phase I and Phase II trials (Desoize, 2004; Abu-Surrah, Kettunen, 2006; Ott, Gust, 2007).

In the search for new agents with anticancer efficacy we have investigated the antitumour activity of more than 50 metal compounds such as:

1. Zn(II), Cu(II) and Co(II) complexes with L picolinic or DL aspartic acid) [28];
2. Zn(II), Cu(II), Co(II), La(III) complexes with cholic, dehydrocholic and lithocholic acids**;**
3. Cu(I, II), Co(II), Ni(II) and Fe(IIm III) complexes (TS1-TS20, Table 1) with Mannich-bases N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP, Fig. 1) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN, Fig.2)
4. Cu(I, II), Co(II) and Fe(II, III) mixed ligand complexes containing BAMP or TAMEN as well as pyrinine (py) or 2,2-dipyridyl (dipy) as a coligand (TS, Table 1).
5. Co(II) complexes with mixed ligands 2-hydroxy-benzophenones and nitrogenous bases enR (still under investigation).

The following model systems were used in our experiments: 1) Permament cell lines from human (breast cancer MCF-7, glioblastoma multiforme 8 MG BA, carcinoma of the larynx Hep-2, hepatoma HepG2, rhabdomyosarcoma RD64, squamous cell carcinoma A431 and erythroleukemia K562), rat (transplantable sarcoma induced by *Rous sarcoma virus* strain *Schmidt-Ruppin*), murine (myeloma P3U1) and chicken (transplantable hepatoma induced by the myelocytomatosis virus Mc29) origin; 2) Primary cultures from 11 day old 15I White Leghorn chickens.

The influence of the compounds on cell viability and proliferation as well as cytopathological changes were studied by MTT test, neutral red uptake cytotoxicity assay, trypan blue dye exclusion technique, colony-forming method, autoradiography, neutral and alkaline variants of single cell gel electrophoresis and acridine orange staining.

The results that we obtained revealed that:

1. According to their ability to reduce cell viability and proliferation, the examined metal complexes with aminoacids were graded as follows:
   Zn(II) complexes > Cu(II) complex s > Co(II) complexes
2. Co(II) and Ni(II) complexes with BAMP expressed more pronounced cytotoxic and antiproliferative properties as compared to TAMEN-containing complexes of the same metals. At the same time Fe(II, III) complexs with TAMEN are found to be active than iron complexes with BAMP.
3. Applied independently, the ligands (BAMP, TAMEN. lithocholic and degydrocholic acids) were found to be less effective cytotoxic and antiproliferative agents as compared to the corresponding metal complexes;
4. The compounds with the most promising antineoplastic properties in cultured tumour cells were found among mixed ligand metal complexes. Thus, the CC_{50} of $Cu_2(BAMP)dipyCl_4$ determined on 24 h were found to vary between 3 μg/ml (LSCC-SF-Mc29) and 34 μg/ml (8 MG BA) and on 48 h between 1 μg/ml (K562) and 23 μg/ml (8-MG-BA). Applied at concentration of 1 to 10 μg/ml this compound completely inhibited the growth of tumor cells in semisolid medium and reduced significantly the incorporation of ^3H-thymidine. Shrinkage of the cell and its nucleus, plasma membrane blebbing, chromatin condensation and segmentation of the nuclei as well as formation of apoptotic bodies were observed after acridine orange staining in the cells treated with ≥ 5 μg/ml $Cu_2(BAMP)dipyCl_4$. DNA damages were found by

single cell gel electrophoresis in 60% of the tumor cells cultured for 48 h in the presence of this compound (10 μg/ml). The mixed ligand copper (II) complex expressed more pronounced cytotoxic and cytostatic activities against LSCC-SF-Mc29 hepatoma and P3U1 mouse myeloma cells than on primary chicken embryo cells and BALB/c 3T3 mouse fibroblasts. Some of the Co(II) complexes with mixed ligands 2-hydroxy-benzophenones and nitrogenous bases enR were found to reduce cell viability by 50% (IC_{50}) when applied at concentrations < 5 μg/ml for 24 h. These compounds were also proved to induce DNA damages (double stranded breaks) in the treated (5, 20 and 50 μg/ml for 24 h) cells.

The need to find a safe and highly effective cure for neoplastic diseases remains a major challenge for modern science. The ideal anticancer treatment by any compound should fulfill certain criteria and exert the following selective effects on malignant cells: 1) reduction of cellular growth rate (antiproliferative effect); 2) cytotoxic action expressed either by necrosis or apoptosis; 3) reduction and/or inhibition of the invasive or metastatic potential of the cells; 4) reduced or absent potency to induce cellular resistance; 5) low side effects (Messori and Marcon, 2004). Developing metal compounds as drugs is not an easy task, because accumulation of metal ions in the body can lead to deleterious effects. Future investigations will clarify the potential antitumour properties and mechanism of action of cobalt and cobalt compounds and will facilitate the design of drugs with improved antineoplastic activity and reduced general toxicity.

Acknowledgements. This study was supported by Grant C1402/2004 from the National Science Fund in Bulgaria; Bilateral project between Bulgarian Academy of Sciences and Aristotle University of Thessaloniki, Greece; Bilateral project between Bulgarian Academy of Sciences and Romanian Academy.

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A STUDY ON THE POSSIBLE PREVENTION OF ENVIRONMENTAL POLLUTION WITH MINING WASTES FROM COPPER MINES THROUGH AGRICULTURAL VALORISATION

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ABSTRACT

We tested the impact of mining wastes from copper mines in southern Banat on wheat, maize, oat, and bean crops. Mining waste analysis results point out that this material can be used for agricultural purposes since heavy metal content is within accepted limits. We applied waste amounts of 1t/ha, 2t/ha, and 3t/ha combined with variable nitrogen rates on a constant phosphorus and potassium agro-fund ($P_{60}K_{60}$)*.*

Key words: agricultural use, mining wastes, copper mine

INTRODUCTION

Our starting point in this study was to see if the thousands of tonnes of mining wastes from the copper mines in the Moldova Nouă and Sasca Montană area can be valorised as a source of oligo-elements and macro-elements in agriculture.

This hypothesis could be tested since the material is not radio-active and heavy metal content is below accepted limits at European level.

The material was found to have an alkaline reaction as a result of high contents of calcium, and because flotation was done with basic elements; this indicates that it is possible to use the material to correct soil acid reaction in the hill area of western Romania.

MATERIALS AND METHODS

Table 1 presents analysis results concerning waste sample chemical element content.

The experiments were bi-factorial, with Factor A represented by mining waste (we tested amounts of 1t/ha, 2t/ha, and 3t/ha) applied on a constant potassium and phosphorus agro-fund ($P_{60}K_{60}$) and with Factor B represented by variable rates of nitrogen, i.e. N_0-N_{150} in wheat, maize, and oat and N_0-N_{90} in bean.

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 113-116, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

The type of soil on which our research was carried out is a typical brown eumeso-basic soil with a pH 5.6, a humus content of 2.61%, and a clayish-sandy texture.

Table 1. The element content of mining sterile from Moldova Nouă

Element	The content in the mining steril store	
	Test 1	Test 2
Si	41	53
Al	2	11
Fe	1,5	5
Ti	0,2	0,25
P	0,08	0.06
Ca	25	32
Mg	2,4	1,7
Mn	0,11	0.09
S	0,30	0,47
As	0,001	0,003
Bi	0,01	0,005
Mo	0,004	0,004
Cu	0,1	0,08
Zn	0.07	0,02
Cr	0,002	0,004
Ni	0,001	0,002
Water	8,0	9,0

There were traces of B, Pb, Cd, Se, and Ag.

RESULTS AND DISCUSSIONS

Results in winter wheat are presented in Figure 1, which show that the smallest crops and, therefore, the lowest valorising of fertilisers were on an agro-fund with no mining wastes. On the agro-funds treated with 1.2 and 3 t/ha of mining wastes, fertiliser valorisation was higher, as a result of the positive impact of the mining wastes neutralising soil acid reaction, and of the fact that micro- and macro-elements improved plant nutrition.

For a rate of N_{150}, yields obtained on the agro-funds treated with 2 t/ha and 3 t/ha were pretty close, which means that increasing waste is not motivated. At the same time, this allows avoiding the danger of increasing heavy metal content in the soil.

Figure 2 shows that the lowest yields in maize were on the agro-fund not treated with mining wastes while the highest yields were obtained on the agro-fund treated with 3 t/ha of mining wastes and fertilised with N_{150}-N_{200}.

Results obtained in oat Figure 4 point out that the highest yields were on the agro-fund treated with 2 t/ha and 3 t/ha of mining wastes and fertilised with N_{150}.

Results obtained in bean Figure 4 show a positive response within the range N_0- N_{100}, on all mining waste funds, with the best results on the fund treated with 3 t/ha.

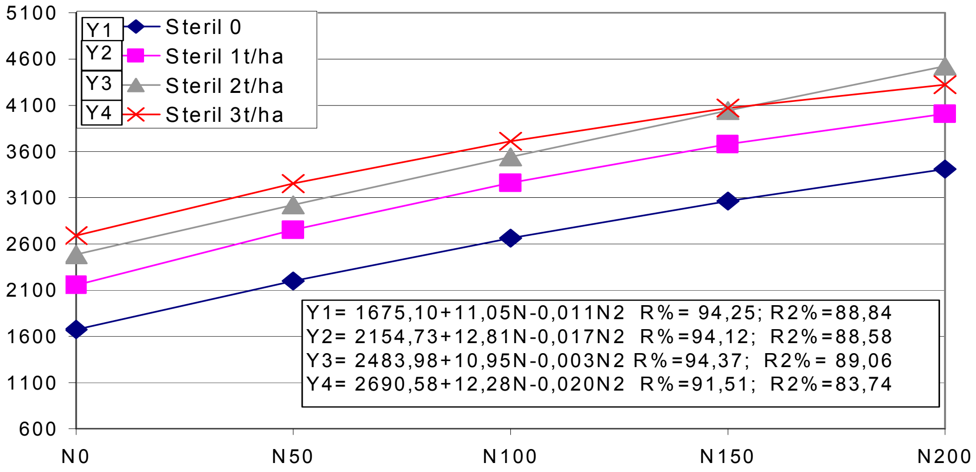

Fig. 1. Response curves wheat, crops to nitrogen fertilisation with variable levels of mining wastes on a constant agro-fund fertilised with $P_{60}K_{60}$

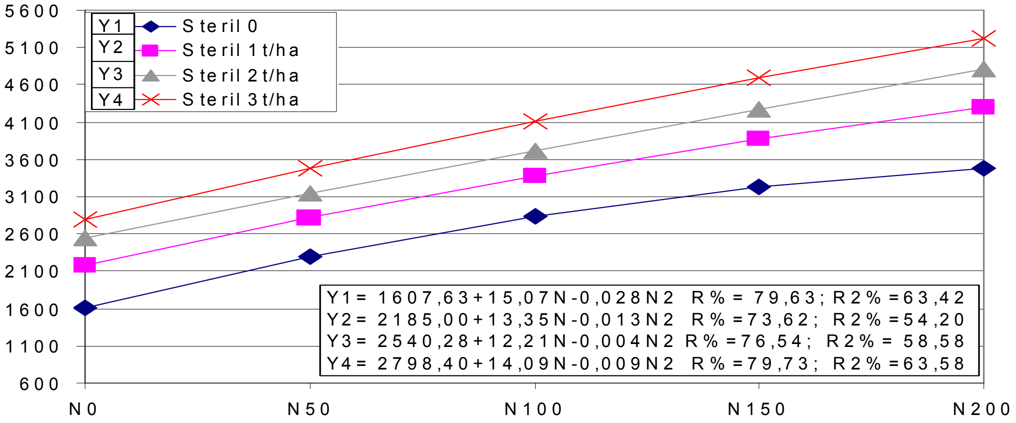

Fig. 2. Response curves maize, crops to nitrogen fertilisation with variable levels of mining wastes on a constant agro-fund fertilised with $P_{60}K_{60}$

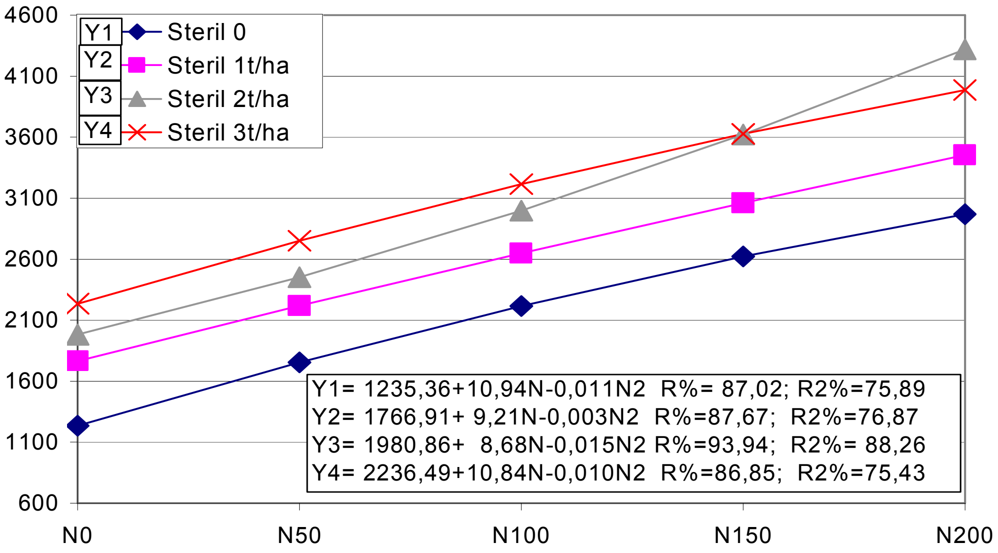

Fig. 3. Response curves oat, crops to nitrogen fertilisation with variable levels of mining wastes on a constant agro-fund fertilised with $P_{60}K_{60}$

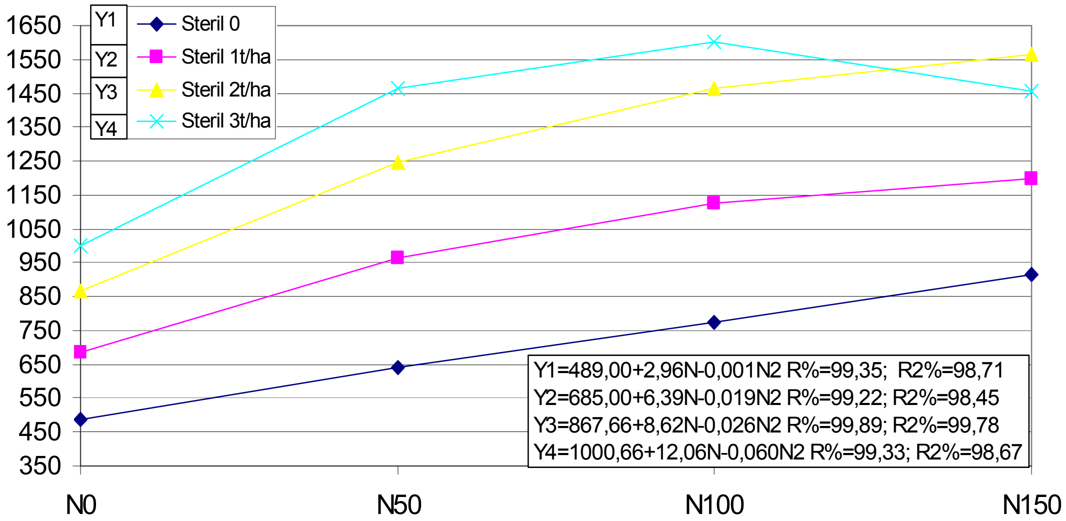

Fig. 4. Response curves of bean, crops to nitrogen fertilisation with variable levels of mining wastes on a constant agro-fund fertilised with $P_{60}K_{60}$

CONCLUSIONS

Mining wastes from the copper mines in the Moldova Nouă and Sasca Montană area are not radio-active and heavy metal content is below European accepted limits.

Alkaline reaction of the mining wastes and their content in micro- and macro-elements make it possible to use them on acid reaction soils.

Applying mining wastes in rates of 2-3 t/ha lead to a better valorisation of the nitrogen fertilizers applied on an agro-fund of $P_{60}K_{60}$.

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STUDY OF THE COMPLEXES FORMED BETWEEN SOME METAL IONS WITH THE CONDENSED PHOSPHATES BY MEANS OF GEL- CHROMATOGRAPHY

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ABSTRACT

The Hummel and Dreyer effect were used to the study of complex formation between Cu^{2+}, Zn^{2+} *and* Fe^{3+} *ions and diphosphate, triphosphate and polyphosphate by steric-exclusion gel-chromatography on Sephadex columns.*

The stability constants of the metal complexe were determined and the effect of pH on the complexation equilibria were investigated.

The method is useful for the physico-chemical analysis of trace minerals in the presence of condensed phosphates.

Key words: complex metal-polyphosphates, gel chromatography, stability constants.

INTRODUCTION

The liniary polyphosphates forms soluble complexes with the divalent and trivalent ions (1,5). The usually study methods for the forming of complexes as the spectrophotometry, nephelometry, potentiometry, conductometry a.o. , being static methods, permited not the simultaneous research of the of the resulted complexes formed by the metal ions with the many mixed ligands.

Hummel and Dreyer (1962) were applied for the first time the sterical exclusion chromatography to the reversible interaction between the molecules and the substances with little molecular weight molecules, method which enable the direct observation of the complex forming which are in equilibrium with the free metal ion, maintained to a predetermined constant value.

Yoza and his coworkers were developped this experiment based on a dynamic method, which were applied for the stability constants determination of the magnesium polyphosphates (Kouchiyama et all., 1978).

In the present study, the complex formation of the copper, zinc and iron, transition metals having trace minerals role in the biological systhems with the diphosphate, triphosphate and the high polycondensed polyphosphate (Kurrol salt) were investigated.

MATERIALS AND METHODS

We used diphosphate, triphosphate (obtained by means of repeated recrystalisations of the technical tripolyphosphate high polymerised), potassium polyphosphate (Kurrol salt) and Blue Dextran 2000.

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 117-122, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

The chromatography were made on Sephadex G – 15, Sephadex G-25 and Seohadex G-50 fine gells columns with 900x15 mm, placed in equilibrium with buffer solutions used for the samples elution, in which the metal ion concentration were 5.10^{-5} M. The eluent flow speed were insured with a peristaltic pump. The sample solution of 1 ml. formed of the polyphosphatic ligands in presence of an metal excess were introduced in the top of the column.

The eluent fractions of 2 ml were collected with an fraction collector. The eluated Fe^{3+} fractions were determined photocolometricallly by means of the ammonium sulphocyanure in presence of aluminium nitrate, for the iron delivering from the condensed phosphate (H.E.Wirth, 1942).

The phosphorus were determined with the Mo^{V} – Mo^{VI} reagent (Yoza et all.,1977), using a colorimetrical automatic line.

The copper and zinc were determined by means of an atomic absorption spectrograph. The used eluents were:

0.08 M NH OH – 0,02 M NH_4Cl (pH = 10)
0.01 M HNO - 0,1 $NaNO_3$) (pH = 2)
A and B contained 5.10^{-5} M metall ions.

RESULTS AND DISCUSSIONS

The study of the ferric ions complexes with the condensed phosphates in the fine Sephadex G – 25, pH = 2 make evident the forming of the soluble complexes with the all ligands used. The ferric ions endure hydrolyse, forming insoluble hydrated polymers at pH values upper to 2.5 (Spiro et all., 1966).

When we graphical represent the metal concentration in the eluent as function of the total eluent, we obtain the elution profile, known as the Hummel Dreyer effect,

The elution prophile of a so experiment presents an positive peak for the metal complex ML, which walk speedy together with the ligand, as compared with the free metal ion M, which go slower.

The elution volumes of the free ferric ion and of the iron – polyphosphates complexes decreased in speed in reverse order with their molecular weights: Fe > FeP > FeP > … > FeP .

Yoza, 1975, showed that through the introduction in the sample of a ligands mixture with various molecular weights together with a metal excess, which insure to forming of the all complexes and to remain also an excess, so appear an positive peak at the metall ion elutions position, fact confirmed also diphosphate and triphosphate elution.

In this manner we may study through a single experiment many complexes of an metal ion with various polyphosphatic ligands in mixture., as well as the competitive phenomena which may interweave between many metal ions for the binding site of the same ligand.

In the case when the metal ions are not in excess in the sample, after the peak of the Fe-triphosphate complex, the iron concentration of the elution continues to be constant, and afterwards it decreased at the elution position of the free iron ion, forming an negative peak, which corresponds to the iron amount consummed from the elution solution.

The study of the concentration at the elution of the Fe^{3+} complex of the high polymerised Kurrol salt is useful as a marker for the determination of the zero volume of the fine Sephadex G-25 column, this being excluded from the gel phase at the position of the corresponding position of the Blue Dextran.

The possibility to metal-complexes forming of the polyphosphates with copper ions were studied with the fine Sephadex gel G-25 at pH = 10. The elution prophile make evident also in this case the soluble complexes forming with the studied polyphosphates.

The elution position of the Cu^{2+} -complex with the Kurrol salt were controlled also by means of the phosphorus determination in the eluated fractions, obtaining a perfect concordance. The fine Sephadex G-25 enables the copper complexes separation, formed in the presence of a ligands mixture.

For to render evident the resolution power of the metal complexes with the condensed phospates also on another Sephadex type, we worked at the zinc complexes with a fine gel Sephadex G-15 and at pH = 10. The quantitative valuation of the gel chromatographic behaviour of the complexes needs to know some parameters of the columns as well as the void volume V and the elution position of the free (not complexed) Zn ion. The elution profile of the zinc complex with the Kuroll salt make obvious the fact that the his retain volume are independent of the complexed iron and of the eluent pH-value.

We have studied the Zn- complexes with the the diphoaphate and the triphosphate, in presence of a zinc ions excess in the eluated sample.

When the chromatography were made on a gel with the large inside pores, as well as the fine Sephadex G-50, these enables to separate the Zn-complexes with the studied polyposphatic ligands.

One can observe that in this case an increase the separation space amongst the retention volume of the $Zn\text{-}P_3$ complexes and the exclusion of the ZnP_n complex, fact which enables to separate from a mixture also the metal complexes with ligands hawing a greather condensation degree as three (tetraphosphate, pentaphosphate, hexaphosphate, etc.)

The so kind of oligomeric polyphosphates are encountered in some technical matters, as well as the polyphosphate glasses with trace minerals, as well as the liquid complex fertilizers with trace minerals, based on the ammonium polyphosphoric acids.

The analytical methods presented in this paper may be apply also to the technical analysis of the this industrial products.

The study by means of the sterical gel-exclusion chromatography enable together to characterize the formed complexes, respective the metal-ligands ratio, as well as to determine the stability constants.

The metal-ligand complex formation may be represented by the relation: $M + L = ML$ (1)

The stability constant of the metal-ligand complex are definable by the known relation: $K = [ML] / [M][L]$ (2)

After Yoza,(1977), in the study of the metal-ligand complexes by means of the sterical - exclusion in the gel chromatography, when the complex migration accomplish in the presence of a predicted concentrations an constante value of the stability contant in the eluant, nominated as $[M]_0$ the stability constant value result by means of the relation: $K = [ML] / [M_0]([L_t]-[ML])$ (3)

In which L_t = the total concentration of the introduced ligand in the sample.

Because during the complex migration, it is permanently in contact with the free metal ions $[M_0]$, the complex stability are insured, this being into a permanent dynamic equilibrium.

When the complex ML elution would be effectuate whith an eluant which not contain free M and L, this successive dissociate into his constituants during the migration, to get to a complicated elution profile, depending so from the stability constant, as well as from the kinetical factors. (11)

When in the (3) relation we introduce the notations:
$[ML]$ = Q_{ML} = the ligand amount binded in the total quantity applied in the sample, the relation become: $K = Q_{ML} / [M_0](Q_{Lt} – Q_{ML})$ (4)

The Q_{ML} value were graphical determined from the positive peak area, measurede by means of planimetry, from the basal line of the metal concentration in the eluent and were report to the standardisation area.

The standardisation area we chose in the horizontal portion of the elution profile, in the predecessor zone of the exclusion peack of the columns, at which correspond a known amount of the metal ion (µM) contained in the respectively eluant volume.

The medium number of the binded metal ions by the ligand designate as n are given by the relation: $n=Q_{ML}/Q_{Lt}=K[M]_0/1+K[M]_0$ (5)

The n values of the stability constants and of the log K for the copper, zinc and iron with the diphosphate, tripolyphosphate and the high polymerised polyphosphate, determined by means of the sterical exclusion chromatography are shown in the table 1.

Table 1. Stability constants of the Fe, Cu and Zn complexes

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	Fe-P_2	4,9	0,993	1	0,007	3.1.10^6	6.49	1,738
2	Fe-P_3	4.9	0.972	1	0.021	1.0.10^6	6.02	1.959
3	Fe-P_n	6.1	0.940	4.24	0.287	2.3.10^5	5,35	0.222
4	Cu-P_2	4.8	0.860	1	0.140	1.3.10^5	5.11	1.720
5	Cu-P_3	5.0	0.560	1	0.440	2.6.10^4	4.41	1.120
6	Cu-Pn	5.0	0.360	8.47	5.702	1.3.10^3	3.10	0.043
7	Zn-P_2	4.6	0.438	1.47	0.562	1.7.10^4	4.23	0.617
8	Zn-P_3	5.9	0.660	1	0.340	3.3.10^4	4.52	0.660
9	Zn-P_3	4.8	0.159	1	0.431	2.4.10^4	4.37	0.854
10	Zn-Pn	4.6	2.294	8.47	0.933	5.4.10^4	4.73	0.271

The stability constants calculated for the mentionated complex ions with the diphosphate and triphosphate are comparable with the data from the bibliography sources, which are determined for the stability comstants by means of the other physico-chemical methods (Bobtelski and Kertes, 1955, Martell and Calvin, 1956).

In the case of the metal complexes with the Kurrol salt, the stability constants are smaller as compared with these for the diphosphate and triphosphate. The calculated values of the n for the copper and iron complexes with the diphosphate and the triphosphate shows a metal-ligand ratio equal to 2:1, and for the zinc complexes with the same ligands, the ratio of 1:1.

In the case of the Kuroll salt complexes with the metal ions, the n values are very small.

Supposing that the molecular weight of the Kroll salt are 10^6 (Kouchiyama and Dreyer, 1978), the medium number of the binded metal ions to a Pn molecule (not to a PO_3 unit), results in the case of the copper a value of 390; results in the case of the zinc of 161 and in the case of iron of 845. These values being smaller as compared with the 1440 value obtained of Yoza for the magnesium complex with the Kurrol salt.

For the iron complex with the Kurrol salt realized at the pH value of 2 for the eluant, results from the graph of the per cent concentration for the various diphosphate specimen as function of the pH value, calculated by means of the diphosphoric acid dissociation constants, results that the complexed iron specia at this pH value are $H_2P_2O_7$.

The stability constants determination by means of the steric exclusion chromatography in gel, give the possibility to study also of the polyphosphate complexes with a condensation degree upper to the studied, and which may be obtained in very small quantities, only by the chromatography in column of the ion exchangers, quantities which are insufficients for the other usually determination methods.

CONCLUSIONS

1. The sterical exclusion gel chromatography enable to study the forming of the metal ions complexes with the oligomeric polyphosphates also in the case of the transition metals with the trace element role in the biological systhems.
2. The method insure a good separation of the complex compounds, by means of a judicious choose the type of the Sephadex gel, as function of the middle molecular weight of the ligands used for the complexation.
3. This method give the possibility to determine the stability constants values and the ratio between the metal and the ligand ratio.
4. To know the metal-ligand ratio in the complexes enable a indirect dosage of the polyphosphoric mixtures in solution, replacing the phosphorus dosage, fact which increased the speed, the sensibility and the precision of the dosage by mean of the metal ions from the automatic dosage by means of the eluated fractions by mean of the others methods, as well as the atomic absorption spectrography.

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REFINED SUNFLOWER OIL: AN EXCELLENT VEHICLE FOR IODINE FORTIFICATION

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ABSTRACT

Iodine is an essential element for normal physical and mental development. More than 2 billions individuals worldwide have insufficient iodine intake. Iodine deficiency disorders (IDD) is a public health priority not only as a main cause of preventable brain damage in children, but also because of its consequences on social and economic development through limitation of human resources potential. Salt iodization is the most common strategy for eliminating iodine deficiency and was mandatory or voluntary implemented in some countries. Because of increased concerns related to salt consumption as a risk factor to cardiovascular diseases, countries are encouraged by WHO to reduce salt consumption and identify other vehicles but salt for iodine fortification (WHO, 2008). A promising option is refined sunflower oil enriched in iodine. Given in complement to iodized salt, it can substantially contribute to increase iodine intake and therefore improve iodine status of the population. It also has the advantage over salt to provide other nutrient beneficial to health such as unsaturated fatty acids and vitamin E.

***Key words**: Iodine deficiency, food vehicle for iodine fortification, iodine status, iodized salt, refined vegetable iodized oil, unsaturated fatty acid

INTRODUCTION

Regarding iodine deficiency the followings are mentioned :

a) 40 million people are at risk of iodine deficiency in Europe (both Eastern and Western)

b) In Europe, iodine deficiency disorders (IDD) is usually moderate or mild with limited manifestations such as poor pregnancy outcome, reduced physical growth or mental impairment: 10-15 IQ points may be lost in iodine-deficient communities!

c) Subtle degrees of mental impairment as observed in mild forms of IDD may lead to poor school performance, reduced intellectual ability and impaired work capacity

d) The fact that some countries have been successful in eliminating IDD over the last few years such as Romania does not preclude that some areas within the country might be still affected by moderate or even severe iodine deficiency.

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e) Even in countries that have achieved iodine sufficiency, the iodine nutrition status of pregnant and lactating women, and infants may still be inadequate

National commitments towards adequate iodine nutrition and its prevention is much weaker in most European countries than elsewhere in the world.

According to WHO, “iodine deficiency and its many consequences are still a public health problem in much of Europe".

1. FOOD FORTIFICATION - BASIC PRINCIPLES

1.1. Food fortification is a public health approach to the correction of micronutrients deficiencies

Food fortification is justified where there is “a demonstrated need for increasing the intake of an essential nutrient in one or more population groups. This may be in the form of actual clinical or sub clinical evidence of deficiency, estimates indicating low levels of intake of nutrients, or possible deficiencies likely to develop because of changes taking place in food habits” (Codex Alimentarius, 1994).

The goal of fortification is to increase the micronutrients intake of an acceptable proportion of the population to an adequate level, without causing an unacceptable risk of excessive intake

1.2. Oil is an appropriate vehicle for food fortification because it:

- Can be distributed widely to reach key target groups throughout the country.
- It is consumed daily in fairly constant amounts.
- Has no objectionable change in taste, appearance or color after fortification.
- Retains an appropriate level of the nutrient after further processing or cooking.
- Is not consumed in amounts that would expose to the risk of over consumption and therefore of iodine intakes beyond the safe level.
- Is an homogenous product

2. REFINED SUNFOWER OIL ENRICHED IN IODINE:

2.1. Product description :

Floriol Iod Protect launched in Romania in 2006

Its Ingredients are: Refined sunflower oil; Ethyl esters of iodized fatty acids; The iodine concentration is 1 mg/kg and two tablespoons (20g) of Floriol Iod Protect bring 15% of daily recommended intake in iodine

2.2. Nutritional information

A tablespoon of this product brings a few calories into the organism. It contains more unsaturated fatty acids than saturated ones and is rich in vitamin E. Details are given in Table 1.

Table 1. Composition of the iodine enriched sunflower oil

Mean nutritional value	**10 g (one table spoon)**	**100 g**
Energetic value	90 kcal or 370 kJ	3700 kJ
Proteins	0	0
Carbohydrates	0	0
Fats	10 g	100 g
Saturated fatty acids	1.2 g	12 g
Monounsaturated fatty acids	2.6 g	26 g
Polyunsaturated fatty acids	6.2 g	62 g
Cholesterol	0 g	0 g
Fibers	0 g	0 g
Natrium	0 g	0 g
Vitamin E	7.0 mg	70 mg
Iodine	10 mcg	100 mcg

2.3. Health claims on Floriol Iod Protect

‘’Recent studies shows that IDD is a worldwide public health problem, including in Romania.

Iodine is an important element for maintaining health, contributing to healthy physical and mental development.

Iodine is an essential nutrient during the overall life cycle especially in adolescent girls as future mothers, in pregnant and lactating women and in young children. Iodine deficiency may result in irreversible brain damage during fetal development and infancy.

CONCLUSIONS

The refined sunflower oil enriched in iodine:

1. Has a similar behavior as regular sunflower oil during storage and cooking;

2. Due to covalent bonds iodine-fatty acids, is very stable during cooking and frying; the losses are much smaller comparing with iodized salt (aprox.10%).

3. Is a complementary source of iodine for optimum iodine nutrition especially in pregnant women and young children;

4. Is a naturally source of essential fatty acids and vitamin E;

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PHOSPHONATE LIGANDS GRAFTED ON STYRENE-DIVINYLBENZENE COPOLYMERS USED IN METAL IONS SEPARATION FROM WATER

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ABSTRACT

Phosphonates grafted on styrene-divinylbenzene supports were prepared and their use in Cu(II) separation from aqueous solutions was investigated. The polymeric materials were characterized by determination of the functionalization degree based on the phosphorus content and thermal stability by thermogravimetric analysis. The sorption capacity of the resins functionalized with phosphonate pendant groups towards Cu(II) was studied.

Keywords: phosphonates, styrene-divinylbenzene microbeads, removal of metal ions

INTRODUCTION

The synthesis of new polymeric matrix based sorbents, incorporating ligands which are immobilized by chemical bonds and are highly selective towards metal ions, received lately great interest due to their potential use in the industrial separation and concentration of metals and in trace metal analysis.

Nonspecific sorbents, such as activated carbon, metal oxides, silica and ion-exchange resins have been used (Reed B.E., 1993). Specific sorbents consist in ligands (e.g. ion-exchange materials or chelating agents) interacting specifically with the metal ions, and a carrier matrix which may be an inorganic material (e.g. aluminum oxide, silica or glass) or polymer microbeads (e.g. polystyrene, styrene copolymers, cellulose, poly(maleic anhydride) or polymethylmethacrylate (Kantipuly et al., 1990; Goto et al., 1993; Popa et al., 2003; Popa et al., 2004; Popa et al., 2007; Popa et al., 2008).

Classes of ligands that have been developed for this purpose include amines, amidoximes, phenols and their derivatives, and phosphonates. Phosphoric acid derivatives are especially versatile, displaying a wide range of ionic selectivities in direct relationship with the ligand structure.

The determination of Cu(II) in natural waters is important whereas this trace element constitutes an environmental problem and there is a lack of information on the behaviour and toxic potential of this metal on the metabolism of living organisms (Korn M.G.A. et al., 2004).

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 127-132, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

In this work we describe the multiphase system synthesis of phosphonates grafted on styrene-divinylbenzene copolymer-supports and their characterization (degree of functionalization and thermal stability).

The aim of this work was to asses the possibility of application of chelating resins obtained by functionalization of styrene-divinylbenzene copolymers with phosphonate groups in copper separation and preconcentration. Atomic absorption spectrometry is nowadays the most common method for trace metal determination. The quantitative analysis of metal ions was carried out by atomic adsorption spectroscopy on SpectrA 110 coupled with GTA-110.

MATERIALS AND METHODS

The functionalization with phosphonate pendant groups was realized by the polymer-analogous Michaelis-Becker reaction presented in Scheme 1.

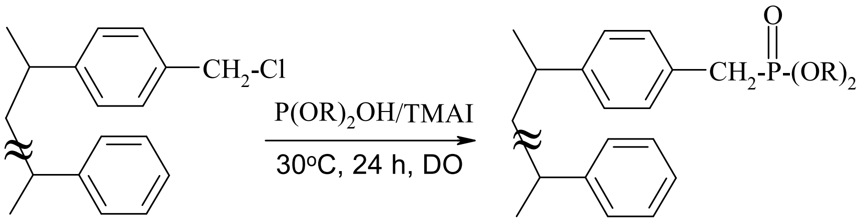

Fig. 1. Michaelis-Becker polymer-analogous functionalization reaction. Phosphite used: $(CH_3O)_2POH$, $(C_2H_5O)_2POH$, $(i\text{-}C_8H_{17}O)_2POH$, $(C_2H_5\text{-}C_6H_{12}O)_2POH$

Synthesis of the phosphonates grafted on styrene-7% divinylbenzene copolymers

The synthesis of the phosphonates grafted on a macroporous styrene-7%divinylbenzene copolymer-support was performed by the method previously described (Popa A. et al., 2003; Popa A. et al., 2004). A 5-g sample of chloromethylated styrene-7%divinylbenzene copolymer and 100 ml of solvent (1,4 - Dioxane) were added into a round bottom flask fitted with reflux condenser, mechanical stirrer and thermometer. Then, the necessary amounts of phosphite and sodium carbonate were added in order to achieve the molar ratio of chloromethyl:diphosphite:sodium carbonate = 1:1.5:1.5 Tetramethylammonium iodide was used as catalyst. The mixture was maintained under stirring for 24 h at 30°C. After cooling, the copolymer beads were separated by filtration, washed with ethanol (2x50 mL), 1,2-dichloromethane (2x50 mL) and ethyl ether (2x50 mL), and dried at 50°C for 24 h.

Procedure for Cu(II) removal from aqueous solutions using phosphonates grafted on polymeric supports (Popa A. et al., 2007; Popa A. et al., 2008)

Cu(II) ion separation from aqueous solutions was performed by a batch technique at room temperature. 0.5 grams of of styrene-7%divinylbenzene copolymer functionalized with phosphonate pendant groups were added to a volume solution containing 100 mg Cu(II) in 0.1 N HNO_3 and maintained under mechanical stirring for 7 hours. The residual Cu(II) content in solution was determined on samples (1 mL) taken from the mixture as follows: at 10 min. interval in the first hour, at 15 min.

interval in the second hour and at 30 min. interval in the last 5 hours. Each sample was diluted (ratio 1:25) and the concentration of Cu(II) was determined by atomic adsorption spectroscopy on SpectrA 110 coupled with GTA-110.

RESULTS AND DISCUSSIONS

The main characteristics of the copolymer functionalized with phosphonates groups are presented in Table 1.

Table 1. Characteristics of the styrene-7%divinylbenzene copolymers grafted with phosphonate groups obtained by polymer-analogous Michaelis – Becker reaction.

Product code	**Phosphite**	**P (%)**	**G_F (mmoles phosphonate/g of copolymer)**
Me-PS	$(CH_3O)_2POH$	5.49	1.78
Et-PS	$(C_2H_5O)_2POH$	6.09	1.98
i-Oct-PS	$(C_8H_{17}O)_2POH$	4.37	1.40
EtHex-PS	$(C_2H_5\text{-}C_6H_{12}O)_2POH$	4.13	1.33

One of the key properties of a polymeric chelating agent used in metal ions separation is its thermal stability. The thermal stability of the original copolymer support (PS) and that of the copolymers functionalized with phoshonate groups was evaluated by thermogravimetric analysis on a Netzsch TG 209, in nitrogen atmosphere, at a heat rate of 5K/min from 20ºC to 600 ºC. The results are presented in Table 2.

Table 2. Weight loss of the polymeric materials

Product	**Weight loss (%)**			**$T_{inflexion}$ (ºC)**
	20-200ºC	**20-300ºC**	**20-400ºC**	
PS	3.25	14.25	23.72	427.7
Et-PS	4.57	11.20	94.72	366.7
i-Oct-PS	5.27	11.91	44.60	399.1
EtHex-PS	5.11	17.99	40.85	404.3

As can be seen the weight loss up to 200ºC is minor, indicating a thermal stability qualifying the products for use in small and large scale separation processes. This type of behavior is characteristic for syrene-divinylbenzene copolymers. At higher temperatures the weight loss is larger indicating significant degradation of the polymeric network. The thermal stability is affected by the nature of the phosphonate ligands. In all cases, a decrease of the thermal stability was observed as a result of the functionalization with phosphonate pendant groups in comparison with the starting chloromethylated copolymer.

The ability of the functionalized copolymers to act as specific sorbents/chelating agents for Cu(II) ions from aqueous acidic solutions (pH=1) was investigated.

The concentrations of Cu(II) (mg/L) in solution were determined by atomic absorption spectrometry on a Varian SpectrAA 110 coupled with GTA-110, at the wavelength λ= 324,8 nm.

The amount of Cu(II) separated, q, was calculated as:

$$q = \frac{C_o - C_t}{G} \cdot V \cdot 10^{-3} \quad \text{mg Cu(II)/g of copolymer}$$

where: C_o is the initial concentration of Cu(II) in solution (mg/L);
C_t is the concentration of Cu(II) in solution after t minutes (mg/L);
V is the volume of the solution (mL);
G is the mass of resin sample (g).

The adsorption rates of of Cu(II) ions by the polymer-grafted phosphonates with different functional groups are shown in Figure 3.

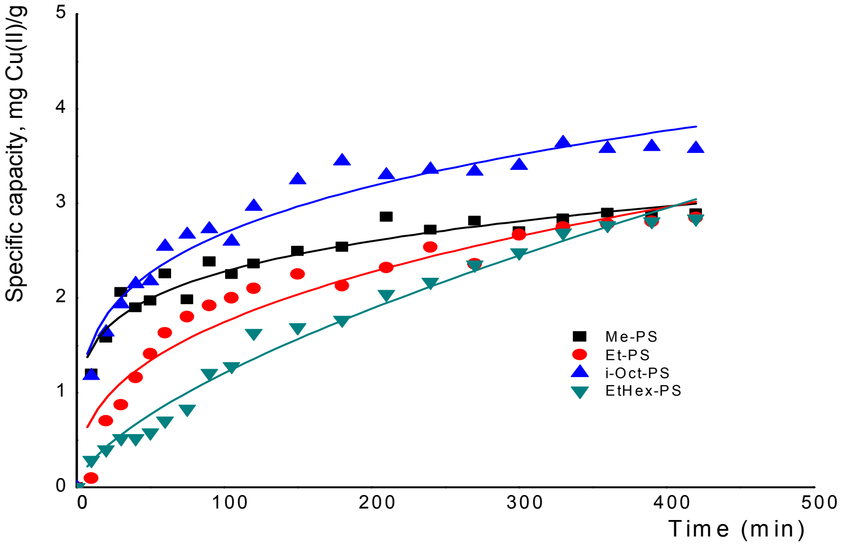

Fig.3. Specific adsorbtion capacity as a function of time, for the resins bearing phoshonate groups, at pH=1 and 25 °C.

As can be seen, the kinetic curves are Langmuir type isotherms, suggesting adsorption in monomolecular layer followed by saturation of the active centers. Such a behaviour was expected due to the fact that water is not a good swelling solvent for the polymer support. In such conditions only the active centers located at the surface of the polymer beads or, eventually, in the larger pores are available.

The amount of Cu(II) ions adsorbed per mass unit of copolymer (*ie.* adsorption capacity) increase with the exposure time and finally reach a constant value corresponding to the saturation of the active centers located in the porous structure of the polymer beads.

The total adsorption capacity of the resins functionalized with phosphonate groups (in mg. Cu(II)/g of copolymer) was: 2.90 mg/g for Me-PS, 2.78 mg/g for Et-PS, 3.58 mg/g for i-Oct-PS and 2.77 mg/g for EtHex-PS and was attained in all cases after around 360 minutes of exposure.

The efficiency of metal retention ability (mmoles Cu(II)/mmole phosphonate) of the functionalized polymers was found to depend on the nature of the phosphonate group, as shown in Figure 4.

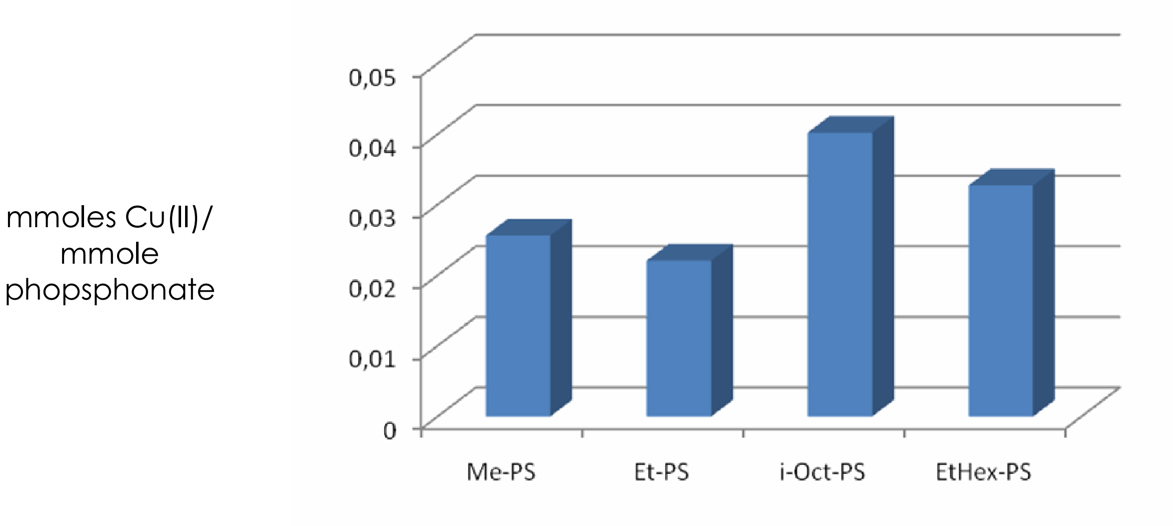

Fig. 4. Efficiency of the retention ability of the polymers bearing phosphonate pendant groups (mmoles Cu(II)/mmole phosphonate).

The efficiency was better in case of the phosphonates with large, bulky or branched alkyl groups. The best results were obtained with the copolymer functionalized with izo-octylphosphonate groups.

CONCLUSIONS

All the copolymers functionalized with phosphonate groups reported in the present study were proved to be efficient sorbents for Cu(II) ions from aqueous solutions.

The polymer-grafted chelating agents investigated are thermally and mechanically stable.

The specific retention capacity of Cu(II) was suggesting clasical monomolecular layer adsorption.

The efficiency of metal retention by the functionalized polymers was found to depend on the nature of the phosphonate group.

Further studies will focuss on the retention capacity of polymer-grafted phosphonates towards other types of metal ions and evaluation of the polymeric sorbents in column separations.

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DEVELOPMENT OF FUNCTIONAL FOODS FROM SOY EXTRACTS ENRICHED WITH MAGNESIUM IN BREAST CANCER

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ABSTRACT

Reactive oxygen species (ROS) play a key role in pathogenesis of degenerative diseases. Evidence offered by antioxidant dietary supplements is intriguing. Vitamin E, ascorbic acid and carotenoids were inversely associated with cardiovascular and cancer mortality in some studies, while negative results have been reported from intervention trials on beta-carotene and vitamin E. Metabolically, nutrients act in combination at physiologic doses, whereas pharmacological doses of a single nutrient, like those used in the above studies, may provoke a nutritional imbalance. Pharmacologic doses of a single carotenoid, such as beta-carotene, can result in competition at the receptor site with the other 50 naturally occuring dietary carotenoids and thus inhibition of their protective effects. The available data justify the conclusion that a balanced mixture of natural antioxidants provides the best protection against cancer and cardiovascular disease. Integral antioxidative capacity and antiradical properties of various extracts from plants can be measured by DPPH (11-diphenyl-2-picril-hydrazyl) scavenging test in order to obtain the most potent mixtures. These can be incorporated as bioactive components in functional foods when prescribing healthy diets. We have studied four soy extracts in order to find the most potent one to be incorporated in foods prepared for patients with breast cancer, preferrablz breakfast cereals with high magnesium content by adding nuts and raisins and and dried buckthorn berries for high vitamin C and E content.

Key words: functional foods, soy extracts, breast cancer

INTRODUCTION

According to data officially reported by the WHO Regional Office in 2002, cancer is responsible for close to one fifth of all deaths, ranging to about 30% in Western European countries (13). The most common cause of death from cancer in males is lung cancer, while in females it is breast cancer. Over the last 150 years, fat

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 133-140, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

consumption has increased substantially in Western and Eastern European countries, which also coincides with a dramatic increase in cancer incidence. A review of epidemiological studies strongly suggests an inverse relationship between the consumption of fresh fruits and vegetables and the incidence of cancer. Fruits and vegetables are rich in fiber, antioxidants, and other plant-derived substances, or phytonutrients, that are thought to have cancer-protective properties (1).

Psychological stress effects, which are often underestimated, should be taken into account among patients bearing free radical-mediated diseases. Psychological stress gives rise to a prooxidant state. Radical oxygen species (ROS) are implicated in mediation of all degenerative diseases, including the atherosclerotic process of coronary heart disease, cancer and neurodegenerative diseases (7). Preservation of key target functions, in the reversible phase of promotion of carcinogenesis, is the major strategic goal of defense against ROS. The human diet contains an array of different compounds that possess either antioxidant activities or the apparent ability to scavenge ROS (11). Examples of opportunities for modulation of target function by candidate food components, as well as possible markers related to defence against ROS are summarized in Table 1.

Table 1. Opportunities for modulation of target function by food components in the defense against ROS

Target functions	**Possible markers**	**Candidate food components**
Preservation of structural and functional activity of DNA	Measurement of damaged DNA components	Vitamin E, vitamin C Carotenoids, polyphenols including flavonoids
Preservation of structural and functional activity of polyunsaturated fatty acids	Measurement of lipid hydroperoxides or derivatives	Vitamin E, vitamin C Carotenoids, polyphenols including flavonoids
Preservation of structural and functional activity of circulating lipoproteins	Measurement of lipid hydroperoxides and oxidized apoproteins	Vitamin E, vitamin C Carotenoids, polyphenols including flavonoids
Preservation of structural and functional activity of proteins	Measurement of damaged proteins or components	Vitamin E, vitamin C Selenium Carotenoids, polyphenols including flavonoids

Reproduced from: Consensus Document: Scientific Concepts of Functional Foods in Europe, BJ Nutrition, 81, 1999 (2).

Diet is estimated to contribute to up to 50% of all newly diagnosed breast cancer cases (3,10). Consumption of phytoestrogens, particularly soy products, as well as vegetables, is higher in Asia than in the Western world. Soy-based diets are high in genistein (4, 5, 7-trihydroxyisoflavone), which has been widely studied for its potential anticancer properties (4,5,9,12). Daidzein and genistein are isoflavone phytonutrients derived from soybeans. Laboratory tests have found that selenium and magnesium reduce the incidence of new breast cancers when taken with adequate dietary supplies of vitamins A, C, and E.

The aims of our research were:

1. Development of functional foods from soy matrixes with nuts and raisins for high magnesium content
2. Analysis of antioxidant activity of functional foods (DPPH)

EXPERIMENTAL PART

Obtaining the extracts from soybeans.

Four types of soybeans extract were used:

- degreased soy extract containing glucosylated isoflavonoids,
- hydrolyzed soy extract containing isoflavonoid aglycons,
- extract of hydrolyzed soy pellets containing isoflavonoid aglycons,
- non-degreased soy extract obtained through the following process: 50g of ground soybeans were mixed with 150ml methanol solution (120 ml MeOH, 30 ml H_2O, 0,2 ml HCl concentrate) and then centrifuged. Supernatant was used as an extract.

Obtaining the soy texturate

The soy texturate sample was obtained as follows: 10g of sample with 50 ml 0.1% HCl in methanol 80% were sonicated for 60 minutes, after which the extract was filtered and its volume measured. 50ml more extraction solvent were added to the sample, 60 minutes sonication, then filtering. The two volumes of solvent cumulated, and the final extract volume was concentrated on rotavapor half way through. For all samples, the UV-Vis spectrum within the range of 200-600mm was recorded.

Obtaining the functional food as breakfast cereal

The soy texturate was enriched with raisins for higher magnesium content and dried buckthorn berries for vitamin C and E content.

Determining the antioxidant activity by the DPPH method

One of the highlighting methods for the *in vitro* antioxidant activity, namely the one of flavonoids, is the DPPH method. It consists of the ability of antioxidants (flavonoids) to eliminate the free radical $DPPH^{\cdot}$ (11-diphenyl-2-picril-hydrazyl). This is a paramagnetic free radical, relatively stable, which accepts an electron from a hydrogen radical in order to form a stable diamagnetic molecule (Diagram 1). The antioxidant flavonoids donate the hydrogen, thus reducing the radical, a phenomenon perceived through the changing in colour from violet to yellow. The decrease in optical density within the characteristic wavelength range during the radical-antioxidant reaction is due to the fact that the $DPPH^{\cdot}$ radical absorbs at 515nm, while after it being reduced, this absorption disappears.

The activity of in vitro free radical scavenging by use of the DPPH method was at first determined with flavonoid standards (quercetine, genistein and daidzein) then compared to the soy extracts.

Chemical reagents and standards used:

- 2,2-dyphenil-1-picril-hydrazyl (DPPH)-0,031g of DPPH reagent were disolved into 99,969ml of distilled water (a concentration of 80μM DPPH was obtained). Then 1 ml of this solution was mixed with 9ml methanol and sonicated in order to produce stable free radicals.
- Genistein - the following concentrations were used: 500 ppm,100 ppm and 50 ppm.

- Daidzein - the following concentrations were used: 4mg/100 ml; 2 mg/100 ml.
- Quercetine - the following concentrations were used: 2mM, 1mM, 0,5mM and 0,25mM.

Method: 2,8ml of the DPPH radical solution were mixed in turn with 400 μl of isoflavone standards (genistein and daidzein) of different concentrations, and with the soy extracts. The mixture was energetically agitated then kept in the dark for 30 minutes, after which the absorbance at 515nm was measured with a spectrophotometer (JASCO V-530). For the blank, 2,8ml of DPPH radical solution were used in mixture with 400 μl of methanol.

The degree of discoloration of DPPH (%) was calculated according to the following formula:

$$\%discoloration=[1-A_{sample}/A_{blank}]x100$$

Where:

A_{blank} – absorbance of the blank solution;

A_{sample} – absorbance of standard solution and soy extracts with DPPH after 30 minutes.

RESULTS AND DISCUSSIONS

The degree of discoloration of the DPPH by the isoflavonoid standards (genistein and daidzein), as well as the soy extracts, are all presented in Table 2.

Table 2 . Values of absorbance (A_{sample} and A_{blank}) and degree of DPPH discoloration for standards and soy extracts.

Specification	**Dilutions**	***A_{sample}**	**A_{blank}**	**%discoloration DPPH**
Genistein	500 ppm	0,4450	0,6520	31,74
	100 ppm	0,5246		19,53
	50 ppm	0,5311		18,54
Daidzein	4 mg/ 100 ml	0,4787		26,5
	2 mg/100 ml	0,5554		14,81
Quercetine	2mM	0,0426	0,6596	93,54
	1mM	0,0315		95,22
	0,5mM	0,0281		95,73
	0,25mM	0,0268		95,93
Degreased soy extract		0,1535	0,6271	75,52
Non-degreased soy extract		0,1393	0,7112	80,41
Hydrolyzed soy extract		0,0967	0,6271	84,57
Extract of hydrolyzed soy pellets		0,2121		66,17

* absorbance after 30 minute

The DPPH method is used for investigating the activity of radical scavenging of several natural compounds such as the fenolic compounds, or by some plant extracts. The DPPH radical is being scavenged by the antioxidants through their capacity of giving away one hydrogen, with DPPH-H formation. The change in colour from dark violet to yellow after reduction can be quantified by the decrease of absorbance to 515nm.

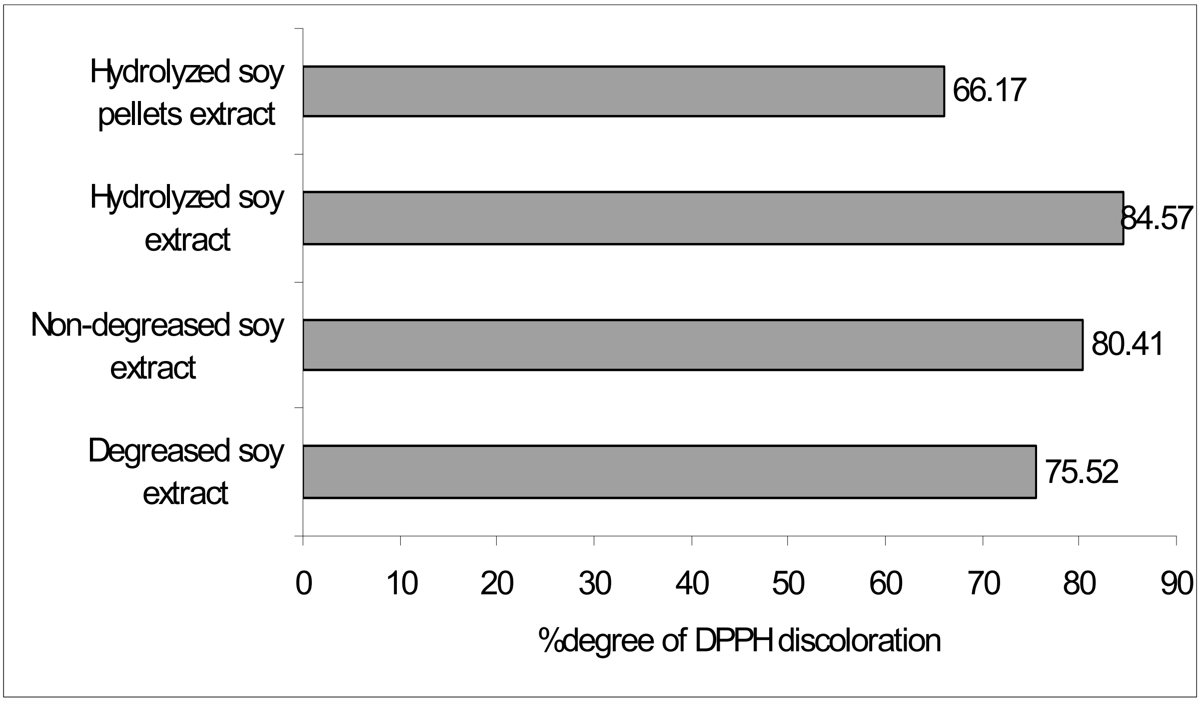

Fig. 1. Various degrees of DPPH discoloration by the four soy extracts studied

In figure 2, the various degrees of DPPH discoloration by the soy extracts are presented comparatively. The highest degree of DPPH discoloration was shown by the hydrolyzed soy extract. This extract was characterized by TLC, by highlighting the presence of genistein and daidzein. The capacity of DPPH radical inhibition by the isoflavonoid aglycons is very small in comparison with the one of the extracts containing these isoflavonoids.

While characterizing TLC extracts, other unidentified compounds were highlighted, which could be responsible for the high antioxidant activity of the extracts. Moreover, the degreased soy extract is shown to have less antioxidant activity than the non-degreased one. Through degreasing, phytosterols – bioactive substances with antioxidant activity – are being eliminated.

The antioxidant activity also depends upon the concentration and structure of isoflavonoids. For example, the glucose binding in isoflavonoid aglycons will result in a decrease of antioxidant activity (6).

The soy beans contain approximately 2% isoflavonoids in the form of aglycons, thus the non-hydrolyzed extracts will present a less intense antioxidant activity than the hydrolyzed ones.

The relationship between the chemical structure of quercetine and isoflavonoids and their antioxidant activity.

In Table 3, on the first column, the structural characteristics that need to be fulfilled by a flavonoid in order to function as an antioxidant are being shown, while in the other columns the structural characteristics fulfilled by the studied flavonoids (quercetine, genistein and daidzein) will be ticked.

Table 3. Comparison between the structural characteristics of quercetine and isoflavonoids regarding their antioxidant capacity

Structural characteristics of flavonoids in free radical scavenging	Quercetine	Genistein	Daidzein
Cathecol group in ring B	√	-	-
Pyrogalol group in ring B	-	-	-
The double bond between carbon atoms 2 and 3	√	√	√
Ketonic group in position 4	√	√	√
–OH group in position 3	√	-	-
–OH group in position 5	√	√	-
–OH group in position 7	√	√	√

Out of the three flavonoids studied here, quercetine fulfills almost all conditions (with the exception of the presence of pyrogalol group in ring B) in order to be considered a powerful antioxidant, followed by genistein and daidzein. In Table 4 it shows that quercetine has the highest capacity of DPPH discoloration (ranging from 95,93% to 93,54%, according to concentration levels). Genistein fulfills only 4 out of the 7 conditions necessary in order to be a good antioxidant, also reflected in the degree of DPPH discoloration (ranging from 31,74% to 18,54%, according to concentration levels). The weakest antioxidant is daidzein, as it fulfills only 3 out of the 7 necessary conditions, with a DPPH discoloration degree between 14,81% and 26,5%.

HPLC chromatographic analysis of soy samples

Genistein being the most hydrophile, it will easily absorb into the thin intestine as aglycon after the hydrolysis of glycosilate conjugate, with exceptional therapeutical effects: estrogen-like activity, breast, colon or prostate cancer prevention, antioxidant activity, prevention of premenopause and osteoporosis symptoms as well as cardiac diseases (8).

The analysis of isoflavones is usually done with the help of chromatographic methods: thin layer chromatography and HPLC -High Performance Liquid Chromatography (Figure 3).

The genistein quantity oscilates between 15-45 mg/100 g, while the daidzein will range between 9-30 mg/100 g. The Daidzein:Glycitin:Genistein ratio can oscilate according to the soy variety from 1.2:1:2.1 to 35.4:1:60.4 .

UV-VIS Spectrophotometric analysis of soy samples

Figure 4 presents the comparative image of the spectras at different dilutions of the soy texturate. Specific peaks ensuring the recognition of characteristic phytochemical compounds (spectral markers) for 250-265/abs.2,4 bandwidth (amino-acid-peptides) were identified.

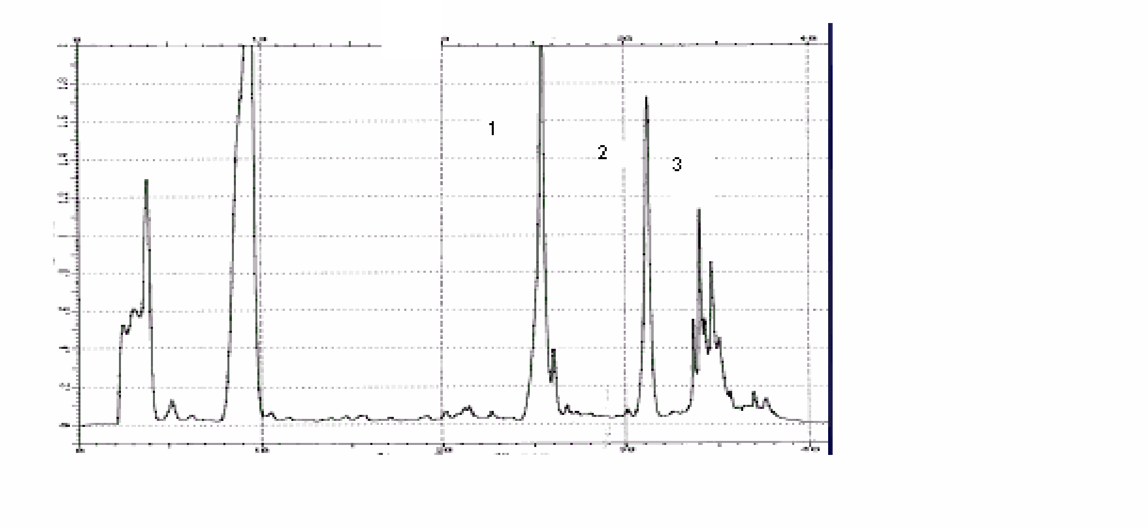

Fig. 2. HPLC chromatogram of a soy extract: 1- genistein; 2-glycitin; 3-daidzein.

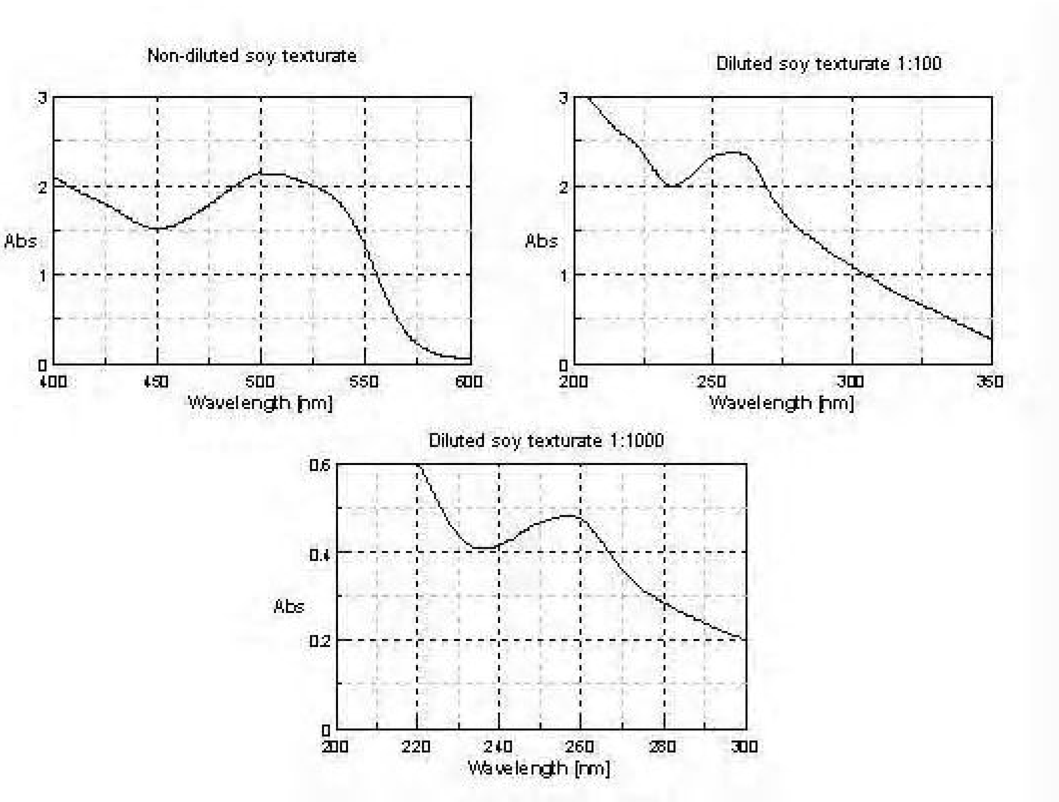

Fig. 3. Comparative image of spectra at different dilutions of soy texturate

CONCLUSIONS

1. Based on data in the literature, it appears that genistein can act as both an estrogen and an antioxidant and an anti-proliferative agent. These effects may be both dose and tissue dependent. Incorporation of natural active components with therapeutic effects in functional foods, based on results of multidisciplinary research will be a challenge for both food and drug industries.
2. It is clear from epidemiologic data that Asian women living in Asia (where a diet high in soy is consumed) have a decreased risk for breast cancer. It is also

possible that soy contains other factors besides genistein that oppose the estrogenic effects of genistein and actually reduce breast cancer risk. Additionally, other environmental or lifestyle factors may be more related to the low breast cancer risk seen in this population than soy/genistein consumption. Fortunately, genistein continues to be an active area of research interest, and therefore an explanation for the dual nature of genistein may not be too far away.

3. The adding of soy flours to the wheat flour for bread as a functional food, as well as the use of soy texturate incorporated in cereal flakes enriched with nuts, raisins and buckthorn berries will give them a pleasant, nutty taste, a brownish colour and will maintain humidity for longer; also, as soy is gluten-free, it is highly recommended to women who are allergic to gluten. The volume of the bread prepared with soy will be less, but the freshness will maintain for longer.

Acknoledgments : This investigation was supported by grant CNCSIS 275/2005

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BIOLOGIC EVALUATION OF PLATINUM AND PALLADIUM COMPLEXES USING THE STATIC CYTOMETRY

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ABSTRACT

The present paper examines the CellScan technique utilization in cancer research in order to evaluate the antiproliferative activity of novel synthesized metal-based compounds and with the purpose to improve treatment of malignant diseases with standard platin-based drugs. The CellScan is a laser scanning static cytometer which allows repetitive spectroscopic measurements in fluorescent marked cells. It was demonstrated the potential of CellScan technology to detect the in vitro effects of the chemotherapy on tumor cells, making this method a valuable tool for chemosensitivity tests. The detection of cell viability and apoptosis is possible based on the transformations which occur in the cytoplasm matrix of tumoral cells influenced by cytotoxic compounds. The measurement of fluorescence changes due to this phenomenon is possible with the CellScan system. The potential of this technology to detect the in vitro effects of the inhibitory molecules on tumor cells was demonstrated, making this method a valuable tool for chemosensitivity tests.

Key words: chemosensitivity, CellScan, fluorescence polarization, platinum complexes, cytotoxicity

INTRODUCTION

Metal-based chemotherapeutic agents, above all platinum complexes, are an important class of antitumour compounds that have had significant impact on the treatment of a variety of tumours for nearly 30 years. The unique efficiency observed with standard platinum-based drugs such as cisplatin, *cis*-diamminecyclobutanedicarboxylato platinum(II) (carboplatin) and 1,2-diaminocyclohexaneoxalato platinum(II) (oxaliplatin) in cancer treatment fuelled the development of new platinum analogues (DeVita, 2004). In the last decade, it was demonstrated that several newly synthesised platinum compounds exhibit biological activity against tumoural cells, and their antimitotic properties were proved by several procedures (Fukuda,1995; Barbara,2006; Carland,2005; Akamatsu,1993; Gay, 2005; Robillard,2003; Cemazar,2006).

One of the major objectives of the cancer research is to find the best therapeutically scheme for the cancer patient and in this trend we performed chemosensitivity tests "*ex vivo*" on the tumoral cells harvested from the patients in order to establish the most efficient drug among the compounds recommended by the international treatment protocols and to improve the remission of the disease and the survival of the patient.

Another important goal is to develop new compounds with antiproliferative effect and to predict their prospective application as anticancer drugs. We synthesised and we tested "*in vitro*" three platin complexes and three palladium complexes with organometal ligands with the intention to characterize their antimitotic potential. Tertiary arsine complexes of platinum and other metals were recently reported (Moldovan, 2008; Phadnis 2005; Kwong, 2005) but there is no information regarding their biological activity. Among phenylarsines, information on biological activity was reported for phenylarsine oxide and dichlorophenylarsine (Frost, 1990; Geran, 1972; Takahashi, 2003; Begun, 1994; Verspohl, 2006), and the phenylarsine compounds attached to biomolecules like peptides and enzymes were studied (Schmidt, 2007; Wang, 2007). Phenylarsine oxide derivates are well known as anti-trypanosome agents and they are used as drugs (Mottram, 1985; Fairlamb, 1989; Berger, 1994). We can achieve both aims using the static cytometry. The detection of fluorescence changes due to this phenomenon is possible with the CellScan system. The

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp.141-146, Publishing House "Eurobit" Timişoara, 2008

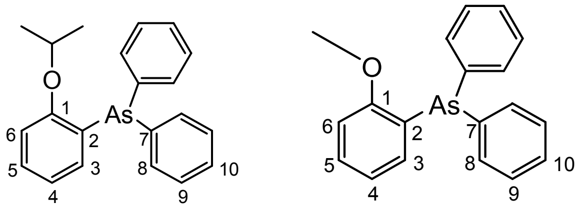
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

specificity and the sensitivity of the test made by CellScan are greater than those with other methods. Simplicity, rapidity and non-invasive character of these methods make the system to be a new perspective in clinical studies and also in the basic research areas.

MATERIALS AND METHODS

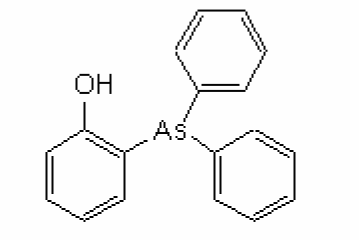
We analyzed the effect of three standard antineoplastic drugs: cisplatin, carboplatin and oxaliplatin on primary cell lines obtained from the biopsies of cancer patients. These drugs are used in the therapeutic scheme of several localizations, among which we select ovary and digestive tumors.

Also we synthesised a new group of platinum and palladium complexes with general formula $[MX_2L_2]$ where M= Pt, Pd; X = I, Cl; L = diphenylarsine ligand, in order to determine their potential biological activity. These complexes have organometallic ligands with structures which lead us to presume that they exhibit biological effects: $2\text{-Pr}^i\text{OC}_6\text{H}_4\text{AsPh}_2$ (**L1**), $2\text{-MeOC}_6\text{H}_4\text{AsPh}_2$ (**L7**) and $2\text{-HOC}_6\text{H}_4\text{AsPh}_2$ (**L11**) [Fig.1 and 2]. Each structure contain two identical tertiary arsine groups bonded to platin, respectively to palladium, the only difference between these groups being one moiety, which is isopropyl for compound L1, methyl for L7 and hydroxyl for compound L11, molecular structures of the six complexes are analogous.



(2-isopropoxyphenyl)diphenylarsine **(2-methoxyphenyl)diphenylarsine**

Fig .1. Structure of ligands L1 and L7



(2-hydroxyphenyl)diphenylarsine

Fig. 2. Structure of ligand L11

We performed cell cultures using MLS tumoral ovary cell line and Hfl-1 normal human lung fibroblast cell line. Primary cell cultures were performed using ovary tumor biopsies obtained with the patient informed consent. To obtain the primary cell suspensions, we performed mechanic-enzymatic disaggregating procedures using the Medimachine equipment. Cell cultures were performed in class II laminar hoods, cells were kept in sterile conditions in incubators with stable temperature (37°C), humidity and CO_2 concentration (5%). For the experiments, tumoral and normal cells were placed on 6-well and 96-well plates and they were treated with the novel synthesized compounds, and with the metal-based drugs, respectively. Measurements were made after 24 hours of incubation with the platinum and palladium complexes.

Cell line Hfl-1 was acquired by Sigma Aldrich Corporation from European Collection of Cell Cultures; MLS ovary cell line was a donation of Dr. Yael S. Schiffenbauer from MedisEl Company, Lod, Israel.

Cell cultures were perform using Dulbecco's Modified Eagles Medium, RPMI 1640 respectively F-12 cell growth media from Sigma Aldrich Corporation. Phosphate Buffered Salt Solution, Trypsine EDTA, MTT salt, Ethidium bromide, Low Melting Point Agarose and Type I Agarose, FDA and Rh 123 fluorescent dyes were provided also by Sigma Aldrich. NaCl p.a, NaOH p.a., EDTA·2 HCl p.a.was obtained from Merck&Co. Inc., and Trizma HCl from Fluka Analytics. Supplier of all disposables used for the biological experiments was Nalgene Nunc International. Chemotherapeutical drugs carboplatin (Carboplatin) and oxaliplatin (Eloxantine) were procured from Sindan Pharma.

Instrumentation:

For this purpose we used the CellScan system, which permit detection of fluorescence intensity (FI) and polarization (FP) of different cell types. There is a strong correlation between inhibition of cell growth induced by different chemotherapeutic agents and the resultant changes in FI and FP. Thus, changes can be revealed by staining treated cells with fluorescent dyes such Fluorescein diacetate (FDA) and Rhodamine 123 (Rh123). Hyperpolarization of the fluorescence intensity detected by the CellScan system can identify apoptotic cells within a complex cell population; it is a marker of early apoptotic processes.

The CellScan apparatus is a laser scanning, static, multiparameter cytometer (Harel, 2005), measuring optical parameters: fluorescence intensity (FI) and polarization fluorescence (FP) of individual, fluorescent-labelled cells. By using this system in cancer research, many diagnosis and chemosensitivity tests became available. The simplicity of this method makes the system to be a new perspective in clinical studies and also in the basic research area.

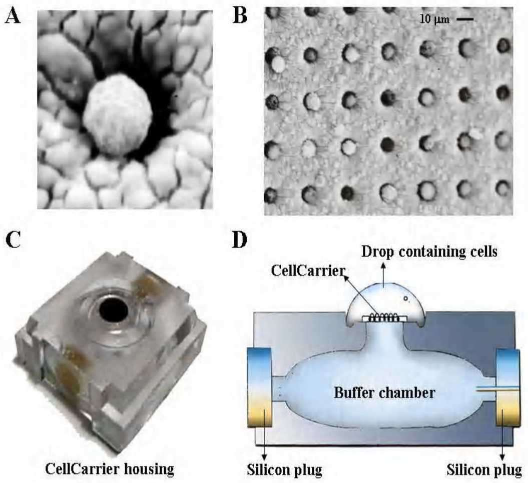


Fig. 3. The cell carrier: A. Tumor cell trapped in the hole of the cell carrier. B. Arrangement of apertures on the cell carrier surface. C. The cell carrier is placed on a plastic grid. D. The carrier can be loaded using pressure gradient. Image kindly provided by Medis El Technologies.

The CellScan system enables repetitive spectroscopic measurements in intact living cells within a population under physiological conditions. It incorporates a unique "cell carrier" [Fig.3] which allows repeated high-precision measurements by fixing the location of the cell in the cell carrier. The "core" of the carrier is a 2 mm squared grid of precisely dimensioned conic apertures : 5-8 µm in diameter, spaced at 20 µm , which can hold 10000 cells, each loaded and maintained in a separate trap. Using the cell carrier, the system can define and retain the locations of individual cells and the resulting measurements (Deutsch, 1994)

The most widespread equipments which quantify optical properties of cells are spectrophotometers, flow cytometers, and confocal laser scanning microscopes. It is possible to make an analogy with the well-known flow-cytometer; both are able to study the functions of the fluorochrome-labelled cells. The differences are that the CellScan have a static configuration, provide the results for each individual cell, not only for the whole population, fewer cells are enough to make a determination, and the measurement is repeatable in the same cells (Deutsch, 1996; Rahmani, 1996, Zurgil, 2002).

This device includes an advanced optical system. The individual trapped cells are illuminated by a He-Cd laser beam source which scans each hole and displays the spread of intensity (FI) on the x-axis and polarization (FP) on the y-axis on the screen. The measurements are made using a preset photon counting technique, in which the same number of photons is counted for each cell, regardless emission's intensity; so that the measurement has the same precision both for the weakly or strongly emitting cells.

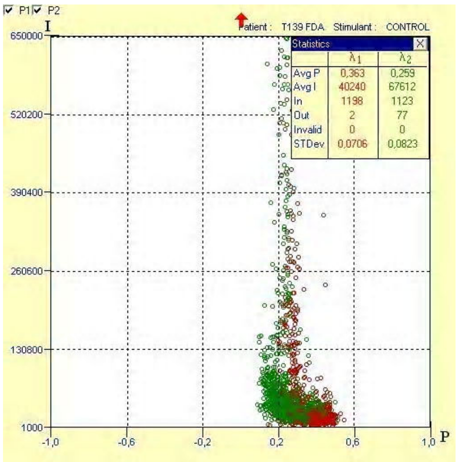
RESULTS AND DISCUSSIONS

We performed static cytometry measurements on primary tumor cultures treated with platin-based drugs, and both on secondary tumor and normal cell cultures, treated with the novel synthesized compounds, but also with the chemotherapeutic drugs, to compare their antiproliferative effect with the standard drugs.

The fluorescence polarization (FP) indicates the entropy, the relative rotational freedom of the fluorescent molecules. The FP of a fluorochrome increases with viscosity or with the restrictions of molecular rotation. Cell activation and apoptosis involve conformational changes of cytosolic enzymes and proteins, the intracellular matrix reorganization. The more the molecule rotational movement is restricted, the higher is the FP value, and vice versa. The fluorescence intensity (FI) of the fluorochrome reflects intracellular content.

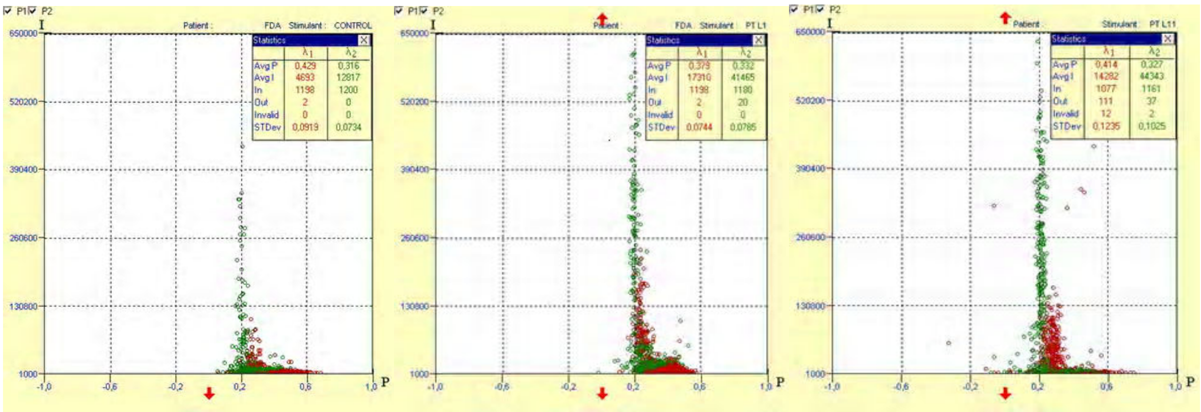
The dyes used are Fluorescein diacetate (FDA) and Rhodamine 123 (Rh123). Fluorescein diacetate (FDA) is a non-fluorescent dye, which is converted through enzymatic hydrolysis in its fluorescent form only in the living cells. It is an indicator of cell viability. Rh 123 is a lipophilic cationic fluorochrome that selectively accumulates in the mitochondria of the living cells and whose specific uptake or release depends upon mitochondrial membrane potential, thus reflecting mitochondrial activity (Zurgil, 1996). A drop in the FI of Rh123 was attributed to early apoptosis (Kaplan, 1997; Fixler, 1997).

The CellScan system provides us histograms [Fig.4] representing the polarization and the intensity of every single tested cell from the cell population of the culture.



	λ1	λ2
Avg P	0,363	0,259
Avg I	40240	67612
In	1198	1123
Out	2	77
Invalid	0	0
STDev	0,0706	0,0823

Fig. 4. Histogram provided by the CellScan: the FP and FI for each cell, measured at two wavelengths it is represented by red and green dots. Here are showed untreated (control) cells.



	λ1	λ2
Avg P	0,429	0,316
Avg I	4693	12817
In	1198	1200
Out	2	0
Invalid	0	0
STDev	0,0919	0,0734

	λ1	λ2
Avg P	0,379	0,332
Avg I	17310	41465
In	1198	1180
Out	2	20
Invalid	0	0
STDev	0,0744	0,0785

	λ1	λ2
Avg P	0,414	0,327
Avg I	14282	44343
In	1077	1161
Out	111	37
Invalid	12	2
STDev	0,1235	0,1025

Fig. 5. Histogram provided by the CellScan: the polarization and the intensity for each cell, measured at two wavelengths are represented by red and green dots. In figure .a untreated (control) MLS cells are shown. The figure b and c represents FI and FP of MLS cells treated with complex PtL1, respectively PtL11.

In clinical oncology drugs are used in concordance with the international chemotherapy protocols for a certain type of tumor (Schiffenbauer, 2002). A recent study showed that FDA fluorescence hyperpolarization is a measure of apoptosis. Alterations of the mitochondrial structure and function are the early events in apoptosis in different cell types (Green, 1998; Lizard, 1995; Zurgil, 2000). These alterations were characterized by distinct changes in the mitochondrial membrane, associated with the dissipation of the membrane potential.

The Cell Scan can detect changes induced by anti-neoplastic drugs in the fluorescence intensity and polarization of fluorescent-labelled tumour cells in order to investigate the effect of anti-neoplastic drugs on cancer cells. T47D and T80 human breast cancer cell lines were exposed to Navelbine and to 5-Fluorouracil and the fluorescence properties of the treated cells, stained with FDA and Rh123, were measured by the CellScan.

A strong correlation was found between the inhibition of cell growth induced by the two drugs and the hyperpolarization and the changes in fluorescence intensity. The T80 human breast cancer cells showed after *in vitro* treatment a significant reduction in FI, directly proportional with the exposure and concentration of anti-neoplastic drug, while T47D cells, known as 5Fu-resistant cells, does not show a significant drop in the FI. Changes in FP are also relevant and well correlated with the drug efficacy. An increase in average FP of treated T80 cells (stained either with Rh123 and FDA) versus untreated control was observed simultaneously with the FI decrease when the cells are responding to the drugs.

Another study pointed out the novelty and the advantages of the method which may be used to assist in the prediction of the response to anti-cancer agents (Chaitchik, 2005). This procedure requires only a 48 hours tumoral primary cell culture, and it is independent of proliferation and colony-formation assays, long and difficult. This test has a significantly lower duration as the previous chemosensitivity tests and enables more samples to be tested in a shorter period of time. The test requires low number of cells, which happens frequent in the case of tumour biopsies.

Using this method, it is possible differentiation between necrotic and apoptotic cells. The CellScan test can detect in this manner the pre-lytic changes induced by the drugs and can measure the sensitivity of cancer cells to them.

In our laboratory we performed studies on tumors prevealed from cancer patients (Soritau, 2005) demonstrating that the CellScan technique is adequate for chemosensitivity tests. This gives a possibility to eliminate ineffective drugs of chemotherapeutic protocols and thus to improve the treatment efficacy. Eighty-eight tumors were tested for *in vitro* response to drugs, using the fluorescence intensity and polarization measurements. Tumors were mechanically dissociated, exposed to different drugs for 24 hours, and stained with two different markers of apoptosis: FDA and Rh123. The FI decrease observed simultaneously with FP increase of the stained cells indicates an inhibition in cell proliferation. The patients were treated with standard protocol regimens. For the patients with advanced cancers (stage III and IV), a correlation between measurements using FDA fluorochrome and the clinical result of the treatment was found.

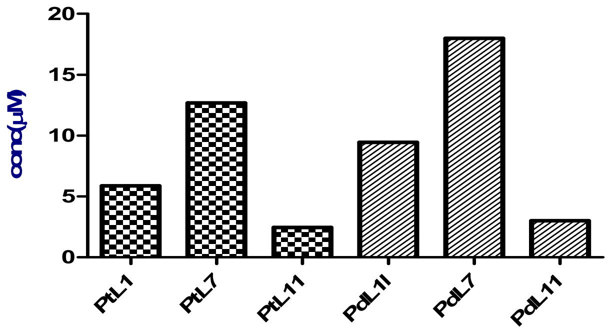


Fig. 6. IC50 values for the MLS tumor cells treated with platinum and palladium complexes

For the novel synthesized compounds we establish the IC50 value, the concentration which inhibits cell proliferation with 50% [Fig. 6], and the correspondent polarization values using the static cytometry. In compare with the untreated, control cells, hyperpolarization of PtL1 treated cells was 5,1%, for the PtL7 treated cells 2,5% and for the PtL11 3,4% (Fischer, 2008). Compounds PtL7 and PdL7 showed a very good potential to induce early apoptosis, their polarization values obtained for the Rh123 dye was 7,8 [Table1], and 8,3%, respectively. These two compounds, containing the L7 ligand present the best potential to induce programmed cell death.

Table 1. Polarization values for tumoral MLS and normal Hfl-1 cells treated with novel synthesized compounds.

Stimulant	FP values [a.u.]	
	MLS cells	Hfl-1 cells
Control	0.362	0,293
Oxaliplatin	0.358	0,310
Carboplatin	0.398	0,345
PtL 1	0.371	0,317
PtL7	0.391	0,305
PtL11	0.352	0,322

To verify the accuracy of the new method, we correlated the results obtained in this way with those determined with customary procedures such as the MTT (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) viability test (Mosmann, 1983) and one of the standard methods for assessing DNA damage, namely, single-cell gel ("comet") assay (Tice, 2000; Collins,2004). Data are correlated with the values obtained using the alternative methods.

CONCLUSIONS

The Cell Scan technology is a valuable tool in cytometry assays, superior in many aspects to the precedent scanners and comparable in efficiency to the well-known flow cytometers. It enables repetitive measurements on the same cells and makes possible the follow-up of a cell population in time. The cell carrier gives the CellScan the capability to investigate the same individual cells after one or several desired manipulations, facilitating the study of cell dynamics and simplifies repetitive examination, even when a small number of cells are available.

The significance of the test lies in its technical simplicity, rapidity, accuracy and the possibility to repeat the measurements in the same cells. The SCM test's aim is to distinguish between patients with neoplastic diseases and healthy controls. Moreover, it can suggest the localization of the cancer completing in this way the other investigations and can precede many clinical and imagistic procedures. The SCM test is a potential tool for screening and early detection of cancer, which in turn may lead to a significant reduction in mortality rates.

The specificity and the sensitivity of the tests made by CellScan were greater than those performed using the spectrofluorimeters. The kinetic information we can obtain is useful in understanding the process of lymphocyte activation and has clinical relevance as well. The results showed the possibility of a different, high specificity method for detection of cancer by a blood test.

The six novel synthesized platinum and palladium compounds are biologically active against tumoral cells; their cytotoxicity is comparable with standard drugs. Measurements using the CellScan technology are well correlated with the results provided by other bioassay methods. The studied compounds, especially PtL7 and PdL7 are suitable to be tested as new drugs.

It was demonstrated the potential of the CellScan system to detect the in vitro effects of the chemotherapy performed on cancer cells. The system can be used to assess drug sensitivity of cancer cells derived from solid human tumours, biopsies in order to predict the in vivo effects of the treatment. The test has the ability to differentiate between drug sensitivity and resistance in cancer cells**.**

Acknowledgements: The present study was founded by CNCSIS Complex Exploratory Research Project “Metallomics”. We appreciate the support of Cell Kinetics Department from MedisEl Company, Lod, Israel.

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FOOD SUPPLEMENTS WITH CHROMIUM AND SELENIUM CONTENT

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ABSTRACT

Food supplements, according to Directive 2002/46/EC means foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities; "nutrients" means the following substances: (i) vitamins, (ii) minerals. This Directive was transposed also by the Romanian sanitary authorities and decided to start monitoring and inspecting food supplements with vitamins and minerals at national level. In this context, the specialists from the Institute of Public Health Timişoara elaborated a methodology which was transmitted to each County Directorate of Public Health and of Bucharestts. The aim of this work was to identify at national level the number of manufacturers, importers, wholesalers and retailer units of food supplements. In the present paper there will be discussed only the food supplements with chromium and selenium content.

Key words: chromium, selenium, food supplements

INTRODUCTION

Mineral insufficiency and trace element insufficiency states are actually more likely to occur than are vitamin insufficiency. Those at increased risk of such insufficiencies include people who eat low-calories diet, the elderly, pregnant women, people taking certain drugs (e.g. diuretics), vegetarians and those living where the soil is deficient in certain minerals. Because of differing geologic conditions minerals and trace elements may scare in the soil of certain region and rich in those of other regions (DiSilvestro, 2001; O`Dell and Sunde, 1997; Garban, 2004).

Any sort of stress deplete the vitamin and mineral stores of organism more rapidly. With age the organs tend to function less efficiently: the metabolisms slows, physical activity diminishes, food intake goes down.

Various diseases reduce also the mineral nutrients intake and especially those that interfere with the ingestion, digestion, absorption and requirement of nutrients: celial disease, Crohn disease, irritable bowel syndrome, lactose intolerance, bacterial, viral ands parasitic infections.

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 147-152, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

Evidence from recent studies reveal that mineral trace element supplementation may help to prevent various forms of cancer, heart disease and some other degenerative processes.

In this context the importance of food supplements intake with mineral content is discussed.

MATERIALS AND METHODS

At national level the Ministry of Health of Roumania adopted a law concerning food supplements which was based on the EU directive. After this directive the health authorities decided to start in the framework of the National Health Programme 1.4 the monitoring and inspecting food supplements at national level. In this context, a methodology was elaborated by the specialists from the Institute of Public Health Timişoara and widespread to 41 County Directorate of Public Health and to the Directorate of Public Health of Bucharest. The task of specialists was to found out the number of manufacturing units, importing units, wholesalers and retailers of food supplements. Thenafter, to identify the composition of food supplements based on vitamins and minerals (Garban, 2006; Garban, 2008).

According to the methodology the specialists sent the obtained data to the Institute of Public Health where they were processed.

RESULTS AND DISCUSSIONS

Data were received from 36 County Directorate of Public Health and the Directorate of Public Health of Bucharest. The total number of identified food supplements was 916 but only 121 were in accordance with the national law (Ordin MSP 1069/2007) referring to food supplements which transpose EU Directive (Garban, 2008).

According to the Directive 46/2002/EC only a limited number of vitamins (13) and minerals (15) are accepted as ingredients in food supplements –Table 2.

Table 2. Vitamins and minerals allowed by law in food supplements

Vitamins	Minerals
Vitamin A (μg RE)	Calcium (mg)
Vitamin D (μg)	Magnesium (mg)
Vitamin E (mg α-TE)	Iron (mg)
Vitamin K (μg)	Copper (μg)
Vitamin B1 (mg)	Iodine (μg)
Vitamin B2 (mg)	Zinc (mg)
Niacin (mg NE)	Manganese (mg)
Pantothenic acid (mg)	Sodium (mg)
Vitamin B6 (mg)	Potassium (mg)
Folic acid (μg)	Selenium (μg)
Vitamin B12 (μg)	Chromium (μg)
Biotin (μg)	Molybdenum (μg)
Vitamin C (mg)	Fluoride (mg)
	Chloride (mg)
	Phosphorus (mg)

Minerals are essential nutrients to life and are needed in small amounts and beside vitamins they constitute the class of micronutrients.

These nutrients participate in a multitude of biochemical and physiological processes necessary for the maintenance of health. For the human organism there are essential about twenty eight elements, e.g. Ca, Mg, Na, K, Zn, Fe, Cu etc.

As to minerals in food supplements one have to mention that there are a lot of data concerning the products with calcium, magnesium, iron, copper, iodine, zinc, manganese etc. content. But, food supplements with chromium, selenium, molybdenum are less known.

Among the identified food supplement there were found 75 containing chromium, without specification of their chemical form – details in Table 2.

Table 2. Food supplements with Cr and Se content on the Romanian market

Specification	**Single component**	**With other minerals and vitamins**	**With minerals vitamins and other substances**	**Total**
Cr containing food suplements	4	23	48	75
Se containing food supplements	1	30	72	103

Chromium is an essential mineral found in very low concentrations in the human body. This mineral is a component of the compound, "glucose tolerance factor" (GTF) in humans. GTF is critical for the maintenance of proper serum levels of blood glucose, or insulin metabolism, and its many diverse actions. GTF plays integral roles in the metabolism of other nutrients as well. Fatty-acids and carbohydrates are metabolized by this compound. GTF is also necessary for the activation of enzymes that aid in the digestion of various food sources, and in cholesterol synthesis, which results from daily food consumption (Scwartz and Mertz, 1959; Mertz, 1993).

Chromium is found in the highest concentrations in those organs that provide for metabolic activity. These include the liver, kidney, spleen and even bone tissue(s). Many medical practitioners label chromium as an "ultra" trace mineral because it is found in such diminutive concentrations throughout the body. The amount of chromium stored in the body is in direct correlation to one's diet. It is estimated that Americans store approximately 6 milligrams of chromium in various metabolic tissues.

Chromium is commercially available in several forms including chromium nicotinate, chromium picolinate, chromium-enriched yeast, and chromium chloride. Chromium is available as part of many multivitamins or alone in tablet and capsule forms. Studies suggest that chromium supplementation may be helpful for the following conditions: diabetes , high cholesterol, obesity, strength training, high blood pressure (Lukaski, 1999; Vincent, 2003).

As to food supplements with selenium content a number of 103 products were identified. Most of them are imported from USA and other EU Member States.

Selenium is a trace mineral that is essential to health maintenance but required only in small amounts (Thomson, 2004). Selenium is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. Free radicals are natural by-products of oxygen metabolism that may contribute to the development of chronic diseases such as cancer and heart disease. Other selenoproteins help regulate thyroid function and play a role in the immune system (Arthur, 1991; Combs and Grey, 1998; McKenzie, 1998; Goldhaber, 2003).

Selenium content of foods can vary according with the soil concentration of selenium. There is evidence that selenium deficiency may contribute to development of a form of heart disease, hypothyroidism, and a weakened immune system. There is also evidence that selenium deficiency does not usually cause illness by itself. Rather, it can make the body more susceptible to illnesses caused by other nutritional, biochemical or infectious stresses.

Three specific diseases have been associated with selenium deficiency: Keshan disease, which results in an enlarged heart and poor heart function, occurs in selenium deficient children; Kashin-Beck disease, which results in osteoarthropathy; Myxedematous endemic cretinism, which results in mental retardation

Studies indicate that death from cancer, including lung, colorectal, and prostate cancers, is lower among people with higher blood levels or intake of selenium. Some population surveys have suggested an association between lower antioxidant intake and a greater incidence of heart disease. Selenium is one of a group of antioxidants that may help limit the oxidation of LDL cholesterol and thereby help to prevent coronary artery disease.

Recently were established the new values of the Recommended Daily Allowances for vitamins and minerals (Table 3).

Table 3. Recommended Daily Allowances (RDAs) for minerals (established in October 2008)

Minerals	RDA
Calcium (mg)	800
Magnesium (mg)	375
Iron (mg)	14
Copper (µg)	1
Iodine (µg)	150
Zinc (mg)	10
Manganese (mg)	2
Sodium (mg)	-
Potassium (mg)	2000
Selenium (µg)	55
Chromium (µg)	40
Molybdenum (µg)	50
Fluoride (mg)	3.5
Chloride (mg)	800
Phosphorus (mg)	700

Today, minerals are used to correct subtle nutritional deficiencies in relatively healthy people; to compensate for increased mineral needs caused by health problems; to enhance exercise training etc.

CONCLUSIONS

1. Food supplements marked at national level are mostly with complex composition, containing beside vitamins and minerals herbal extracts, proteins, probiotics etc.
2. Among the 916 identified food supplements a great number contained chromium (75 products) and selenium (103 products).
3. Most of food supplements had complex composition, meaning that in their composition contains not only vitamins and minerals but also other substances.

Acknowledgements. The author want to express her gratitute to all specialists from the Counties Directorate of Public Health from Roumania who participated and send data for the National Synthesis - Monitoring and Inspecting Food Supplements in Romania 2007 in the framework of the National Health Programme 1.4. This work was financially supported by the Roumanian Ministry of Public Health.

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Dedicated to the memory of
Professor Petru Dragan (1932-2007)

ADDUCTS OF DEOXYRIBONUCLEIC ACID WITH METALS „AD LIMINA" OF INTERDISCIPLINARITY METALLOMICS AND PROTEOMICS

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ABSTRACT

Biogenesis of adducts between deoxyribonucleic acid and micromolecules presents a special interest because these are studied both in biochemistry and xenobiochemistry within a dual framework: biochemistry / patobiochemistry, physiology / physiopathology, morphology / morphopathology. If we circumscribe the problem of adducts between DNA and metal ions (M^{n+}) to the domains of major interest for life sciences, we can state that, in facto, there are approached problems of metabollomics and proteomics at their very frontier.

In order to have a global image are discussed aspects related to adducts' biogenesis in biochemistry and xenobiochemistry as well as, in a succint manner, peculiarities of metallomics and proteomics regarded as far-reaching domains that address the relation chemical structure – biological activity, evidently with implications in biochemical homeostasis, heterostasis, homeorhesis as mechanisms of the regulation and control systems in the biologic processes developed in vivo and even in vitro. Some of these aspects present interest for modern biotechnologies, too, often applied on DNA molecules.

Key words: DNA adducts, metallomics, proteomics

1. BIOGENESIS OF DNA ADDUCTS IN BIOCHEMISTRY AND XENOBIOCHEMISTRY

The term "DNA adduct" is generally used to describe a covalent binding of a chemical compound to the macromolecule of deoxyribonucleic acid. The chemical compound can be a biological substance (e.g.: biometals) but also a xenobiotic derivative (e.g. food additives with metals, chemotherapeutic drugs etc.) .

Investigations on the compounds resulted from the interaction of the DNA macromolecule with small molecules were made for the first time in the sixth decade of the 20th century, and the results lead to the idea of appearance of some bioincompatible structures that were named for the first time "molecular complexes".

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 153-162, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

It was also used the term “molecular associations” (Bergmann, 1968) but both denominations were improper with the characteristics of the studied chemical structures.

After years of physico-chemical investigations and mecanic-quantum calculations on the electronic density in the donor-acceptor relationship between DNA and small molecules, the term “adducts” was preferred (Grunberger and Weinstein, 1979; Stich, 1988). Therefore this denomination will be used also in this paper to describe “molecular complex” resulted from the covalent binding of a chemical substance to the DNA macromolecule.

The biogenesis of DNA adducts is important for the domain of Biochemistry and Xenobiochemistry. Biometallic ions like Mg and Zn have an important role in the stabilization of the DNA macromolecule. The negatively charged phosphate backbone of DNA (with polyanionic characteristics) prevents the formation of the duplex without the positively charge metallic ions that have the ability to neutralize the charge of the phosphate bases. The metallic ions that have this ability are usually bivalent ions and they attach to two consecutive phosphate bases on the same strand. These interactions are specific for the domain of biochemistry.

Foreign chemical substances, also called xenobiotics, or their metabolites, may react with DNA macromolecules, too. The resulting DNA-adducts can disrupt the biologic activity of DNA, leading to the perturbation of replication, transcription and even DNA-repair process. Xenobiotics that can lead to DNA adducts, and therefore have carcinogenic potential, are divided in two categories: a) indirect-acting carcinogens – xenobiotics that require metabolic activation prior to DNA adduct formation and b) direct-acting carcinogens – xenobiotics that can damage DNA directly, without needing a metabolic activation in order to generate adducts. These interactions with the DNA macromolecule make one of the object of xenobiochemistry.

2. METALLOMICS AND PROTEOMICS – INTERACTIVE ASPECTS

2.1. Metallomics

The term metallome has been introduced by R.J.P. Williams (2001) from Oxford University and afterwards by Hiroki Haraguchi (2004) from Nagoya University by analogy with proteome, as distribution of free metal ions in every cellular compartments. It refers to the complete complement of metals and metal moieties in a biological cell, tissue, or system. This definition is similar to that of the genome (gene), proteome (proteins), and metabolome (metabolites).

Metallomics can be defined as “the study of metals and metal species as well as of their interactions, transformations and functions in biological systems” (Koppendaal and Hieftje, 2007).

In a larger context, metallomics is defined as “comprehensive analysis of the entirety of metal and metalloid species within a cell or tissue type” (Szpunar, 2005).

In this acception “metallomics” can be considered as a branch of “metabollomics” although metals are not typically metabolites.

While traditional bioinorganic chemistry has focused on the role and interactions of a single (or few) metals in a protein or enzyme system, metallomics studies global, multi-element interactions and relationships.

Metallomics gained importance as an emerging science because about 30% of proteins contain metals, such as metalloproteins and metalloenzymes. There also are numerous metabolites originating from carbohydrates and lipids that are bond to metals.

Metallomics is distinct from other notions (such as speciation) because it has a global role, dealing with the investigation of metals and metalloids (non-metallic elements) from nature, predilectly from biological systems.

It is necessary to mention that metallomics is a new domain, which could develop due to the progresses made in the field of atomic absorption spectroscopy

2.2. Proteomics

The proteome is the entire complement of proteins expressed by a genome, cell, tissue or organism. More specifically, it is the set expressing proteins at a given time under defined conditions. Taking into account the concept of proteome, the term proteomics can be defined as the study of the set of proteins produced by an organism, tissue or cell, with the aim of understanding the behaviour of these proteins under varying environments and conditions. In proteomics are studied, on a large scale, structural and functional peculiarities of the proteins. Generally, in life sciences, is considered that proteomics is the next step in the investigation of biologic systems, after genomics.

It is estimated that proteomics is more complex as compared to genomics because a proteome differs from cell to cell and changes constantly by the biochemical interactions with the genome and the environment. The protein expression differs in function of life cycle (Belhajjame et al., 2005).

At the beginning of the third millenium the evolution of knowledge about genome led to the intensification of researches of molecular biology and genetics that imposed the new term *„genomics”*.

2.3. Ad limina metallomics – proteomics

In the domaine of life sciences the problem of interdisciplinarity at the frontiers (ad limina) of metallomics and proteomics, can be registered and explained numerous aspects with interest for biochemistry, patobiochemistry, citostatic chemotherapy (as elective domaines discussed in this paper).

The biogenesis of compounds that can be studied by metallomics and proteomics is in fact of interest for numerous domaines such as: nutrition, pharmacology, enzymology, biotechnology a.o.

Genomics - is a distinct field of life sciences studying the entire genome of an organism. It also includes studies of intragenomic phenomena and other interactions between loci and alleles within the genome.

The genomics is included in the more complex problem of *proteomics.*

Genomics and proteomics are considered fundamentally important scientific fields, because genes (DNAs and RNAs) contain the genetic information codes to synthesize various proteins. However, genes and proteins cannot be synthesized without the assistance of metalloenzymes containing zinc and other metals. The term metallomics therefore was suggested to be considered at the same level of scientific significance as genomics and proteomics (Haraguchi and Matsuura, 2003).

Currently in the domaine of *genomics* one has in view (as in proteomics) the duality structure-function, knowing that: a) *structural genomics* - is the branch of genomics that determines the three-dimensional structures of proteins; b) *functional* *genomics* - is the branch of genomics that determines the biological function of the genes and their products.

In a larger frame is remarkable the observation regarding *genetics vs. genomics*. The two terms are sometimes used interchangeably. However, *genetics* usually refers to the study of single genes, while *genomics* refers to the study of all the genes in a person or organism.

The goal of *genomics* is to promote the understanding of the structure, function, and evolution of genomes in all kingdoms of life and the application of genome sciences and technologies to challenging problems in biology and medicine.

In order to function properly an organism must regulate transcription, translation, assimilation and incorporation of the necessary biometals. Therefore the biochemistry of the cell must be characterised not only by its genome or its proteins content (proteome) but also by the distribution of the metals and metalloids among the different species and cell compartements (metallome). The term that would therefore refer to the totality of individual metal species in a cell including the inorganic (free and complexed) element content and the assembly of metallic complexes with biomolecules (especially with proteins) is metalloproteome (Szpunar, 2004).

Most of the biological substances (nutrients) as well as metal ions are transported inside the cell through the membrane. In general, the biotransformation of these substances is occurring inside the cell and also often in the cell membrane. The processes by which the nutrients are transported in and out of the cell and converted in biological active forms are called metabolism. Biological substances, which are usually small molecules such as amino acids, organic acids and metal ions produced in metabolism, have been called metabollomes or metabolites and are the subject of the field called metabollomics (Lindon et al., 2003).

3. ADDUCTS OF DNA MACROMOLECULE WITH METALLIC IONS

3.1. Characteristics of DNA adducts in biochemistry and molecular biology

Metal ions (M^{n+}) as chemical agents, determine specific interactions based on ionization processes occurring at different levels of the DNA macromolecule followed by the metal ions coordination. In the double-stranded and even simple-stranded structure of DNA appear changes caused by the formation of adducts (DNA-M^{n+}).

The action of metals on biologic systems is realised during certain processes involved in chronobiochemical mechanisms. Implicitely, the reaction between DNA and metals is submitted to cronobiochemical mechanisms, fact that can be explained by the modifications of hepatic DNA synthesis conditioned by the factor time (Garban, 1984).

The interaction of DNA with metal ions is studied by various physico-chemical methods including nuclear magnetic resonance, optical rotatory dispersion, spectroscopy (UV, VIS, IR, Raman), circular dichroism.

Bindings of divalent ions depend both on the structure and configuration of the macromolecule, as well as the electronic configuration of M^{2+} .The distinctive electron provides special peculiarities to the interaction causing changes of the steric parameters followed by biological consequences (fig. 1).

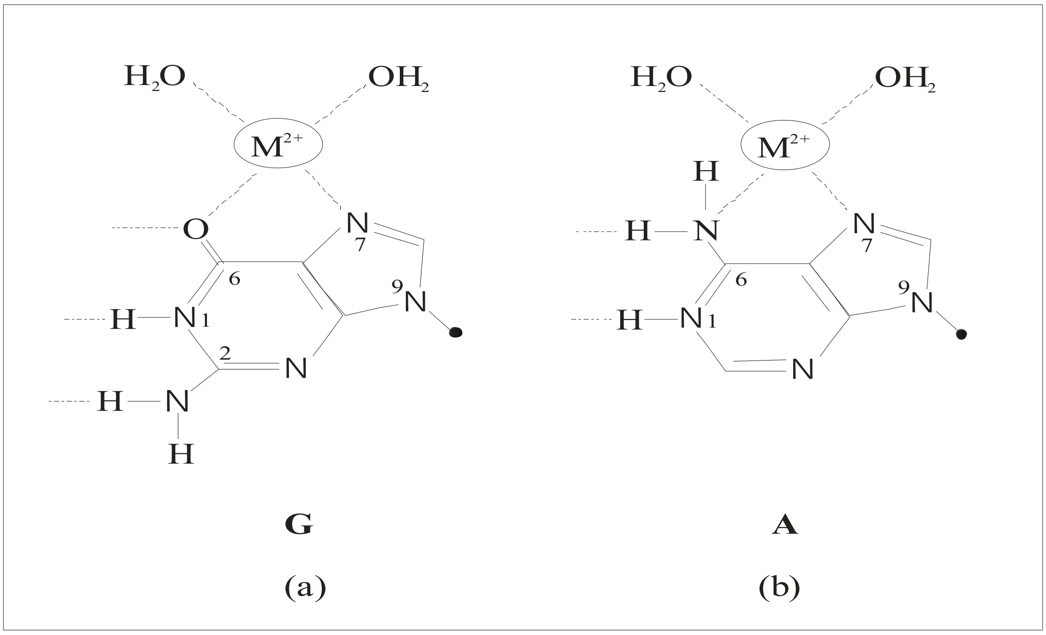

Fig. 1. Binding of M^{2+} to DNA nucleobases: a) M^{2+}-guanine; b) M^{2+}-adenine

The electron donor groups of DNA determine the binding at the level of nitrogenous nucleobases. Guanine is often affected as it has the N_7 position, being the center with the highest density of electrons

Alkaline-earth ions bind electrostatically at the phosphodiester groups of DNA. Experimentally have shown that Mg^{2+} binds more strongly to native DNA than to denaturated DNA (fig. 2)

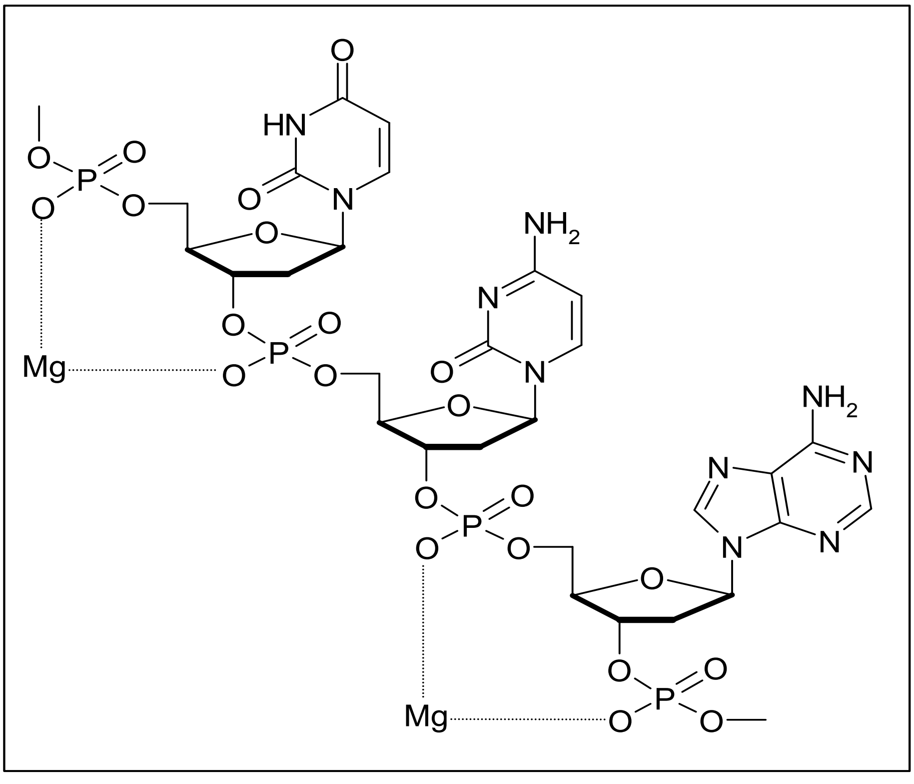

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Fig. 2. Binding of Mg^{2+} to phosphate groups from DNA macromolecule

The electrostatic nature of the binding is explained by the existence of a diffuse electrostatic change effect. Binding can take place at the phosphate groups from the DNA macromolecule which has the character of macromolecular polyanion.

Transition metals form covalent bindings with the nitrogen atoms present in the nucleobases. The destabilization of the double helix depends on the molar ratio M^{2+}/DNA-P, the G-C content and the ionic force of the environment.

The effect of biometals studied in vitro and in vivo, e.g. Zn^{2+}, Cu^{2+} can reveal, at certain concentrations (in excess), modifications of the DNA structure that can precede the biochemical injury (Garban et al., 1994).

A number of studies have shown the destabilizing effect on nucleic acids due to the interaction of divalent cations with π-systems of nucleobases (Garban, 2005).

The location of actions between phosphodiesteric groups is carried out by chelation of the phosphorus group with N_7 of purine nucleobase of GMP.

Intercalation between complementary intrastrand nucleobases shows an intrahelical disposition of M^{2+}. There are also remarked “sandwich”-type bindings at the level of two nucleobases placed on the same strand (Garban, 2004).

Interaction of metal ions with biologic systems and mostly the interaction of nucleic acids with metal ions (and small molecules) led to numerous studies that followed the mechanism of action and the relation structure-activity (Sigel and Sigel, 1996).

A special mention refers to the fact that there are adducts of DNA-M^{n+} type used in technical domaine. Such adducts have been obtained with Zn^{2+} ion. It has to be mentioned that recent studies revealed the semiconductor properties of the DNA-M^{2+} structures or the possibility to use M-DNA as a biosensor to predict diagnosis (Lee, 1993)

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3.2. Characteristics of DNA adducts in pathobiochemistry

Generally, the interactionsproduced between the DNA macromolecule and metal ions can be of interest both for biochemistry and patobiochemistry, being conditioned by the metal ion that is present in the cell and often facilitated by an environment where effects took place on the double helix leading to molecular chain clivage that can be caused by physical factors (e.g. radiations) or even biological ones (e.g. oncoviruses). In such cases, having in view the primar denaturation, the interaction with DNA becomes a biochemical injury (Garban, 2004).

Studies of the relation chemical structure-biological activity revealed also the consequences of interaction and the mechanisms of adduct biogenesis in patobiochemistry. Investigations have been extended over the biomedical, pharmacological aspects, their genotoxicity and also involvement in chemical homeostasis.

The interaction of DNA with M^{n+} ions without toxic potential (e.g. Hg^{2+}, Cd^{2+}) produces modifications in structure-activity relationship with undesirable biological effects. Consecutive effects lead to the appearance of chromosomal modifications as well a mutagenic, oncogenic and teratogenic effects (fig. 3).

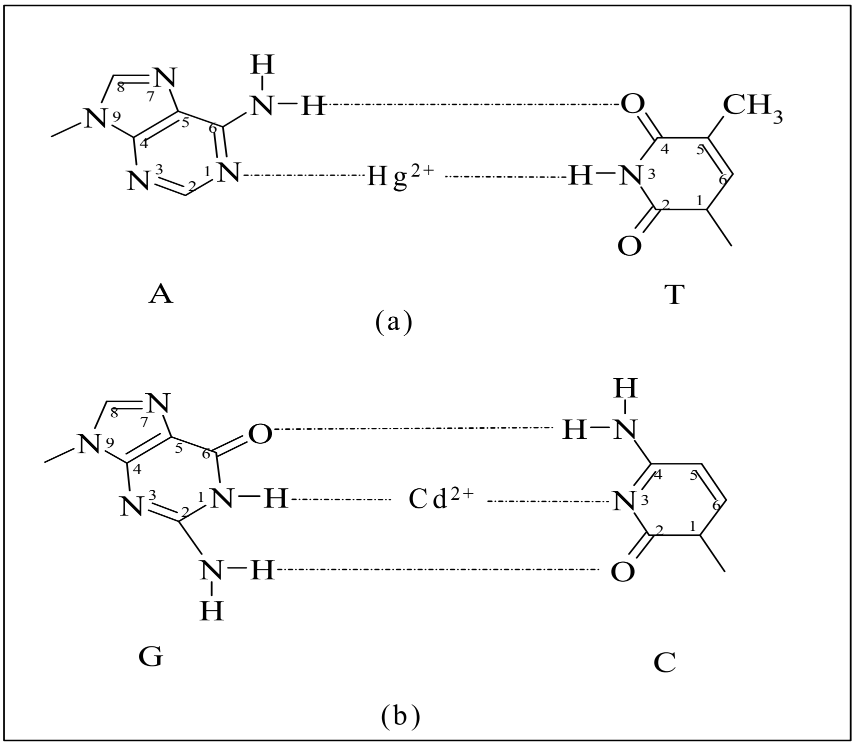

Fig. 3. Internal chelatization of DNA nucleobases with metallic ions (a) Hg^{2+} - A-T nucleobases pair; (b) Cd^{2+} - G-C nucleobases pair

Binding of metals with toxicogen potential (e.g. Hg^{2+}) can be made too, at the guaninic rests from the nucleosidmonophosphates present in pairs of nucleobases disposed on neighbouring landings in the double helix (fig. 4).

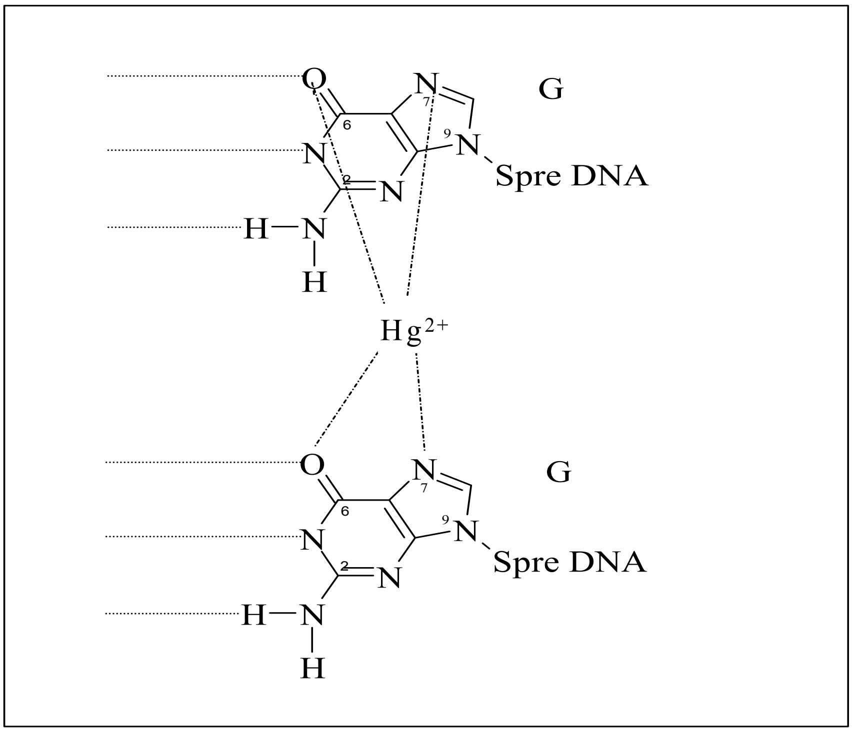

Fig. 4. The binding of Hg^{2+} ions to guanine adjacent nucleobases

Carcinogenic metals such as Cd, Cr, Ni inhibit DNA repair damaged by direct genotoxic agents.

3.3. Characteristics of DNA adducts in chemotherapy

It is known that metals interact with biologic systems representing compounds at the origin of some mutagenic and cancerigenic processes (Beyersmann, 1994). In this situations are the ions Hg^{2+}, Zn^{2+} etc.

The interaction between cytostatic chemotherapics and biologic tissues is extremely complex and covers big domaines of the chemistry of organometallic compounds (Haiduc and Silvestru, 1989, 1990). There can be mentioned the numerous aspects in connexion with the role of DNA and of the interaction with cytostatic chemotherapics among which cis-platinum plays an esential role (Lippert, 1999; Garban et al., 2000).

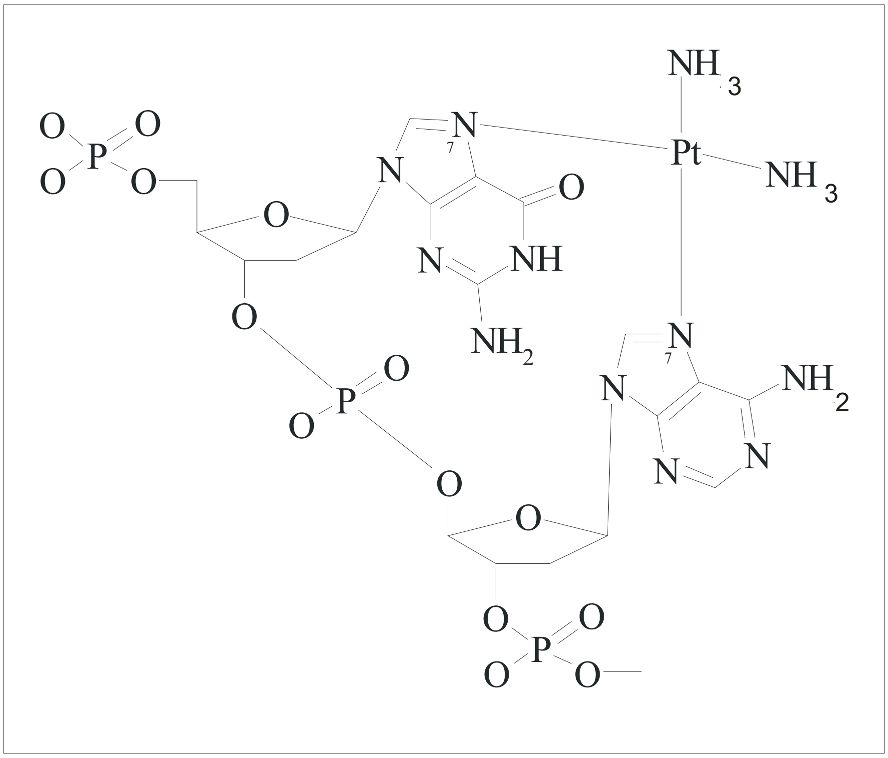

Fig. 5. Structural formula of cisplatin-dApdG adduct

There are chimiotherapic drugs whose action is based on formation of adduct with DNA macromolecule or with its precursors. There are exemplified cyclophosphamide, cis-platinum.

In case of cisplatin (cDDP) binding, the interaction can take place at one chain or at both chains.

Such an example is given in fig. 5 where the binding of cis-platinum is made at two nucleobases on neighbouring landings. The nucleobases are on the same chain. Figure 6 presents the formation of adducts on one chain - the so called monodentate binding or on both chains – the so called bidentate binding.

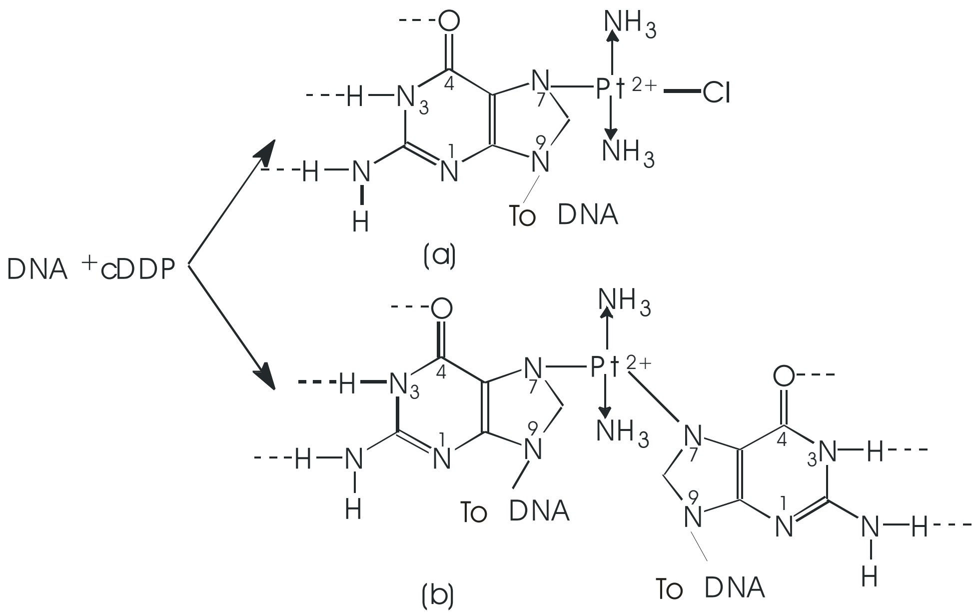

Fig. 6. Biogenesis of adducts (a) monodentate binding of cDDP to the guanine of DNA ; (b) bidentate binding of cDDP to the guanine of DNA

Interaction of DNA with cis-platinum is also afected by chronobiochemical modifications that take place in the body. It was observed the existence of homeostatic modifications in case of the interaction of cis-platinum at the level of the liver (Garban, 1989). Generally, in chimiotherapics, the problem of adducts formation and that of the cytostatic effects are approached in a larger context in correlation with the cell cycle, more exactly in citokinetics (kinetics of the cell cycle).

CONCLUSIVE ASPECTS

Adducts' biogenesis presents a particular interest in case of their study in circumscribed domains such as: biochemistry and molecular biology, patobiochemistry, chemotherapy.

The manner in which the thematic approach on adducts focused on metallomics and proteomics has been shortly presented, can offer a more larger framework both to investigations and interpretations that can follow the more thorough defining of the mechanisms of action during the building phase of these compounds.

It is also important to remark that some information obtained in vitro could lead to predictability for in vivo effects. Also can afford a monitorization, by non-invasive means, of certain processes in vivo (e.g. testing of chemotherapeutic drugs, prediction regarding the possibility of appearance of dyshomeostasic effects.

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HEPATIC METALLOGRAMS IN RABBITS AFTER SODIUM NITRATE EXCESS IN DRINKING WATER

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ABSTRACT

Sodium nitrate was administrated in the tap water of rabbits in order to determine the potential effects on hepatic metallograms. The hepatic metallograms were obtained using the atomic absorption spectrophotometry technique and were used to evaluate the effects of nitrates on the biochemical homeostasis, regarding especially the variations of some macro- and trace elements in liver. Sodium nitrate was administered in high concentrations compared with the reference value equal to the maximum contaminant level (MCL) in drinking water set by Environment Protection Agency. The investigation followed the effects of sodium nitrate - $NaNO_3$ administered on two groups of rabbits in concentrations of 20 x MCL and 40 x MCL. The analytical determinations underline the effects of nitrates on biochemical homeostasis of macro elements (Na, K, Ca, Mg) and trace elements (Fe, Zn, Cu, Mn, Ni) by determination of their concentrations in the liver of rabbits.

Key words: sodium nitrate, liver, metallograms

INTRODUCTION

Nitrates are natural components of the soil derived from the demineralization of the organic matter which play an essential role in the process of plants development. The extensive agriculture which utilizes at large scale fertilizers based on nitrates and the industrial pollution often leads to the accumulation of high levels nitrates in the environment (Prakasa and Puttanna, 2002). From the environment nitrates accedes in plants and finally in the organism of animals and humans where they exert toxicogenic effects by generating nitrites with methemoglobinizing effects (Speijers et al, 1989). Also if certain conditions are met (i.e.: the presence of nitrites and amines) nitrates consumption may lead to carcinogenic nitrosamines (Vermeer et al., 1998; Rostkowska et al., 1998; Gârban, 2007). In experiments on rats with dietary sodium nitrate administration (using pellets) liver tumors were induced (Aoyagi et al, 1980).

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 163-168, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

Nitrate administration is known to cause disturbances of kidney and liver function and also to alter the biochemical parameters of blood serum (urea, cretinine, uric acid), but the major manifestation of their toxic effects remains the generation of nitrites which react with hemoglobin and cause methemoglobinemia (Zabulyte et al., 2007; Ghibu et al, 2008a). Another aspect which is often neglected in the scientific literature is the fact that nitrates, being salts of nitric acid, also have in their chemical structure metallic ions. These ions are released inside the organism and may interact with other bioconstituents and interfere in the absorption and biodisponibility of other biometalic ions (Ghibu et al, 2008b).

Sodium nitrate is one of the most important compound from the class of nitrates due to its utilization at large scale in both agriculture and food industry. In agriculture sodium nitrate is utilized as fertilizer. Though sodium nitrate is used in agriculture, it is more expensive than ammonium-type fertilizers which are therefore preferred. Ammonium nitrate, ammonium sulfate, urea and anhydrous are the most frequently used fertilizer.

In food industry sodium nitrate is used especially due to its property to generate sodium nitrite which maintains the red color of the meat and meat products and also prevents the growth of certain pathogen bacteria (Clostridium botulinum) – Banu et al, 2000.

MATERIALS AND METHODS

Experimental model. Sodium nitrate - $NaNO_3$ was added in the tap water of rabbits. The water was administrated “ad libitum” and the effects of nitrates consumption on the mineral metabolism of rabbits were studied. The reference value chosen in the preparation of nitrates solutions was the maximum contaminant level (MCL) admitted in drinking water, a value established by the Environment Protection Agency (EPA) from United States Department of Agriculture (USDA) to 10 mg/L nitrogen nitrate (usually noted as $N\text{-}NO_3$) and 1 mg/L nitrogen nitrite (usually noted $N\text{-}NO_2$).

In this study rabbits (Oryctolagus cuniculus) with the age of 30 days and the average weight of 700 ± 25 g were used. The rabbits were included in two experimental groups: $E_{A(1)}$ and $E_{A(2)}$ and a control group. All groups were comprised of 10 animals (5 males and 5 females). Animals were fed with VivaBio - a granulated fodder for rabbits produced by Freeman S.R.L (content: 14.94% protein, 2.86% fat and 8.51% cellulose and necessary minerals). Animals from control group received tap water, while animals from $E_{A(1)}$ group received $NaNO_3$ solution with a concentration equivalent with 20 x MCL and $E_{A(2)}$ group received $NaNO_3$ solution with a concentration equivalent with 40 x MCL established for nitrite in drinking water. Nitrates solutions were prepared using tap water from the same source as the control group.

Before the beginning of the experiment, a quarantine period of 10 days was kept during which the health status of rabbits was observed. Also in this period the rabbits were accommodated with the experimental laboratory environment and with the diet made of granulated fodder. After the quarantine, the experiment has started and lasted 20 days.

For the liver sample collection to be made, the subjects were killed according to the procedures recommended by O.G. nr. 37/2002. On this purpose ketamin was administered intravenously. The liver samples were than taken in concordance with the techniques of laboratory animal’s necropsy and stored in 25 ml glass bottles which were placed in a refrigerator until the biochemical investigations were performed.

Biochemical investigations. Tissue was prelevated from each animal from the control and experimental groups. The determinations of macro elements (Na, K, Ca, Mg) and trace elements (Fe, Zn, Cu, Mn, Ni) were performed in the "Laboratory of molecular and atomic spectroscopy" of the Faculty of Food Products Technology from Timişoara.

The liver samples were weighted and then calcinated at a 700 ºC temperature for a 3 hours time. The obtained ash was mineralized with nitric acid (0,5 N) and brought in gradated flasks of 50 ml. The obtained solutions were analyzed using a spectrophotometer with continuous atomic absorption. The model of the spectrophotometer was Analytik Jena ContrAA 300. Analytical data were expressed in μg/g wet weight.

Statistical analysis. Mean values (X) and standard deviation (SD) were determined for each parameter obtained. Also the Student test was performed using the software Origin 6.0.

RESULTS AND DISCUSSIONS

In order to investigate the influence of the concentration of the nitrate solution compared the results from the $E_{A(1)}$ and $E_{A(2)}$ groups. The comparison shows the different effects of $NaNO_3$ at 20xMCL and 40XMCL concentrations. The results were divided in different tables for the maco elements and for trace elements.

The macro elements investigated in this paper were: Na, K, Ca and Mg. The homeostatic modifications produced by sodium nitrate solutions administrations in different concentrations (20xMCL and 40xMCL) are presented in table 1.

Table 1. The quantum of hepatic macro elements in rabbits after administration of $NaNO_3$ solutions in different concentrations

Group	M.U.	n	**Na** $\bar{X} \pm DS$	**K** $\bar{X} \pm DS$	**Ca** $\bar{X} \pm DS$	**Mg** $\bar{X} \pm DS$
C	μg/g	10	887.35 ± 130.99	3018.18 ± 122.86	21.14 ± 1.73	193.36 ± 12.45
$E_{A(1)}$	μg/g	10	913.76 ± 120.42	2785.46 ± 116.58*	22.93 ± 2.54	174.06 ± 12,73*
$\Delta X_{A(1)}$			+26.41	-232,72	+1.79	-19.30
$E_{A(2)}$	μg/g	10	937.72 ± 58.24	2696.07 ± 131.66*	23.74 ± 2,02**	164.08 ± 12.26*
$\Delta X_{A(2)}$			+50.37	-322.11	+2.60	-29.28

*p<0.01; **p<0.05

The resulted analytical data revealed that sodium nitrate affects the hepatic metalograms. An increase of Na and Ca concentration followed by a decrease of K and Mg concentration shows an alteration of the extracellular ions (Na, Ca) an intracellular ions (K, Mg) whit possible implications in the function of "Na-K pumps" and "Ca-Mg pumps" at the membrane level. These modifications are probably a result of the increased sodium intake.

The liver is an organ with a known role in regulating the water and electrolytes balance in the organism, having the capacity of retaining a part of the water and some ions excess. The retention of sodium in the hepatic tissue after an excess was experimentally demonstrated (Terner and Aizman, 1984).

The sodium excess leads to the perturbation of the physiological equilibrium between extra- and intracellular electrolytes. Hepatic dysfunctions like cirrhosis are known for their ability to affect the metabolism of sodium and other electrolytes and the origin of these effects is the onset of sodium retention (Murakami et al, 1996; Yu et al., 2005).

Significant variations were found in the case of K, Mg and in the case of Ca from $E_{A(2)}$ group. The results suggested that between the magnitude of the dyshomeostatic effects and the concentration of sodium solution there is a direct proportionality relation.

The homeostatic modifications produced by nitrates on trace elements are presented in table 2 taking as reference the same substance (sodium nitrate) in two different concentrations: 20xMCL - $E_{A(1)}$ and 40xMCL - $E_{A(2)}$.

Table 2. The quantum of hepatic trace elements in rabbits after administration of $NaNO_3$ solutions in different concentrations

Group	**M.U.**	**n**	**Fe** $\overline{X} \pm DS$	**Zn** $\overline{X} \pm DS$	**Cu** $\overline{X} \pm DS$	**Mn** $\overline{X} \pm DS$	**Ni** $\overline{X} \pm DS$
C	µg/g	10	75.57 ±9.10	24.60 ±4.39	3.77 ±0.85	2.04 ±0.14	0.19 ±0.05
$E_{A(1)}$	µg/g	10	67.66 ±6.56	23.37 ±1.93	3.25 ±0.62	1.91 ±0.21	0.20 ±0.01
$\Delta X_{A(1)}$			- 5.91	- 1.23	- 0.52	- 0.13	+ 0.01
$E_{A(2)}$	µg/g	10	64.79 ±11.40	22.44 ±1.46	2.83 ±0.88**	1.82 ±0.67	0.22 ±0.06
$\Delta X_{A(2)}$			- 8.78	- 2.16	- 0.94	- 0.22	+ 0.03

**$p<0.05$

The analytical results show a decrease in concentration in all the analyzed trace elements except for nickel. Significant variations are found only in the case of copper at the $E_{A(2)}$ group. The hepatic concentrations variations of trace elements are directly proportional with the concentration of the sodium nitrate solution.

From the result shown in table 2 we can see the high concentration of iron which is specific to the liver. The liver is an organ with an important role in iron metabolism. Iron can be stocked in high concentration in liver.

Ferritin is an iron storage protein which can play the role of depositing iron at the hepatic level. After the nitrates solutions were administrated the iron level decreased probably due to the formation nitrites which interact with hemoglobin generating methemoglobin. The decrease of hepatic concentration of iron was experimentally observed after the administration of sodium nitrite to rats (Roth and Smith, 1988).

CONCLUSIONS

1. Macro elements show in the case of sodium nitrate administration at different concentration an increase of Na and Ca (bioelements with extracellular distribution) and a decrease of K and Mg (bioelements with intracellular distribution). The variation of macro elements concentration in liver is directly proportional with the sodium nitrate solution concentration.
2. The studied trace elements concentration in liver decrease after $NaNO_3$ administration, with the exception of Ni which shows an increase in concentration. The trace elements variation is directly proportional with the concentration of $NaNO_3$ solutions.

Acknowledgements: The authors express their thanks to Assoc. Prof. Ana Driha for the support given during experimental laboratory phases.

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SODIUM, POTASSIUM, CALCIUM AND MAGNESIUM CONTENTS – ARE INDICATORS FOR GEOGRAPHYCAL ORIGIN OF BEE'S HONEY FROM BANAT COUNTY?

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ABSTRACT

This scientific paper presents our researches regarding the usage of Na, K, Ca, Mg contents and Principal Component and Classification Analysis for differentiate the honey samples purchased from two different villages from Romania: Berini (Timis District) and Cornea (Caras-Severin District). The Na, K, Ca and Mg contents were determinated by flame atomic absorption spectrometry (F-AAS) with high-resolution continuum source ContrAA 300 spectrometer.

Key words: macro-minerals, honey, flame atomic absorption spectrometry, multivariate analyses

INTRODUCTION

Honey represents a sweet natural substance produced by the bees from flowers nectar or other sweet secretions of plants [Nanda et al., 2003]. After transport and transformation processes the nectar are stored like honey in the comb cells and it is used for bee nutrition (Bura et al., 2005). The nectar processing by bees refers to invert the saccharose in fructose and glucose, and the water content decreased at 18-20% (Özcan et al., 2006).

Honey can be easy assimilating, being a valuable nutritive product for children, athletes and convalescents. It is a good source of antioxidants, macro- and micro-minerals, monosaccharides, B vitamins, and enzymes (Juszczak et al., 2009). It contains potassium in higher quantity than sodium, that's way has a diuretic effect, being indicated in kidney diseases. Other macro-minerals from honey, like magnesium (with energetic roll) and calcium (muscular stimulator) are necessary in carbohydrates metabolism, in rahitism, spasmophily, and the water repartition in human's body (Socaciu, 2003). The sources of the mineral components of honey are its raw materials (nectar, honeydew) and pollen grains (Hernández et al., 2005), not bee organisms, as some researches suggest. Also it is a biological product of the bees, obtained without human interference. Its quality is unaffected by changes

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 169-172, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

which is possible to appear during technological processes. That's way the mineral composition of honey can be used to determine its geographical origin.(Madejczyk and Baralkiewicz, 2008).

The aim of this work was to differentiate the geographical origin of honey samples purchased from two different villages from Romania (Berini and Cornea) using Principal Component and Classification Analysis and the Na, K, Ca and Mg contents.

MATERIALS AND METHODS

The analysed samples were different types of honey from Berini (Timis District) and Cornea (Caras-Severin District). Honey samples (10 g) were dry burning in quartz capsules at 650^{o}C for 4 hours. After complete burning a nitric acid 0.5 N solution was added up to 50 mL. The obtained solutions were used for Na, K, Ca and Mg contents determination by flame atomic absorption spectrometry (F-AAS).

The multi-elements standard solution (1000 mg/L), used to obtain the calibration curves, was purchased from Riedel de Haen (Germany). The nitric acid 65% solution used was of ultra pure grade (Merck, Germany). All solutions were prepared using deionized water. All analyses were made in triplicate and the mean values were reported in mg/kg fresh product (ppm).

The determination of Na, K, Ca and Mg contents were made with ContrAA-300, Analytik-Jena device, by flame atomic absorption spectrometry (FASS) in air/acetylene flame.

Statistical interpretation of obtained data was made using Principal Component and Classification Analysis implemented in Statistica-6 software.

RESULTS AND DISCUSSIONS

The Na, K, Ca and Mg contents in analysed honey samples are presented in Table 1:

Table 1. The Na, K, Ca and Mg contents in analysed honey samples

No.	**Honey samples**	**Na ppm**	**K ppm**	**Ca ppm**	**Mg ppm**
1	Mixed flowers honey Berini 2005	34.4	325.1	12.9	16.3
2	Mixed flowers honey Berini 2006	31.6	323.5	39.3	25.7
3	Acacia honey Berini 2005	64.0	485.4	17.5	34.7
4	Acacia honey Berini 2006	60.8	930.9	22.7	35.9
5	Acacia honey Cornea 2006	52.5	**1251.9**	148.4	130.1
6	Acacia honey Cornea 2007	58.6	1246.4	147.5	130.6
7	Lime honey Cornea 2007	**82.7**	1241.9	**226.4**	**132.6**

The major macro-mineral in analysed honey samples was potassium (325.1 - 1251.9 ppm), followed by calcium (12.9 – 226.4 ppm), magnesium (16.3 – 132.6ppm) and sodium (31.6 – 82.7 ppm).

Between the samples from Berini the acacia honey has higher content in sodium, potassium and magnesium than mixed flowers honey (64.0 ppm, 930.9 ppm, respectively 35.9 ppm). Mixed flowers honey from Berini (2006) has the highest calcium content (39.3 ppm).

Lime honey from Cornea (2007) has the highest sodium (82.7 ppm), calcium (226.4 ppm) and magnesium (132.6 ppm) contents among all analysed samples. The highest potassium content was determinated for acacia honey from Cornea (2006).

In Figure 1 was represented the distribution of analysed honey samples depending on the Na, K, Ca and Mg contents. It is possible to observe that sodium, potassium, calcium and magnesium can be used to discriminate between the honey samples from Berini than those from Cornea. The first group is formed by acacia honey (2006 and 2007) and lime honey from Cornea; witch has the highest contents in potassium, calcium and magnesium between all analysed samples. In the first group it is possible to observe that sodium content can differentiate the lime honey, which has the highest content (82.7 ppm) than acacia honey samples from the same village (Berini). The second group includes the mixed flowers honey (2005 and 2006) and acacia honey (2005 and 2006) from Berini, which have smaller concentrations in potassium, calcium and magnesium than the honey samples from Cornea.

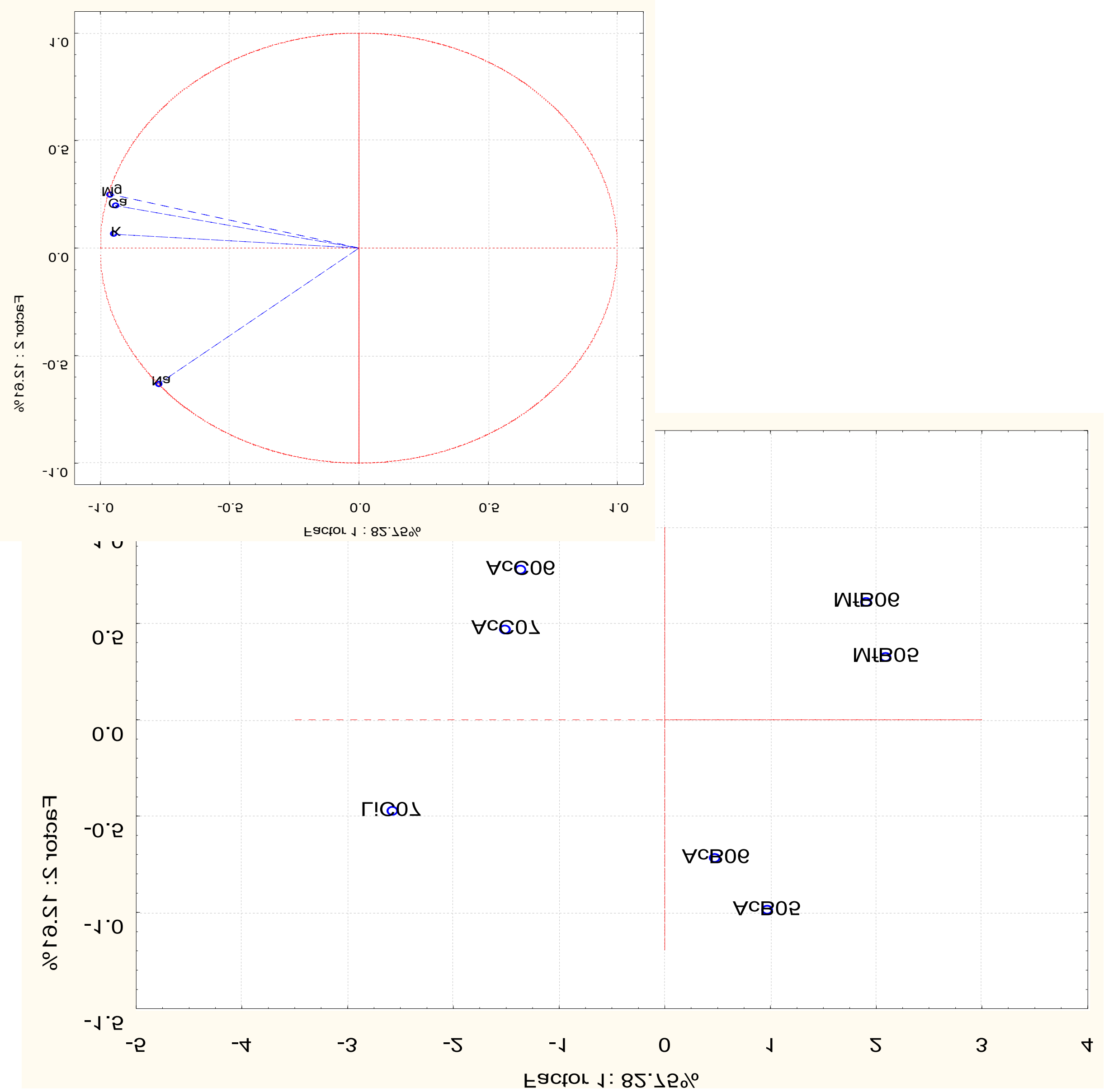

Fig.1. The distribution of analysed honey samples function the Na, K, Ca and Mg contents

CONCLUSIONS

The results of this research have shown that using Principal Component and Classification Analysis and the macro-minerals contents (sodium, potassium, calcium and magnesium) it is possible to differentiate between the honeys samples purchased from Berini than those from Cornea.

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MINOR AND TRACE ELEMENTS IN PARSLEY (*PETROSELINUM SATIVUM* HOFFM.) FROM THE WEST SIDE OF ROMANIA

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ABSTRACT

The paper presents a study on the variation of the concentration of main elements from the parsley's pulp, shell and leaves harvested from the west side of Romania. The results were compared with those obtained for the similar samples harvested from the hungarian region (close to the border). The representative samples were collected from different sides of West Romania or from the East Hungary and prepared according to AOAC Official Methods of Analysis. The concentrations of main elements (Na, K, Fe, Cu, Zn, Mn, Pb, Cd, and Ni) were determined by Atomic Absorption Spectroscopy.

Key words: parsley, spice, metals, nonmetals, atomic absorption spectroscopy, ecology.

INTRODUCTION

Vegetables are vital for the human diet, and particularly spices and aromatic herbs are very important for seasoning the food, providing the well-known trace elements. Some spices exhibits antioxidative properties (Zöchling S. et. al., 2002; Demo A. et. al., 1999; Belitz H.-D. et. al.,1987) or antimicrobial activity (Arora D.S. et. al., 1999; El Hamss R. et. al., 2003; Nielsen, P.V. et. al., 2000). Parsley (*Petroselinum sativum*, Hoffm.) is among the most valuable vegetables and it is a very rich source of vitamin C, β-carotene and mineral constituents (Lisiewska Z. et. al.,1997 ; Mohamed A.E. et. al., 2003). Minor elements such as Ca, Fe, K, Mg, and Na as well as trace elements such as Co, Cu, Mn, Ni, Pb, and Zn, were determined using the atomic absorption spectrophotometry (Mohamed A.E. et. al., 2003). A number of elements such as As(III), Al, B, Be, Cd, Co, Cr(IV), Cu, Mo, Ni, Se(IV), and Ti can be harmful to plants and humans even at quite low concentrations (Bowen H.J.M., 1979). These elements occur in variable concentrations in living tissues and are believed to be acquired by the body through environmental contamination (air and soil pollution). The aim of this work are to (1) assess the minor and trace elements in core, shell and leaves of parsley harvested from the west side of Romania for bioavailability or toxicity to humans, and (2) study of the correlational

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 173-180, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

parameters between the concentrations of these elements and the part of parsley or the harvesting region.

MATERIALS AND METHODS

Sample collection.

Five samples of parsley (*Petroselinum sativum*, Hoffm.) were collected from different region of Timiş (Cenad, Ghiroda, Moşniţa Nouă, and Utvin) and from the east side of Hungary (close to Romanian boundary).

Sample preparation.

The samples were washed with water several times, finally with distilled water and dried at room temperature. The core, shell (maximum 0.5 mm thickness) and leaves were collected in duplicates. These samples and a blank were put in porcelain crucibles and ashed in a furnace oven at 550°C for 48 h. The white residues were moisted with 5 ml 37% HCl (analytical grade, Merck Chemical Co.), boiled 2 minutes, evaporated to dryness on a hot plate, and heated on steam bath for 3 h. The residues were moisted with 5 ml 37% HCl, boiled 2 minutes, and then 50 ml distilled H_2O were added; the clearly solutions were heated on a water bath for 5 minutes, filtered and washed with distilled water. The filtrate was diluted to 200 ml with distilled water.

AAS Analysis.

Atomic absorption spectroscopic standard solutions (1-100 mg/l for Na and K, 0-15 mg/l for Fe, 0-6 mg/l for Cu and Mn, 0-1.5 mg/l for Zn, and 0-7.5 mg/l for Pb, Cd, and Ni, Merck&Co. Inc.) were used for calibration and for standard curves. A Varian-1100 atomic absorption spectrophotometer was used for the determination of element concentrations. The mean of two determinations was used for statistical analysis. Element hollow cathode lamps were used at recommended conditions.

Statistical analysis.

Statistical analysis of the database includes ANOVA, cluster analysis and principal component analysis (PCA), performed with *in house* programs. The data include the elements compositions.

RESULTS AND DISCUSSIONS

Minor elements.

From the atomic absorption spectrophotometric data, the most concentrated minor elements were Na and K, with values between 748 to 17062 mg/kg raw sample for the first, and between 600 to 58657 mg/kg raw sample for the second element. The upper limits for Na appear for samples harvested from Utvin (both core and shell), and the lower ones for samples from Cenad. By far, the most concentrated sample in element K was that from Cenad (especially for core part), and that less concentrated was the sample from Ghiroda (for core and shell).

Element Fe exhibits values in the range 507 – 542 mg/kg, the most concentrated sample being from Utvin (shell and leaves parts) and the lower limit for this element was 7.3 and 7.7 ppm (sample from Cenad – core and leaves). Major samples contains element Fe in the range of 7 to 541 ppm.

The atomic absorption spectrophotometric data are indicated in Table 1, and the relative concentration of minor elements in parsley are shown in Figs 1, 2, and 3.

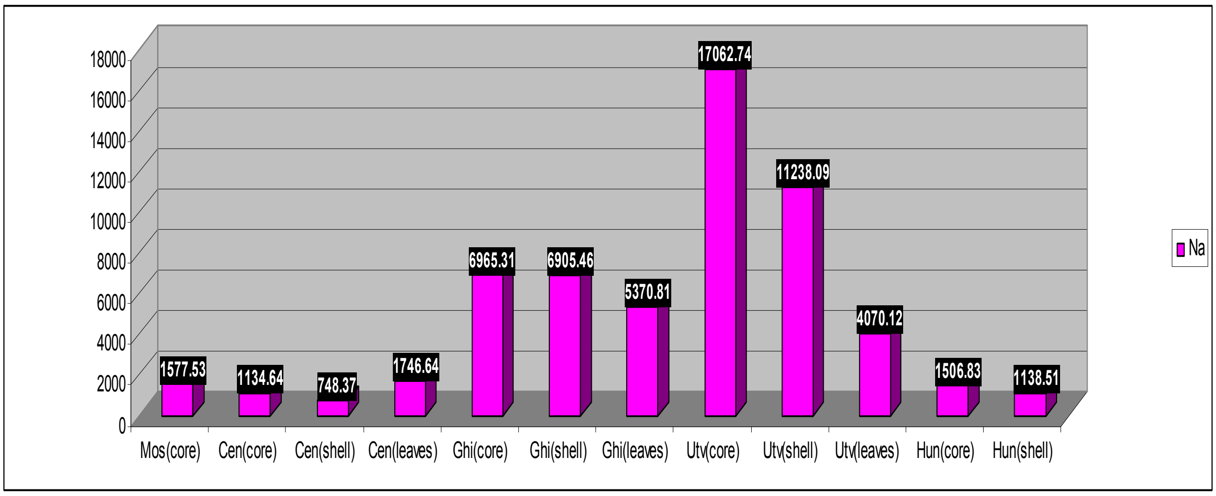

Fig 1. Minor element (Na) concentrations (ppm) in parsley

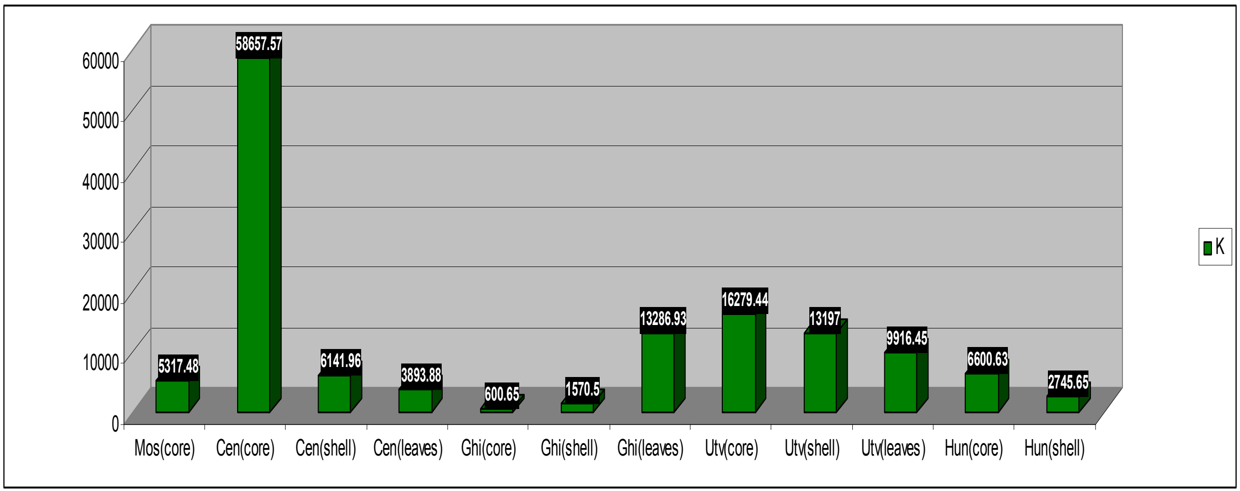

Fig. 2. Minor element (K) concentrations (ppm) in parsley

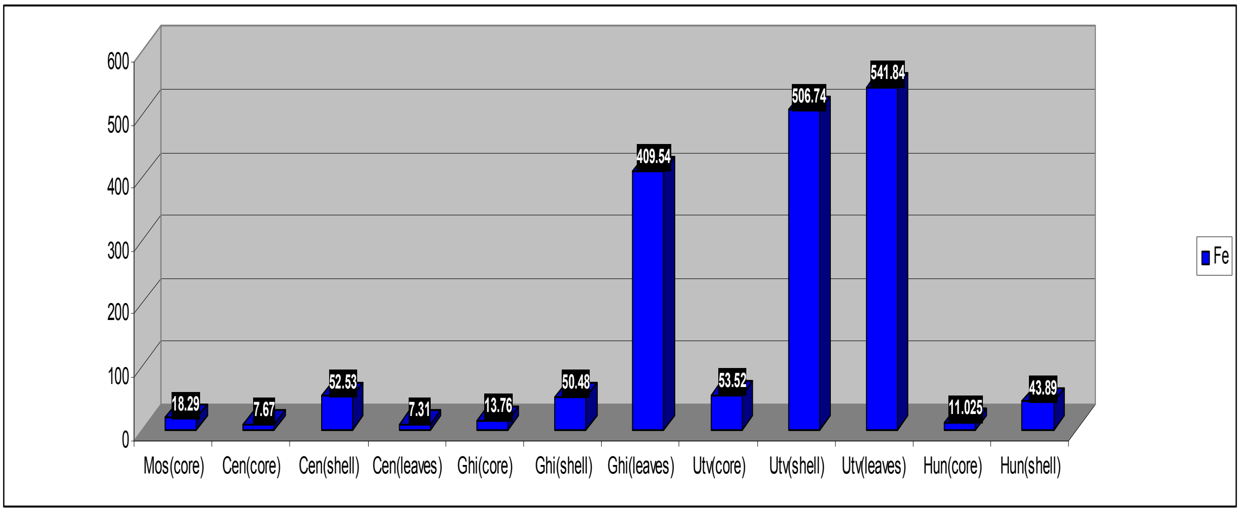

Fig. 3. Minor element (Fe) concentrations (ppm) in parsley

Table 1a. Minor element (Na, K and Fe) concentrations (mg/kg raw sample or ppm) in parsley from west side of Romania

Sample name	Code	Na	K	Fe
Parsley Moşnița Nouă (core)	**Mos(core)**	1578±1414	5317±67	18.3±1.4
Parsley Cenad (core)	**Cen(core)**	1135±71	58658±141	7.7±0.7
Parsley Cenad (shell)	**Cen(shell)**	748±8	6142±42	52.5±3.5
Parsley Cenad (leaves)	**Cen(leaves)**	1747±14	3894±21	7.3±0.7
Parsley Ghiroda (core)	**Ghi(core)**	6965±7	601±2	13.8±2.8
Parsley Ghiroda (shell)	**Ghi(shell)**	6905±71	1571±30	50.5±3.5
Parsley Ghiroda (leaves)	**Ghi(leaves)**	5371±45	13287±23	410±35
Parsley Utvin (core)	**Utv(core)**	17063 ±216	16279±424	53.5±2.1
Parsley Utvin (shell)	**Utv(shell)**	11238±424	13197±283	507±131
Parsley Utvin (leaves)	**Utv(leaves)**	4070±21	9916±28	542±78
Parsley Hungary (core)	**Hun(core)**	1507±92	6601±4	11.0±1.1
Parsley Hungary (shell)	**Hun(shell)**	1139±63	2746±70	43.9±1.7

Table 1b. Trace element (Cu, Mn, Zn, Pd and Cd) concentrations (mg/kg raw sample or ppm) in parsley from west side of Romania.

Sample name	Code	Cu	Mn	Zn	Pb	Cd
Parsley Moşnița Nouă (core)	**Mos(core)**	1.68±0.4	4.2±0.6	3.1±0.1	2.5±0.1	1.2±0.23
Parsley Cenad (core)	**Cen(core)**	1.44±0.3	3.4±0.7	2.5±0.6	1.55±0.5	1.67±0.3
Parsley Cenad (shell)	**Cen(shell)**	1.9±2.3	8.7±0.7	3.3±0.9	1.8±0.1	1.15±0.2
Parsley Cenad (leaves)	**Cen(leaves)**	0	0	0	0	4.2±0.5
Parsley Ghiroda (core)	**Ghi(core)**	0.66±0.1	4.0±1.1	2.4±0.3	0.7±0.1	1.1±0.1
Parsley Ghiroda (shell)	**Ghi(shell)**	0.8±0.08	3.6±0.6	2.8±0.1	0	1.0±0.07
Parsley Ghiroda (leaves)	**Ghi(leaves)**	0	7.8±0.2	17.1±0.3	0	5.06±0.1
Parsley Utvin (core)	**Utv(core)**	5.08±0.5	22±3.0	10.8±0.8	0.9±0.03	2.6±0.04
Parsley Utvin (shell)	**Utv(shell)**	5.9±0.4	13.1±2.3	14.0±1.1	0	1.3±0.1
Parsley Utvin (leaves)	**Utv(leaves)**	18.6±1.6	18.7±1.9	41.1±3.1	0	1.4±0.5
Parsley Hungary (core)	**Hun(core)**	2.68±0.1	16.0±2.5	4.9±0.3	3.7±0.2	1.5±0.2
Parsley Hungary (shell)	**Hun(shell)**	0.71±0.7	4.8±0.4	3.1±0.04	0	0.9±0.04

Trace elements

For the trace elements (Cu, Mn, Zn, Pb, Cd, and Ni) the concentrations are about 1000-fold lower than minor elements. The most concentrated is Cu (up to 18.6 ppm), Mn (up to a range 22 ppm), and Zn (up to 41.1 ppm). The lower concentrations were found for Cd and Pb, even if these harmful elements exhibit quite higher values. The higher concentration for Cu, Mn, and Zn were found for the samples from Utvin (especially in leaves), but the Pb concentration in these samples was lower. The core part of parsley is generally most concentrated in Pb, than the shell part. Cd was most concentrates in leaves, in almost all cases, and Ni was not found in any part of parsley (under detection limit). The AAS data are indicated in table 1(a,b) and Figs 4 and 5.

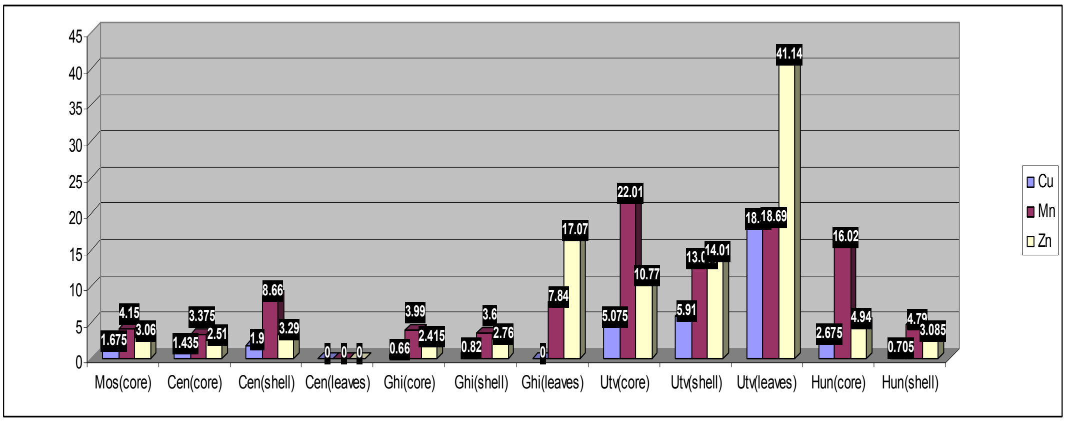

Fig. 4. Trace element (Zn, Mn, Cu) concentrations (ppm) in parsley

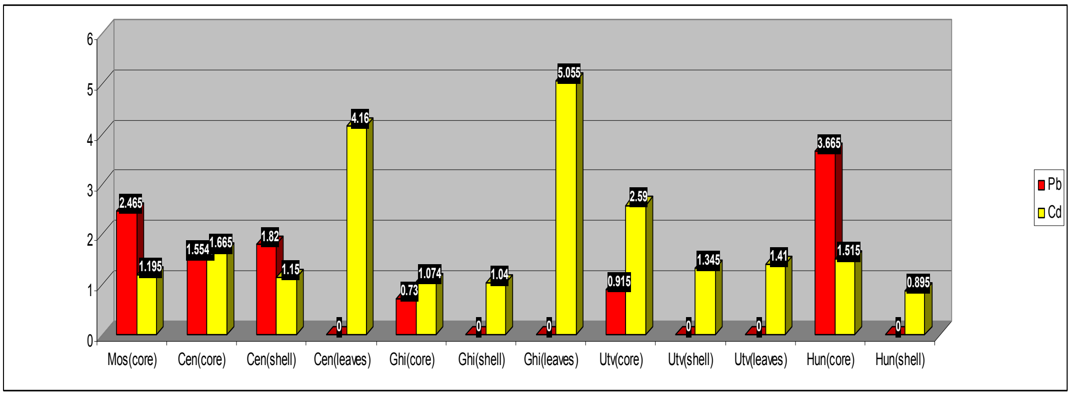

Fig. 5. Trace element (Pb, Cd) concentrations (ppm) in parsley

Statistical analysis

The multivariate analysis by PCA (principal component analysis), using all data (Table 1), indicate that the samples from Utvin and Ghiroda were classified in the upper side of the score plot (Figure 6), and the Moşniţa, Cenad, and Hungary samples in the lower side, closely grouped (exception the Cenad core sample). The Na and K concentrations were responsible for this classification (loadings plot). The explained variance of the data was 89% for PC_1 and 11% for PC_2.

The samples were classified by core, shell and leaves parts only by PCA analysis using the concentrations for trace elements (Mn, Zn, Pb, and Cd). The samples containing leaves were localized in the lower side of the score plot, and the samples containing core or shell were classified in the upper side (Figure 7). The explained variance of the data was 79% for the first principal component and 18% for the second one. The main elements for this classification were Mn and Zn.

From hierarchical cluster analysis of all data (minor and trace elements), the similarity of the samples was relatively good, excluding the core sample from Cenad (with a very high level of K). Very similar were the samples from Moşniţa, Cenad and Hungary. The samples from Utvin and Ghiroda were also similar (Figure 9).

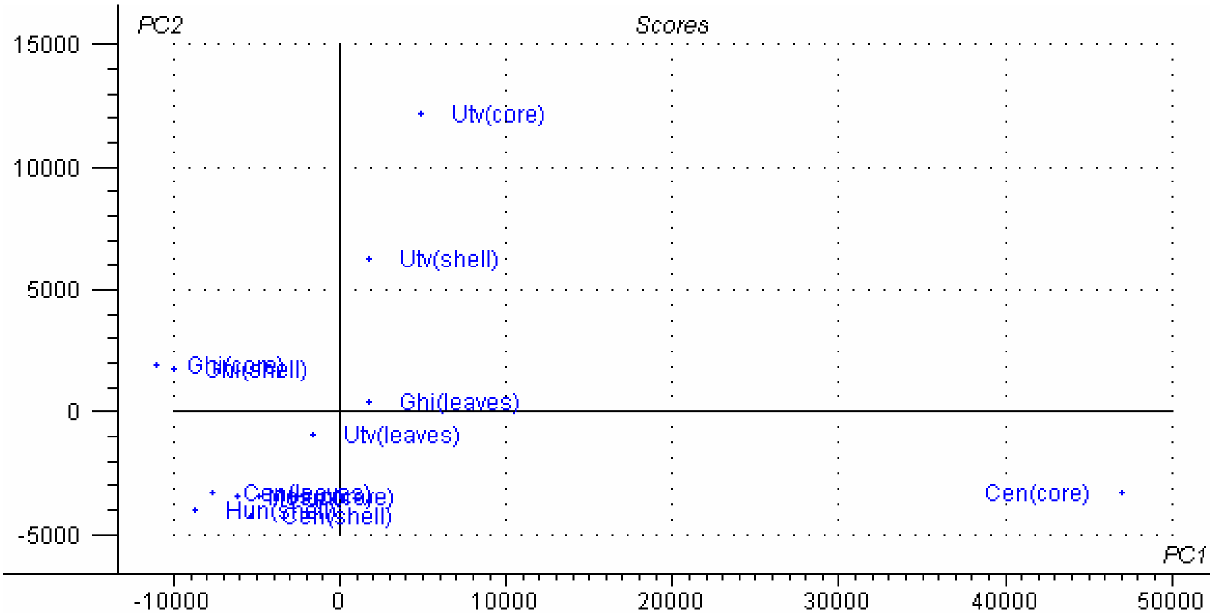

Fig. 6. Score plot for all data from PCA analysis

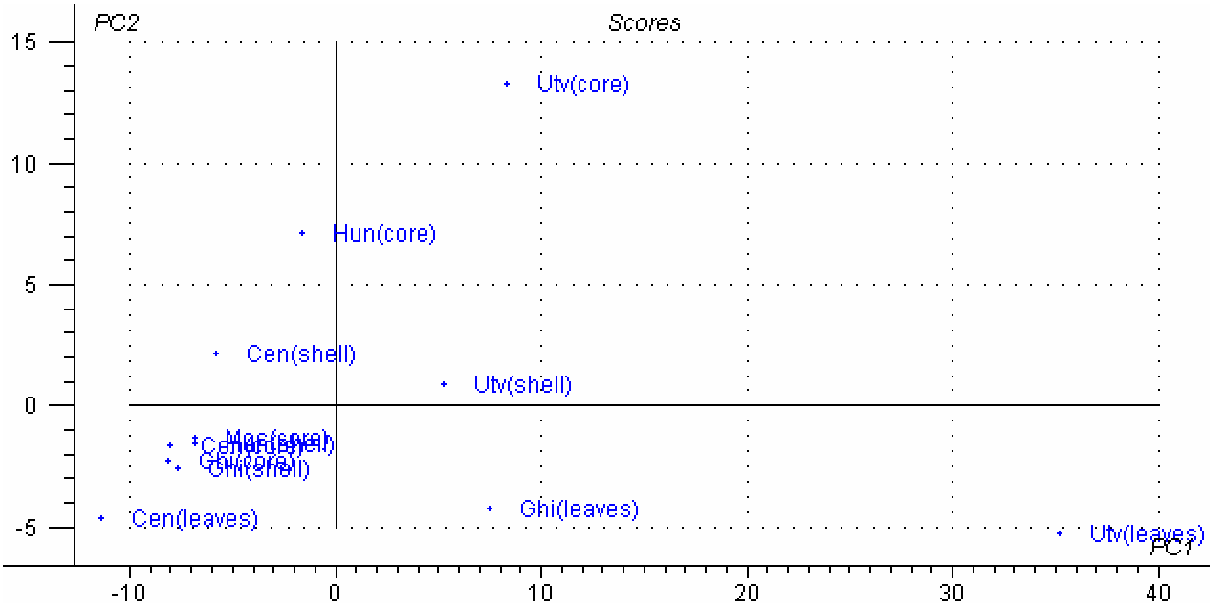

Fig. 7. Score plot for trace element data from PCA analysis

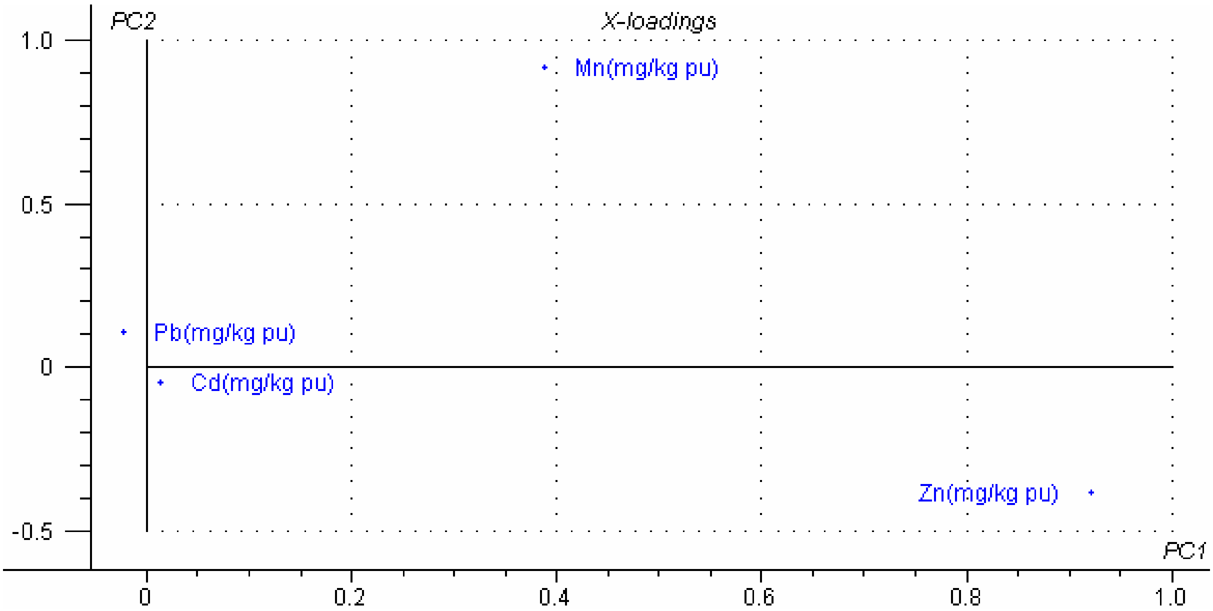

Fig. 8. Loading plot for trace element data from PCA analysis

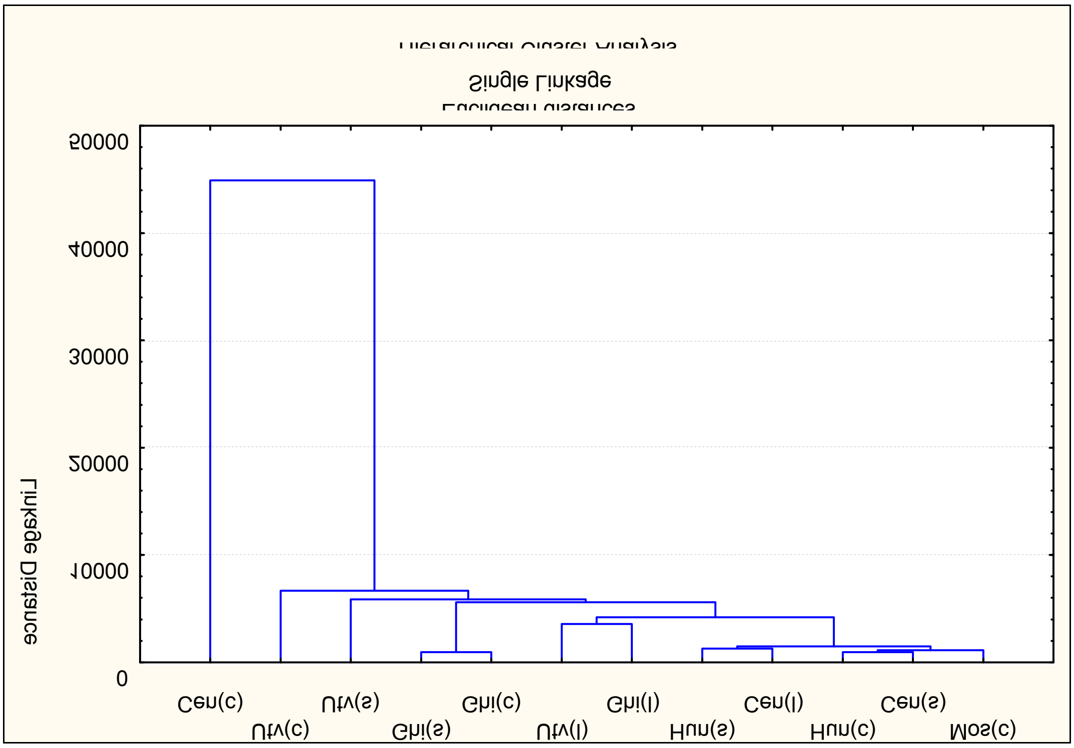

Fig. 9. Hierarchical cluster analysis for parsley samples

CONCLUSIONS

The concentration of trace elements in vegetables is very important for our health and from environmental ecology point of view. The samples generally exceed the maximum concentration values for almost all elements. For Cu, this maximum concentration (5 mg/kg) were exceeded for samples from Utvin, Mn and Zn are more concentrated in samples from Utvin and Ghiroda (leaves), and more concentrated in Pb are samples from Hungary. Cd concentration exceeded the maximum concentration limit (0.05 mg/kg) for all samples, more concentrated being the leaf part of parsley.

These results are good correlated with de distances from Timişoara (industrial city) to the region from where the parsley samples are harvested. Utvin and Ghiroda are more closed to Timişoara city (up to 5 km).

By multivariate analysis (principal component analysis, PCA, and hierarchical cluster analysis, HCA) the samples (harvested sites) have been classified in two main groups: Utvin - Ghiroda group (close to Timişoara city) and Cenad – Hungary group (close to Romanian-Hungary boundary).

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CONCLUSIONS CONCERNING THE SOURCE AND THE ENVIRONMENT CONCENTATION WITH HEAVY METALS ON THE ETNO-HISTORICAL BANAT

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ABSTRACT

The Banat soil between the Carpathians, Tisa, Mureş and Danube have now a relative high content in heavy metals. Because of the lack of industrial pollutants, the causes which have favoured the accumulation of heavy metals can be put only on the mountain zone as well as the modality of their transport towards the west, towards the piedmont areas and the plain ones.

In the long singenetical process, under the action of the weathering solutions, both in the previous geological periods and in the present period, there have been a lot of continuous processes of delivering the constituent elements. This delivering depends in general on the solubility of the chemical compounds, their valence and the climatic and litological circumstances.

The researches followed the understanding on the geochemical importance of the soils from the etno-historical Banat.

The explication is based on the native occurence of these elements in the rocks of south-western Romanias' Montains. The more humid climate (of oceanic influence and more warmer (the Mediteranean influence) has favoured the balteration and the Panonic fore deep which was a lake in this first phase and a swamp later, favoring the accumulation. In the last accumulative medium, the concentration of metal elements have increased also on the base of the sulfur of organic matter in an anoxic medium.

Key words: heavy metals, pollution, Banat, Mureş-Tisa-Danube Euregion.

INTRODUCTION

The geogenous and the anthropogenous abundance of heavy metals in Banats'rocks and soils (between the Carpathians-Tisa River-Mureş River and Danube) is conditioned by the paleogeographical evolution, by the type and the direction of weathering that occured especially during the Quaternary as well as by the direction and the characteristics of the pedogenetical processes.

The Banat soils do now have a high content of heavy metals that are almost always above the background domains. In the absence of predominant industrial poluters the causes that have favourised the accumulations of heavy metals can only be explained by the high geochemical backgrounds of the rocks and of the parental materials of the eastern mountain zone and by the manner of transport

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 181-188, Publishing House "Eurobit" Timişoara, 2008
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towards these areas and the modifications that occured in the chemistry of the soils during the pedogenesys process.

The researches done took into consideration the knowledge about the geochemical endowment of Banat in order to appreciate the capacity of the soils of being able to reach or not the extraloadings of heavy metals. The identification of heavy metals on levels of content and their map matherialisation is allowing the characterisation of the place, the appreciation of their origin (autochtonous, alochtonous or anthropogenous) as well as the establishing of some correlations between the total forms and the mobile ones of each studied element.

The exploration on the Serbian Banat fields and the conclusions results, made on the Serbian Banat fields, is just a hardship. Confirmation or confutation emisses assumption result thoroughgoing study or reorientation of investigation.

MATERIALS AND METHODS

Towards estimating the geochemical background of the etno-historical Banat, within the surface of the Romanian Banat there have been taken about 400 soil samples from the horison placed at different depths (from 0-150 cm), from over 100 places. The analysis methods relied on the weathering of the prevaled samples with a mixture of nitric, sulphuric and perchloric acids, in a 2 : 0,1 : 1 (I.C.P.A. - 1986) proportion and spectometrical atomical absorbtion dosation in variant of air-acetilene flame atomisation.

Te manner in which the position carthogrames were made presenting the area disposition of the researched oligoelements relied in the supraposition of the following maps: the map of main geomorphological units of Banat (Ianos, 1985), the geological map (Ianos, 1987), the map of the rocks and of the parenthal matherials, the map of the intensly mineralised materials (fig.1), the map of the soil asociations (Ianos, 1985), the map of Banat along XVIII and XIX century (Ianoş-1997), the map of the depth of the underground waters (Ianoş 1997), the map of the humus supplies (0-50 cm)(fig.2), the map of the soil acidity (fig.3).

Functions of the particularities of occurance of the researched metal microelements (Cd, Co, Cu, Cr, Fe, Mo, Mn, Ni, Pb, Zn) in the subiacent and parental rocks, of the environment and the manner in which these elements are taken out of rocks by weathering, of the behaviourism of these rocks during the transportation as well as of the situation created during the sedimentation there have been delimitated new areas with similar concentrations on content levels. On the basis of the above presented considerations to which there can be added lithological, gliptogenetical or pedogenetical particularities, in the zones where there were not gathered and analysed samples the conclusions that could be drawn were extrapolated to that the future analytical determinations to confirm or to deny the formulated suppositions

RESULTS AND DISCUSSIONS

1. The conditions of occurance of the heavy metals in the rocks and soils of Banat

In its eastern part, Banat is limited by more or less homogenous mountain massifs. Created during the alpine orogenesys, they have taken at the same time or after an intense magmatic activity generated by the Postlaramic tectonical movements after which on the fault line there were pooshed towards the surface the Banatitic intrusions especially formed on granodioritic type of rocks (Oncescu-1965, Folea-1980). In paralely there have been taken place large processes of contact metamorphism and selective metasomatoza and as a result there were replaced especially by transformation of the limestones the skarns (Gheorghiță-1975, Petrulian-1979).

Fig. 1 Areals with anomale mineralizations in the mountain Banat region

Legend; **1**. Complex sulphates; **2**. Anomal areals with mineralizations; **3.** Direction of speading for the erroded materials; **4**. General direction of transport and sedimentation of the intense mineralized materials.

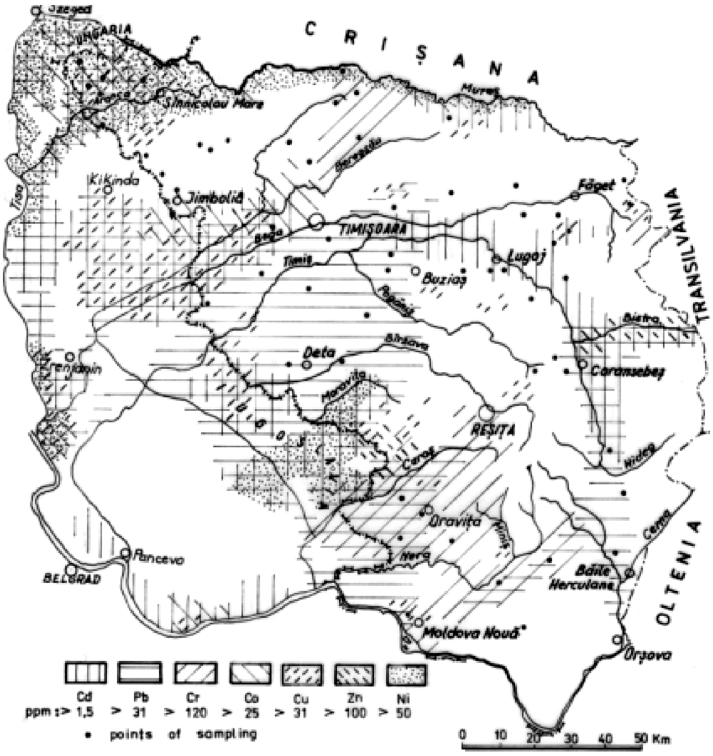


Fig. 2
Values of attentions of the heavy metals concentration in the soils of the Ethno-historical Banat

Later, the endo and exogene skarns were influenced by a hydrothermal metamorfism (Folea-1980) with the making of polimetal sulphurs (pyrite, blende, calcopyrite, galena, aikinit, bismuthit, cerussite, crocoite, piromorphite, molybdenite etc).

A short inventary of the areas with intense mineralisation are indicating us a zone of positional accumulation in the nord-west part of Banat (Metaliferi Mountains) where there are predominating the polisulphurs of plumb, copper, zinc (fig.4). Towards west and south west Savu (1970) mentioned rich mineralisations of mollibden and manganese or iron in the eastern part of Zarand Mountains. From both zones, the Mureş River and its tributarries have taken denudated materials and put them towards west building the hills and the plains in the central and western part of Banat. There follows, on the south, the metalogenetical areals in the mountain massifs Poiana Rusca-Ruschița (lead, cooper, zinc, molybdenum), Dognecea (iron, copper, chromium), Anina-Sasca (cobalt, nickel), Almăj-Moldova Nouă (copper) (fig.1).

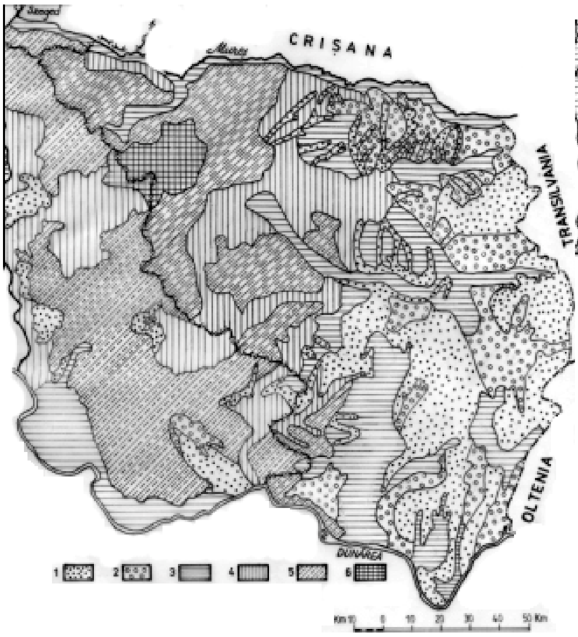


Fig. 3 Humus reseve of Banat Soils on the depth of 0-50 cm (t/ha)

Legend: **1.** < 60; **2.** 61-120; **3.** 121-160; **4.** 161-200; **5.** 210-300; **6.** > 301.

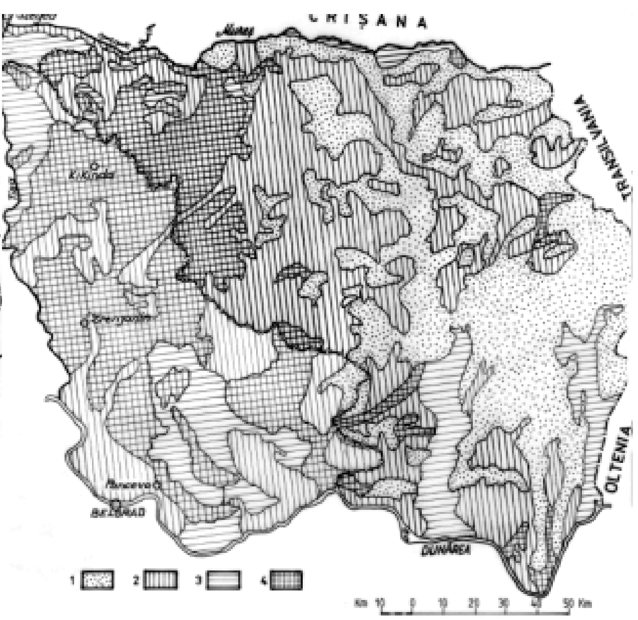


Fig. 4 Soils reaction in Banat (pH units) on the depth of 0-29 cm.

Legend: **1.**< 5,80 (strong medium acid);**2.** 5,81-6,80 (weak acid);**3.** 6,81-7,20 (neutral); **4.**> 7,21 (alkaline).

During the geological time, but especially during the Quaternary, when the frequent climatical oscilations have been favourising an intense physical weathering in stages and a pronounced chemical weathering in the interstages, from the rocks of the mountain massifs there was released a part of the constituent chemical elements.

The positive epirogenetical movements of the Carpathians and the drainage of the Panonic lacustrine domain by Danube pearcing through the Iron Gates have been accelerating the denudational processes, those of transport of the materials resulted as well as an accelerated sedimentation in the piemountaneous zones and then on the plain zones in the east-west direction. On the basis of these considerations we have to underline the fact that not only the superficial lithological layers in the plain or piedmountain zones are loaded with heavy metals but also thick packs of rocks placed on deep depths. This fact is explaining some overloadings with arsenium of the depth waters (60-200 m), there were registered in the northern part of the Mureş River that are also continuing in Hungary.

A last stage in the process of rocks and soil loadings with heavy metals is conditioned by the filling with alochtonous materials of the vast areas of subsidence in the eastern Banat, zones occupied by the end of the XVIII-th Century by large swampy domain where there existed a varied and rich hydrophile fitocenosys that was decomposed and there resulted rich amounts of sulphur (H_2S). Because the waters of the main rivers that were erroding Banat used to carry in suspension fine particles of erroded material or ions of the heavy metals in solution and as the majority heavy metals have a special affinity for the sulph, at floods these elements were bloked in the fine texturated materials loaded with sulphur compounds, fact that leads to high occurances of chemical elements in the clayed rocks, especially the smectical ones.

The tectonic and climatic stability at the beginning of the holocene marked a diwindling of the errosional processes, of the sedimentation, as well as an amplification of the solification processes function of the time lift from the beginning of the solification process of the granulometrical making of the parental rocks and of the soil, of the amount of humified organical matter, of the pH and rH. of the solutions that are circulating in the soil, heavy metals placed here together with the solification materials have been suffering concentration modifications by lateral or vertical movements ascendent or descendent.

On the basis of the analytical data there can be inferred that in the Banat soils the metal microelements that were researched are especially to be found in the structure of the primary and secondary minerals as well as kept by different physico-chemical mechanisms by the organical coloides and the mineral ones. The total supplies and the degree of mobility is conditioned by nature of the solification rock and by the processes of pedogenesys (eluvial-iluvial, bioacumulation, oxido-reducing). Generaly in reduced and moderate weathering conditions, the influence of the rock is determinant and in the conditions of advanced weathering the main role goes to the pedogenetical processes.

Generaly as regarded and correlated with the data obtained by Lăcătuşu (1992) for Romania or by Adriano (1986), Alloway (1990) for other zones of the world, the microelements determined in the Banat soils are in a concentration (mean values) of 1.2 to 3 times higher, the maximum values being characteristic especially for the lead, cadmium, cobalt and chromium.

The distribution of the main heavy metals (cadmium and lead) in Banats'rocks and soils

In order to give examples, there have been selected two chemical elements with high toxic values. Other seven elements were selected and presented in publications made in last years (Ianoş, 1999).

The cadmium. The high release of the cadmium from the primary rocks in the special conditions of transport towards the western accumulative areas make the Banat soils contain important amounts of cadmium (1.0-1.8 ppm) with a mean value of 1.3 ppm. In comparison with the value of the clark, the mean cadmium content in the Banat rocks in 10 times higher and in comparison with the general mean content in the soils, 4.3 times higher (Lăcătuşu-1993).

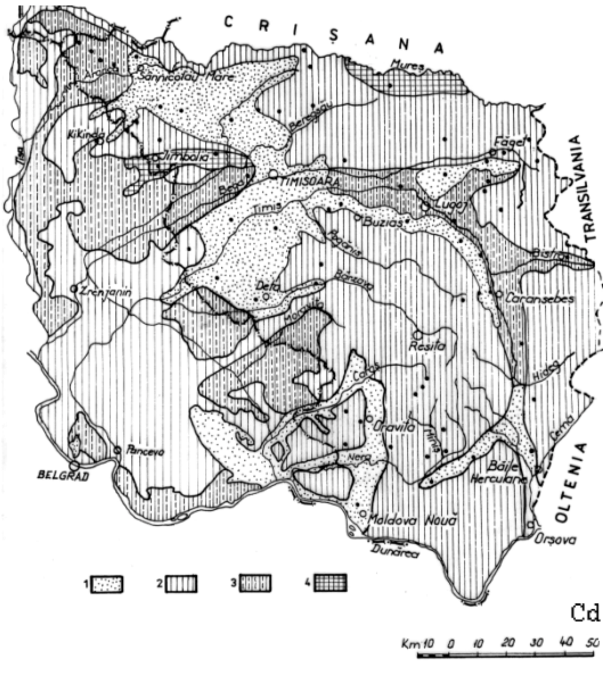
The long weathering conditions makes that in rezistants, cadmium be almost totally missing and its affinity for the sulphur determine an enriched precipitation and concentration in the hidrolizate especially in the areas that do predominantly have oxide-reducing processes (the clayed muds in the subsidence zones). The uniformity of cadmium concentrations can be owed to the values generally reduced of the cadmium in the rocks (less than 2 ppm) or on the working methodology, the determination device not being able to sustain the exactness of some determinations at low amounts.

The smallest cadmium concentrations (less than 1 ppm) are to be found in the evaluated soils on the bulk deposits on the dejection cones of Mureş, Timiş, Bârzava, Caraş, Nera Rivers (fig.5). In the remaining ones the cadmium contents are slightly higher (1.3-1.8 ppm).

The later improvement with cadmium of the soils especially of those in the podzolite series in the piedmountain areas can be explained by the absorbtion of the element in the oxidazed sediments here manganese rich.

On the genetic types of soil there can be seen a moderate degree of variation with a slight tendancy of concentration in argiluvisols. During the background domain (1.2-1.5 ppm), the cadmium is presenting a moderate degree of variation with a slight tendancy of concentration in argiluvisols and the clayed soils (vertisols). Beneath these limits there are to be found the chernozems, the cambisols and fluvisols in the Mureş, Timiş, Bârzava Plains and in the Lugoj Hills, Pogăniş Hills and the Almăj, Oravița, Moldova Nouă Depresions.

The lead. Released by the rocks as a result of some weathering process the plumb is oxidated and reduces its mobility especially in the presence of the suplh equalising "in situ" the lead concentrations of the eluvial layers. Taken by the groover waters and then by the running waters rich in calcium cations the lead will make new combinations especially with $CaCO_3$ especially in the sediments and soils of the depressionary corridores (>30 ppm). In the subsidence areas with sediments rich in organic sulphurs, the lead finds storage conditions in the extremely clayed soils in the Aranca Plain in the inferiour sector of the Timiş Plain or Caraş Depression (fig. 6).



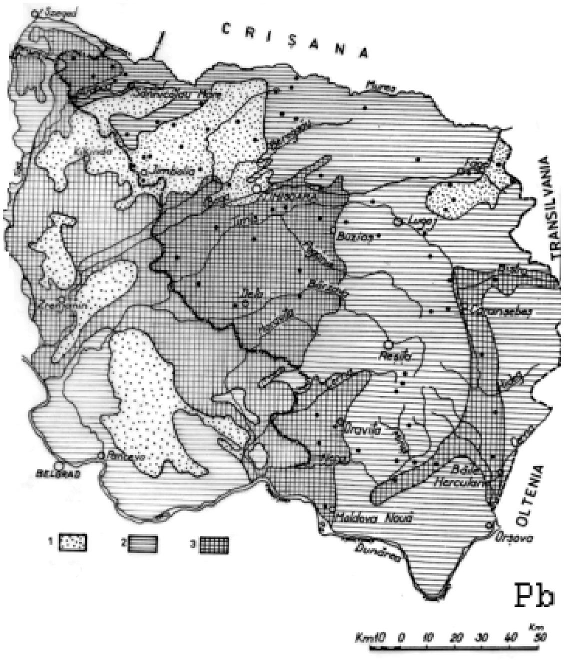


Fig. 5 The state of assurance with cadmium (total forms) of Banat soils (depth 0-40 cm)

1. little (<1,0 ppm); **2.** medium (1,1-1,5 ppm); **3**. great (1,6-1,9 ppm); **4.** very great (>2.0 ppm);

Fig. 6 The state of assurance with lead (total forms) of Banat soils (depth 0-40 cm)

1. little (<20 ppm); **2**. medium (21-30 ppm); **3.** great (>31 ppm); **4**. sampling stations.

On the background of some more reduced sulphur concentrations of the porosity and of an enriched permeability in the loessic plains, covered with chernozems, the lead will easily realise bicarbonate compounds easily removable by vertical or laterally migrations situation that in this areas presents more reduced concentrations of total lead (<20 ppm). In this conditions the lead is oscilating with a large interval of values between 15 and 40 ppm with a medium value of 28.5 ppm, concentrations that are also over the values of the clarks and of the mean contents of other similar zones in the country and in the world.

CONCLUSIONS

1. The heavy metals in the rocks and soils of Banat come from the eastern mountain zone by weathering processes, desintegration, transport and resedimentation.

2. The enrichment with heavy metals of the soils in the Banat 'hills and plains is conditioned by the reaction of the transporting solutions, of the sedimentation background as well as of the sulphur content.

3. Function of the conjunctural conditions of releasing transport and sedimentation most of the heavy metals presented in the Banat soils have a superior abundance in comparison with the neighbouring areas or other parts of the world.

4. Areas of anomal concentrations are placed in the subsidence zones occupied by the vertisols or at the content between the piedmountain and the mountain formations.

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METAL ION COMPOSITION OF STINGING NETTLE (*URTICA DIOICA* L.) LEAVES IN VIEW OF THE NUTRITIVE VALUE

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ABSTRACT

The aim of our investigations was to determine the macro and micro element components in leaves of common nettle (Urtica dioica L.) of different origin and the total phenol content and antioxidant capacity (by FRAP method) of extracts from them. Plant materials were collected from a meadow near the city Paks (Hungary) and from along a roadway near Paks. These samples were compared to commercial teas (drug from pharmacy, and teas from conventional and organic collection). Well detectable differences were obtained for the investigated parameters between the samples. Our results showed that the regular control of element composition of teas is very important from the viewpoint of health protection because the place of growing has a big influence on the accumulation of some elements in the nettle plant. Closed relations could be found between the antioxidant capacity and concentrations of some metal elements, which influence the nutritive value of herbs

Key words: nettle, *Urtica dioica L.,* metal element, antioxidant capacity

INTRODUCTION

Medicinal plants are very important for our health, in addition to active ingredients and vitamins, mineral elements also have a big influence on the human organism. Positive or negative effects of plant antioxidants depend also on the macro and micro element content since complex binding and the too low or too high uptake of elements modify the biochemical processes, not to mention the uptake of toxic elements (Szentmihalyi and Then 1999, Blazovics et al. 2003).

Abiotic environmental factors have a high influence of nutritive value of the plants. Lead, cadmium, copper, zinc and nickel are metals frequently reported to have the highest impact on organisms. The metal uptake from the soil by plants is an introductory pathway for heavy metals to enter the foodchain. Plant species differ in ability to absorb and allocate nutrients. *Urtica dioica* L. is not considered as hyperacumulator and does not exhibit heavy metal tolerance but it has been mentioned many times as a good bioindicator (Sawidis et al. 1995, Singh et al. 1995, Tack and Verloo 1996, Prasad and Freitas 2003).

Leaves of stinging nettle (*Urtica dioica* L.) are a rich source of trace elements, absorbing and accumulating them. Due to its active ingredients nettle has

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 189-194, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

anti-arthritic, antispasmodic, diuretic, astringent, tonic and expectorant effects. Dried nettle leaves are ordinarily used in herb tea mixtures. Fresh leaves also can be consumed as a vegetable similarly to spinach, and this usage begins to spread in Hungary nowadays. (Neumayer 2000, Dudas 2008)

MATERIALS AND METHODS

The aim of our investigations was to compare the macro and micro element composition of common nettle (*Urtica dioica* L.) leaves of different origin. Plant materials were collected from a meadow near the city Paks (Hungary) and from along a roadway near Paks. These two nettle populations were definied as the same genotype. Samples were formed from 10 subsamples each collected from a 1 m^2 area. Plant material was harvested in July and dried at 30°C. Samples were compared to commercial teas (drug from pharmacy, teas from conventional and organic collection).

For analysis of element composition, 0,2 g of dried and pulverized samples (duplicated) were taken. The following elements were determined by ICP -OES (Thermo Jarrell Ash Co, ICAP 61): Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Si, Sr, Ti, V, Zn. The concentration of metal ions is given as mg/kg dry weight.

Extracts were prepared from 1 g of dried leaves which were infused with 100 ml boiling water or aqueous ethanol (25°C; water/ethanol 80/20, v/v). The ethanolic and aqueous extracts were stored at room temperature for 72 and for 24 h. After centrifugation (13000 rpm, 10 min), the supernatants were analysed.

Total phenol content was measured using the method of Singleton and Rossi (1965). The phenol content. For determining the antioxidant capacity the FRAP method was used (values are given in mmol ascorbic acid / l) (Benzie and Strain 1966).

RESULTS AND DISCUSSIONS

Metal ion concentrations in nettle leaves vary between wide limits in the different studies (Dudas 2008). Concentrations measued by us generally were in the higher ranges.

Table 1 shows the element compositions in dry leaves of *Urtica dioica* L. The concentrations of Cd, Co, Cr, Hg, Pb and V were under the detection limit in each samples, these elements are not indicated in the table.

The concentration of Al and Ba was significantly the lowest in the sample from the meadow. In teas from conventional and organic collection relativly high concentrations of Al were found.

On the bases of previous studies it can be said that the concentration of Ca shows a high variability. In our investigations the lowest concentration was obtained for the drug from the pharmacy and the highest for the sample collected from along the roadway.

Some differences were found in the concentrations of Fe, nettle leaves from the meadow had the lowest Fe content among the investigated plant materials.

The concentration of K was almos equal, no differences could be detected between the samples.

The Mg content was significantly lower in the leaves from along the roadway than in the other samples.

Mn seems to be accumulated in a high volume in leaves of nettle. The concentation of this element was significantly higher in the obtained samples (except of sample from along the roadway) than it has been reported in the literature.

In the case of Na the two commercial teas had significantly the highest concentrations.

Table 1: Element concentration of *Urtica dioica* L. (mg/kg dry weight)

	Meadow	**Roadway**	**Pharmacy**	**Tea**	**Eco tea**
Al	2,50	48,47	84,41	158,1	138,2
As	12,09	7,56	7,56	4,43	2,91
B	37,84	36,42	48,84	46,86	46,75
Ba	16,49	45,72	35,45	34,58	42,17
Ca	32810	37690	27740	35687	35411
Cu	14,02	16,69	12,99	12,14	11,84
Fe	98,35	185,10	188,70	208,54	162,52
K	23250	23750	24390	24795	26207
Li	1,202	1,213	1,282	0,262	<dl
Mg	5231	3117	5385	5108	5435
Mn	116,5	33,6	224,6	172,5	177,7
Mo	2,29	4,33	1,42	4,80	2,17
Na	118,0	146,5	152,4	325,3	396,8
Ni	<dl	0,53	2,34	1,42	1,08
P	4927	6229	3877	5010	4648
Se	26,03	25,76	27,83	<dl	<dl
Si	140,8	146,3	137,1	425,5	427,5
Sr	73,41	125,4	109,8	107,6	116,7
Ti	1,57	3,65	2,86	2,35	1,73
Zn	25,75	27,72	22,04	27,04	25,11

<dl: under detection limit

The highest phenol content was measured in aqueous extract of commercial tea (292,07 µg/ml) and the lowest value was found in nettle leaves from the meadow (92,98 µg/ml) (Fig. 1).

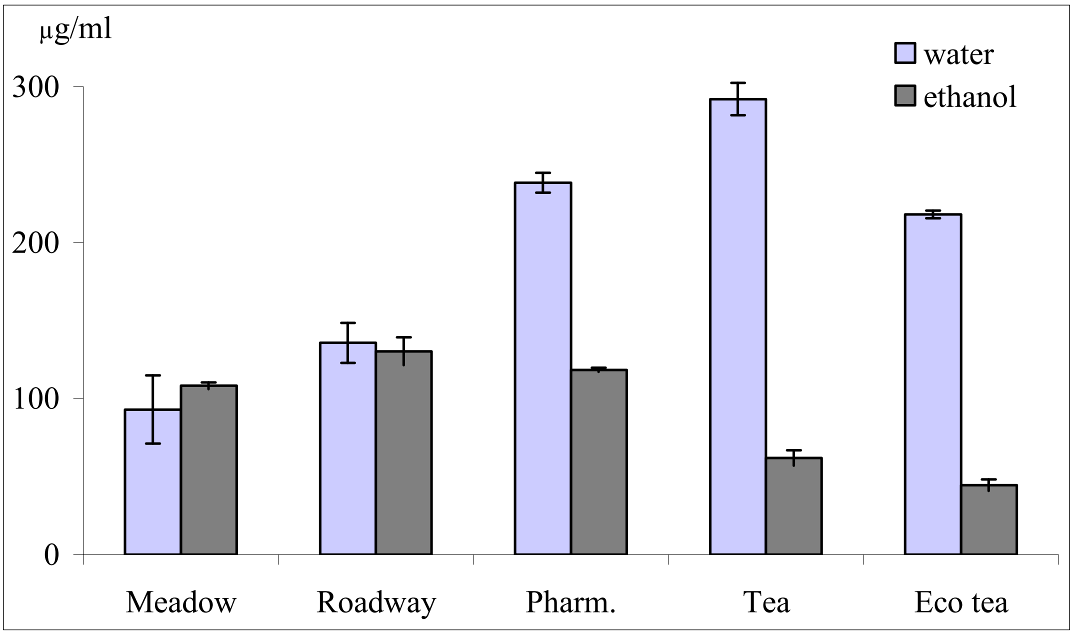

Fig. 1: Total phenol content of extracts of *Urtica dioica* L. leaves

In the case of ethanolic extracts the highest phenol content could be detected in the sample from along the roadway (130,3 µg/ml). The lowest phenol content had in our investigations the tea from organic collection (44,56 µg/ml).

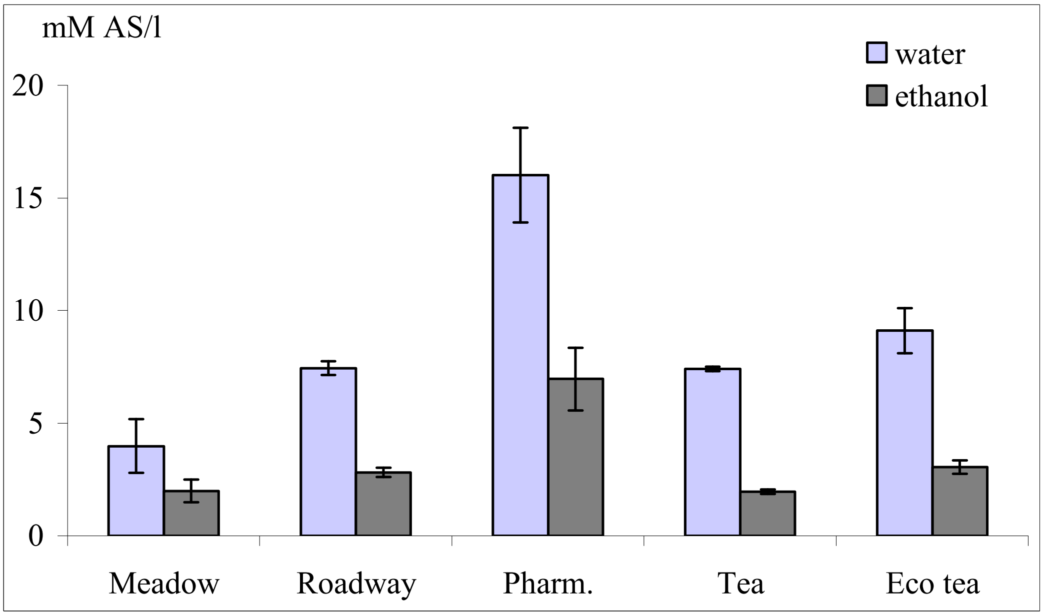

Fig. 2. Antioxidant capacity of extracts of *Urtica dioica* L. leaves

The antioxidant capacity was significantly higher in the aqueous extracts than in the ethanolic extracts (Fig. 2). In the case of aqueous extracts the drug from the pharmacy had the significantly highest antioxidant capacity (16,01 mM AA/l) and the sample from the meadow showed the lowest antioxidant capacity (3,98 mM AA/l).

Well detectable differences could be obtained also for the antioxidants capacity in the ethanolic extracts: the highest value was found for the drug from the pharmacy (6,95 mM AA/l) and the lowest (1,85 mM AA/l) for the commercial tea from conventional collection.

CONCLUSIONS

1. *Urtica dioica* L. accumulates some metal elements in excessive amount in its leaves.
2. There are big differences in element concentrations between samples of different origin. The growing place play an important role in accumulation of metal ions.
3. Antioxidant capacity was in a good accordance with the phenol content of the samples. Significant differences could be detected for the phenol content and the antioxidant capacity between the aqueous and ethanolic extracts.
4. There are closed relations between the antioxidant capacity and concentrations of some metal elements, which influence the nutritive value of herbs.
5. Place of collection of wild growing medicinal plants should be chosen cautiously and heavy metal concentration of herbs should be controlled regularly before being marketed.

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STUDY REGARDING THE HEAVY METALS CONTENTS IN SOME VEGETABLES CULTIVATED IN THE FIELD IN ROMANIAN WEST AREA

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ABSTRACT

In this paper was analyzed the some heavy metals (Co, Ni, Pb and Cd) accumulated in some vegetables (pepper, tomatoes, cucumber and eggplants) cultivated in field, in Romanian west area. The specific climatic factors from this areal create conditions for normal uptake of the heavy metals.

The experimental results revealed that the distribution of the heavy metals in vegetables from this areal are in the range of normal limits concentrations.

Key words: vegetables, heavy metals

INTRODUCTION

Vegetables are a source of important vitamins, minerals and carbohydrates. Heavy metals, such as cadmium, copper, lead, chromium and mercury, are important environmental pollutants, particularly in areas with high anthropogenic pressure.Their presence in the atmosphere, soil and water, even in traces, can cause serious problems to all organisms. Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effects on food quality (safety and marketability), crop growth (due to phytotoxicity) (Ma et al., 1994) and environmental health (soil flora/fauna and terrestrial animals). Metal contamination of garden soils may be widespread in urban areas due to past industrial activity and the use of fossil fuels (Sánchez-Camazano et al., 1994).

Gardeners should avoid placing a vegetable garden near an older painted building or on a site where a building once stood. If this cannot be avoided, have the soil tested for lead and other heavy metals and contaminants. Soil also may be contaminated with heavy metals from automobile and equipment exhaust and pesticide use in old orchards. Vegetables growing on contaminated, but otherwise fertile soil, are contaminated mainly by dust on the leaves. Washing vegetables before consuming them negates this potential problem. Some vegetables absorb some of the heavy metals and become contaminated, posing a threat to the health of children, the elderly and ill people who eat them (www.hcs.ohio-state.edu).

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 195-198, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

Cobalt (Co) is a naturally-occurring element that has properties similar to those of iron and nickel. Small amounts of these chemical compounds can be found in rocks, soil, plants, and animals. Cobalt is even found in water in dissolved or ionic form, typically in small amounts. A biochemically important cobalt compound is vitamin B_{12} or cyanocobalamin. Vitamin B_{12} is essential for good health in animals and humans (www.atsdr.cdc.gov). Cobalt is essential in trace amounts for human life. It is part of vitamin B-12, and plays a key role in the body's synthesis of this essential vitamin. Cobalt has also been used as a treatment for anemia, because it causes red blood cells to be produced. The major source of cobalt is vegetables. The toxicity of cobalt is quite low compared to many other metals in soil. Exposure to very high levels of cobalt can cause health effects. Effects on the lungs, including asthma, pneumonia, and wheezing, have been found in workers who breathed high levels of cobalt in the air (Erdogrul, O, 2006).

Nickel (Ni) Small nickel particles in the air settle to the ground or are taken out of the air in rain. Much of the nickel in the environment is found with soil and sediments because nickel attaches to particles that contain iron or manganese, which are often present in soil and sediments (Erdogrul, O, 2006). Nickel is a component of some enzymes, this element playing an important role in all plant species which contain these enzymes (some cucurbitaceous plants) (Barry T.,2006).

Lead (Pb) In general, plants do not absorb or accumulate lead. However, in soils testing high in lead, it is possible for some lead to be taken up. Higher concentrations are more likely to be found in leafy vegetables and on the surface of root. Since plants do not take up large quantities of soil lead, the lead levels in soil considered safe for plants will be much higher than soil lead levels where eating of soil is a concern. Generally, it has been considered safe to use garden produce grown in soils with total lead levels less than 300 ppm. The risk of lead poisoning through the food chain increases as the soil lead level rises above this concentration. Even at soil levels above 300 ppm, most of the risk is from lead contaminated soil or dust deposits on the plants rather than from uptake of lead by the plant (Rosen, C., 2002).

Cadmium is the most element amoung all analyzed heavy metals.Cadmium is concentrated in particular parts of plants. As a general rule, leaves contain the most, followed by storage roots and tubers, seeds or grain and fleshy fruits (Murtaza, G., 2008).

MATERIALS AND METHODS

Were used chemicals and reagents from Merck; deionized water.

Soil samples were taken (0-25 cm depth) before vegetables cultivation.

Heavy metals determination: 20 g fresh vegetables were dried at 105^0C to 3 hours, calcinated at 650^0C for 3 hours; added 10 mL HNO_3 0.5N solution and to run dry. The mineral residue were solubility in 25 mL HNO_3 0.5N (MAIA, 1983). Determination of heavy metals were determinate by atomic absorption spectroscopy using Continuum Source Atomic Absorption Spectrometer contrAA®300 by Analytik Jena.Using standard work conditions - flame type: C_2H_2/air; flame height: 6mm; air flow: 568 L/h; acetylene flow: 50 L/h. The wavelength dominate (λ) for each chemical elements: Cd – λ=228 nm, Co – λ= 240 nm, Ni – λ= 232 nm, Pb – λ= 217 nm.

Were used the work protocol that is stipulated in the AOAC standards.

All determination was repeated for three times.

RESULTS AND DISSCUSIONS

The experience was done in a cambic cernosium soil, with low acidity reaction and the high natural fertility potential favorable vegetables cultivation. Experimental data of heavy metals content in soil are presented in table 1.

Table1. Heavy metals content in soil

Heavy metals	**Co [mg/kg]**	**Ni [mg/kg]**	**Pb [mg/kg]**	**Cd [mg/kg]**
Averange values	4.8	14.8	10.3	0

In comparison with official limits, the values for heavy metal content in soil are in the normal range for all elements. Cd is not present in soil. Maximum limits accept in Romanian legislation for heavy metals in soil: Pb and Ni-20.0 mg/kg dry matter, Cd - 1.0 mg/kg (Ordinance 756/1997).

In table 2 was presented the metals content in vegetables.

Table2. Heavy metals content in some vegetables cultivated in west area.

Analysed product	**Co [mg/kg]**	**Ni [mg/kg]**	**Pb [mg/kg]**	**Cd [mg/kg]**
Pepper	0.05	0.22	0.02	0.02
Tomatoes	0.05	0.07	0.00	0.01
Cucumber	0.05	0.10	0.02	0.02
Eggplants	0.05	0.11	0.01	0.02

Maximum limits accept in Romanian legislation for heavy metals in vegetables: Pb-0.5 mg/kg fresh matter, Cd, Co and Ni -0.1 mg/kg (Ordinance 975/1998).

The European Community legislation impose lower values for these limits: 0.1 mg/kg for vegetables (Directive 2001/22/EC).

The cobalt concentration was found in the same values (0.05mg/kg) for these vegetables.

Ni is present only in trace, in the range of 0.07-0.25 mg/kg for vegetales.

Lead is a heavy metal with a higher toxic potential, the maximum admissible limits being 0.5 mg/kg for fresh vegetables. Pb is not present in tomatoes fruit. In the study lead was identified in relatively lower concentrations, which are in the maximum admissible limits from our country, but higher than the limits imposed by the European Community. The most contaminated vegetables with lead is pepper and cucumber (0.02 mg/kg).

In all product samples the concentration of cadmium was very small, these concentrations were in the range from 0.01 to 0.02 mg/kg.

CONCLUSIONS

The lowest concentrations were determined for lead and cadmium.

Middle concentrations were determined for cobalt, and nickel.

The studied areals do not presents any risk of pollution/contamination with this heavy metals.

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MICROELEMENT CONTENT IN FRUITS OF FIG GENOTYPES GROWN IN HUNGARY AND IN COMMERCIALLY AVAILABLE DRIED FIGS

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ABSTRACT

Our research was carried out to determine the antioxidant capacity, total phenolics content and nutrient element contents in the fruits of fig (Ficus carica L.) grown in Hungary as well as in dried fruits commercially available in the Hungarian market. In this study, the possibility of fig cultivation was investigated. The climate changing of the Carpathian Basin may favour an increase in the cultivation of this species all around this region. Our results show that fig fruits are naturally rich sources of minerals particularly as concerns elements K, Mg, Ca, Na, and P. In addition, we found a high antioxidant capacity, especially in dried figs. This might be due to a relatively gentle way of processing by preserving the heat sensitive compounds and a resultant increase in the dry mass. Our study demonstrated that fig might be considered as a perspective fruit with putative application under special diet requirements by providing high amounts of polyphenols and nutrient elements.

Key words: antioxidants, Ficus carica, fig, macro and microelements, polyphenolics

INTRODUCTION

The Hungarian climate is regarded continental temperate. According to the four seasons, there are huge differences in it: January is the coldest and July is the warmest month. The annual median temperature is approximately 12°C, the maximum is 38 °C, while the minimum is -10 °C. Spring starts in early April with big rainfalls. Summers are dry and hot. Autumns are chilly, misty and wet and winters are moderately cold and usually dry, but sometimes sunny. The annual rainfall is around 550-580 mm. According to the forecasts of scientists, the average temperature of the Carpathian basin is expected to increase by 1.5-8^{o}C in the next 100 years. That kind of climate-changing would be able to make the opportunity to increase the importance of fruits, which had difficulties in the adaptation to previous ecological conditions.

Fig (*Ficus carica* L.) might be one of these plants. Figs have been known in Hungary for centuries as a fruit-bearing plant of upland historical wine-growing districts or as an ornamental and fruit-bearing plant with an exotic appearance cultivated in sheltered gardens (Reszelő, 2005). Because of its climate requirements (temperature and light), it could have been grown safely exclusively on hills in

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 200-206, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

southern exposition, because its canes might be damaged in winter below an average temperature of -15^{o}C (Jeszenszky, 1959, 1963). In Hungary, there are some fig clones (local cultivars) which have been adapted to the Hungarian climate. These belong to the group of common or Adriatic type figs. This group has seedless fruits and sets without fertilization.

These local cultivars have been established in Hungary since the Turkish occupation and could have been remained over time, because they can be easily propagated by layering and by softwood or hardwood cuttings (Schmidt and Tóth, 1996).

MATERIALS AND METHODS

In this study, dried fruits of fig from different southern country (Turkey, Iran, and Syria) and fresh fruits of different Hungarian fig clones were examined. Two of the three fresh pear-shaped figs were characterised by green skin colour and the remainder was purple (Table 1).

Table 1. The codes used to label fresh and dried fig samples.

	Genotype, extraction	Code
Fresh figs	Almási fehér, 70% MeOH	**FFAF A**
	Almási fehér, water	**FFAF V**
	Almási lila, 70% MeOH	**FFAL A**
	Almási lila, water	**FFAL V**
	Házi 70%, MeOH	**FFI A**
	Házi, water	**FFI V**

	Country of origin, extraction	Code
Dried figs	Assyria, 70% MeOH	**AFA A**
	Assyria, water	**AFA V**
	Iran, 70% MeOH	**AFI A**
	Iran, water	**AFI V**
	Hungary, 70% MeOH	**AFM A**
	Hungary, water	**AFM V**
	Turkish, 70% MeOH	**AFT V**
	Turkish, water	**AFT A**

The element contents were determined from freeze-dried samples with ICP-OES IRIS Thermo Jarrel ASH instrument.

Total soluble phenols were determined using Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965). The content of soluble phenols was calculated from a standard curve obtained with different concentrations of gallic acid.

Antioxidant power was determined by the FRAP (Ferric Reducing Ability of Plasma) method at λ=593 nm (Benzie and Strain, 1996). Deionized water or 70% (V/V) methanolic extraction was performed from the homogenized samples. After centrifugation (4 ^{o}C, 35-min, 18,750 g_n) clear supernatants were utilized for redox analyses.

Dry matter content was measured to facilitate comparison between dried and fresh fruits.

RESULTS AND DISCUSSIONS

The results of the experiments carried out on fresh and dried fig samples are presented with their codes listed in Table 1. Table 2. shows the dry matter content of each sample in percentage. According to these data, dry matter contents of dried figs are approximately 4-4.5 times higher than those of fresh fruits. Thanks to the low water and high sugar contents due to water evaporation, dried figs can last long, and hence this technique have been utilized for thousand years.

Table 2. Dry matter content of fresh and dried fig samples.

Dry matter content %	**Fresh fig**				**Dried fig**			
	FFAL	**FFAF**	**FFI**		**AFA**	**AFI**	**AFM**	**AFT**
	23,28	34,01	19,34		96,65	88,31	90,32	96,29

Among the mineral elements, those having a value below the detection limit were not included (Table 3). Huge differences in element contents in fruits originating from Hungary or the Middle East may have several explanations. The influencing effects of different soil characteristics and a wide range of biochemical factors may be involved as it was previously shown (Stefanovits-Bányai et al., 2005, 2006; Hegedűs et al., 2008). In addition, discrepancies in genetic constitution may be also notable since genetic diversity in the Middle East region was shown to be high for several fruit species (Ercisli, 2004; Halász, 2007a,b; Ercisli et al., 2008).

Table 3. Nutrient element content of fresh and dried figs (μg/g).

Elements	**Apple**	**Fresh fig**				**Dried fig**			
μg/g	**(avarage)**	**FFAL**	**FFAF**	**FFI**		**AFA**	**AFI**	**AFM**	**AFT**
Al	5,985	19,91	10,34	12,48		18,07	15,1	17,68	**24,66**
B	21	7,099	9,194	11,68		17,42	**20,63**	11,81	15,05
Ba	1,8	9,193	0,8021	1,736		5,789	4,238	7,071	**9,806**
Ca	58	1473	1657	3100		2315	**3553**	1979	3254
Cu	12,1	6,916	6,55	6,557		9,164	7,725	4,581	**11,09**
Fe	2,5	18,64	25,68	21,18		23,36	18,64	25,22	**26,51**
Ga	x	x	2,051	0,8205		**9,22**	0,6154	x	4,965
K	1220	**11977**	10943	9554		11223	8725	7729	10888
Mg	403	536	837,1	1055		892,5	1021	675,8	**1138**
Mn	x	1,393	1,393	3,018		4,45	4,992	5,815	**6,164**
Na	12	99,76	127,2	123,2		1447	606,8	411,7	**2828**
Ni	x	1,384	1,109	1,274		2,171	1,274	2,153	**2,195**
P	120	987,5	**1114**	970,7		1110	692,1	618,3	1103
Si	x	74,49	72,11	74,43		87,3	98,08	86,9	**100,8**
Sr	x	4,05	4,445	9,024		9,553	**49,83**	10,5	14,99
Zn	6,1	**20,14**	10,85	9,552		12,97	8,152	5,275	13,85

Compared with apples, figs generally contain significantly higher amounts from Ca, Fe, K, Mg, and Zn. The dried fig samples originating from the Middle East have extremely high contents from such elements.

RDA% (Recommended Daily Allowance) were calculated for microelements of fresh and dried figs compared with a common apple and these data are represented on Table 4.(Belitz et al., 2005).

Table 4.:RDA% of microelements of the fresh and dried figs

Elements		Al	Ca	Cu	Fe	K	Mg	Mn	Na	Ni	P	Si	Zn
Fresh fig	RDA% / 100mg	36,39	5,31	13,64	3,72	7,00	5,17	1,41	0,10	116,66	2,62	5,62	2,76
Dried fig		175,37	25,78	60,50	14,51	22,68	21,64	14,21	4,24	658,15	8,18	25,87	7,48
Apple		7,78	0,075	12,58	0,22	0,40	1,31		0,01		0,16		0,63

In case of both total antioxidant capacity and total soluble polyphenol content, big differences were detected between the fresh and dried fig samples. Moreover, dried figs showed 4-4.5 times higher values than fresh fruits (Fig. 1., Fig. 2.).

However, the differences among dried figs may come from the different drying technologies used and from the distinct features of specific plant genotypes. The correlation between total soluble polyphenolics content and FRAP values seems to be close (R=0.89284), so it might be supposed that in case of figs total soluble polyphenolics content has the most decisive contribution to the total antioxidant capacity.

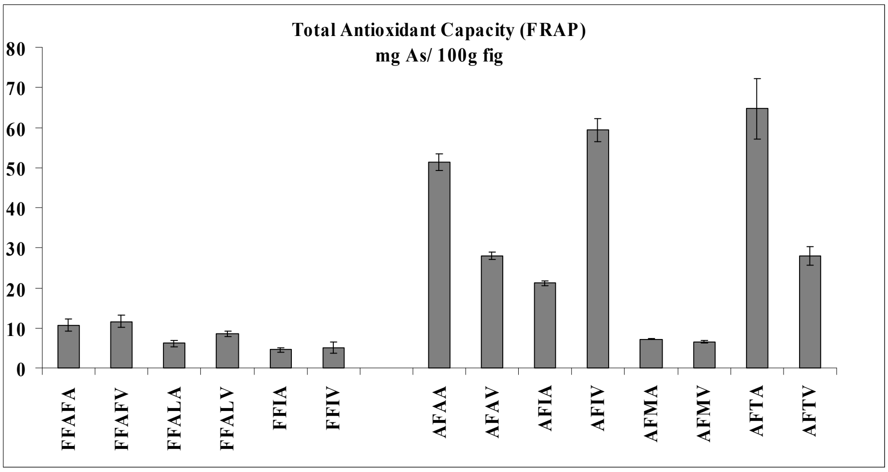

Fig. 1. The total antioxidant capacity of water and methanolic extracion from fresh and dried figs (mg AS/100g).

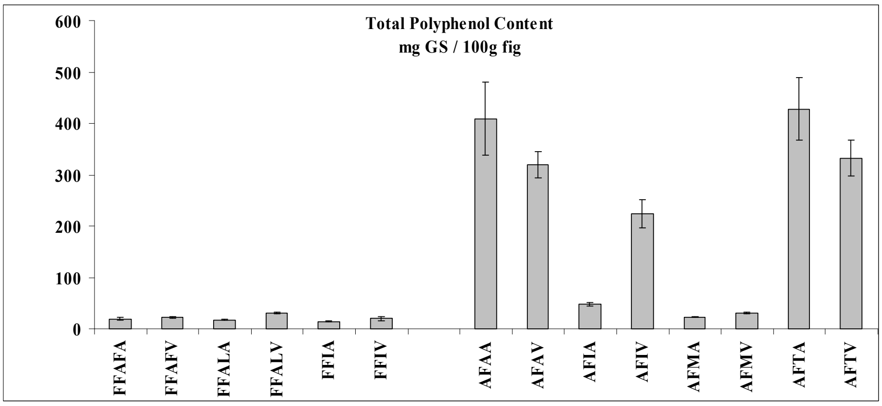

Fig. 2. The total polyphenolics content of water and methanolic extracion from fresh and dried figs (mg GS/100g).

CONCLUSIONS

This study demonstrated that fig might be considered as a perspective fruit with putative application under special diet requirements since it provides high amounts of polyphenols and nutrient elements. Combining the results for ICP and redox analyses, we can conclude that higher element contents of dried figs may not only be attributed to the different soil conditions in foreign countries, but also a putative higher chelating capacity in these fruit samples due to their high polyphenolic contents. Even if flavonoids are devoted to metal chelation they retain their radical scavenging capacity (Lugasi et al., 2003) and can have a crucial contribution to the antioxidant capacity of fruits.

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BODY ZINC LEVEL, EATING HABITS AND SOME NUTRITIONAL PARAMETERS IN AN ELDERLY GROUP

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ABSTRACT

Elderly people is an important population group affected by the zinc deficiency. One of the cause of this deficiency may be the nutrition. The aim of the study was to search the relation between the zinc level and some eating habits and between the zinc level and some nutritional parameters in a group of elderly (60 – 85 years) comparing with a group of adults (20 – 59 years). We found in all subjects an insufficient energy intake and some nutritional imbalance. In elderly serum zinc positively correlated with ingested energy and negatively with ingested animal fats. The frequency of food consumption generally did not correlate with zinc level. Investigated biochemical parameters correlated with serum or erythrocyte zinc level both in older and younger subjects, emphasizing the implication of the metal in protein, lipid and glucose metabolism.

Key words: zinc, elderly, eating habits, biochemical parameters

INTRODUCTION

Zinc plays an essential role in living bodies, being indispensable for cellular functions. Zinc deficiency affects at least 1 in 5 people worldwide (Maret & Sandstead, 2006) and among the implicated population groups elderly people are an important one. It is assumed that zinc deficit gradually occurs; its biodisponibility for intracellular enzymes decreases, and the ageing process is initiated. It was suggested that intracellular zinc deficiency may be the first biochemical event that starts the whole spectrum of functional changes that lead to the loss of division capacity and to the cellular death in cells culture in vitro (Hayflick, 1982). There are many causes of zinc deficit in elderly, one of them being nutrition. Today, the elderly tend to avoid meat and other high zinc content food due to fears of cholesterol. Rather, they increase consumption of refined wheat products. Much more important than the quantity of ingested zinc are factors that condition zinc absorption. The proportion of the absorbed zinc may vary from 20 to 80 % of the ingested zinc. Phytic acid, vegetal fibres, calcium and copper decrease zinc absorption, while animal proteins, picolinic acid, some amino acids and peptides increase zinc absorption (Bulboaca, 2005).

„Metal Elements in Environment, Medicine and Biology”, Tome VIIII, pp. 205-210, Publishing House “Eurobit” Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

The aim of the study was to search the relation between the zinc level and some eating habits and between the zinc level and some nutritional parameters in a group of elderly comparing with a group of adults.

MATERIALS AND METHODS

Study was performed in a group of persons selected from the consulting rooms of two general practitioner physicians from the Iasi city. Initially, the elderly group (60 - 85 years) comprised 139 subjects that were compared with a group of 104 younger subjects (20 – 59 years). After removing the subjects suffering of diseases (stroke, allergy) and taking medicines (diuretics) that showed associations with the zinc level, the elderly group had 65 subjects and the younger group had 60 subjects.

The body zinc level was determined by assessing serum, erythrocyte and urine zinc using atomic absorption spectrophotometry. Serum glucose, total proteins, albumin, total cholesterol, HDL-cholesterol, triglycerides were determined. LDL-cholesterol was calculated. Eating habits were investigated by applying a questionnaire including weekly frequency of food consumption (14 items) and 24h recall of food intake. Total energy and the main nutrients were estimated. Anthropometric variable (weight and high) were registered and Body Mass Index (BMI) was calculated. Statistical analysis was carried out using Epi Info programme (Pearson correlation index , one way ANOVA test, “t” test).

RESULTS AND DISCUSSIONS

Some characteristics of the elderly were compared with those of the adults (Table 1).

Table 1. Characteristics of the study sample

Variable	**>= 60 years (n = 65)**		**< 60 years (n = 60)**	
	%	**Means +/- SD**	**%**	**Means +/- SD**
Age (years)		69.4 +/- 6.4		49.0 +/- 9.1
Weight (kg)		74.4 +/- 13.4		80.4 +/- 21.2
BMI (kg/m^2)		28.6 +/- 5.0		28.6 +/- 6.3
Income (low, medium, high)	45, 45, 10		39, 44, 17	
Education (low, medium, high)	43, 28, 29		17, 43, 40	
Living alone	20		2	
Smoking (never, previous, current)	65, 24, 11		60, 13, 27	

Table 2 shows the zinc levels of the two groups. One can see a significant difference between sex in elderly regarding serum and erythrocyte zinc. Subjects >= 60 years had serum and erythrocyte zinc levels nonsignificantly lower.

Table 3 shows an ingested energy under the recommended values (>= 2100 kcal for adults in Romania) (Ionuţ, 2004), a carbohydrate intake closed to low limit (50 – 60 % recommended) and a total lipid intake over the upper limit (24 – 34 % recommended) for the younger women. One can see a decrease of energy intake in old women, reflected especially by the decreasing of the proportion of ingested total lipids and the amount (g) of carbohydrates.

Table 2. Mean values of zinc

Specification		>= 60 years		< 60 years	
		Women	**Men**	**Women**	**Men**
Serum zinc	μg/dl	75.3 +/- 12.6 a: **p<0.05** b: p>0.05	86.3+/- 13.7 c: p>0.05	80.3 +/- 16.7	92.3 +/- 16.3
Erythrocyte zinc	μg/ml	11.7 +/- 1.4 a: **p<0.05** b: p>0.05	13.6 +/- 1.1 c: p>0.05	12.3 +/- 2.0	14.0 +/- 2.1
Urinary zinc	μg/l	544 +/- 425 a: p>0.05 b: p>0.05	701 +/- 561 c: p>0.05	472 +/- 287	842 +/- 650
Urinary zinc/ creatinine	μg/g	687 +/- 599 a: p>0.05 b: p>0.05	562 +/- 420 c: p>0.05	469 +/- 286	561 +/- 346

a: comparing with the older men; b: comparing with the younger women; c: comparing with the younger men

Table 3. Estimated daily energy and nutrients intake

Specification		>= 60 years		< 60 years	
		Women	**Men**	**Women**	**Men**
Energy	kcal	1545 +/- 474	2065 +/- 697	1836 +/- 730	1923 +/- 749
Total proteins	g	60.5 +/- 25.0	69.9 +/- 29.7	62.4 +/- 33.7	65.9 +/- 34.0
	% from energy	15.9 +/- 4.1	13.9 +/- 3.6	13.8+/- 4.3	14.0 +/- 3.7
Animal proteins	g	35.0 +/- 25.9	34.7 +/- 28.0	33.4 +/- 25.8	35.5 +/-27.8
	% from total proteins	50.0 +/- 30.7	45.0 +/- 26.9	47.7 +/- 22.8	49.1 +/-18.9
Total lipids	g	57.0 +/- 30.5	81.1 +\- 40.4	82.0 +/- 66.7	68.0 +/- 38.7
	% from energy	32.9 +/- 10.5	35.7 +/- 10.8	42.2 +/- 39.3	31.8 +/- 11.6
Animal lipids	g	26.3 +/- 22.8	30.8 +/- 27.3	31.8 +/- 24.9	31.6 +/- 23.7
	% from total lipids	44.1 +/- 31.1	36.3 +/-28.2	42.1 +/- 24.5	49.2 +/-27.3
Carbohydrates	g	186.8 +/- 61.4	252.1 +/- 102.0	220.8 +/- 88.2	247.6 +/- 100.3
	% from energy	51.0 +/- 12.6	50.2 +/- 11.2	50.4 +/- 11.3	54.2 +/- 13.4

Table 4 shows the frequency consumption of food. Older women ate less meet and vegetables than the younger ones and than the men. Younger men ate less dairy products and sweets than older men and than the women. The men consumed fewer eggs than women and the elderly people less than the young people. The old women ate more fish, pasta and pastry products comparing with the other groups. Older men eat less dry legumes than women and than younger people. Older women consumed fewer vegetables than the younger women and than the men. The consumption of vegetal fats and potatoes was comparable in all the groups. The consumption of animal fats was much higher in young men comparing with the other groups. The women drank alcohol beverages less than men and the older people less than younger people. The women drank coffee more than men and the younger people more than the older ones. One can note generally a reduced consumption of fish and vegetables and a high consumption of animal fats and coffee.

Table 4. Frequency of food consumption for a week (%)

Specification	**Frequency**	**>= 60 years**		**<60 years**	
		Women	**Men**	**Women**	**Men**
Meat	<= once	24	18	15	16
	2 – 3 times	38	25	22	32
	>= 4 times	38	57	63	53
Dairy products	<= once	18	11	25	42
	2 – 3 times	38	36	23	16
	>= 4 times	54	54	52	42
Eggs	<= once	51	64	40	47
	2 – 3 times	35	18	50	16
	>= 4 times	14	18	10	37
Fish	<= once	78	86	87	89
	2 – 3 times	22	14	13	11
Potatoes	<= once	30	10	25	26
	2 – 3 times	40	54	48	42
	>= 4 times	30	36	27	32
Dry vegetables	<= once	78	89	85	79
	2 – 3 times	22	11	15	21
Vegetables	<= once	30	11	22	11
	2 – 3 times	22	11	5	21
	>= 4 times	48	78	73	69
Fruits	<= once	13	7	17	16
	2 – 3 times	14	7	10	21
	>= 4 times	73	86	73	63
Sweets	<= once	59	56	48	68
	2 – 3 times	16	21	12	16
	>= 4 times	24	32	40	16
Pasta, pastry products	<= once	13	68	60	58
	2 – 3 times	65	25	28	37
	>= 4 times	22	7	12	5
Vegetal fats	<= once	78	64	70	74
	2 – 3 times	13	18	20	21
	>= 4 times	8	18	10	5
Animal fats	<= once	68	57	57	16
	2 – 3 times	24	32	28	63
	>= 4 times	8	11	15	21
Alcohol beverages	<= once	84	65	70	58
	>=2 times	16	36	30	42
Coffee	<= once	35	50	22	42
	>=2 times	65	50	78	58

Total proteins and albumin values did not significantly vary between sex and groups (Table 5). HDL-cholesterol slightly decreased with age being lower in men. Triglycerides and glucose were slightly increased in elderly.

Table 5. Mean values of biochemical parameters

Specification	**Years >=60**		**Years <60**	
	Women	**Men**	**Women**	**Men**
Glucose	90 +/- 31	92 +/- 26	86 +/- 25	91 +/- 38
Total proteins	83 +/- 4	85 +/- 4	84 +/- 4	85 +/- 4
Albumin	48 +/- 5	48 +/- 5	48 +/- 5	48 +/- 6
Total cholesterol	232 +/- 33	224 +/- 37	228 +/- 32	230 +/- 38
HDL -cholesterol	55 +/- 13	50 +/- 12	58 +/- 11	55 +/- 12
LDL-cholesterol	150 +/- 34	144 +/- 39	147 +/- 32	147 +/- 42
Triglycerides	136 +/-46	148 +/-54	124 +/- 52	140 +/- 43

The following of the relationships between zinc and ingested nutrients in elderly, showed a positive correlation between serum zinc and ingested energy, a negative correlation between serum zinc and animal lipids (% from total ingested lipids) and a positive correlation between urinary zinc/creatinine and animal proteins (%) (Table 6). A similar tendency of correlation urinary zinc/creatinine - animal proteins was emphasized in the younger subjects.

Table 6. Correlations between zinc level and ingested nutrients

Age (years)			**n**	**r**	**p**
>= 60	Serum zinc	Energy	64	+ 0.23	**0.017**
	Serum zinc	Animal lipids (%)	62	- 0.29	**0.024**
	Urinary zinc/creatinine	Animal protein (%)	51	+ 0.28	**0.047**
< 60	Urinary zinc/creatinine	Animal protein (%)	46	+ 0.28	0.057

Serum and erythrocyte zinc did not associated with the frequencies of food consumption probably because of many factors that determine zinc status. Only urinary zinc/creatinine positively associated in elderly with eggs consumption (p=0.040).

Serum zinc level positively correlated withy serum total proteins and LDL-cholesterol and negatively with HDL-cholesterol in elderly (Table 7). Also, erythrocyte zinc level positively correlated withy total proteins and albumin and urinary zinc/creatinine positively correlated with glucose.

In younger subjects, we found a positive correlation between serum zinc and total proteins, between erythrocyte zinc and total proteins and triglycerides and a negative correlation with HDL-cholesterol. Urinari zinc/creatinine positively correlated with glucose and negatively correlated with total cholesterol and LDL-cholesterol.

Our results are in agreement with those of Hermann et al. (1993) who found that both plasma total cholesterol and LDL-cholesterol were elevated with lower zinc intakes. Recently Mazzatti et al. (2007) found that many genes involved in lipid and cholesterol homeostasis are differentially regulated by zinc in an age-dependent manner.

Table 7. Relationships between zinc level and biochemical parameters

Specification	**Parameter**	**>= 60 years**			**< 60 years**		
		n	**r**	**P**	**n**	**r**	**P**
Serum zinc	Total proteins	65	+ 0.35	**0.005**	59	+ 0.25	0.055
	HDL-Cholesterol	65	- 0.39	**0.000**			
	LDL-Cholesterol	65	+ 0.26	**0.036**			
Erythrocyte zinc	Total proteins	63	+ 0.44	**0.000**	58	+ 0.30	**0.022**
	Albumin	60	+ 0.34	**0.009**			
	HDL-Cholesterol				53	- 0.30	**0.030**
	Triglycerides				59	+ 0.37	**0.005**
Urinary zinc/creatinine	Glucose	53	+ 0.26	0.061	49	+ 0.48	**0.000**
	Total cholesterol				49	- 0.29	**0.045**
	LDL - cholesterol				45	- 0.29	0.055

CONCLUSIONS

1. We found in all subjects an insufficient energy intake and some nutritional imbalance.
2. Serum zinc positively correlated with ingested energy and negatively with ingested animal fats.
3. The frequency of food consumption generally did not correlate with zinc level.
4. Investigated biochemical parameters correlated with serum or erythrocyte zinc level both in older and younger subjects, emphasizing the implication of the metal in protein, lipid and glucose metabolism.

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EFFECT OF FOLIAR MAGNESIUM FERTILIZATION ON LEAF AND FRUIT MINERAL COMPOSITIONS OF GREENHOUSE GROWN CUCUMBERS

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ABSTRACT

In our experiment we studied the effect of foliar magnesium application (Hydromag, in concentrations of 0.2 and 0.4%, a total of 300 ml solution per plant) on leaf and fruit mineral composition with cucumber plants (Cucumis sativus cv. Mustang) grown in peat in containers under greenhouse conditions. Foliar applications were carried out 5 times, 10 days apart. Plants receiving no foliar treatment were also grown as control. For the purpose of laboratory analysis fruit and leaf samples were collected twice. Fruit mineral composition was determined using the apparatus ICP-OES IRIS Thermo Jarrel ASH, Corp., Franklin, MA, USA. The Mg content of the fresh cucumber fruits did not show any increase in response to the foliar treatments. At the first occasion of measurement, the Mg content of the leaves of the treatments with foliar magnesium application were higher than the value registered for the control, but on the second occasion we obtained results that were just the opposite. The foliar Mg fertilization changed the macro- and microelements concentration of leaves in cucumber fruits.

Key words: cucumber, magnesium, mineral composition, foliar fertilization

INTRODUCTION

Peat is one of the most commonly used root media in the ever more popular container vegetable growing in greenhouse. In the case of this growing method roots have a much smaller volume available as compared to traditional growing in real soil. Therefore a fertilization with a daily frequency is applied using complex chemical fertilizers (Göhler – Drews, 1990). On the other hand, based on the experiences from production, it can be seen that yields and quality can be improved further by foliar fertilization and this way the harmful effect of the nutrient antagonisms occurring in the root medium can also be eliminated.

40-50% of the magnesium absorbed by plants is assimilated by the foliage (Terbe, 1989). The beneficial effects of the application of magnesium as a foliar fertiliser have been demonstrated for several plants (saffron: Takácsné Hájos et al., 2001; vine: Moretti, 2002; tomato: Takács Hájos et al, 2003; pear: Gastol - Domagala-Swiatkiewicz, 2006). Magnesium applied in the form of foliar fertilizer will remain in the leaves and will not move together with plant saps. The young leaves of

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 211-216, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

a healthy cucumber plant contain 0.5-0.8% magnesium, while the elder ones 0.8-2.0%. The efficiency of the fertilizer spraying is highly dependent on the time of application. In general, the best result can be achieved during the period of intensive development (Fazekas et al., 1992).

The importance of magnesium is manifested in the first place in protein synthesis and photosynthesis, as well as in the enzyme reactions of starch formation (Marschner, 1986).

Occurrence of deficiency symptoms can be expected under 0.3-0.4%. As a result of an inadequate supply, magnesium will be mobilized from the older plant organs to the young ones, therefore the chlorosis of older leaves is observable first (Terbe, 1992; Ushijima at al, 2004).

MATERIALS AND METHODS

The experiment was set up at the Experimental Farm of the Faculty of Horticulture, Corvinus University of Budapest in a plastic tunnel greenhouse of Filclair type, under unheated conditions.

The following 3 treatments were applied in 4 repetitions (5 plants/plot):

Mg1 = 0.4 g/l Mg as foliar fertilizer
Mg2 = 0.8 g/l Mg as foliar fertilizer
Control= no treatment (receiving only fertigation)

For the purpose of the foliar fertilizer we used the foliar fertilizer product Hydromag (Phosyn fertilizer family) (Mg concentration: 200 g/l) and in the determination of the concentration we followed the manufacturer's recommendation for the bottom and top limits (0.2 and 0.4%).

The variety Mustang (Cucumis sativus L. cv. Mustang) was chosen as test plant.

Growing was carried out in black buckets of 12 litres having rigid walls in which 10 litre medium was filled, respectively. For the purpose of root medium we used a 1:1 volume mixture of fen and raised bog peat which was given 1.5 kg/m^3 fodder lime in order to set the pH value.

Seeds were sown in 9 cm pots on March 3, in a mixture of peat and sand. Seedlings were transplanted on April 7. 1.95 plants per square meter were arranged at a spacing of 160 x 32 cm. Shoots were trained on a string trellis up to the horizontal wires stretched at 240 cm high. Plants were pruned to the main stem.

After transplanting plants received nutrient solution on a regular basis according to Table 1. The nutrient solution was applied always in the morning hours and if it was necessary some irrigation was done in the afternoon hours using either plain water or nutrient solution and taking care not to leach the nutrient solution out of the medium.

Table 1: Frequencies and concentrations of drip fertigation

Period	**Frequency**	**Amount of Nutrient Solution (l/day/plant)**	**Fertiliser composition (g/1000l)**				
			N	**P_2O_5**	**K_2O**	**Mg**	**Ca**
April 7-8*	Daily	0.5	300	600	300	-	-
April 11-19*	Every two days	1	433	410	260	10	47
April 20 – June 8**	Daily	1	253	105	315	10	47
June 9 – July 7*	Daily	1-1.5	443	245	280	10	47

* 1,000 litre nutrient solution contained also: Fe: 2.6g; Mn: 0.6g; Zn: 0.4g; Cu:0.26g: B: 0.06g; Mo: 0.04g active ingredient.

** 1,000 litre nutrient solution contained also: Fe: 1.95g; Mn: 0.45g; Zn: 0.3g; Cu:0.195g: B: 0.045g; Mo: 0.03g active ingredient.

Foliar fertilization was applied at every 10-14 days, 5 times in all (for the first time on May 13 and for the last on July 1). Using a hand pump sprayer the nutrient solution was applied evenly to every plant leaf in an amount to provide a thin uniform coating over them. The amount applied in this way over the five occasions was a total of 300 ml/plant, i.e. 585 ml/m^2.

For the purpose of the laboratory analysis, leaf and fruit samples were collected twice (May 23 and June 27). The leaves came from the middle region of the plant. The mineral composition of the fruits, after their washing, cutting, homogenisation in a blender and drying and pulverization, was determined using an ICP-OES IRIS Thermo Jarrel ASH, Corp., Franklin, MA, USA. At the same time, we also determined the dry matter content of the homogenized pulp, this way we were able to perform calculations to convert the data registered in the dry samples to fresh weight.

For the statistical evaluation we used the single-factor analysis of variance at 95% significance level.

RESULTS AND DISCUSSIONS

Measurement results relative to the fruit and leaf magnesium contents are illustrated by Table 2. The magnesium content of the fresh cucumber fruits did not show any increase in response to the foliar fertilizer applications. It can be ascribed to the fact that the fruits contained the nutrient examined in an amount that was over the value given for the cucumber in general (9 mg / 100 g) even in the case of no foliar fertilization and this way probably the plants did not assimilate any more magnesium in the fruits.

At the first measurement time, the Mg content of the leaves of the treatments with foliar magnesium application were higher than the value registered for the control, but on the second occasion we obtained results that were just the opposite.

Table 2: Fruit and leaf magnesium contents

Plant part	1st sampling (May 23)			2nd sampling (June 27)		
	Control (µg)	Mg1 (µg)	Mg2 (µg)	Control (µg)	Mg1 (µg)	Mg2 (µg)
Leaf (1 g dry)	8227	9475	8435	12447	12169	11045
Fruit (1 g dry)	2951	2477	2675	2157	2212	2201
Fruit (100 g fresh)	13052	10846	11198	10075	9413	9345

The effect of the treatments on the different nutrients examined is summarized in Tables 3 and 4.

According to the results of our statistical analyses:

1. **leaf Ga content** at the first sampling time was diminished by the foliar application of magnesium, but was increased at the end of the growing season, particularly in response to the treatment Mg2, though there was no significant difference between the two foliar fertilization treatments;
2. **leaf Mn content** was increased by the foliar fertilization, in particular in the case of the Mg treatment with the lower concentration;

Table 3: Changes in leaf and fruit nutrient compositions in response to magnesium fertilization (1st sampling)

Treatment effect	Result of statistical calculations (SD 95%)	Leaf	Dry fruit	Fresh fruit
increase in response to treatment Mg1 (Mg1>Control)	SD exists	Zn		
	no SD	Al, B, Li, Mg, Mn, Mo	Al, B	Al
decrease in response to treatment Mg1 (Mg1<Control)	SD exists		Ba, Ca, Fe, K, Mg, Na, Si, Sr	Ba, Ca, K, Mg, Na, Si, Sr, Zn
	no SD	Ba, Ca, Fe, Ga, K, Na, P, Si, Sr	Mn, Ni, P, Zn	B, Fe, Mn, Ni, P
increase in response to treatment Mg2 (Mg2>Control)	SD exists		Fe	
	no SD	Al, Li, Mg, Mn, Mo, Na, Si, Ti, Zn	Al, B, Ba, Li, Mn, Zn	Al, B, Fe, Li, Zn
decrease in response to treatment Mg2 (Mg2<Control)	SD exists		Ca, K, Na, Sr	Ca, K, Mg, Na, Si, Sr
	no SD	B, Ba, Ca, Fe, Ga, K, P, Sr	Mg, Ni, P, Si	Ba, Mn, Ni, P
increase in response to concentration (Mg1<Mg2)	SD exists		Ba, Fe	Ba
	no SD	Ga, K, Mo, Na, Ni, Si	B, Ca, K, Mg, Mn, Mo, Na, P, Si, Sr, Zn	B, Ca, Fe, K, Mg, Mn, Mo, Na, P, Si, Sr, Zn
decrease in response to concentration (Mg1>Mg2)	SD exists			
	no SD	Al, B, Ba, Ca, Fe, Mg, Mn, P, Sr, Zn	Al, Ni	Al, Ni

3. **leaf Si content** was increased by the foliar fertilization at the second sampling time, in the case of the Mg1 treatment we observed significant increase as compared to the control; at the first measuring time, in response of the lower magnesium doses, the value was lower as compared to the control.
4. **leaf Ba content** at the second sampling time was significantly diminished in the Mg2 treatment as compared to the control and to the treatment Mg1;
5. **fruit Ca, K, Mg, Na, Si, Sr and Zn contents** at the first measuring time were significantly diminished in both magnesium treatments as compared to the control, while at the second measuring time we continued to observe a decrease for these elements (except for Ca and Mg) , but already there was no significant difference between the treatments;
6. **fruit Fe** content was significantly increased at the first measurement time by the treatment Mg2, but this was not observed at the second measurement time and a decrease was observed in this parameter at both measurement times in response to the lower dose;

Table 4: Changes in leaf and fruit nutrient compositions in response to magnesium fertilization (2nd sampling)

Treatment effect	Result of statistical calculations (SD 95%)	Leaf	Dry fruit	Fresh fruit
increase in response to treatment Mg1 (Mg1>Control)	SD exists	Ga, Mn, Si		
	no SD	B, Ca, Fe, K, Mo, Na, P, Ti, Zn	Al, B, Ba, Ca, Mg, Sr	Al
decrease in response to treatment Mg1 (Mg1<Control)	SD exists			P, Zn
	no SD	Al, Ba, Li, Mg, Sr	Fe, K, Mn, Na, P, Si, Zn	B, Ba, Ca, Fe, K, Mg, Mn, Na, Si, Sr
increase in response to treatment Mg2 (Mg2>Control)	SD exists	Ga		
	no SD	Al, Fe, K, Mn, Mo, Na, P, Si	Al, B, Ba, Ca, K, Mg, Sr	Al
decrease in response to treatment Mg2 (Mg2<Control)	SD exists	Ba		P, Zn
	no SD	B, Ca, Li, Mg, Sr, Ti, Zn	Fe, Mn, Na, P, Si, Zn	B, Ba, Ca, Fe, K, Mg, Mn, Na, Si, Sr
increase in response to concentration (Mg1<Mg2)	SD exists			
	no SD	Al, Ga, K, Li, Mo, Na	Al, B, Fe, K, Na	Al, B, Fe, K, Na
decrease in response to concentration (Mg1>Mg2)	SD exists	Ba		
	no SD	B, Ca, Fe, Mg, Mn, P, Si, Sr, Ti, Zn	Ba, Ca, Mg, Mn, P, Si, Sr, Zn	Ba, Ca, Mg, Mn, P, Si, Sr, Zn

7. at the second measurement time we observed that **fruit Zn and P contents** per unit of fresh weight were decreased compared to the control in both treatments receiving foliar application of magnesium.

8. **fruit Ba content** was significantly increased at the first measurement time with the increase in the concentration of the foliar fertilizer, while a slight decrease was observed during the second measurement period.

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BIODIVERSITY AND XENOBIOTICS RELATED TO FOODS OF ANIMAL ORIGIN

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ABSTRACT

Biodiversity is a notion that refers to the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems, and the ecological complexes of which they are part. This also includes diversity within species (genetic diversity), between species and of ecosystems. Biodiversity can be influenced by a number of natural and anthropogenic factors. The chemical composition of the environment and foods can be one of the factors influencing biodiversity. Xenobiotics are a class of chemical that can affect the living organisms and in some cases may interact with the deoxyribonucleic acid macromolecule generating cancerigenic, teratogenic or mutagenic effects. By their action at the level of the DNA macromolecule and also by their presence in the environment in high concentrations as anthropogenic pollutants xenobiotics can dramatically affect the ecosystem and may influence biodiversity.

Key words: biodiversity, animal food products, xenobiotics

1. CONSIDERATIONS REGARDING BIODIVERSITY

Biodiversity is a concept that defines the variation of life forms within a given ecosystem, biome, or the entire Earth. A more concise definition is given by Gaston and Spicer (2004) which define biodiversity as the variation of life at all levels of biological organisation.

This concept regards the world in its complexity, from genes to species, through the broad scale of ecosystems, thus summarizing the problem of "life on Earth" (Gaston, 1996). In this acception the definition of biodiversity is of interest not only for "living organisms" (biochemistry) and their complex interactions but also for the interactions with the environment (geochemistry).

The term „natural diversity" was first used in "The Preservation of Natural Diversity" a study elaborated in 1975 by "The Science Division of The Nature Conservancy". Later in 1985 Rosen uses for the firs time the term "biodiversity" in a planning for the "National Forum on Biological Diversity" organized by the National Research Council (NRC) which was to be held in 1986, and first appeared in a

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 217-224, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

publication in 1988 when entomologist Wilson used it as the title of the proceedings of that forum. After 1986 the term and the concept of biodiversity was used more often by biologists, ecologists, political leaders and peoples involved in the domain.

In 1993, a statistic following the keywords found in Biological Abstracts revealed the fact that the term biodiversity was used 72 times and the term biological diversity was used 19 times.

The first book on biodiversity appeared in 1997 with the title “Biodiversity II” (Reaka-Kudla et al. 1997). The book documents the rapid rise of the term "biodiversity" regarding its theoretical and applicative importance and influence. Also the book traces the study of aspects of biodiversity back to Aristoteles in the Antiquity

After the year 2000 the concept of biodiversity is more often found in the scientific publications, in the thematic of various scientific manifestations, and also appeared an increasing number of work groups and research centers regarding this domain (Bogdan et al., 2008).

2. CHARACTERISTICS OF BIODIVERSITY

2.1. Natural variations

The natural variations are conditioned by the evolution in time and can be classified in 3 subcategories: a) spatial variations; b) functional variations; c) morphological variations.

Spatial variations of biodiversity - may appear due to geological processes or to climacteric changes. The main geological processes that may influence biodiversity are the tectonical processes (tectonically movements, orogenesis, soil corrosion) and the marine processes (marine transgression and regression).

Functional variations - are time related and may refer to: circadian, circamensual, seasonal and circannual variations.

Morphological variations - refer to natural selection associated to geological and climacterical changes

The natural process of new species formation, which is the motor of biodiversity, usually takes place in two ways. The firs way is common to plants and consists in the process of polyploidy. This is a simple multiplication in the number of gene-bearing chromosomes - sometimes within a preexisting species and sometimes in hybrids between two species. A second major process is geographic speciation and takes much longer. It starts when a single population (or series of populations) is divided by some barrier extrinsic to the organisms. The isolated populations then diverge from each other in evolution because of the inevitable differences of the environments in which they find themselves. Since all populations evolve when given enough time, divergence between all extrinsically isolated populations must eventually occur (Wilson, 1988).

In order to have an image of the magnitude of natural biodiversity one must mention that in the year 2000 there were around 1,75 million species named by taxonomists (UNEP-WCMC, 2000).

2.2. Artificial variations

The artificial variations are conditioned by the human intervention on: a) the genome; b) eukaryotic organisms (animals and plants) and prokaryotic organism (microorganisms); c) products of vegetal and animal organisms .

Variations triggered by human interventions on the genome are of interest for medical biology (genic therapy) and for agricultural biology (genetically modified organism).

Those variations conditioned by human interventions on eukaryotic and prokaryotic organisms are regarding the growth-development systems (e.g.: farm animals), the cultivation systems related to hydric regime, temperature and minerals from soil

Variations due to human intervention on products of vegetal and animal origin have as destination: food industry, light industry, pharmaceutical industry, etc

An aspect of the modern science that can affect biodiversity is the genetic engineering that is increasingly used for the genetic improvement of crops. The organisms produced are referred to as genetically modified organisms (GMOs) or living modified organisms (LMOs) and efforts have focused on such crops as tomatoes, grains, cassava, maize and soybeans. Genes from any species of plant, animal, or microorganism can now be moved into a particular plant. Genetic engineering is not without risks and concerns have been raised about potential risks to biodiversity (Committee on Noneconomic and Economic Value of Biodiversity, 1999). In response to this concern, a subsidiary agreement to the Convention on Biological Diversity was negotiated to address the potential risks posed by cross-border trade and accidental releases of GMOs. Adopted in January 2000, the Cartagena Protocol on Biosafety was developed to ensure that recipient countries have both the opportunity and the capacity to assess risks relating to GMOs, and to ensure their safe transfer, handling and use.

3. BIOCONSTITUENTS OF ANIMAL PRODUCTS OF NUTRITIONAL INTEREST

The components of biodiversity (e.g.: plants, animals) are the source for foods, drugs, fibers, biofuels, and industrial products. About 150 species of plants have entered world commerce, and 103 species account for 90% of the supply of food plants by weight, calories, protein, and fat for most of the world's countries (Prescott-Allen and Prescott-Allen 1990). Just three crops - wheat, rice, and maize - account for roughly 60% of the calories and 56% of the protein consumed directly from plants (Yeatman et al, 1985).

General data regarding the composition of natural food products (unprocessed) are given in Table 1.

3.1. General aspects regarding nutrients

Nutrients are the chemical constituents of foods which, after the process of metabolization, ensure the morphophysiological status of the organism and the energy necessary for all vital processes. As a generally accepted classification, nutrients are grouped in: a) macronutrients – carbohzdrates, lipids, protides; b) micronutrients – biominerals, vitamins; other nutrients – water, alimentary fibers, bioactive substances.

Literature presents the complex problems of the mechanisms specific for toxicogenesis with implications on public health (Goudez-Perriere et al.,2003).

3.2. Particularizations for nutrients in foods of animal origin

In foods of animal origin predominate the lipids (acylglycerols, sterides, phospholipids, fatty acids etc.), proteins (aminated acids, holo- and heteroproteides).

Also there are significant quantities of biominerals and water. In more reduced amounts are carbohydrates, vitamins. There are no dietary fibers. Numerous papers present in detail this problem (Watt et al., 1975; Moll and Moll, 2003).

4. BIOCHEMISTRY AND XENOBIOCHEMISTRY IN RELATION WITH BIODIVERSITY

4.1. Comparative aspects

Xenobiochemistry or is a domain of Biochemistry which regards both molecular biology and pathobiochemistry. In order to better understand the field of work of xenobiochemistry, we must first define the term of xenobiotic and make the distinction between xenobiotics and nutrients (Garban, 2005).

Nutrients, as base constituents of aliments, accede into the organism through the digestive way (enteral) or parenteral (in therapeutic conditions). In the organism, the nutrients follow the metabolzation processes generating various metabolits with a great importance in all the functions of the body. The nutrients are involved especially in morphogenetic and energogenetic processes (Dumitrache, 1996; Kastori, 2000).

In the case of nutrients, which are studied by biochemistry, the concept of metabolisation defines their physiologic role in the organism. Metabolisation is characterized by two phases: a) catabolism (the totality of biodegradation reactions peculiar to nutrients); b) anabolism (the totality of biosynthesis reactions peculiar to nutrients).

Xenobiotics (xenos – foreign; bios – life), as non-nutritive substances accede in the organism usually along with the other nutrients and follow, gradually, the biotransformation process.

In the case of xenobiotics, which are studied in xenobiochemistry, the definitory concept is biotransformation. Similar to metabolism, in biotransformation there are also two phases:

a) xenobiodegradation – defined by the totality of biodegradation reactions peculiar to xenobiotics (i.e.: the oxydo-reduction reaction and hydrolyses reaction). After these reactions, the non-polar compounds which are biologically inactive are brought to a polar state and thus activated.

b) xenobiosynthesis – including the totality of biosynthesis reactions which are specific to xenobiotics. The xenobiosynthesis reactions contain conjugation reactions and reaction with adduct formation. The conjugation reactions are specific for the binding of xenobiotics to some endogenous substances e.g.: glucuronic acid, glycocol, acetylated compounds, sulphonic compounds, etc. The reactions that lead to adducts biogenesis take place between various organic and inorganic compounds and deoxyribonucleic acid (DNA).

4.2. Xenobiotics of alimentary interest as disturbants of biodiversity

Chemical xenobiotics of alimentary interest accede in the organism usually along with the nutrients. Once inside the body xenobiotics become part of the biotransformation process yielding various residual compounds which in some cases are electrophyl radicals that can interact inside the cells with the nucleophylic deoxyribonucleic acid (DNA) macromolecule. In the case of xenobiotics - DNA interactions a new complex arises called DNA adduct.

Among the xenobiotics that can lead to DNA adducts formation there are compounds from the following classes: a) metallic ions (e.g.: Hg, Cd); b) nitrosamines, polycyclic aromatic hydrocarbons, steroidal compounds, micotoxins (e.g.: aflatoxines),

Table 1. General chemical composition of the main food products of animal origin

Foods (name and description)			Water (%)	Proteids (g)	Lipids (g)	Carbohydrates (g)		Biominerals (mg)				
						Total	Fibre	Ca^{2+}	PO_4^{3-}	Fe^{2+}	Na^+	K^+
B			C	E	F	G	H	J	K	L	M	N
ovine	Beef meat		80.3	14.7	4.0	-	-	8.0	127	2.0	228	18
	Veal meat		80.7	15.5	2.7	-	-	10	145	1.7	226	21
	Organs	Hearth	77.5	17.1	3.6	0.7	-	5	195	4.0	86	19
		Liver	69.7	19.9	3.8	5.3	-	8	352	6.5	136	28
		Kidney	75.9	15.4	6.7	0.9	-	11	219	7.4	176	22
	Potted meat		60.7	17.5	19.2	-	-	-	-	-	-	-
wine	Fat class		33.4	9.1	57.0	-	-	5	88	1.4	70	28
	Medium fat class		37.3	10.2	52.0	-	-	6	103	1.5	70	28
	Thin class		41.1	11.2	47.0	-	-	6	116	1.7	70	28
vine	Lamb		56.3	15.4	27.1	-	-	9	135	1.1	75	29
	Organs	Hearth	71.6	16.8	9.6	1.0	-	11	249	-	-	-
		Liver	70.8	21.0	3.9	2.9	-	10	349	10.9	52	20
		Kidney	77.7	16.8	3.3	0.9	-	13	218	7.6	200	23
oultry	Turkey		64.2	20.1	14.7	-	-	-	-	-	-	-
	Hen		56.9	17.4	24.8	-	-	10	167	1.4	-	-
	Chicken		73.7	23.4	1.9	-	-	11	218	1.1	50	32
	Goose		51.1	16.4	31.5	-	-	10	176	1.6	-	-
	Quail		65.9	25.0	6.8	-	-	-	-	-	-	-
	Duck		54.3	16.0	28.6	-	-	10	176	1.6	-	-
ish	Cod		81.2	17.6	0.3	-	-	10	194	0.4	70	38
	Crap		77.8	18.0	4.2	-	-	50	253	0.9	50	28
	Herring		79.4	17.5	2.6	-	-	-	225	1.3	74	42
	Salmon		63.6	22.5	13.4	-	-	79	186	0.9	-	-
	Pike		78.3	19.3	1.2	-	-	-	214	0.4	51	31

etc. (Landis and Yu, 1995; Hodgson and Smart, 2001). These compounds are often found as food pollutants and some of them may lead to carcinogenic, mutagenic and teratogenic effects, thus influencing the genome and the biodiversity (Garban, 2008).

Refering at the contamination induced by metals there are important scientific papers that present the problem of their mutagenous and carcinogenous effects (Kazantsis and Lorna, 1979).

Genetic diversity provides the raw material for plant breeding, which is responsible for much of the increases in productivity in modern agricultural systems. Much of the genetic diversity available for crop breeding is now stored in a network of national and international genebanks administered by the UN Food and Agriculture Organization, the Consultative Group on International Agricultural Research, and various national agricultural research programs. The important contribution of genebanks to agricultural productivity has been recognized by government since the 18th century. It led to the rise of botanical gardens and expeditions in search of new plant varieties (Fowler 1994).

Another important aspect of influence of xenobiotics is the use of nitrates in agriculture. Besides their effects as xenobiotics of nutritional interest that range from interactions with hemoglobin up to formation of nitrosamines with cancerigene, teratogene and mutagene effects,nitrites affects the nitrogen cycle with unpredictable outcomes for biodiversity. Nitrogen accumulation in the environment has become a major cause of biodiversity loss. It has increased substantially in recent decades, primarily as a result of an increase in the use of fertilizer and the burning of fossil fuels. Increased nitrogen in soil and water can lead to loss of species and shifts in the species composition of plant communities (Wedin and Tilman, 1996).

5. SUSTAINABLE AGRICULTURE IN RELATION WITH THE PROBLEMS OF BIODIVERSITY AND XENOBIOCHEMISTRY

Sustainable agriculture is a concept defined by Perlas (1983) which in time led to the founding of International Alliance for Sustainable Agriculture (IISA).

The spreading of the studies and the broadening of the knowledge related to sustainable agriculture has lead to the elaboration of a generic model published in the book “Seven Dimensions of Sustainable Agriculture“(Perlas, 1993).

These seven dimensions are correlated with Agricultural biodiversity and Agricultural xenobiology (the xenobiochemistry applied in this field).

In this context one must take into account the following:

1) instead of pesticides, is recommended the use of ecological management principles;
2) replacement of chemical fertilizers with a integrated management of soil fertilization;
3) the use of monocultures is recommended to replace policultures, thus developing the biodiversity;
4) replacement of chemical treated seeds with alternatives strategies of reproduction, strategies that are adopted in the ecological practice
5) instead of soil erosion and water depletion, soil and water conservation

6) replacement of animal growing based on large farms with a zooculture based on growing animals in households or small farms where the ecological measures can be better implemented and supervised;
7) instead of applying techniques based on genes and chemical substances, using the formative energies from nature (i.e. biodynamic preparatives) is recommended

These percepts, even if without a rigorous explanation and without elucidated methods, can be constituted as “operating means” in sustainable agriculture.

Regarding foods, providing an adequate composition of nutrition, without contaminant metals, assures foods safety for the consumer. There are also preoccupations and papers elaborated on this topics (Lee, 1972; Moll and Moll, 2003).

CONCLUSIVE ASPECTS

Biodiversity in the animal reign, for interest in farm animal breeding and valorisation of their products, presents a special interest because almost all products are destinated for foods. One can observe the existence of a biodiversity of composition of foods originating from animals, conditioned by biogeographical conditions, ecologic frame, breeding technologies and evidently, animals feeding.

It must be mentioned the specificity of chemical composition interesting the proteins, lipids, carbohydrates and biominerals present in meat, organs at diverse species but also in animal products.

The paper presents aspects referring to meat and organs, showing data regarding their natural composition. From the point of view of xenobiochemistry this composition can be denaturated by the presence of diverse contaminants of organic nature (polycyclic aromatic hydrocarbons, micotoxins, steroids) or of inorganic nature (heavy metals, nitrates, nitrites).

Beside the contamination that appears in the existential environment of animals, there can also be remarked a contamination produced during processing of animal products in nutritional purpose (e.g. additivation with preservatives, sweeteners etc.).

In this context studies on biodiversity from the last decade approach more and more the problem of contaminats exclusion and the orientation toward an ecological nutrition.

These problems are possible applying the principles of a sustainable agriculture.

Acknowledgements: The authors express thanks to George-Daniel Ghibu – PhD student in chemistry and Cornel Baltă – PhD student in Veterinary Medicine for their participation at documentation in the domaine of xenobiotics of nutritional interest, some mentioned in the present paper.

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SNUTRIENT ELEMENT OF STONE FRUIT SPECIES AND CULTIVARS

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ABSTRACT

Several epidemiological studies revealed that the consumption of antioxidant compounds and the risk of atherosclerosis, increased blood pressure or cancer are inversely proportional. The individual amounts and relative proportions of macro- and micro elements present in food is also of great consideration since these are involved in a wide range of physiological processes including the influence of the redox homeostasis. This study was carried out to survey the antioxidant power and mineral element content in fresh fruits of four stone fruit cultivars. The elemental analysis of fruits was carried out by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). Considerable differences in element contents between cultivars of the same species occurred most often in sour cherries and apricots. Such differences could be primarily attributed to their different genetic constitutions. Japanese plums contained the smallest quantities from most of the elements. It points to their potential use in the treatment of some neurodegenerative diseases, where the restriction of the exposure to Al, Ba, Fe, Na, Mn, Zn is crucial. A detailed knowledge on the nutrient element content of fruits may offer the possibility to construct individual diets in response to the varied requirements arising from specific health conditions and promote the medical treatment and help the patients to get better in a natural way.

Key words: healthy diet, macro and microelements, minerals, Prunus, stone fruit

INTRODUCTION

Hungary has been witnessed a slight increase in the production of main stone fruit crops (eg. cherries, apricot, peaches and nectarines and plums) (Faostat, 2007). Probably the consumption of stone fruits will also rise, because people pay more and more attention for their health. Therefore, natural foods having enhanced functional properties may be very popular in the future.

The increased consumption of fresh fruits has several health benefits. Because of their high antioxidant capacity, they inhibit the harmful oxidative processes, and reduce the risk of several degenerative diseases (i.a. cancer, stroke, cardiovascular diseases) (Liu, 2003; Scalzo et al., 2005; Dauchet and Dallongeville,

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 225-230, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

2008). In addition, minerals have also considerable health effects (Stefanovits-Bányai et al., 2006; Hegedűs et al., 2008). Stone fruits are rich sources of the most important macro- (Na, K, Ca, Mg) and microelements (Fe, Cu, Zn, Mn) that have a considerable contribution to the human redox homeostasis through several mechanisms, e.g. they are indispensable cofactors of antioxidant enzymes. The transient metals, Fe and Cu ions may induce oxidative hazard because they can easily donate electrons to other molecules (Toyokuni, 2002; Valko et al., 2005). The Zn helps to build biomembranes, in absence of this metal element biomembranes may be easily disorganized. Knowledge on the Na/K ratio is indicative, because of the high Na levels in the human diet. Ca and Mg are required for the healthy bones and teeth, normal blood circulation, as well as for the efficient working of nerves and muscles.

The aim of this study was to evaluate the differences in the element contents of stone fruit species and cultivars.

MATERIALS AND METHODS

Fruit samples. Experiments were carried out by using 4 sour cherry ('Érdi bőtermő', 'VN-7', 'Oblacsinszka', 'Csengődi'), 4 sweet cherry ('Karina', 'Celeste', 'Firm Red', 'Sylvia', 'Van'), 5 Japanese plum ('Super Giant', 'Black Amber', 'Black King', 'Fortune') and 3 apricot ('Zard', 'Gönci Magyar', 'Aurórai') cultivars.

Sample preparation. 100 g fruit were homogenized (peel and flesh together) and freeze-dried.

Biochemical assays. 0.2 g of the dried fruit samples were weighted in teflon bomb (PTFE). 2.0 cm^3 nitric acid and 2.0 cm^3 hydrogen-peroxide were added to the samples and they were left standing for a night. Next day the closed teflons were boiled in hot water for half an hour. The digested samples were transfused in volumetric flask and supplemented to the volume of 10 ml with Milli-Q water. This solution was filtered through filter-paper into test tubes. The following elements were determined by ICP-OES (Thermo Jarrell Ash Co, ICAP 61): Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, P, Pb, and Zn.

RESULTS AND DISCUSSIONS

Sodium contents in the analysed fruits showed great discrepancies. In average, Na contents in sweet cherries and apricots were 2-3-fold higher than the levels that sour cherries and Japanese plums had. 'Érdi bőtermő' proved to be an exceptional sour cherry cultivar since its fruits have accumulated the highest quantities of Na. The average potassium content of all tested fruits was similar with only some outstanding values measured for the sour cherry cultivar 'Oblacsinszka' and the three apricots.

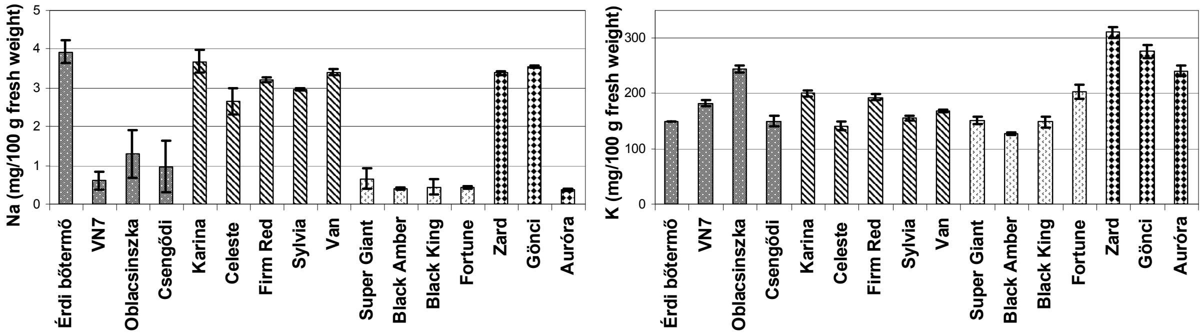

Fig. 1. Na and K contents in stone fruits. The grey, striped, V mottled columns and those with black diamonds refer to sour cherry, sweet cherry, Japanese plum and apricot cultivars, respectively.

From a nutritional aspect, the Na/K ratio may be of crucial importance. In the values there are fairly large margins. The lowest and hence the most favourable ratio was determined Japanese plums and in three of the sour cherry cultivars. These fruits contained the lowest levels of Na and the highest amounts of K. The sour cherry cultivar, 'Érdi bőtermő' proved to be an exception again with an outstandingly high Na/K ratio. Sweet cherries were also characterized by high values. The ratios were also high in case of two apricot cultivars, but were a little bit lower than those for sweet cherries.

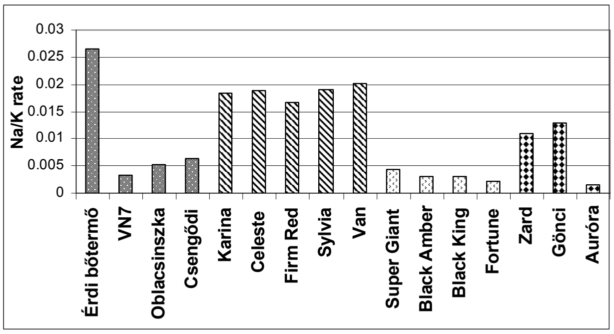

Fig. 2. Na/K ratio in stone fruits. The grey, striped, V mottled columns and those with black diamonds refer to sour cherry, sweet cherry, Japanese plum and apricot cultivars, respectively.

The apricots, sour and sweet cherries contained similar levels of Ca. Japanese plums had significantly lower levels from Ca, while the highest values could have been determined for the sour cherry 'Oblacsinszka', which possessed 22,1 mg Ca/100g fresh weight. Japanese plums also had the lowest quantities of Mg; without notable fluctuations among the tested cultivars. The rest of the fruits showed 2- to 3-fold higher Mg concentrations than plum cultivars. In our study, the 'Oblacsinszka' sour cherry had the highest Mg concentration (14,8 mg/100 g fresh weight).

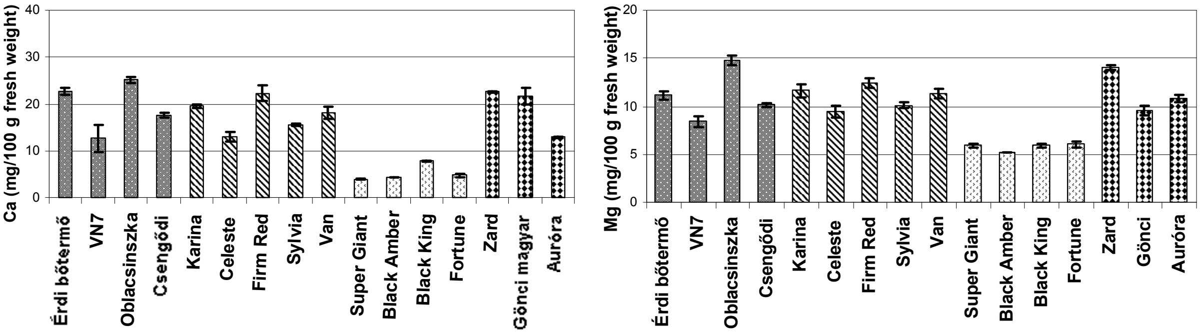

Fig. 3. Ca and Mg content of stone fruits. The grey, striped, V mottled columns and those with black diamonds refer to sour cherry, sweet cherry, Japanese plum and apricot cultivars, respectively.

In average, apricots showed the highest Cu contents, but concentrations were quite similar in the samples. The results ranged from 0.09 to 0.15 mg/100 g fresh weight. Only three cultivars could be identified as accumulating significantly lower values of Cu in their fruits. These include 'Csengődi' sour cherry, 'Celeste' sweet cherry and 'Gönci magyarkajszi'. The highest amount of Fe was determined for

‘Karina’ sweet cherry (0.37 mg/100 g fresh weight). Japanese plums showed the lowest values of Fe, its concentration ranged from 0.15 to 0.19 mg/100 g fresh weight in plums.

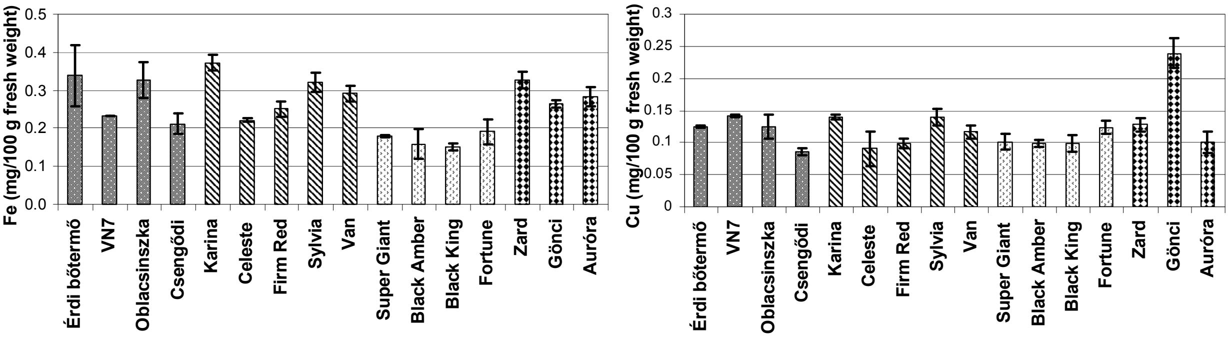

Fig. 4. Cu and Fe content of stone fruits. The grey, striped, V mottled columns and those with black diamonds refer to sour cherry, sweet cherry, Japanese plum and apricot cultivars, respectively.

As considers the Mn concentrations there could be observed only one outstanding value: in average, the ‘Celeste’ sweet cherry showed 2-fold higher element content than the rest of the fruits. From sour cherry cultivars, the ‘VN7’ had the highest values and approached the level determined for ‘Celeste’. The average Zn-content in Japanese plums and the sour cherries ‘Csengődi’ and ‘VN7’ was somewhat lower than the rest of the fruits, while the apricot cultivar ‘Aurora’ accumulate outstanding Zn levels.

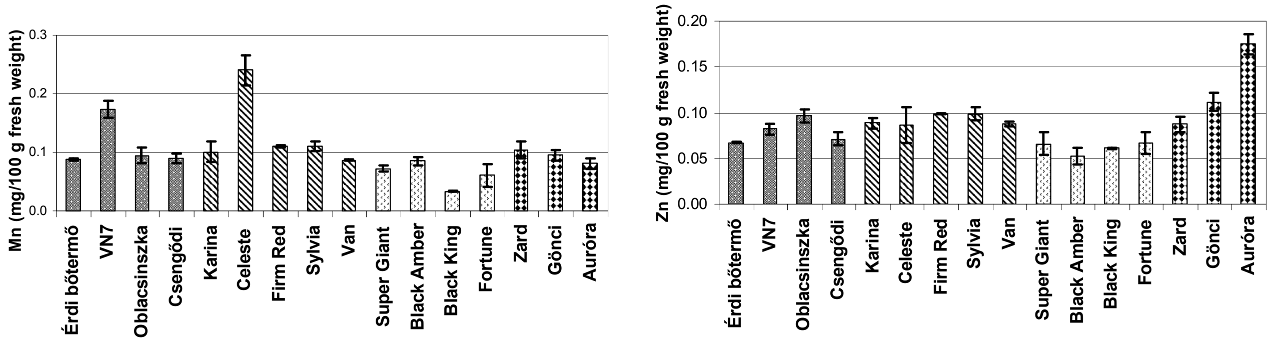

Fig.5. Mn and Zn content of stone fruits. The grey, striped, V mottled columns and those with black diamonds refer to sour cherry, sweet cherry, Japanese plum and apricot cultivars, respectively.

CONCLUSIONS

From the tested macro-elements relevant differences were obtained for the Na/K ratio where obviously the Na- concentration defines the value. For example, foods with high Na-content are not recommended for people who suffer from kidney- or nervous diseases. In contrast, fruits with low Na/K ratio (like Japanese plums) might be useful to compensate for high Na levels in a typical human diet (John et al., 2002). The Ca and Mg-contents were the lowest in the Japanese plums while the other three fruit showed nearly identical values.

The micro-element contents were also different among the species. In average, the Japanese plums accumulated the lowest levels from all four elements. It might point to their use in the treatment of some neurodegenerative diseases, where the decrease of the exposure to Al, Ba, Fe, Na, Mn, Zn is important (Stefanovits-Bányai et al., 2006).The results of the sour and sweet cherries were similar. Their values were in the middle of the range. The element content of the apricots revealed the most interesting tendencies. Their Cu and Zn-concentrations were the highest among all fruits, while their Fe and Mn levels were almost as low as for the Japanese plums.

Considerable differences in element contents between cultivars occurred most often in case of the species, sour cherries and apricots. Since all trees were grown under identical climatic and soil conditions, such differences among cultivars within each species may be attributed to their different origins and genetic constitutions (Halász, 2007a,b; Pedryc et al., 2005; Szabó, 2007; Szilvássy et al., 2008). It might offer the possibility to construct individual diets in response to the varied requirements arising from specific health conditions and promote the medical treatment and help the patients to get better in a natural way.

Stone fruits are characterized by seasonal availability in the markets. The food processing industry should find alternative ways to process these fruits while preserving their beneficial compounds, antioxidants, vitamins and mineral elements. Vegetable and fruit consumption are very important factors of a health-promoting diet, and statistic data show that Hungarian people do not eat enough from these foods. New products, which are rich in health protecting components, together with the required marketing strategy may help to alleviate this problem.

Acknowledgements. This work was co-financed by the Ányos Jedlik programme NKFP06A2-BCETKA06 and the NKTH-OTKA K68921 grant. Attila Hegedűs is grateful for receiving a János Bolyai Scholarship.

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INTESTINE AND LIVER HISTOPATHOLOGICAL CHANGES INDUCED IN FED AND STARVATED GOLDFISH *(Carassius auratus auratus, Cyprinidae, Pisces)* DURING CHRONIC EXPOSURE TO MANGANESE

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ABSTRACT

Two lots of Carassius auratus auratus were exposed to sublethal concentration of manganese for 28 days. A lot of individuals were fed daily to satiation, the other one starved during manganese intoxication. Intestine and liver tissue fragments were used in order to evaluate manganese concentration and structural changes. Metal exposure stress caused no mortality. Nutrition status, fish size and growth rate as well as tissue heavy metal concentration are important data for monitoring assessment. After 28 days of exposure to the pollutant fish exhibited statistically insignificant decrease in their weights. Manganese concentration was higher in intestine compared to liver. Intestine manganese concentration in fed fish was lower than in starved fish. Both stress factors induced histological changes to diminish absorption surface of the intestine. Injuries of epithelial layer, tip of the villi changes, villi fusion, macrophages accumulation and sinusoids capillaries dilatation were frequent.

Key words: manganese pollution, intestine, liver histopathological changes

INTRODUCTION

Manganese was regarded as one of the least toxic elements but more recent experimental and epidemiological studies have shown that exposure to manganese can indeed lead deleterious changes (European Commision, Health and Consumer Protection Directorate General, 2002).

Manganese accumulates in mitochondria, a major source of superoxide, which can oxidize Mn^{2+} to the powerful oxidizing agent Mn^{3+}. Oxidation of important cell components by Mn^{3+} has been suggested as a cause of the toxic effects of manganese.

Teleost intestine surface represents a selectively permeable barrier that allows nutrient absorption, but excludes most toxic substances and pathogenic organisms. Additionally, the intestine is involved in water and electrolyte balance, active transport of salts and water, as part of hydromineral homeostasis, immunity, and regulation of

„Metal Elements in Environment, Medicine and Biology”, Tome VIIII, pp. 231-238. Publishing House “Eurobit” Timişoara, 2008
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digestion and metabolism, the absorbtive transport of nutrients, Intestinal structure responds rapidly and reversibly to changes in dietary inputs.

In order to assess manganese effects on fish digestive system, intestine and liver histological changes and the influences of nutritional status on metal toxicity and accumulation were analyzed.

MATERIALS AND METHODS

Two years old individuals of *Carassius auratus gibelio* were kept in 150 liter tanks for two weeks before metal exposure. Pathological symptoms did not appear and fish were in good state of health. After acclimation period, manganese chloride was added in tap water to a final manganese concentration of 75 μg/L. Ten aquaria of 40 l capacity were used and each one was occupied by 10 individuals. The lots of individuals from five of aquaria were not fed, the other received food during the entire period of the experiment. Two aquaria were occupied by fed control individuals and starved control individuals respectively. Each 7 days, the water was replaced and manganese concentration of 75 µg/ml adjusted. We used a static water system with continuous aeration, 21±2.15 ºC temperature, pH 8.23±0.24, total hardness 1.75 nmol/ in $CaCO_3$, the amount of dissolved oxygen was 6 mg/l. The metal ions content was measured by atomic absorption spectrometry, using a Perkin Elmer AAS 600 graphite oven spectrometer. For manganese ions analysis, manganese solutions of 0 – 40 µg/l concentration were prepared. The following parameters were chosen: λ = 279.5 nm, slot=0.7 nm; two matrix modifiers: Pd and $Mg(NO_3)$; atomization temperature: 1900° C, atomization period: 3 sec. For each sample three replicates were made, repeating the test until standard deviation was lower than 3%. Liver and intestine fragments were removed after 7, 14, 21, and 28 days of exposure to the metal, then fixed in buffered formaldehyde 24 hours long and further prepared for light microscopy and Hematoxylin-Eosin stained. The tissues samples were investigated by Olympus CX 40 light microscope. Weight, standard lenght, total lenght were recorded for all individuals

RESULTS AND DISCUSSIONS

Water samples analysis showed that manganese content ranged between 61 and 80 µg/ml. Chronic exposure of fish to 75μg/L manganese caused no mortality. No weight, standard and total length changes occurred, excepting the lots of fed and starved individuals exposed for 28 days to the pollutant. This group showed an insignificant weight decrease as Javed (2006) noticed during nickel and manganese intoxication.

The main tissues that stored manganese were the intestine (42%) and liver (24.61%). It is known that liver plays an important role in metal sequestration because heavy metals may be bound to metallothioneins or other ligands (Dallinger, 1995). Manganese accumulation was higher in starved individual compared to the fed individuals (Fig.1). A higher metabolism of fed fish and a great efficiency in metal remove could explain the results. Our hypothesis is supported by a slow raise in liver metal concentration after 7 day exposure followed by a decrease due to detoxification processes (Fig. 2) .

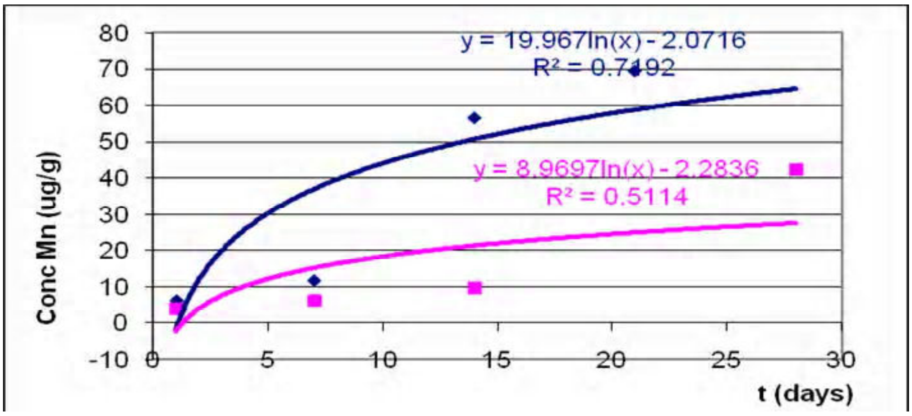

Fig. 1. Intestine manganese concentrations in fed (red line) and starved individuals (blue line

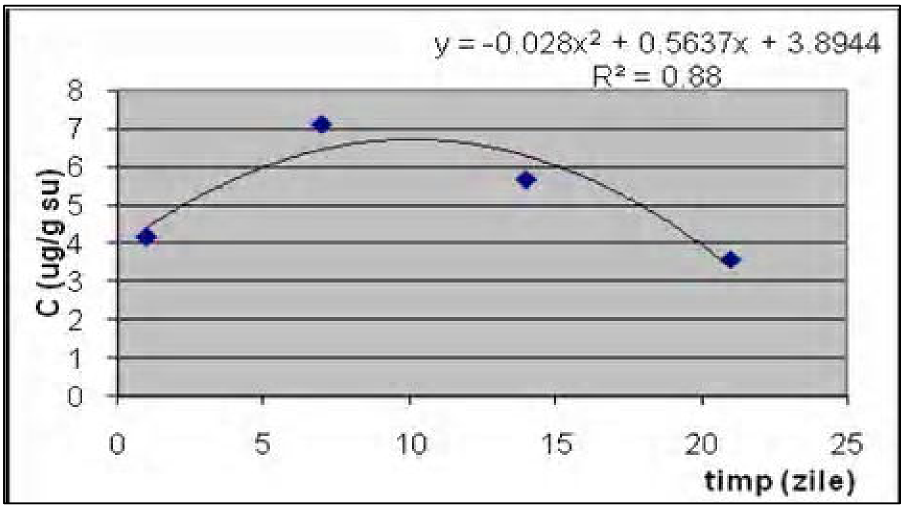

Fig. 2. Liver manganese concentrations in starved individuals

The digestive tract and its associated glands are the first organs impaired by food deprivation (Theilacker, 1978). Following 7 day exposure to manganese higher vacuolization of epithelial cells and epithelial lifting were noticed in starved individuals (Fig. 3B). In both fed and starved fish following 14 day exposure to the pollutant, epithelial layer disorganization in the apical region of intestinal villi, cellular and nuclear hypertrophy, flattening towards the tip of the villi appeared (Fig. 3 C, D).

The 28 day manganese intoxication induced intestinal villi fusion in order to decrease the absorbtion surface and to reduce the pollutant absorption (Fig. 4C, D). Additionally epithelial layer multiplied atypically (Figure 4 B). Reduced nutrient supply in the gut lumen is responsible for significant histopathological changes: mucosal atrophy (McLesse-Moon, 1989), absorptive surface reductions by regression or distortion of villi or microvilli (Gas-Noailac-Depeyre, 1976, Segner et al., 1987; Hall-Bellwood,1995), reduction of mucosal thickness due to decrease heights of epithelial cells (Hall-Bellwood, 1995), widening of intercellular spaces and loosening of cell contacts (O'Connell, 1976; Theilacker, 1978; Peters, 1982; Nonotte et al, 1986; Segnel et al., 1987), cell dissociation and disruption in mucosa (Uriarte-Balbotin, 1987) vacuolization (Peters, 1982; Eckman, 1985; Segner et al., 1987), autophagic process (Gas-Noailac-Depeyre, 1976; Peters, 1982; Segner et al., 1987), villi flattening, hypertrophy of epithelial cells, swelling or oedema of *lamina propria*, and fusion of villi due to excessive hypertrophy, flattening and rupture of villi at tip (Bhatnagar et al., 2007). The liver is known as an integrator of physiological and biochemical functions and alterations in its structure is expected under toxic circumstances (Hinton-Lauren, 1990). After 14 day exposure to the pollutant rare hypertrophied nuclei of hepatocytes were observed (Fig. 5 B) and macrophages aggregates appeared (Fig. 5A). Additionally after 21 days of chronic intoxication intercellular corridors and sinusoid capillaries dilatation were noticed. No other liver histological changes appeared after 21 days (Fig. 5B).

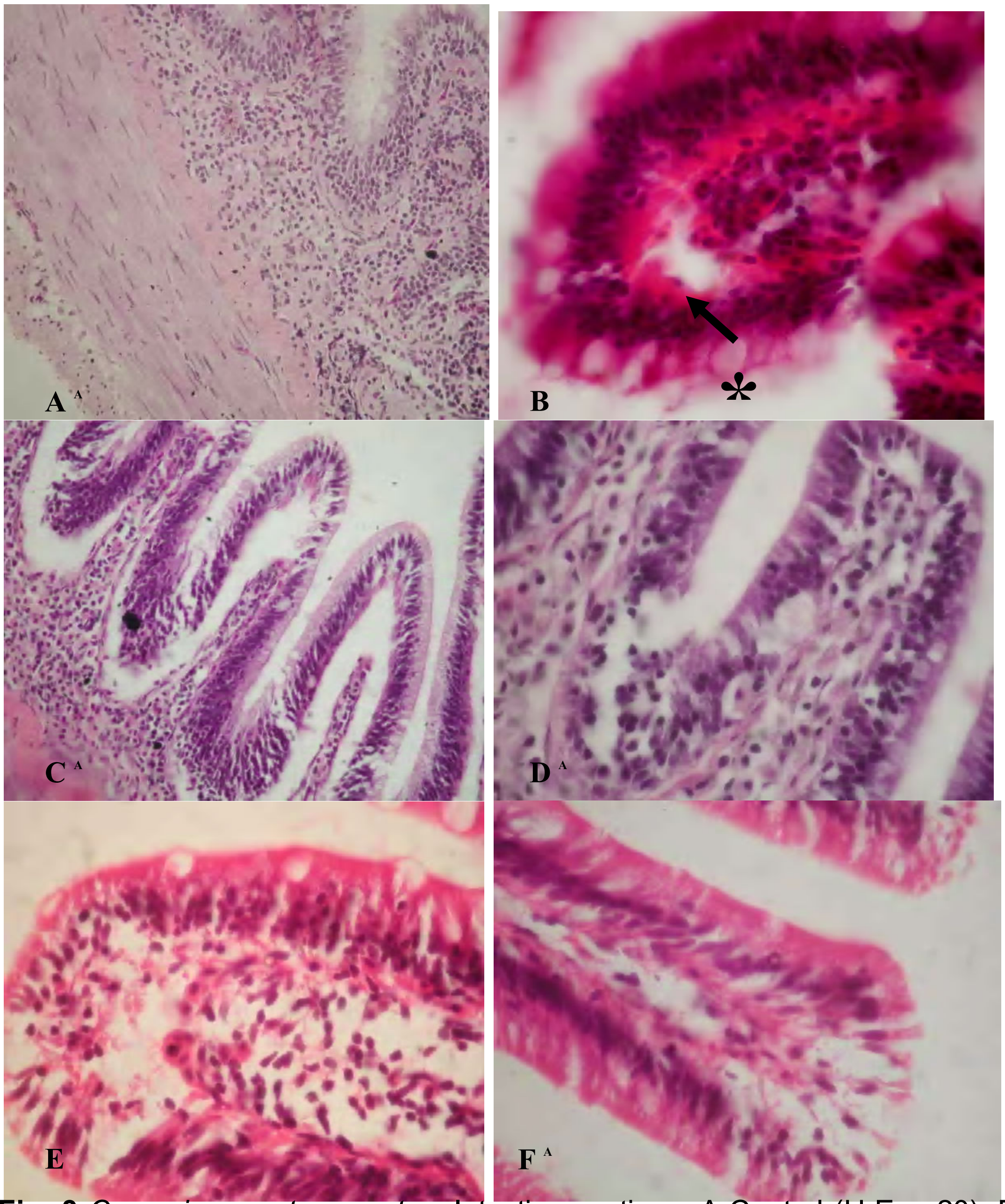

Fig. 3 *Carassius auratus auratus* -Intestine sections; A-Control (H-E, x 20); B-7 day exposure to manganese of starved individuals; cytoplasm vacuolization and hyperactivity of goblet cells, intestinal epithelium lifting in the apical region (arrow) (H-E, x100); C-14 day exposure of starved individuals (H-E; x20); D – 14 day exposure of fed individuals flattening at the tip of villi; nuclear hypertrophy; E, F-21 day exposure to manganese; severe disorganization of intestinal epithelium both in fed and starved fish

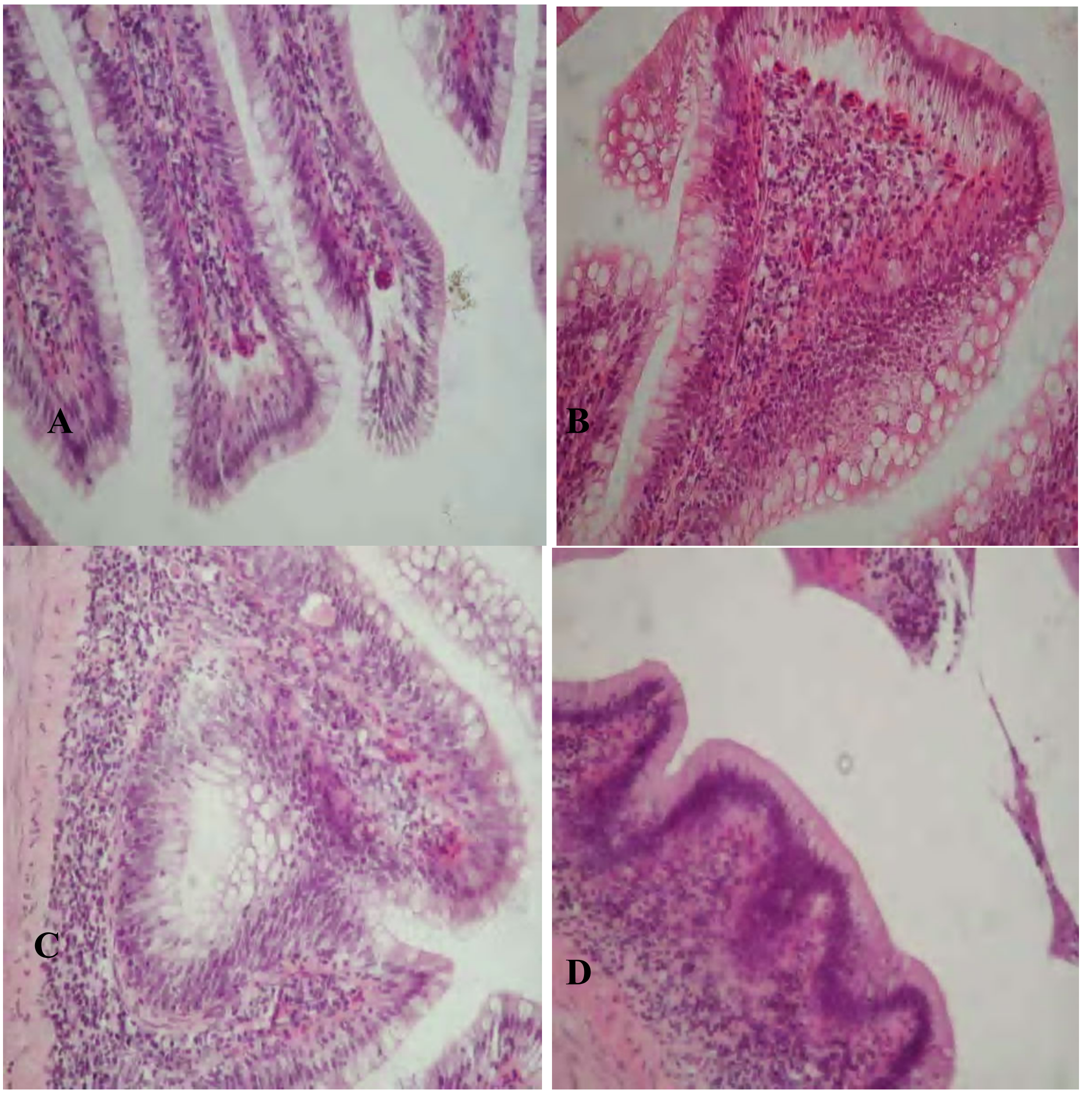

Fig. 4 *Carassius auratus auratus* -Intestine sections; 28 day exposure to the pollutant of the fed fish A-intestinal epithelium rupture, hyperactive goblet cells, blood vessels dilatated at the tip of villi (x100); B, flattening and dilatation of the apical area, goblet cells hypersecretion (x 100); C –fusion of intestinal villi (x 100); D- starved fish, intestine villi fusion (x 100)

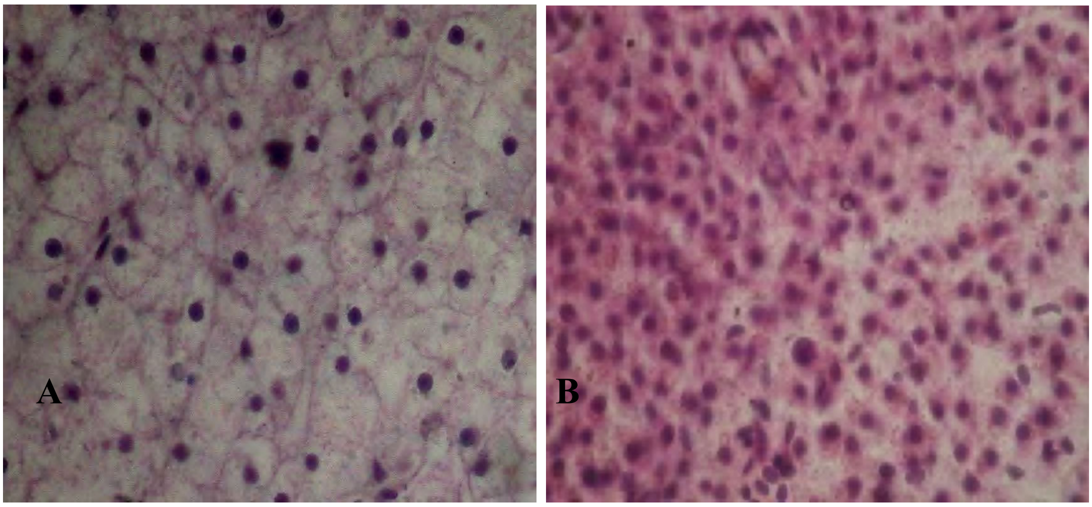

Fig. 5 Carassius auratus auratus; Liver sections; A- Control Liver (x 40); B 14 day intoxicated starved fish (x100); nuclear hypertrophy, large corridors between hepatocytes

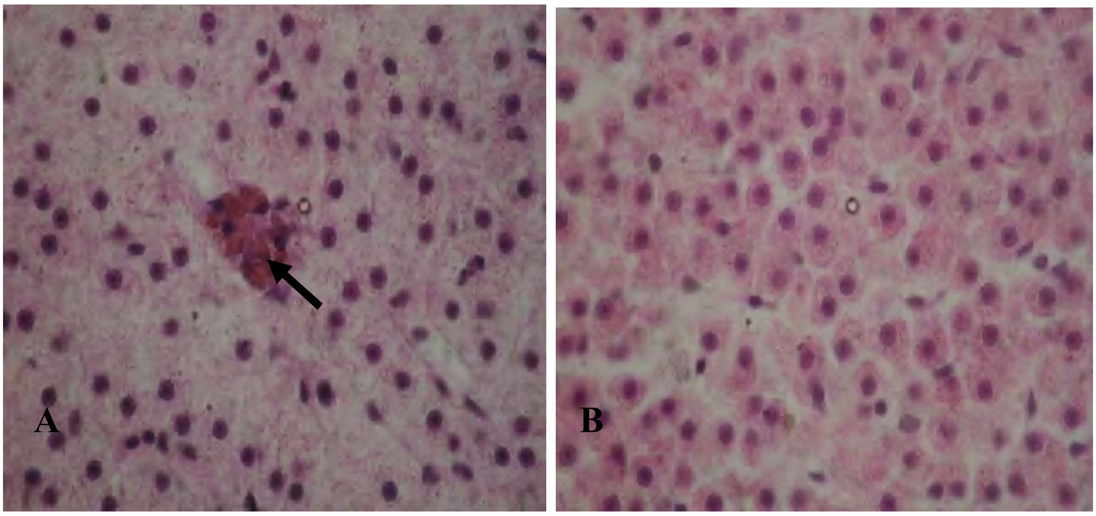

Fig. 6. *Carassius auratus auratus*; Liver sections; A- 14 day intoxication of fed fish ; macrophages aggregates (arrow) (x100; H-E); B- 21 day intoxicated starved fish (x 100)

CONCLUSIONS

1. Nutrition status, fish size and growth rate as well as tissue heavy metal concentration are important data for monitoring assessment.
2. No significant weight, standard and total lennght changes occurred, excepting the lots of fed and starved individuals exposed for 28 days to the pollutant where an insignificant weight decrease occurred.
3. Manganese concentration was higher in intestine compared to liver.
4. Intestine manganese concentration in fed fish was lower than in starved fish.

5. Both intestine and liver histological changes appeared due to manganese exposure and starvation. Both stress factors induced modifications to diminish absorption surface of the intestine.

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ACUTE ELECTROPHYSIOLOGICAL EFFECTS OF A METAL AND A NON-METAL MITOCHONDRIAL TOXIN

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ABSTRACT

Mitochondrial toxins are related both to causation and modelling of central nervous damage. Manganese, often present in occupational settings and as environmental pollutant, inhibits complex 2 and 3 of the mitochondria and is known to cause a Parkinson-like CNS damage. 3-nitropropionic acid (3NP), a neurotoxin of plant and microfungal origin, inhibits succinate dehydrogenase leading to various biochemical and morphological alterations in the brain. Experimental animals treated by 3NP are used to model Huntington's disease. In this work, adult male Wistar rats were acutely prepared for recording spontaneous and stimulus-evoked activity from the projection area of the whiskers and the tail. It was tested whether the dependence of the evoked response on the parameters of stimulation was influenced by the mitochondrial toxins and what correlation existed between the cortical and peripheral responses. Mn intensified frequency-dependent fatigue. 3-NP caused latency decrease with minimal amplitude change, and had less effect on frequency dependence. The observed effects were apparently limited to the brain.

Key words: manganese, 3-nitropropionic acid, evoked activity,
nerve action potential, stimulation parameters

INTRODUCTION

Mitochondrial toxins diminish oxidative energy production by blocking the mitochondrial enzyme complexes. This causes a decline in the cells' reserve energy that can result in acute symptoms and contribute to various diseases.

Manganese is essential for living organisms in small amounts but toxic when overdosed. The presence of Mn in the general environment is partly due to human activity (petrol additive, fungicide, dry cells etc). Inorganic Mn can deposit in the brain (Aschner et al., 1999), causing functional (Shinotoh et al., 1997) and structural (Yamada et al., 1986) damage. The toxin 3-nitropropionic acid (3NP) is naturally present in leguminous plants, occasionally poisoning grazing livestock. Human poisoning may come from consumption of foodstuffs infested with certain moulds producing 3NP (Brouillet et al., 1999). A common effect of both Mn and 3NP is the inhibition of the enzyme complexes responsible for energy production. Mn decreases the activity of all complexes, particularly complex 2 and 3 (Zhang et al. 2003) whereas 3NP irreversibly inhibits succinate dehydrogenase in complex 2 (Coles et al., 1979).

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 239-242, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

Although Mn and 3-NP both are mitochondrial blockers, there are many differences in their intra- and extra-mitochondrial mode of action which leads to completely dissimilar clinical pictures in chronic intoxications. The symptoms of excess Mn intake are, on the whole, called manganism, a state that can be very similar to Parkinson's disease, while 3NP poisoning resembles Huntington's disease and is used in an animal model of that (Brouillet et al., 1999).

Since the function of the nervous system requires lots of energy, the mitochondrial damage is probably reflected in the electrical activity of the brain. Beside damage in energy production, the two substances studied have also more direct effects in the nervous system, so their excessive presence should be detectable by electrophysiology. To study this, we examined the changes of evoked activity in acute experiments in rats.

MATERIALS AND METHODS

Adult male Wistar rats (ca. 300 g) were given urethane (1000 mg/kg b.w.) ip. for anaesthesia. The left hemisphere was exposed by removing a part of the bony skull. Following a short recovery period, silver recording electrodes were placed on the surface of the cortex at the primary somatosensory projection area of the tail and the whiskers for measuring electrical activity. Evoked potentials (EPs) were triggered by 0.05 ms square electric stimuli that were delivered by pairs of needle electrodes placed in the contralateral whisker pad and in the tail base. To record the peripheral nerve responses another pair of needles were inserted in the tail 50 mm distally from the base. See Lukács and Szabó, 2007, for the recording techniques.

First, in 8 rats for both substances, the dependence of the obtained cortical EP on parameters of the whisker stimulation was tested. The just-supramaximal stimulus strength (around 3 - 5 V) was determined first, then trains of 50 stimuli were given at frequency of 1, 2, 5 and 10 Hz. This sequence was done 3 times with 30 min intervals for control. Then the appropriate solution was given in an intraperitoneal injection, and further 4 records were taken. 3NP was given in a dose of 20 mg/kg, or Mn^{2+} (in form of $MnCl_2$) in 50 mg/kg, both dissolved in distilled water. In another experiment both whiskers and tail of 8 rats was stimulated at 1 Hz. Most conditions were the same as in the previous set except that beside the corresponding cortical EPs, the tail nerve response was recorded also. The records were averaged, and their onset latency and peak-to-peak amplitude was measured.

To eliminate individual variation, the latency and amplitude values were normalized to the mean of the control period (first 3 series). From these normalized data, group mean was calculated and plotted against the frequency (1 to 10 Hz) of stimulation, and any difference between the resulting curves before vs. after administration of the toxicant was sought for. In the second experiment, cortical and peripheral response parameters were plotted in the same graph to see their similar or dissimilar time trend, and their correlation was tested.

RESULTS AND DISCUSSION

Before application of either Mn or 3NP, or in the untreated parallel controls, the latency data normalized to control mean and plotted against frequency of stimulation gave a horizontal line, showing that stimulation with 10 Hz vs. 1 Hz had no effect on the latency (Fig. 1). After injection of Mn, the latency started to increase and developed nonlinear frequency dependence with maximum at 2 Hz. Treatment with 3NP induced latency decrease which increased in time (curve 4 to curve 7) and diminished with increasing stimulation frequency.

The time trend of the latency and amplitude of EPs obtained by whisker or tail base stimulation was investigated together and was compared to the trend of the peripheral response (nerve action potential) evoked by tail stimulation.

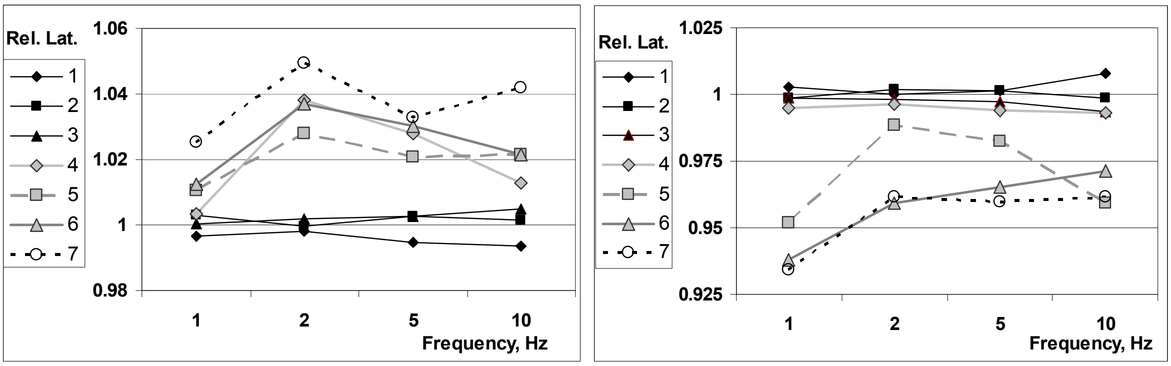

Fig. 1. Frequency dependence of the EP latency before and after administration of Mn (left) and 3NP (right). Curves 1 to 3 show values before, and 4 to 7 after, application. Group means of normalized individual data (see Methods).

In the Mn-treated rats, the latency of the cortical EPs evoked by whisker or tail stimulation had a clear decreasing trend (Fig. 2, top left), while the amplitude of the EPs increased (Fig. 2, top right), but neither parameter of the tail nerve response had a noteworthy change. The correlation diagrams of Fig. 2 show that the two cortical responses changed together but independently of the peripheral response.

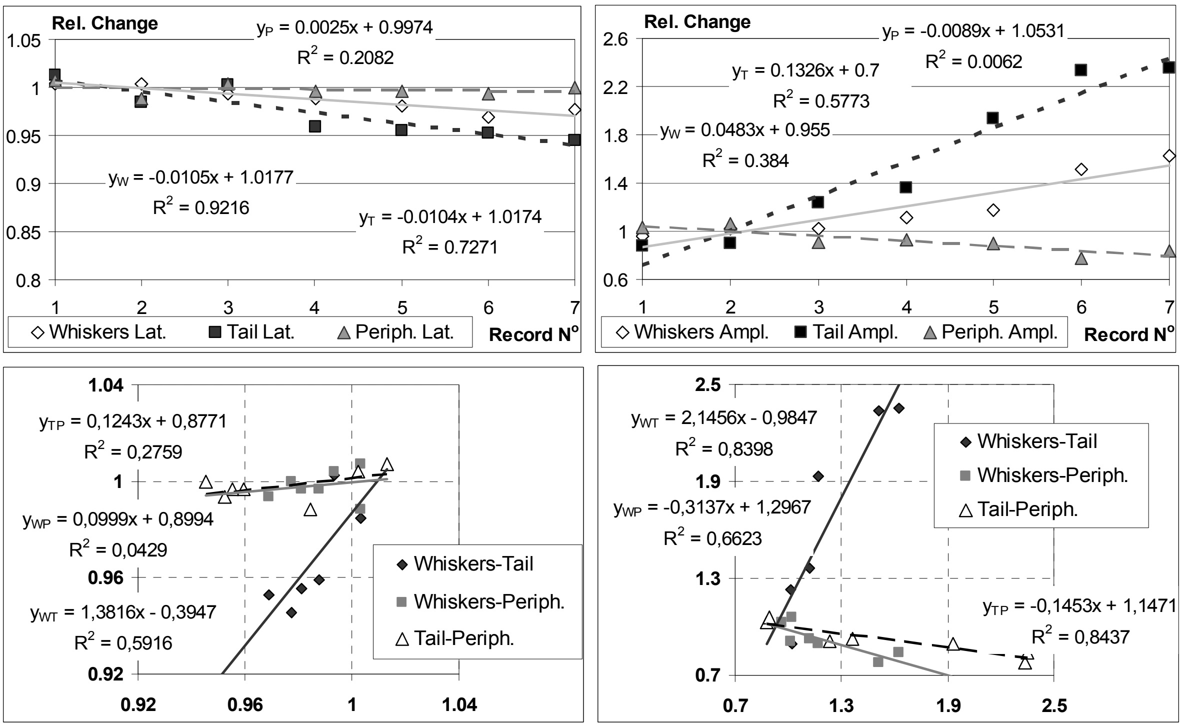

Fig. 2. Top: Relative change (compared to control mean) of the latency (left) and amplitude (right) of the cortical and peripheral evoked responses in Mn-treated rats. Record N° 1 to 3 was take before, and 4 to 7 after, Mn application. Bottom: correlation diagrams of the same data sets. Inserts: equations and correlation coefficients of the fitted straight lines.

In the rats treated with 3NP the effect was highly similar. Latency decrease and amplitude increase of the cortical responses evolved in parallel, shown by the linear trend line standing at about 45 degrees inclination; but there was no change in the tail nerve response, as shown by the nearly horizontal trend lines. (Fig. 3).

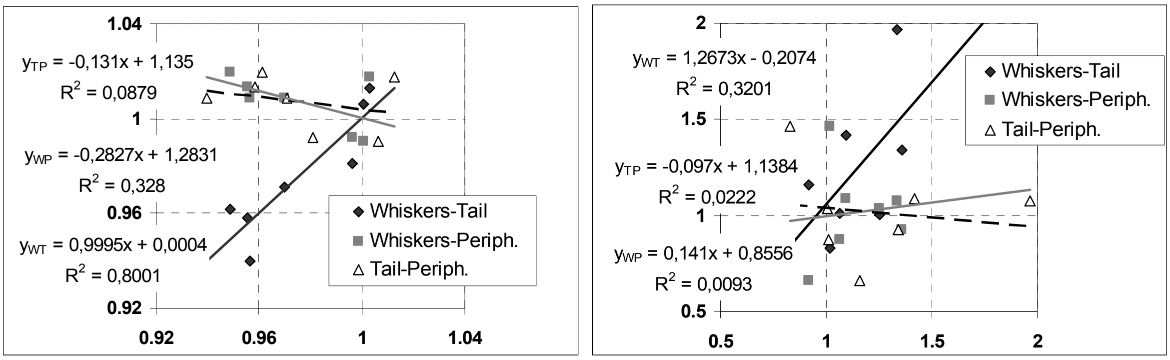

Fig. 3. Correlation diagrams of the latency (left) and amplitude (right) of cortical and peripheral responses obtained in 3NP-treated rats. Displayed as in Fig. 2.

Mitochondrial inhibition is the obvious common point in the action of Mn and 3NP, and the cortical activity changes in inherited mitochondrial encephalopathy are known (Smith and Harding, 1993). The correlation diagrams suggest, however, that a cortex- or at least CNS-specific mechanism played a role, most probably the disturbance of glutamate turnover (Mn: Hazell and Norenberg, 1997; 3NP: Tavares at al., 2001). Electrophysiological recording may be of use in the study of mitochondrial toxins.

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IMPACT OF SOME MINERAL ELEMENTS FROM INDUSTRIAL WASTE ON WHEAT PLANTS

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ABSTRACT

The present paper follows the cultivation of green wheat plants in low acid soils, which were previously treated by different doses of mineral waste from the magnesium products industry. The two experimented industrial addings, one from the industrial process (A) and the second resulted as crusts deposed on the equipment walls (B), can be used in certain doses as soil amendment for low fertile acid soil and fertilizer for plant nutrition. The presence of magnesium and calcium in the waste composition represents a source of macroelements for plant nutrition. The enhance of the trace elements in soil is representative for iron, manganese and zinc. At harvest time, the size of green wheat plants treated with addings doses was taller comparative to the control alternative. The nitrogen supplementation increases their dry weight and dry matter, especially for the alternatives with waste B.

Key words: mineral supplementation, waste, soil, wheat plant,
essential and trace elements

INTRODUCTION

At all times, the life of human beings was connected with the soil in which grow and develop plants. The soil composition has a decisive influence on the yield and the crops quality. Therefore, it is obvious that the mineral deficiencies of soil can generate diseases of animals and men. Besides the geochemical problems existing in certain areas, the man intervention could also contribute to the disturbance of nutritive elements equilibrium, even without intention; for example, by inadequate fertilization is possible to attend lacks of mineral elements in soil content (Kiss, 1978).

In this context, extensive studies had put into evidence the influence of mineral elements, magnesium especially, on the wheat characteristics and fall resistance of plant stalk (Kiss, 1984).

The magnesium products industry, magnesium carbonate and oxide obtained by carbon dioxide leaching of dolomites, generates a waste with an important mineral content. The composition of this waste includes calcium carbonate, precipitated magnesium carbonate (in ratio 3:1) and as impurities certain compounds of iron,

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 243-248, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

manganese, copper, zinc (Radulescu et al., 2006; Radulescu et al, 2007; Taubert, 2001; Taubert, 2002). This waste was tested in order to be valuated in agriculture as soil amendment and tested for green oat cultivation (Taubert et al., 2008).

The present paper follows the cultivation of green wheat plants in low acid soils, which were previously treated by different waste doses, without or with nitrogen contribution.

MATERIALS AND METHODS

The soil of luvosoil type, having a pH_{H2O} of 6.85 and pH_{KCl} of 5.60 and a rather low fertility was collected, air-dried, crushed, mixed and put into pots, each containing 1 kilogram soil. Two types of addings were experimented, one from the industrial process (A) and the second resulted as crusts deposed on the equipment walls (B), having the composition presented in Table 1. The soil was treated with the two types of industrial addings in different amounts.

Table 1: Composition of the two experimented industrial addings

Specification	Waste A	Crusts B
Ca, %	28	19
Mg, %	7	14
Na, %	0.77	
Fe, mg/kg	1850	880
Cu, mg/kg	1.9	51
Mn, mg/kg	136	51
Zn, mg/kg	2.6	50

The experimental alternatives pursued by this research consist of four different doses for each adding (A_1-A_4, B_1-B_4) and also a control alternative (C_0) representing untreated soil.

Table 2: Description of the experimental alternatives

Experimental alternative		N contr., mg/kg	Mineral supplement / soil							
			Dose, mg/kg	Ca, mg/kg	Mg, mg/kg	Na, mg/kg	Fe, mg/kg	Mn, µg/kg	Zn, µg/kg	Cu, µg/kg
A_1	R	-	179	50	13	1.38	0.33	24.3	0.47	0.34
	R_N	134	179	50	13	1.38	0.33	24.3	0.47	0.34
A_2	R	-	357	100	25	2.76	0.66	48.7	0.93	0.68
	R_N	134	357	100	25	2.76	0.66	48.7	0.93	0.68
A_3	R	-	714	200	50	5.51	1.32	97.4	1.86	1.36
	R_N	134	714	200	50	5.51	1.32	97.4	1.86	1.36
A_4	R	-	1429	400	100	11.02	2.64	194.7	3.72	2.72
	R_N	134	1429	400	100	11.02	2.64	194.7	3.72	2.72
B_1	R	-	263	50	37		0.23	13.4	13.2	13.4
	R_N	134	263	50	37		0.23	13.4	13.2	13.4
B_2	R	-	526	100	74		0.46	26.8	26.4	26.8
	R_N	134	526	100	74		0.46	26.8	26.4	26.8
B_3	R	-	1053	200	147		0.93	53.6	52.6	53.6
	R_N	134	1053	200	147		0.93	53.6	52.6	53.6
B_4	R	-	2105	400	295		1.85	107.3	105.2	107.3
	R_N	134	2105	400	295		1.85	107.3	105.2	107.3

All the experimental alternatives took place in two replicates, one being without nitrogen treatment (R) and the other (R_N) - treated with 134mg N/kg soil as ammonium nitrate (see Table 2).

All the pots were sown with thirty wheat grains. The vegetation period was that of green plant, pursued for 6 weeks. Along the vegetation period, some morphological parameters, like number of risen plants, plant size, fresh and dry plant weight were pursued. At harvest time, soil samples were collected in order to establish the impact of waste treatment on soil fertility. Essential and trace elements were analyzed; the metal element content in soil at harvest time was established by AAS - ICP method.

RESULTS AND DISCUSSIONS

The impact of soil treatment by different waste doses on the macroelements content is shown in Table 3.

Table 3: Impact of waste treatment on soil macroelements content

Experimental alternative		Ca content		Mg content		K content		Na content	
		g/kg	%	g/kg	%	g/kg	%	g/kg	%
C_0	R	2.434	100	0.418	100	0.125	100	0.149	100
	R_N	2.295	100	0.381	100	0.091	100	0.145	100
A_1	R	2.475	102	0.422	101	0.115	92	0.138	93
	R_N	2.390	104	0.401	105	0.095	104	0.135	93
A_2	R	2.572	106	0.436	104	0.114	91	0.136	91
	R_N	2.476	108	0.418	110	0.088	97	0.141	97
A_3	R	2.111	87	0.365	87	0.097	78	0.132	89
	R_N	2.151	94	0.339	89	0.079	87	0.137	92
A_4	R	2.126	87	0.367	88	0.081	65	0.122	82
	R_N	1.732	75	0.315	83	0.061	67	0.119	82
B_1	R	1.979	81	0.346	83	0.086	69	0.096	64
	R_N	1.532	67	0.284	75	0.065	71	0.087	60
B_2	R	2.381	98	0.413	99	0.105	84	0.116	78
	R_N	2.339	102	0.387	102	0.081	89	0.121	83
B_3	R	2.451	101	0.425	102	0.101	81	0.115	77
	R_N	2.319	101	0.409	107	0.090	99	0.114	79
B_4	R	2.024	83	0.403	96	0.080	64	0.101	68
	R_N	2.056	90	0.423	111	0.076	84	0.114	79

Analysing the calcium content, an increase was established only for the two lowest doses of waste A and B. Nitrogen contribution enriches calcium soil content once with the growth of waste A and crusts B doses.

A growth of the magnesium soil content once with the increase of the waste doses was more evident for crusts B, which contains more magnesium in its composition. The supply of nitrogen generates for both waste apports an evident increase of magnesium soil content.

The potassium soil content established for all experimental alternatives is lower as that of the control alternative. The nitrogen contribution generates an evident increase of the potassium soil content for both waste apports.

The sodium soil content is lower for all alternatives in comparison with the control alternative. The sodium values are lower for crusts B and nitrogen contribution does not enrich evidently the sodium soil content.

Table 4: Influence of waste type and doses on soil trace elements content

Experimental alternative		**Fe content**		**Mn content**		**Zn content**		**Cu content**	
		µg/g	**%**	**µg/g**	**%**	**µg/g**	**%**	**µg/g**	**%**
C_0	R	379.6	100	52.07	100	35.04	100	3.768	100
	R_N	381.2	100	43.55	100	48.30	100	3.543	100
A_1	R	391.8	103	54.74	105	37.15	106	3.730	99
	R_N	410.8	108	45.45	104	28.76	60	3.786	107
A_2	R	422.3	111	48.26	93	35.82	102	3.908	104
	R_N	424.9	111	49.14	113	38.26	79	4.010	113
A_3	R	393.8	104	50.60	97	30.20	86	3.871	103
	R_N	384.3	101	46.75	107	40.84	85	3.750	106
A_4	R	336.4	89	39.13	75	33.87	97	3.186	85
	R_N	410.6	108	48.98	112	33.70	70	3.740	106
B_1	R	385.9	102	63.36	122	44.47	127	3.701	98
	R_N	374.0	98	42.71	98	47.16	98	3.570	101
B_2	R	403.8	106	50.64	97	32.68	93	3.554	94
	R_N	416.8	109	55.21	127	47.74	99	3.671	104
B_3	R	422.0	111	52.51	101	30.36	87	3.738	99
	R_N	395.1	104	42.84	98	35.94	74	3.358	95
B_4	R	384.5	101	51.21	98	32.56	93	3.459	92
	R_N	395.4	104	53.61	123	35.94	74	3.336	94

Because of their trace elements content, the soil treatment with waste A and B generates a different trace element level in comparison with the control alternative. Results are given in Table 4.

A proportional increase of the iron soil content once with the growth of the waste dose was established only for the alternatives with waste B. The apport of nitrogen generates an increase of the iron soil content only for the alternatives with waste A.

An evident increase of the manganese soil content took place only for the lowest dose of waste B. However, the supplimentary apport of nitrogen increases in all cases the manganese content.

For all alternatives the zinc soil content is low. There is an exception for the lowest dose of waste B. Nitrogen contribution decreases for all alternatives the zinc soil content.

The copper content established for all the experimental alternatives is low. However, the apport of nitrogen increases the copper content in comparison with the untreated alternatives.

The effects of soil treatment with waste A and B show an evident influence on the development of green wheat plants by analysing some vegetation characteristics (Table 5). Adding waste A and B in soil, a beneficial effect was established for plant germination and a higher number of risen plants was determined. Adding nitrogen to soil, the number of risen plants increased especially for the alternatives with crusts B.

Table 5: Influence of addings on vegetation characteristics of wheat

Experimental alternative		**Risen plants**		**Size green pl.**		**Fresh weight**		**Dry weight**		**Dry matter**
		nb.	**%**	**cm**	**%**	**mg/p.**	**%**	**mg/p.**	**%**	**%**
C_0	R	28	93.3	31.2	100	212	100	30.7	100	14.5
	R_N	30	100	32.6	100	207	100	40.8	100	19.7
A_1	R	30	100	31.6	101.3	205	96.7	33.0	107.5	16.1
	R_N	30	100	33.2	101.8	171	82.6	42.8	104.9	25.0
A_2	R	30	100	30.6	98.1	182	85.9	31.1	101.3	17.1
	R_N	30	100	36.2	111.0	251	121.3	42.4	103.9	16.9
A_3	R	28	93.3	32.3	103.5	139	65.6	32.0	104.2	23.0
	R_N	28	93.3	38.8	119.0	241	116.4	53.3	103.6	22.1
A_4	R	29	96.6	33.6	107.7	233	109.9	35.6	116.0	15.3
	R_N	30	100	38.3	117.5	237	114.5	53.6	131.4	22.6
B_1	R	29	96.6	31.2	100	163	78.9	39.1	127.4	24.0
	R_N	29	96.6	36.6	112.3	156	75.4	51.8	127.0	33.2
B_2	R	27	90.0	31.0	99.4	143	67.5	35.3	115.0	24.7
	R_N	30	100	35.3	108.3	155	74.9	48.5	118.9	31.3
B_3	R	28	93.3	32.6	104.5	152	71.7	36.3	118.2	23.9
	R_N	30	100	34.6	106.1	169	81.6	48.5	118.9	28.7
B_4	R	29	96.6	31.3	100.3	241	113.7	37.8	123.1	15.7
	R_N	29	96.6	34.5	105.8	203	98.1	55.2	135.3	27.2

Adding waste A and B in soil, a beneficial effect was established for plant germination and a higher number of risen plants was determined. Adding nitrogen to soil, the number of risen plants increased especially for the alternatives with crusts B.

Green wheat plants grew taller once with the increase of the waste dose for both waste. Adding nitrogen an increase of the plant size was established, especially for the alternatives with waste A.

At harvest time, only the green wheat plants treated with the highest doses of waste A and B had a higher fresh weight towards the control alternatives. Nitrogen contribution improved the values of weight only for the alternatives with waste A.

Nitrogen contribution improves the values of dry weight and dry matter especially for soil treated with waste B.

CONCLUSIONS

1. Considering the obtained results, the two experimented industrial addings can be used in certain doses as soil amendment for low fertile acid soil and fertilizer for plant nutrition.
2. The presence of magnesium and calcium in the waste composition represents a source of macroelements for plant nutrition.
3. The enhance of the trace elements in soil is representative for iron, manganese and zinc (without nitrogen contribution).
4. At harvest time, the size of green wheat plants treated with addings doses was taller comparative to the control alternative. Nitrogen contribution increase their size. 5. The nitrogen supplementation increases their dry weight and dry matter, especially for the alternatives with waste B.

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LACTATE DEHYDROGENASE ACTIVITY AND HISTOPATHOLOGICAL CHANGES IN Cu^{2+} INTOXICATION OF *CARASSIUS AURATUS GIBELIO* LIVER AFTER SHORT AND LONG TERM EXPOSURE

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ABSTRACT

The pathological effects of two sublethal concentrations (100 µg/l and 250 µg/l) of copper ($CuSO_4$ x $5H_2O$) on goldfish Carassius auratus gibelio were studied for short (24, 48, 72 hours) and long term exposure (7, 14, 21 days). The specific activity of LDH in liver, the first organ exposed to toxic compounds, were assayed. In both experiments we registered increased LDH specific activity compared to the control. This increase induced a hypoxic condition but also stimulated the glycolysis rate. The elevation in LDH activity may reflect a high dependence on anaerobic carbohydrate metabolism of the liver exposed to copper. Generally, the histopathological changes were related to the toxicant dose and exposure time.

Key words: *Carassius auratus gibelio*, copper toxicity, LDH, histopathology.

INTRODUCTION

The aquatic environment has suffered due to the growing number of xenobiotics such as heavy metals and pesticides. Copper (Cu^{2+}) is a trace metal , classified as an essential element for most living organisms but, in high concentrations, it can be a toxic pollutant. It reaches the aquatic environment through wet or dry deposition, mining activities, domestic and agricultural waste disposal. The toxic effect of Cu is related to its capacity for catalyzing oxidative reactions, leading to the production of reactive oxygen species (Lopes et al., 2001). These highly reactive compounds may also include tissue alterations and physiological derangement in fish (Varanka et al., 2001).

The application of environmental toxicology studies among nonmammalian vertebrates is rapidly expanding, and for aquatic systems, fish have become indicators for the evaluation of the effects of noxious compounds.

Lactate dehydrogenase is an oxydoreductase that catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD^+ as hydrogen acceptor. This enzyme activity is present in all cells of the body and is invariably found only in the cytoplasm of the cells. In toxicology and clinical chemistry, LDH is widely used as marker of

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 249-254, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

organ or tissue lesions. Therefore, LDH has also been used as an indicator of hypoxic conditions in organisms and plays an important role in glycolysis (Das et al., 2004).

In this study liver histopathology and LDH enzymatic activity were investigated in *Carassius auratus gibelio* exposed to copper intoxication.

MATERIALS AND METHODS

Freshwater goldfish *Carassius auratus gibelio* of length 13.5-16.5 cm and weight 20.0-30.0 g were obtained from Nucet Fishery Research Station and kept in three glass tanks (each of 60 l) at 20-21^{0}C. Prior to exposure, fish were held for 15 days for acclimatization and evaluation of overall health under laboratory conditions. The water from the control and experimental tanks was changed every 3 days. For the intoxication experiments the fish were divided in three groups: one as control and the others two as experimental groups. In both experimental tanks, concentrations of 100 µg Cu^{2+}/l or 250 µg Cu^{2+}/l were used. In the short term exposure the fish were intoxicated for 24, 48 and 72 hours, while in the long term exposure they were intoxicated for 7, 14 and 21 days. During the experiment the fish were not fed. After the selected periods, the liver was excised and prepared for biochemical and histological analysis.

Enzymatic assay. LDH activity was determined by the rate of oxidation of NADH measured by the change in absorbance at 340 nm (Bergmeyer and Bernet, 1974).

Protein concentration was determinated according the method of Lowry (1951) with bovine serum albumin (BSA) as standard.

Histological procedures. Small pieces of gills and intestine were immersed in Bouin's solution (overnight), dehydrated in ethanol, cleared in xylene and embedded in paraffin. The sections were stained with hematoxylin-eosin. The photos were made with an Olympus B061 Microscope.

RESULTS AND DISCUSSIONS

Liver is the central organ of metabolism and acts as an organ of storage. Metabolic action by the hepatic parenchyma cells has been regarded as an important defense system against toxicants. The great susceptibility of liver to be damaged by chemical agents is presumably a consequence of its primary role in metabolism of xenobiotic substances.

Several liver enzymes are generally associated with cellular metabolic activity, which is inhibited or elevated under oxidative stress, especially, after exposure to heavy metals. LDH is used as a biochemical marker for assesing liver function (Das et al., 2004).

In our short term experiment, in the case of the first concentration (100 µg Cu^{2+}/l) LDH specific activity increased by 72% after 24 h, by 151% after 48 h, respectively by 162% compared to control (Fig. 1A). This increase could be due the Cu^{2+} induced mitochondrial dysfunction which has generated hypoxia and stimulated glycolisis rate. LDH acts as a pivotal enzyme between the glycolityc pathway and the tricarboxylic acid cycle. Thus, the nonavailability of oxygen and simultaneous elevation of LDH may suggest a bias towards the anaerobic glycolitic pathway in order to mitigate the energy crisis for survival. The same type of increase was recorded by Das et al. (2004) in the liver of three species of Indian major carp (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) due to nitrite toxicity.

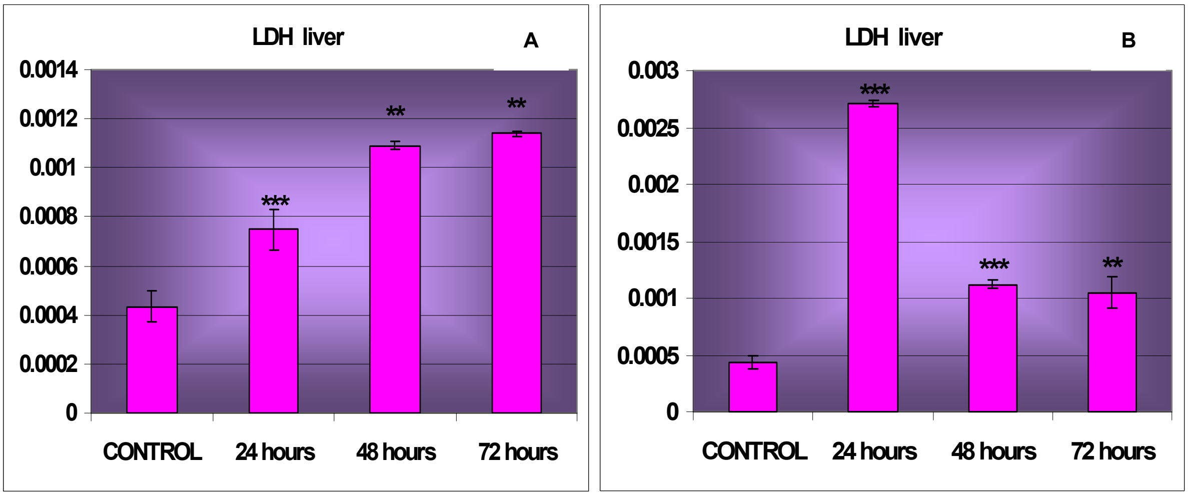

Fig. 1. The variation of LDH specific activity in liver of *Carassius auratus gibelio* exposed to 100 µg Cu^{2+}/l (A) and 250 µg Cu^{2+}/l (B).

At the second concentration (250 µg Cu^{2+}/l) the LDH enzymatic activity increased very significantly after 24 h, by 523%, compared to the control, but decreased after 48 and 72 h, by 58.6%, respectively 61.3%, compared to the first period of exposure (Fig. 1B). This decrease can be due to the copper induced oxidative stress in the fish, which could generate an oxidative damage at proteins level (Stadtman and Levine, 2001). Sastry and Gupta (1980) postulated that inhibition of LDH activity may be due to ion imbalance or to intracellular action of metal subsequent to initial cell membrane damage.

In our long term exposure in the case of the first concentration (100 µg Cu^{2+}/l) the specific activity of LDH increased, by 103%, after 7 days, compared to the control, inducing a hypoxic condition. After 14 and especially 21 days the activity decreased, by 17.7%, respectively 52.6%, compared to 7 days intoxicated liver and as a consequence the glycolysis rate could be diminished (fig. 2A).

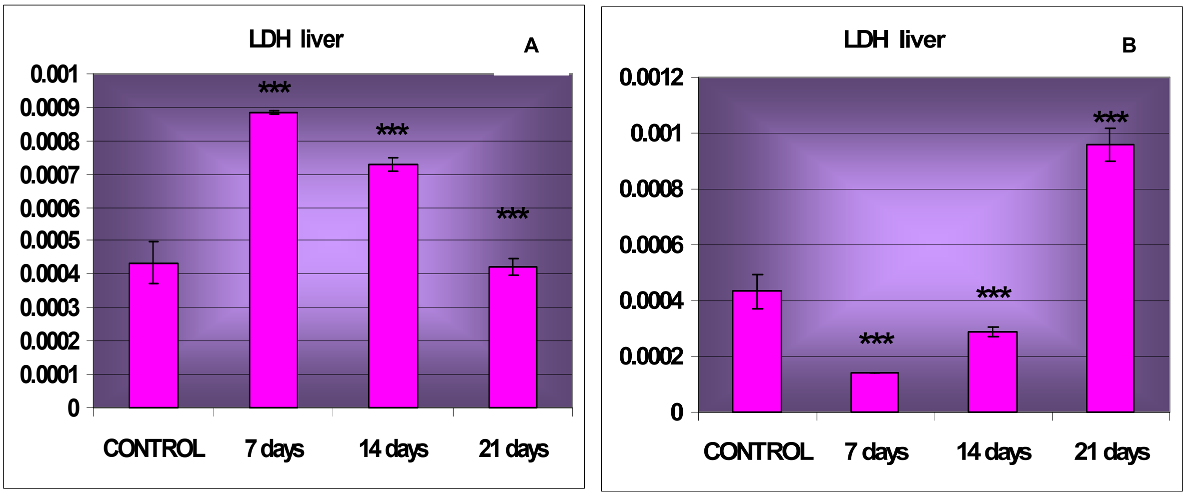

Fig. 2. The variation of LDH specific activity in liver of *Carassius auratus gibelio* exposed to 100 µg Cu^{2+}/l (A) and 250 µg Cu^{2+}/l (B).

In consistence with our results Mishra and Shukla (1997) reported that endosulfan (an insecticide) inhibited the LDH activity in the liver of *Clarias batrachus,* while Elumalai et al. (2002) recorded inhibition in LDH activity in the crab *Carcinus moenas* due to exposure to Cu, Cr or a mixture of both. In the same way Sastry and Rao (1984) and Almeida et al. (2001) recorded inhibition of LDH activity in *Channa punctatus* due to exposure to mercuric chloride and in Nile tilapia *Oreochromis niloticus* due to exposure to Cd.

It can be seen that in the second concentration (250 µg Cu^{2+}/l) the enzymatic activity decreased after 7 days, by 67%, compared to the control, but increased after 14 and 21 days, by 101%, respectively 568%, compared to the first period of exposure (Fig. 2B). The lower LDH specific activity after 7 and 14 days of exposure to 250 µg Cu^{2+}/l could be explained by the marked inhibition of hexokinase and moderate one for pyruvate kinase (Lai and Blass, 1984). The important increase of this activity after 21 days treatment it is possible to be due to the high need of ATP for survival in the oxidative stress conditions.

These sublethal levels of Cu affect also the morphology and cause pathological changes in the liver, comparatively with liver control (Fig. 3C). So, in our short term exposure in the case of the second concentration (250g Cu^{2+}/l), after 72 h of treatment, the main alterations found in the liver were: irregular-shaped nuclei, nuclear hypertrophy, or nucleus in a lateral position, close to the cell membrane. Anomalies such as cytoplasmic vacuolation and nucleus in a lateral position were also described in the siluriform *Corydoras paleatus* contaminated by organophosphate pesticides (Fanta et al., 2003). At the same concentration we observed melanomacrophage centres (MMG). Several studies have demonstrated that fish livers from contaminated areas show occasional MMG centres and granulomas (Teh et al.,1997).

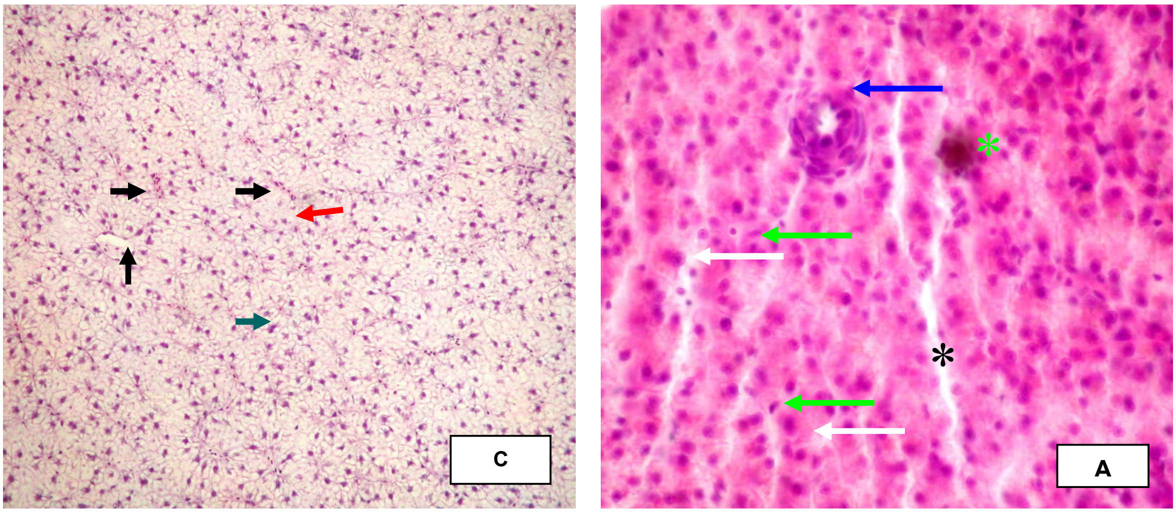

Fig. 3 - Liver **control (C)** – hepatocytes (red arrow), nucleus (green arrow), blood sinusoids (black arrows), x 20; **(A)** *Carassius auratus gibelio* liver intoxicated with 250µg Cu^{2+}/l (short term exposure) - **72 hours**: nuclear hypertrophy (white arrows), irregular - shaped nuclei (green arrows), billiary duct (blue arrow), melanomacrophage centres (*), intercellular spaces (*), x 40.

Although high prevalence of hepatic MMG centres may occur in fish collected in contaminated sites due to the storage of foreign material, they can not be linked to direct contaminant exposure since they are also involved in other metabolic functions (Stentiford et al., 2003).

In our long term exposure, at 100 µg Cu^{2+}/l, the connective tissue around the large blood vessels increased in thickness, after 14 days of treatment. But the most severe histopathological changes induced by copper intoxication registered after 21 days of treatment in the second concentration (250g Cu^{2+}/l). These consisted of cytoplasmic vacuolation, irregular shaped cells, cellular rupture, blood vessels congestion, which impair the normal functioning of the hepatic tissue.

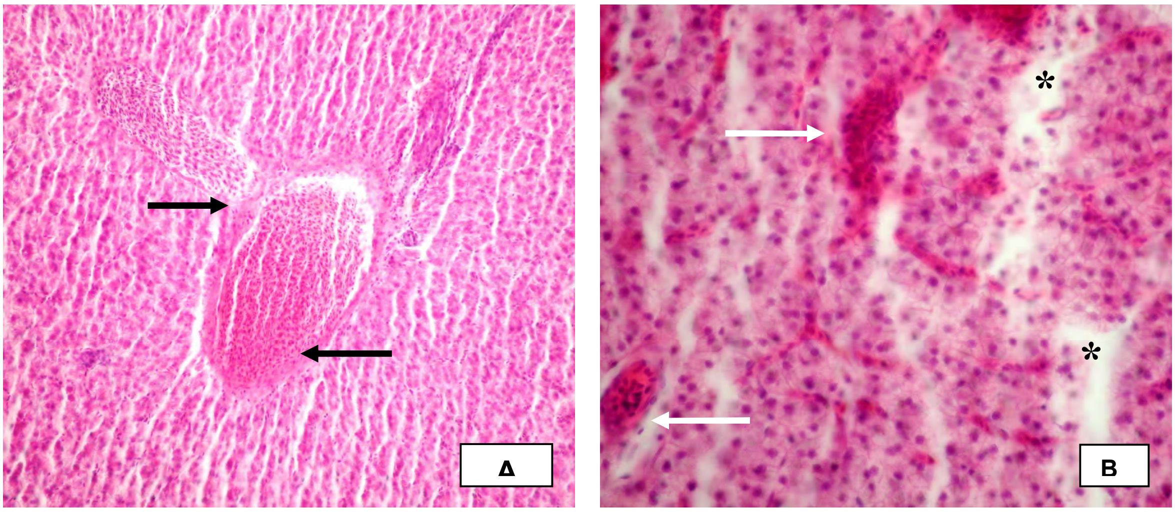

Fig.4 - **(A)** *Carassius auratus gibelio* liver intoxicated with 100µg Cu^{2+}/l (long term exposure) – **14 days**: large thickness of connective tissue around blood vessels (black arrows), x 20; **(B)** *Carassius auratus gibelio* liver intoxicated with 250µg Cu^{2+}/l (long term exposure) – **21 days**: blood congestion (black arrows), large intercellular spaces (*), x 40.

Thus, sublethal levels of copper sulphate affect the efficiency of tissue metabolites and cause pathological changes in the liver of *Carassius auratus gibelio*.

CONCLUSIONS

1. The goldfish showed various stress responses to 100 µg Cu^{2+}/l and 250 µg Cu^{2+}/l exposure.

2. Taking in to account that the liver is the first organ exposed to toxic compounds, the biochemical and histological alterations produced by Cu^{2+} were studied.

3. The increase in LDH activity may reflect an increased dependence on anaerobic carbohydrate metabolism by the liver exposed to copper.

4. Our results provide evidence that LDH can be used as a sensitive indicator of aquatic pollution.

5. Generally, the histopathological changes were related to the toxicant dose and exposure time. Thus, sublethal levels of copper caused pathological modifications in the liver of *Carassius auratus gibelio*.

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K^+-SELECTIVE ELECTRODE BASED ON A CALIX[6]ARENIC COMPOUND

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ABSTRACT

A calix[6]arenic compound (C6Cr3Am3) was tested as ionophore for cation-selective electrodes (ISE), based on PVC membrane. Potentiometric measurements were performed in Na^+, K^+, Ca^{2+}, Mg^{2+} and Ba^{2+} standard solutions. The best response was recorded for K^+: a low detection limit and large linear range were obtained.

Key words: calix[6]arene, PVC-based ISE, potassium selective electrode

INTRODUCTION

Ion-selective electrodes (ISE) are well-known for their wide applications in important fields such as clinical, food and environmental chemistry (Bakker et al., 1999). For example, it was estimated that over a billion clinical analyses are performed annually in laboratories all over the world using ISE (Bakker et al., 1997).

ISE are usually made of a polymer matrix incorporating an ionophore. Macrocyclic compounds are among the most frequently used ionophores, they selectively bind different ions by entrapping them in their cavity. The selectivity of the membrane is thus strongly influenced by the size match between the guest ion and the host cavity. A very widely used class of ionophores are calixarene derivatives, due to their cup-like shape (Arora et al., 2007, McMahon et al., 2003, Ludwig 2002), as shown in figure 1.

This paper aims at testing a new calix[6]arenic compound as ionophore for cation-selective membranes. The structure of the ionophore, p-tert-butyl-tricrothyl-triamide-calix[6]arene (C6Cr3Am3), is shown in Figure 2.

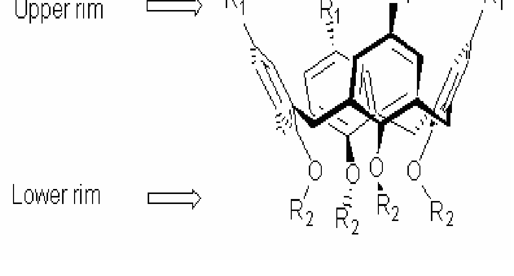

Fig. 1. The general structure of a calix[4]arene compound

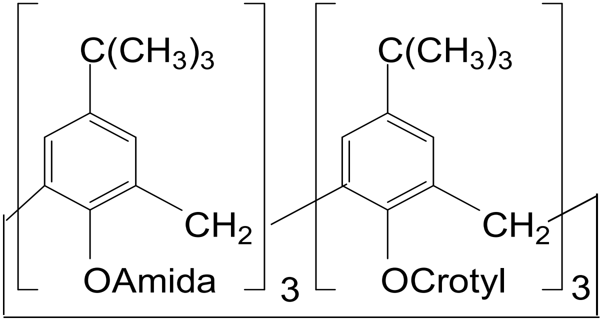

Fig. 2. The structure of the C6Cr3Am3 ionophore. Crothyl: $-CH_2-CH=CH-CH_3$; Amide: $-CH_2-C(O)N(CH_2CH_3)_2$

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MATERIALS AND METHODS

Materials

The C6Cr3Am3 ionophore was kindly provided by dr. Jeanne-Elisabeth Popovici from the Institute of Chemistry "Raluca Ripan" Cluj-Napoca (Romania). All used reagents were of analytical grade. Calcium chloride, ammonium chloride, lithium acetate, 2-nitrophenyloctylether (NPOE), high molecular weight polyvinyl chloride (PVC) and tetrahydrofurane (THF) were purchased from Fluka (Buchs, Switzerland). Potassium chloride was from Riedel-de Haën (Seelze, Germany), magnesium chloride was purchased from Chimopar (Bucharest, Romania) and sodium chloride was from Merck (Darmstadt, Germany). Barium chloride was from Silal Trading (Bucureşti - România).

Membrane preparation

The investigated cation-selective membrane contained 1% (w/w) C6Cr3Am3 as ionophore, 33% (w/w) PVC as polymer matrix and 66% (w/w) NPOE as plasticizer. The membrane weighted ~0.3 g.

The ion-selective membrane was prepared according to the following procedure. The ionophore, plasticizer and polymer matrix were successively dissolved in THF, under stirring. After dissolution, the mixture was poured into a glass cylinder, in THF atmosphere, in order to avoid pores formation. The membrane was dried and stored in dark. Before use, it was conditioned for at least 24 hours in the solution containing the cation to be determined.

Experimental setup

Measurements were performed using a PC-controlled setup (Dorneanu et al., 2005). The system control, as well as data acquisition, was performed using the LabView 5.1 software. Data treatment was done by using the Origin 5.0 software.

Two similar electrodes were prepared by fixing an 8 mm diameter disc membrane at the bottom end of a plastic syringe body. As internal reference, an Ag/AgCl system was used. The inner electrolytes contained 5 mM NaCl. Both electrodes were tested simultaneously, and each measurement was repeated at least two times in the same working conditions. A double-junction saturated calomel electrode was used as external reference. The external liquid junction was filled with CH3COOLi 0.1 M.

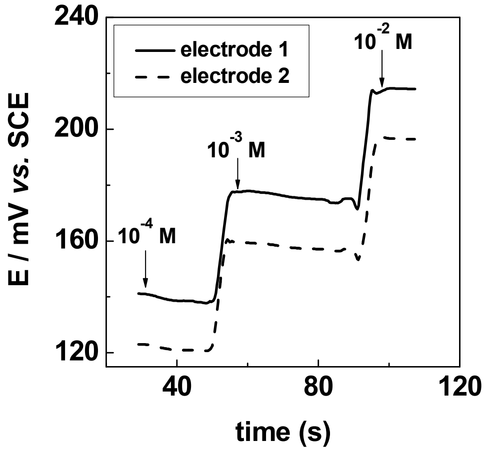

Fig. 3. Records of electrodes potentials during additions of standard solutions of investigated cations

Experimental procedure

The experimental procedure consisted in "batch" potentiometric measurements, performed using addition of standard solutions of the investigated cations. In order to assess the ISE sensitivity towards Na^+, K^+, Ca^{2+}, Mg^{2+} and Ba^{2+} cations, the ISE potential was recorded for three concentrations (10^{-4} M, 10^{-3} M and 10^{-2} M), and the sensitivity was estimated as the potential difference observed between 10^{-3} M and 10^{-2} M (see figure 3). Then, calibration curves were drawn only for the cation for which a cvasi-nernstian response was obtained. The calibration curves, served also for evaluation of the linear domain and the detection limit.

RESULTS AND DISCUSSIONS

ISE sensitivities for Na^+, K^+, Ca^{2+}, Mg^{2+} and Ba^{2+}

As a preliminary test, the sensitivities of the electrode, based on C6Cr3Am3 ionophore, were evaluated for various cations, using the method described in the previous section. The reported values are the mean for the two measurements performed with the two different ISEs, in similar experimental conditions (Table 1). As can be observed, a quasi-nernstian response was obtained only for K^+. All other cations gave under-nernstian responses.

Table 1: Sensitivities for different cations recorded for C6Cr3Am3-based electrode.

Cation	Sensitivity (mV/decade)
Na^+	44.0 ± 2.0
K^+	53.8 ± 1.2
Ca^{2+}	8.0 ± 0.0
Mg^{2+}	7.5 ± 0.5
Ba^{2+}	11.5 ± 1.0

Based on the preliminary tests, the calibration curves were drawn for K^+ cation. In order to maintain a constant ionic strength, the calibration solutions contained 1 mM NaCl. An example of calibration curves is given in figure 4. The ISE sensitivity for K^+, recalculated from the calibration curve, was 50 mV/decade, the mean detection limit was $5 \cdot 10^{-1}$ mM, and the mean linear range was ~2.5 decades.

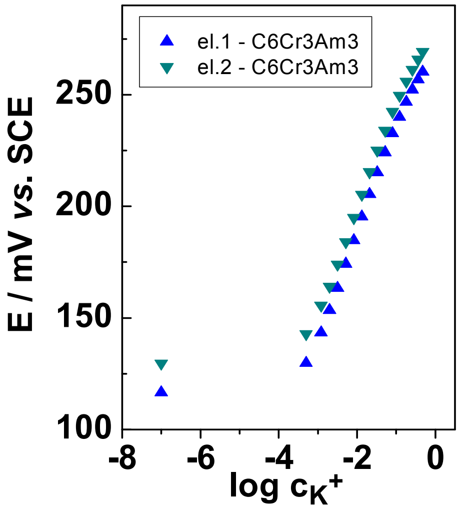

Fig. 4. Calibration curve recorded for K^+ using C6Cr3Am3-based ISE

Electrodes reproducibility

Two types of reproducibility were studied: (1) inter-electrodes reproducibility, i.e., the reproducibility observed between two measurements performed with similar electrodes in the same experimental conditions; (2) inter-measurements reproducibility, i.e., the reproducibility observed for three measurements performed with the same electrode, in the same experimental conditions. As figure 5 shows, in both cases a very good reproducibility was observed.

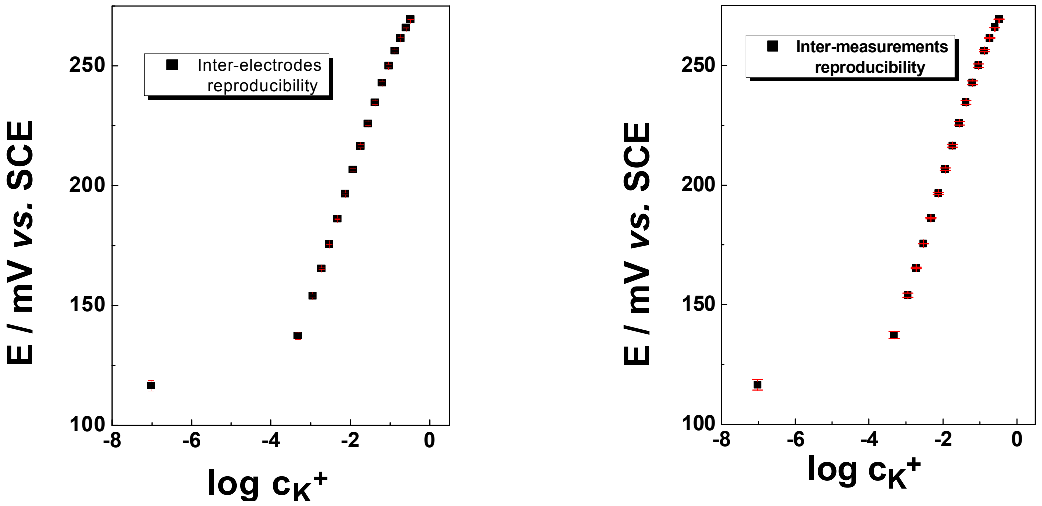

Fig. 5. Inter-electrodes and inter-measurements reproducibility. The error bars correspond to the standard deviations of the measurements.

CONCLUSIONS

1. This study aimed at characterizing the calix[6]arene compound C6Cr3Am3 as ionophore for potentiometric ISE based on a PVC membrane.
2. A preliminary study was performed to assess the ISE sensitivies for different cations: Na^+, K^+, Ca^{2+}, Mg^{2+} and Ba^{2+}. Results showed a quasi-nernstian response only for K^+.
3. Calibration curves were recorded for K^+ and good electroanalytical parameters were obtained: a mean slope of 50 mV/decade of concentration, a linear range of ~2.5 decades and a detection limit of $5*10^{-1}$ mM.
4. The obtained results suggest that C6CrAm3 could be used as ionophore for K^+-selective ISE. A study to evaluate the interference of cations, usually accompanying K^+, is under progress.

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IN VIVO EFFECTS OF CIS-PLATINUM ON MAGNESIUM LEVELS IN BLOOD, MUSCLES AND HEART SAMPLES

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ABSTRACT

The purpose of this study was to evaluate the concentration of magnesium in blood, muscles ad heart samples during cis-platinum administration on Wistar rats. For this experiment, we used adults animals from Wistar rats strain divided in two experimental groups and one control group. Wistar rats from the experimental groups were injected intraperitoneally (i.p.) with the drug called Sin-Platinum (doses 2.0 mg/kg b.w. respectively 8.0 mg/kg b.w.), and the animals from control group were injected with saline solution (placebo). The results showed that the concentration of metal elements from blood and tissues samples is changed proportionally with the administrated doses.

We have observed the consequences of accumulation of cis-platinum in several different samples, (in this experiment blood samples, muscles and heart tissues) and we evaluated the modifications which appeared after repeated doses simulating chemotherapeutic treatment in tumor-free rats.

Key words: cis-platinum, Wistar rats, magnesium

INTRODUCTION

Cis-platinum, as a compound was the first time described by Peyrone in 1845, and the structure was elucidated in 1895 by Alfred Werner. In 1960s, Rosenberg and he's team research discovered that electrolysis products from a platinum electrode inhibited mitosis in Escherichia coli bacteria (Rosenberg, 1985). Cis-platinum is known as a chemotherapeutic agent often used in the treatment of cancer. Literature data show that cis-platinum administration affects the biochemical homeostasis (Velciov et al., 2007). Studies regarding the consequences of cis-platinum administration in blood serum and some tissues samples in case of experimental animals, present interest for biochemistry, nutrition, pharmacology and toxicology.

Cis-platinum (cis-diamminedichloroplatinum) abbreviated cDDp or cis-DDP is a planar complex containing a central platinum atom surrounded by two ammonia

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 259-262, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

molecules and two chloride atoms arranged in cis position (Riley and Sternson, 1985, Lippert, 1999, Threkeld, 1999). Cis-platinum mechanism of action is based on binding to DNA which determined intra-strand cross—links and formation on DNA adducts.

This drug has a broad spectrum of activity against many different solid tumors such as lung, ovary, testis, bladder and head and neck cancers and is also an effective agent in treatment of some hematological malignancies such as refractory lymphomas (Lippert, 1999; Baba, 2002). The adverse effects of c-DDP include nausea, nephrotoxicities, ototoxicities, neurotoxicities and myelosupression.

Many nutritional problems in cancer patients are caused by alterations following chemotherapy. During cytostatic therapy the metabolism of carbohydrate, lipid and protein is disturbed. Metal elements are implicated in maintenance of acido-basic balance, osmotic and colloid-osmotic balance. Without an adequate supply of magnesium, the toxic effects cannot be prevented (Links and Lewis, 1999; Ghizdavu, 2000; Garban, 2005b).

MATERIALS AND METHODS

For the experimental part, we used adults Wistar strain rats, maintained on pathogen-free conditions, at 22–25°C room temperature, at 55–65% relative air humidity, fed on normal rhythm and standard breeding food and water.

The animals were randomly divided in three groups: one control (C) and two experimental groups (E_1 and E_2). Each group contained 10 animals (males and females) with an average body weight (b.w.) of 200 ± 20 g.

Cis-platinum used for this study was the commercially available Sin-Platin (Sindan, Romania). The animals of experimental group E_1 were injected i.p. with 2.0 mg/kg b.w. Sin-Platin and with 8.0 mg/kg b.w. the animals of E_2 group. The animals from C group, were injected i.p. with saline solution.

On the 15th day of the experiment, after 12 hours of fasting (overnight), and Ketanest anesthesia, the rats were killed, and blood and tissue samples from skeletal muscles and heart were removed for analyses. The blood samples were taken after laparotomy and puncture of vena cava caudalis. Magnesium concentration in blood was determined by using an analyser Hospitex Screen Master. The contents of magnesium in tissues were determined by flameless atomic absorption spectrophotometry of samples. Results are expressed as means (X) and standard deviations (SD) and to calculate the statistical significance, the *t* test was used as apppropiate and *p* values.

RESULTS AND DISCUSSIONS

Macrobioelements and trace elements have a very important role in material and energetic metabolism, and are responsible for biochemical homeostasis (Ghergariu, 1980, Garban, 1999).

The studies regarding chemotherapy are developed especially using experimental studies on laboratory animals (Ciudin şi Marinescu, 1996).

Our results concerning the concentration of magnesium in blood, skeletal muscle and heart samples after cis-platinum administration in Wistar rats are presented in Table 1.

Table 1. Mean concentration of Mg in blood, muscles and heart samples of Wistar rats after cis-platinum administration.

Specifications	n	Mg in blood mg/dL	Mg in muscle (μg/g tissue)	Mg in heart (μg/g tissue)
		$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$
Group C	10	2.12 ± 0.18	258,28 ±43,01	235,91 ± 39,20
Group E_1	10	0.93 ± 0.59	248,56 ± 41,42	227,41 ± 37,15
$\Delta\overline{X}$		-1.19	-9,72	-8,50
Grup E_2	10	0.52 ± 0.06	227,38 ± 37,58*	207,61 ± 33,60*
$\Delta\overline{X}$		-1.60	-30,90	-28,30

n– number of animals in case of each group

*p< 0,01;** p<0,05

From this data it is obviously that mean value of Mg in blood after administration of cis-platin are lower compared to the values of control group (Sartori, 1991) – see fig. 1.

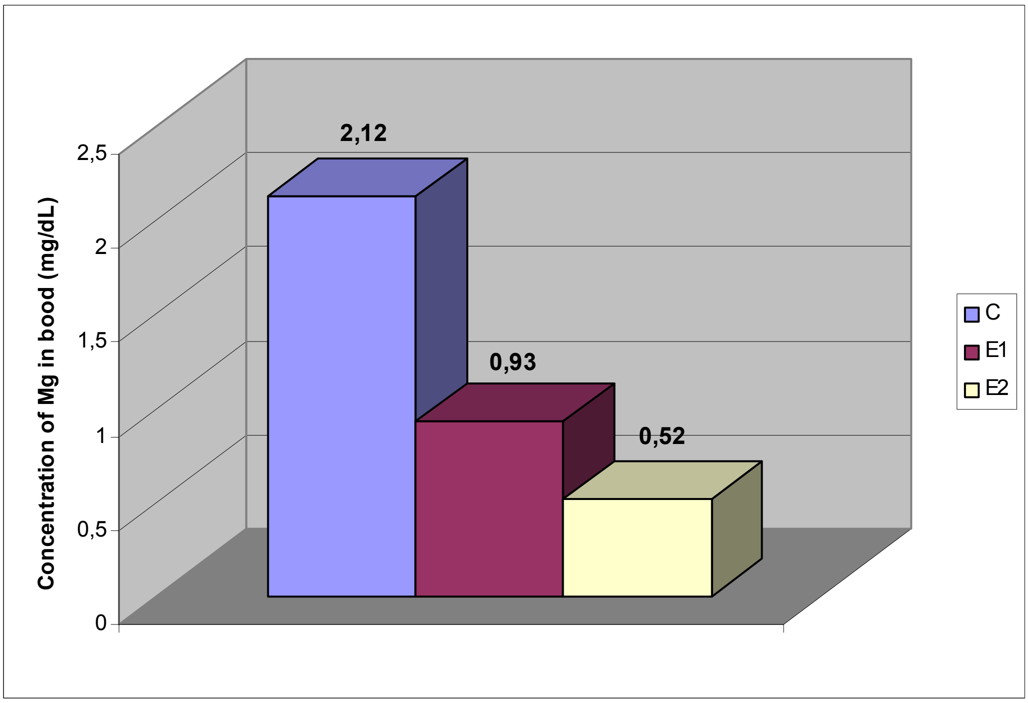

Fig. 1 Concentration of magnesium in blood of Wistar rats

Depressions of trace elements can be correlated with some changes of acid - base balance, osmotic and osmotic – colloidal balance (e.g. magnesium can realise stabile combinations with aminoacids).

From the obtained data we can observe that Mg concentration in experimental groups is lower as compared with the values obtained in the control group. Magnesium plays a structural and regulatory role in living organisms (Ghergariu, 1980, Buckley et al., 1984, Seelig M., 1993, Lajer et al., 2003, Garban et al., 2005). It has an essential role in metabolism of several minerals, through he's function as an activator of all enzymatic systems. Magnesium deficiency is linked with cardiovascular, renal digestive, neurological and mostly muscular alterations (Garban, 1999) – see fig. 2.

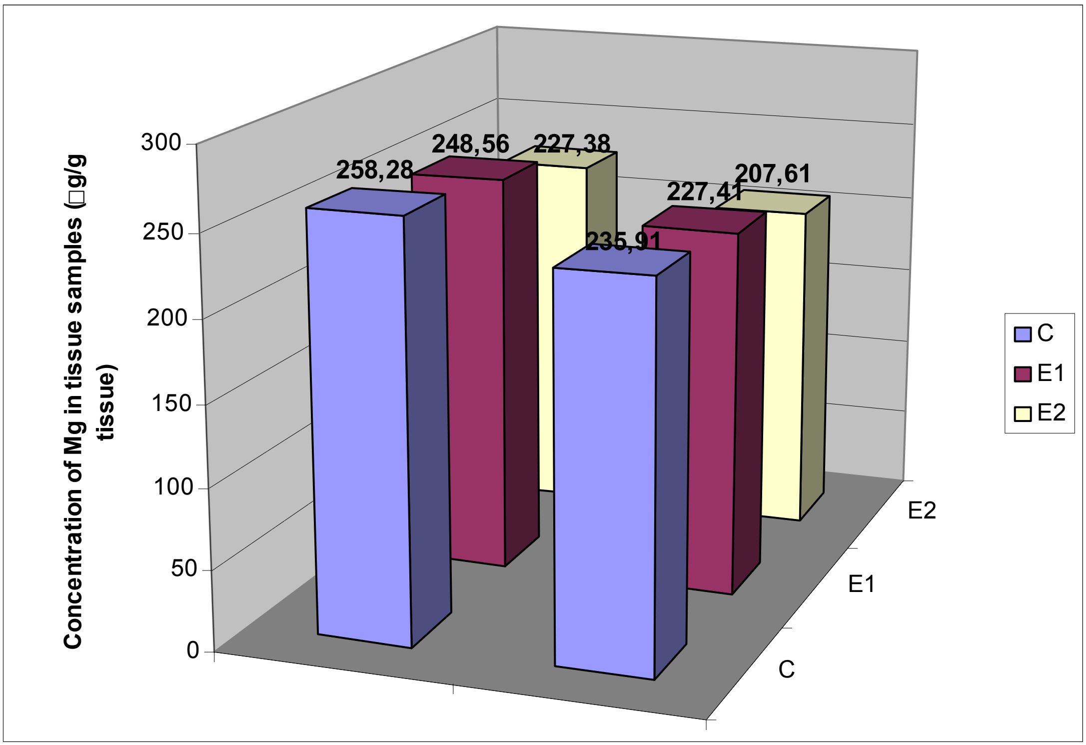

Fig. 2. Concentration of magnesium in tissue samples of Wistar rats

In case of heart tissues samples magnesium values are lower in experimental groups as compared to control groups, and in case of experimental group (E_4) these difference are significant ($p< 0.01$).

CONCLUSIONS

1. Studies regarding concentration of some metal elements from blood and tissue samples are very important for defining the influence of chemotherapy on normal health status.
2. In case of blood samples, we can observe a depression of magnesium levels, some even significant, in experimental groups compared to control groups.
3. Administration of cis-platinum in Wistar rats is characterized by magnesium depression in skeletal muscles indirectly proportional with administrated doses.
4. The concentration of magnesium metal elements in heart tissue are decreased after cis-platinum administration maybe because toxic effects of the metallic compound, on particularly nephrotoxic effects, which cause severe depletion magnesium.

5. Metal elements are involved in biochemical homeostasis, which is very important for the normal development of physiological processes, such as: acido-basic balance, osmotic and colloid-osmotic balance. Without an adequate supply of metal elements the toxic effects cannot be prevented.

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MAGNESIUM IN DRINKING WATER OF SOME PUBLIC SYSTEMS AND WELLS IN IASI DISTRICT AND THE DAILY MAGNESIUM REQUIREMENT

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ABSTRACT

Tap water was more effective in meeting magnesium requirements than dietary supplementation. The mineral macroelements concentration: Mg, Ca, Ca/Mg, fixed residuum and total hardness was measured in 62 samples of the drinking water, 38 of the public systems and 24 samples of wells in two periods of the year, July and October in 5 citis of Iasi district. The chemical analyse of the samples point out a low degree of the mineralization and fixed residuum in average below 500 mg/l, variations of the Ca, Mg, total hardness concentration. Mg average concentration was higher in the well water of Tg. Frumos and Hirlau city and lower in depth water of the city Pascani and Tg. Frumos; the mean concentration of the total hardness in drinking water of the public system, was ranged between 12.02 – 16. 28 ^{O} G, average was higher in the well water of the four citys. Ca/Mg ratio was higher in the drinking water of the public system with variation between 2, 91- 16, 84. In our study, in 5 citis with an total of 388. 650 inhabitants, the Mg content of drinking water may provide below 10% of the daily Mg requirement in public system and between 10,78 % – 48,5 % in wells, with importants variations depending on the soil.

Key words: magnesium, daily Mg requirement, Ca/Mg, total hardness, drinking waters

INTRODUCTION

Several previous epidemiological studies have shown a relation between drinking water quality and death in cardiovascular disease (Rylander, Arnaud, 2004).

Many studies support a statistic correlation between a reduced level of the magnesium and total hardness in the drinking water and the higher cardiovascular diseases mortality (Alexa L., 1996, Cuciureanu R., 2002, Gavăt V., 2007, Ionuț C., 2004, Nebrand C., 2003).

It is possible, then, that waterborne magnesium could correct an insufficient dietary magnesium level.

Magnesium - rich water may contribute to coverage of magnesium requirements by providing significant amounts of natural, energy-free, bioavailable magnesium. Waterborne magnesium could correct an insufficient dietary magnesium level (Durlach, M.J., 1985, Rylander, Arnaud, 2004, Sabatier M., 2002, Seeling M.S., Rosanoff A., 2003)

„Metal Elements in Environment, Medicine and Biology”, Tome VIIII, pp. 265-270, Publishing House “Eurobit” Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

The aims of the study were to analyse the Mg, Ca, Ca/Mg and total hardness in the drinking water of the towns areas of Iasi county, for evaluate their health hasard. In the same time, were to realise an useful presentation for the therapy of the mean Mg concentration depending of a person's daily magnesium requirement.

MATERIALS AND METHODS

The mineral macroelements concentration: Mg, Ca, Ca/Mg, fixed residuum and total hardness was measured in 62 samples of the drinking water, 38 of the public systems and 24 samples of wells in two periods of the year, July and October in Iaşi, Pascani, Hirlau, Tg. Frumos and Podu Iloaie (*with an total of 388. 650 inhabitants*) areas of Iasi district.

The calculus of probability to determinate the size of the water sample in the four towns was used. On used the standardisation methods.

RESULTS AND DISCUSSIONS

Magnesium concentration in drinking water

In the drinking water of the public system of the 5 citis magnesium average concentration was higher at Hirlau (mean 23,74 mg/l in july and 28,75 mg/l in octobre) and in order at Podu Iloaie city (mean 27,18 mg/l on july and 14,33 mg/l in octobre), Iasi city (mean 19,25 mg/l in surface water Prut and 9,46 mg/l in groundwater Timisesti), at Pascani city (mean 12,23 mg/l on july and 13,52 mg/l on octobre), at Tg. Frumos (mean 10,28 mg/l on july and 5,36 mg/l on octobre). Results are given in Table 1.

In the drinking water of the wells, of the 5 citis, magnesium average concentration was higher in well water of Tg. Frumos, Podu Iloaie and Hirlau city and lower in wells water at the Pascani city (table 1).

It is advisable to have 30 mg/liter of Mg in drinking water (Durlach, 1989).

Table 1. The mean Mg concentration (mg/l) in the samples of the drinking water of the 5 cities in the public system and wells

Town	Public System		Wells	
	july	octobre	july	octobre
Iasi	19,25 mg/l in surface water Prut 9,46 mg/l in grandwater Timisesti		-	-
Pascani	12,23	13,52	37,93	26,6
Hirlau	23,74	28,75	94,36	74,5
Tg. Frumos	10,28	5,36	102,95	101,56
Podu Iloaie	27,18	14,33	94	98

In some geographic areas, the magnesium content of drinking water may provide 20% to 40% of a person's daily magnesium requirement (Sabatier, 2002).

In our study the Mg content of drinking water may provide below 10% of a person's daily magnesium requirement from the water of the public system (with variations between 9,62 – 2,17) (fig 1) and between 10,78 % – 48,5 % from the water of wells, with importants variations depending on the soil for the depth water in differents citis (fig 2).

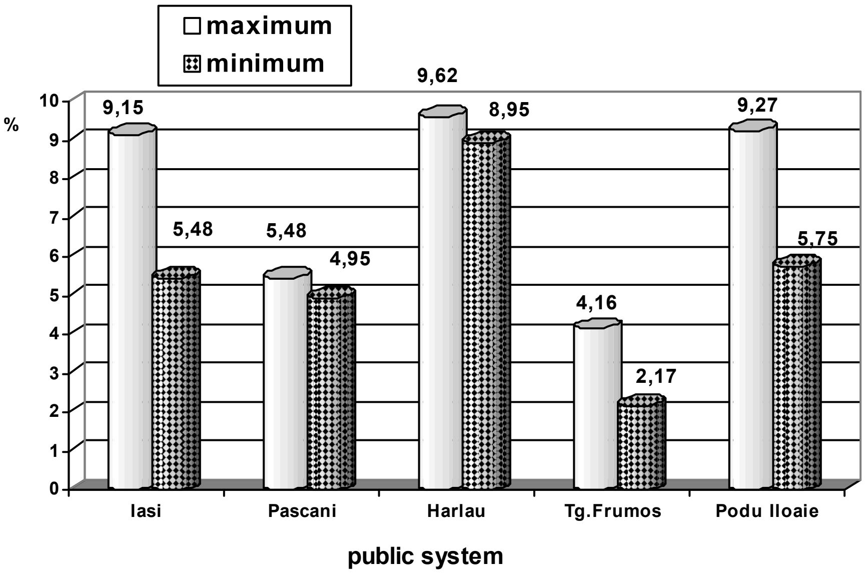

Fig.1. Percent provide of the person`s daily magnesium requirement (370 mg/day) through drinking water of public system

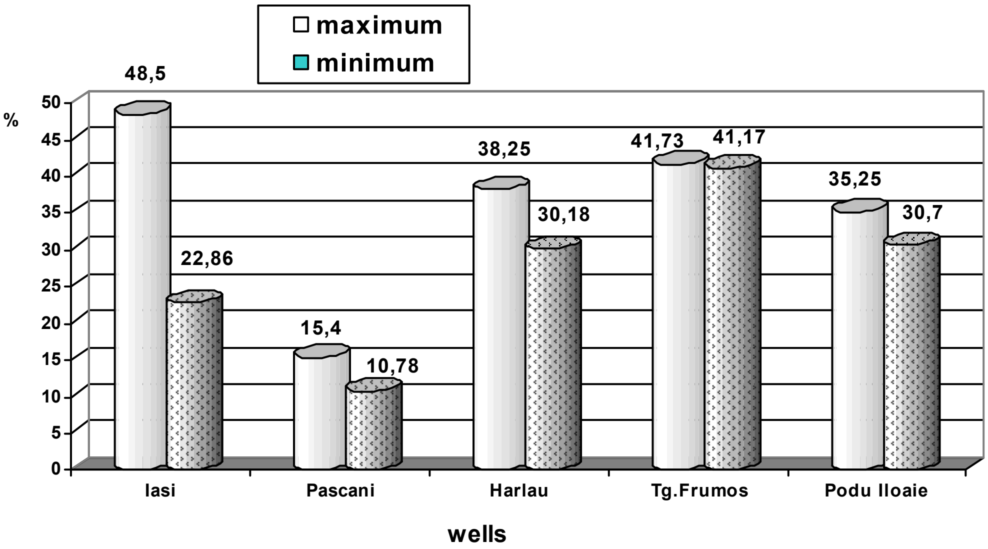

Fig. 2. Percent provide of the person`s daily magnesium requirement (370 mg/day) through drinking water of wells

For example, a 1and1/2 liter of water with a magnesium content of 100 mg per liter contains 40,54 % of the daily magnesium requirement of 370 mg per day. However, a liter of water that is low in magnesium (eg, <1O mg per liter) provides less than 3% of the daily requirement.

In a previous study on rural area of Iasi county, (430.402 inhabitants in 2007) the results showed that Mg $^{++}$ in wells water was the only mineral wich proved to have a protective effect against cardio-vascular diseases; the interrelation between the ratio of Mg and the mortality from cardiovascular disease was statistically significant (Alexa, 1996).

We found important variation of the Mg and Ca concentration depending of the season.

Corrosivity of the water in public sysem is important. Magnesium appears as a competitive inhibitor of the two main noxious polluting agents: Pb and Cd.

Mg and Ca/Mg ratio in drinking water

An optimal water would also have a ratio of Ca/Mg of not more than 2:1.(Durlach, 1985). Results are given in Table 2.

Table 2. The mean Ca/Mg ratio in the samples of the drinking water of the 5 cities in the public system and wells

Town	Public System		Wells	
	july	octobre	july	octobre
Iasi	4,63 in surface water Prut 4.5 in grandwater Timisesti		-	-
Pascani	8,55	11,96	3,46	8,49
Hirlau	5,13	2,91	1,37	2,04
Tg. Frumos	11,79	16,84	0,7	0,75
Podu Iloaie	6,3	8,4	1,3	1,8

Total hardness in drinking water

Cardiovascular diseases rates may be inversely related to water hardness. Rates of cardiovascular mortality and sudden death are 10% to 30% greater in soft water areas (low in magnesium or calcium) than in hard water areas (high in magnesium or calcium).

Total hardness is made up by Ca and Mg compounds/ salts of other metals (Al, Ba, Fe, Mn, Sr).

The mean concentration of the total hardness in drinking water of the public system, was ranged between 12.02 – 16. 28^{O}G (table 3). We found important variation of the total hardness (Mg and Ca too) concentration depending of the season (table 3 and 4).

Table 3. The mean, maximum and minimum of the total hardness (grd. G) in the samples of the drinking water of the 4 cities on the public system

Town	july			octobre		
	mean	maximum	minimum	mean	maximum	minimum
Iasi* Iasi**	12,02 13,10	17,78 14,11	9,32 11,42	- -	- -	- -
Pascani	13,33	15,03	12,12	16,28	25	13,5
Hirlau	15,66	20,2	13,91	15,15	19,5	12,6
Tg. Frumos	14,09	15,93	9,14	13,2	14,6	11,9

* surface water Prut; **Groundwater Timisesti

The total hardness was higher in the wells water with variations of the mean concentration between 20, 36 – 38, 37 grd.G(table 4).

Table 4. The mean, maximum and minimum of the total hardness (grd. G) in the samples of the drinking water in the wells of the 3 cities

Town	**july**			**octobre**		
	mean	maximum	minimum	mean	maximum	minimum
Pascani	23,78	33,43	13,91	20,36	33,9	13,5
Hirlau	38,37	55,65	22,89	31,2	55,4	20,6
Tg. Frumos	32,91	42,64	7,28	34,21	44,9	17

The chemical analyse of the samples point out a low degree of the mineralization and fixed residuum in average below 500 mg/l.

CONCLUSIONS

In our study, in Iasi county, in urban area (5 citis with an total of 388. 650 inhabitants) the Mg content of drinking water may provide below 10% of the RDI in public system and between 10,78 % – 48,5 % of the RDI in wells, with importants variations.

Mg need to be taken into account as a protectiv factor in cardiovascular diseases when represent quantitatively a significant part of the Recommended Dietary Allowances or if, quantitatively constitute a higher bioavailable intake.

In the drinking water of the public system of the 5 citis magnesium average concentration was higher in Hirlau (mean 23,74 mg/l on july and 28,75 mg/l on octobre) and in order in Podu Iloaie city (mean 27,18 mg/l on july and 14,33 mg/l on octobre), Iasi city (mean 19,25 mg/l in surface water Prut and 9,46 mg/l on groundwater Timisesti), in Pascani city (mean 12,23 mg/l on july and 13,52 mg/l on octobre), in Tg. Frumos (mean 10,28 mg/l on july and 5,36 mg/l on octobre).

In the drinking water of the wells, of the 5 citis, magnesium average concentration was higher on well water of Tg. Frumos, Podu Iloaie and Hirlau city and lower in depth water of the city Pascani

Because Mg concentration in the drinking water in the public system of the towns in Iasi county is below 30 mg/l, Mg is not competitive inhibitor of the two main noxious water polluting agents: Pb and Cd.

The mean Ca/Mg ratio in the water of the public system in urban area of Iasi county varied between 2,91- 16,84 and in wells water between 0.75- 8.49 (optim 2/1).

The chemical analyse of the samples point out a low degree of the mineralization and fixed residuum in average below 500 mg/l.

The mean concentration of the total hardness in drinking water of the public system, was ranged between 12,02 – 16, 28^{O}G. The total hardness was higher in the wells water with variations of the mean concentration between 20,36 – 38,37 grd. G.

We found important variation of the total hardness (Mg and Ca too) concentration depending of the season.

It is also the possibility to supplement the drinking water of the public system with magnesium.

Tap water was more effective in meeting magnesium requirements than dietary supplementation.

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THE REDOX REACTIVITY OF GLOBINS: THE CHICKEN AND EGG PARADOX

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ABSTRACT

The role of globins in oxygen transport and storage is well established and has long been known. However, more recently discovered members of the globin family appear to clearly have functions in vivo that do not involve oxygen storage and transport. Such are the globins found in inferior organisms, for which nitric oxide dioxygenase as well as peroxidase functions have been proposed; the roles of neuroglobins, cytoglobins and leghemoglobins are yet to be fully established, with nitrite reduction among the possible candidates. With globins present in organisms that seem to precede aerobic metabolism, it appears most likely that all of these redox activities, which may appear at least exotic in relation to hemoglobin and myoglobin, do in fact precede, in an evolutionary sense, the dioxygen transport function in the globin family.

Key words: globins, oxidative stress, nitrosative stress

Globins are mostly known for their functions in dioxygen transport (hemoglobin) and storage (myoglobin). However, more recently a few other types of globin reactivities, mostly of redox and enzymatic nature, have been found to have biological/physiological/medical relevance. These reactivities are the focus of the current presentation; whereas in relation to the dioxygen handling function they appear as rather exotic and perhaps even fortuitous, the redox reactivities are now well accepted as physiological functions for a range of globins.

The globin family has in recent years expanded to include a number of new members. Among these, **neuroglobin** has been demonstrated to be expressed in human brain.[1] Unlike hemoglobin and myoglobin, neuroglobin in its deoxy (ferrous, not bound to oxygen) state appears hexacoordinated endogenously, which suggests its function is distinctly not one involving ligand binding – hence, not dioxygen storage or transport; the two axial ligands are, as expected, the homologues of the famous hemoglobin proximal and distal histidines.[1] Moreover, even the ferric form is still hexacoordinated, as a further argument against ligand binding playing any role in neoroglobin's physiological function. On the other hand, both dioxygen and CO can, in vitro, displace the 'distal' histidine; however, the oxygen affinity is too low for this reactivity to be relevant under the low dioxygen concentrations found in neuronal cells, and the autooxidation rate is also distinctly higher than that of myoglobin.[1-7] It is thus generally accepted that neuroglobin's in vivo function is not dioxygen binding; neuroglobin is overexpressed under hypoxia and ischemia, and exerts a protective

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„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 271-276, Publishing House "Eurobit" Timişoara, 2008
Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

effect under these conditions.[4-7] Ferric, but not ferrous, human Ngb has been shown to bind to heterotrimeric G proteins, acting as a guanine nucleotide dissociation inhibitor, eventually protecting against neuronal cell death; within this framework, Ngb would be acting as a redox sensor.[1] A physiological role for the ferric (met) form of a globin is certainly at considerable distance from everything known about the 'traditional' members of this family – hemoglobin and myoglobin.[1] On the other hand, a role in reactive nitrogen species scavenging (e.g., peroxynitrite) has also been proposed for neuroglobin.[8]

Closely related to neuroglobin is **cytoglobin**. It, like neoroglobin, appears hexacoordinated in the deoxy state. However, its dioxygen affinity falls within the physiological range, and subject both to cooperativity (cytoglobin is a dimeric protein) and to control via a disulfide bridge. Interestingly, however, is that cytoglobin appears localized exclusively in cell nuclei, which suggests that its function, while related to dioxygen metabolism, is not simply one of storage, and may in fact be one of transcriptional regulator, still under some redox (via the cysteine disulfide bridge) control.[4,5]

A family of slightly smaller ('truncated') globins, occasionally condensed with a flavin-containing redox partner, has been identified in inferior organisms, such as *E. coli*. Of these, **flavohemoglobin** (Fhb) has probably received most of the attention. One of the roles proposed for Fhb has been the reaction with lipid hydroperoxides; indeed, this protein is reactive towards such peroxides more so than towards H_2O_2; moreover, a possible role for the ferrous form of the protein has been invoked in this reaction – which would be at odds with all other reactions of heme proteins with peroxides, known in general to involve ferric, not ferrous, hemes.[9,10] In any case, dioxygen storage is distinctly not a physiological function, in view of the high autooxidation rate.[11-13] On the other hand, Gardner at al have demonstrated that Fhb protects *E. coli* against toxic levels of nitric oxide, under non-anaerobic conditions, and that, moreover, expression of this protein is induced under these conditions.[11-13] The proposed mechanism of this process involves an iron(II)-O_2 + NO reaction[14-25], with initial attack of NO on the (formally) iron(III)-superoxo heme, forming an iron(III)-peroxynitrite species, $[\text{Fe-OONO}]^{2+}$ (Reaction 1). Formation of this intermediate is controlled only by diffusion of NO into the heme pocket, in line with the superoxo character of the dioxygen ligand; free superoxide indeed reacts with NO at rates approaching the diffusion limit.[26] The peroxynitrite adduct then decays to resting iron(III) heme, liberating nitrate.

$$\text{Fe(II)-O}_2 + \text{NO} \rightarrow \{\text{Fe(III)-OONO}^-\} \rightarrow \{\text{Fe(III)-NO}_3^-\} \rightarrow \text{Fe(III)} + \text{NO}_3^-$$

Reaction 1

An alternative mechanism for flavohemoglobins, with O_2 attacking an Fe(II)-NO heme adduct to produce an N-bound iron(III)-peroxynitrite intermediate,[27] is disfavoured by DFT calculations.[10] Moreover, in mammalian myoglobins, the reaction of Fe(II)-NO with dioxygen is rate-limited by dissociation of nitric oxide and a subsequent mechanism cf. Reaction 1.[28]

In relation to the peroxidase function proposed for flavohemoglobin, we and others have demonstrated that **myoglobin** as well as **hemoglobin** exhibit an ascorbate peroxidase reactivity, which is entirely enzymatic in nature as characterized within the Michaelis-Menten formalisms.[29,30] In fact, the Km values for ascorbate in this process are in the low-micromolar range for hemoglobin, and well within the range of physiological concentrations of ascorbate, suggesting that hemoglobin is especially designed to interact with ascorbate, and in fact to our knowledge it is the one protein with the highest affinity for ascorbate, of all those known to us so far, from any species.[29] Figure 1 illustrates the type of reactivity entailed by globin reaction with peroxides.

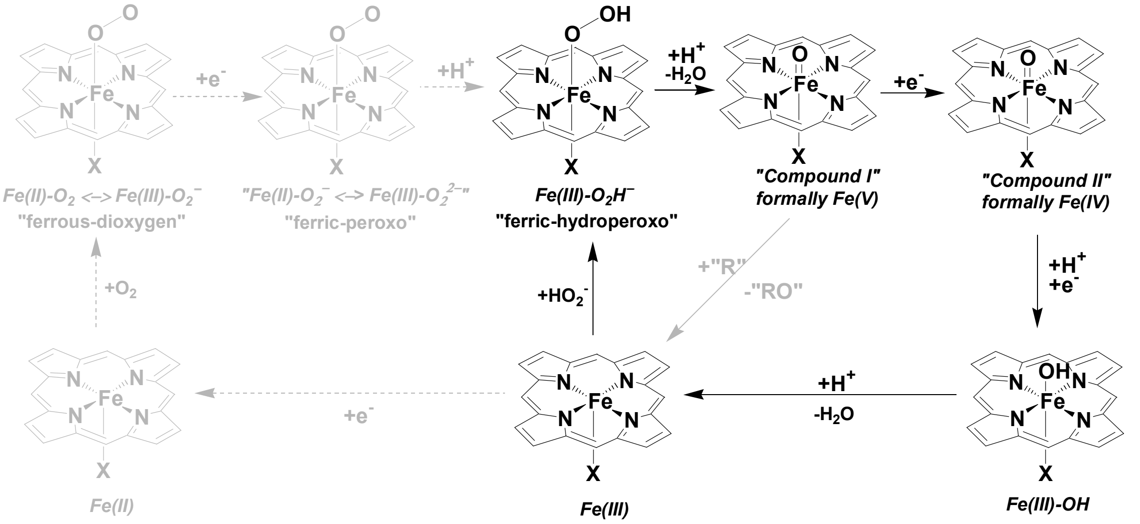

Fig. 1. Shown in black and with solid arrows is the peroxidase general mechanism. The electrons required for reduction of Compound I and Compound II are supplied by a reducing substrate (typically a small organic molecule, ascorbate in the globin case). Shown in gray and with a solid arrow is the peroxygenase variation of the catalytic cycle, which is less common in globins; here, 'R' is an organic substrate and 'RO' is its monooxygenated form. Shown in gray and with dashed arrows is the oxygenase variation, which again is even further less common in globins. 'X' denotes the protein-derived axial ligand which, in globins, is the proximal histidine.

A final type of reactivity taken into discussion here is that of nitrite reductase. This reaction, occurring via mechanisms illustrated in Fig.2, liberates nitric oxide, known for its physiological role in blood among other things.[31,32]

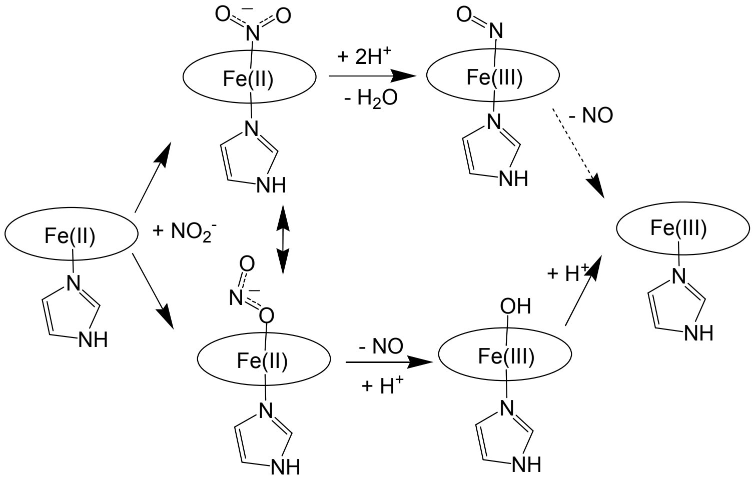

Fig.2 Mechanism of nitrite reductase reaction

Of particular interest to us is the occurrence of nitrite linkage isomerism in this reaction, which was introduced by us based on density functional theory (DFT) calculations, and has since been demonstrated experimentally by crystal structures.[33] On the other hand, with this reaction contributing to the regulation of the NO concentration in blood, significant interest has recently been shown towards the possibility of nitrite being used as a drug for cardiovascular issues.[34-41] The key importance, and medical relevance, of this and of the peroxidase reactivity of globins,[29,30] do question any attempt to view these redox reactivities as mere essentially fortuitous side-reactions in hemoglobin and myoglobin. Moreover, as the redox reactivity appears as a predominant function in globins found in inferior organisms, in metabolic systems that are likely to have preceded aerobic life, it appears that the functions of dioxygen transport and storage in globins must have evolved subsequent to those of redox nature, and that, in chronological order, reversible dioxygen handling is in fact a side-reaction of the initial, redox based, physiological function(s) of globins in ancient organisms.

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THE SIGNIFICANCE OF CYTOMORPHOMETRY IN PERITONEAL EFFUSIONS CYTOLOGY PRACTICE

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ABSTRACT

The cytomorphological evaluation of effusion is a challenging aspect of non-gynecology cytology. The definite diagnosis of malignant effusion is often complicated by the presence of inflammation, exuberant mesothelial hyperplasia and a paucity of malignant cells. Computerised image analysis can rapidly digitise the image of the cells and thereby can estimate various morphometric parameters of the cell. Cytological samples suitable for image were obtained from cytocentrifuge. The previous study showed that existed significant differences between morphometric variables derived from nuclear and cytoplasmic outlines in benign and malignant effusions. In this retrospective study based on 58 available cases with a histological diagnosis, we have examined the peritoneal effusions from routine morphologic features. Cytologically, 34 (58,62%) peritoneal fluids smears were classified as benign (negative cytology), 18 (31.03%) were malignant (positive cytology) and 8 (13.79%) were considered suspicious.The various cellular parameters that were measured by cytomorphometry included nuclear as well as cytoplasmic diameter, area, ratio of nucleus to cytoplasmic area. Cytomorphometry hepls in better and quicker sorting for malignant cells. Finally, 34 cases were found to be benign, 22 malignant and 4 in the category of atypical cases.

Key Words: cytomorphometry, peritoneal effusios, benign, malign

INTRODUCTION

Because of its complexity, cytopathologic evaluation of effusions fluids can be challenging. However, because effusions fluids are relatively easy to collect, any pathology laboratory may be confronted with such specimens, regardless of its level of expertise. In comparison to other common specimens, effusions fluids need special and unique approaches for cytopathologic evaluation.

The cytological diagnoses of serous effusions are usually made by routine cytomorphology with certainty, allowing treatment decisions. Various studies have shown a sensitivity of 57.3% and specificity of 89% by conventional cytology for the detection of malignant cells in effusion samples (Thunnissen et al., 1993). Studies have shown that positive and negative predictive values for detection of malignancy by cytomorphology are 89.3% and 69.4% respectively. However, a grey zone always exists, where the cytopathologist encounters problems in determining the nature of the cells whether reactive, atypical, or beyond doubt malignant (Bedrossian, 1994;

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 277-284, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

Motherby et al., 1999). Therefore various ancillary techniques should be used to increase the diagnostic accuracy of malignancy in serous effusions.

The conventional cytology rate for identification of neoplastic cells in effusions is about 60%. The rate of diagnostically equivocal effusions in routine cytology is dependent on the volume of effusion examined, type of preparation and staining, experience of the examiner, and application of ancillary methods. Peritoneal effusions are a frequently encountered clinical manifestation of metastatic disease, with breast, ovarian, and lung carcinomas and malignant mesothelioma leading the list (Redman et al., 1991; Creasman and Rutlegge, 1971).

Neoplastic cells that disseminate into cavities containing effusions are highly metastatic and possess a strong autonomous proliferative drive while concurrently being stimulatory of exudative effusions.

The diagnosis of a malignant effusion signifies disease progression and is associated with a worse prognosis regardless of the tumor site of origin. Furthermore, cancer cells of different origins differ considerably in their biology and have unique phenotypic and genotypic characteristics (Johnson, 1966).

Primary cytomorphologic criteria of malignancy include cellular aggregates, pleomorphism (variable cellular appearance), anisocytosis (variation in cell size), anisokaryosis (variation in nuclear size), multinucleation, prominent to irregular nucleoli, increased nuclear to cytoplasmic ratio, monomorphic cellular appearance, and increased mitotic figures. Hyperplastic mesothelial cells also may exhibit anisocytosis, anisokaryosis, increased nuclear to cytoplasmic ratio, binucleate and multinucleate, and scattered mitoses. Any situation that results in fluid accumulation within the body cavities can induce mesothelial cell hyperplasia and exfoliation with an abnormal cellular morphology (Runyon, 1999). Therefore, the differentiation between mesothelial cell hyperplasia and mesothelioma may be difficult or impossible.

This study was carried out with the aim to analyse not only the mean nuclear variables from the sample of cells in each specimen, but also the outlying values, represented by the largest nucleus found in each, and to to confirm the role of cytomorphometry on peritoneal effusions.

This study used computerized interactive morphometry to evaluate the differential characteristics of mesothelial normal cells, mesothelial hyperplastic cells, and carcinomatous cells.

MATERIALS AND METHODS

This was hospital based prospective study carried out in Emergency Clinical Hospital of Constanta – Pathological Anatomy Department (SCJUC) from January 1, 2008 to 31 Octobre, 2008. Clinical charts of all the patients whose peritoneal fluid samples were sent for cytological examination during the study period were retrieved for relevant information.

Histopathologic examination of tissue was used as gold standard to diagnose malignancy, in cases where this was available. Where histopathological examination was not done, diagnosis of malignancy was based on strong clinical suspicion, radiological methods and direct visualization of tumour.

The fluid for cytological analysis was collected during laparotomy from the abdominal cavity. If no fluid was present, the peritoneal cavity was lavaged with saline solution, and the fluid was then collected for analysis.

Follow-up data were obtained from the Tumor Registry at SCJUC.

For small fluid accumulations the entire specimen was submitted for laboratory evaluation. For larger effusions, 50-200 mL of well-mixed fluid was sent for cytologic examination; however, the entire specimen is also acceptable.

Giemsa stained and Papanicolau stained slides were prepared from sediment obtained by centrifuging the peritoneal liquid samples at 1500 rpm for 5 minutes, using Shandon Cytospin preparations. After the centrifugation, the stained is fixed using alcohol (95% ethyl alcohol) as the fixative.

The Papanicolaou stain uses a standard nuclear stain, hematoxylin, and two cytoplasmic

Counterstains, OG-6 and EA. The outcome of this method is crisp nuclear detail and transparency of the cytoplasm, which allows the examiner to clearly visualize cellular morphology. The analysis of fals positive and false negative peritoneal fluid sample shows the importance of using both the Giemsa stain and the Pap stain.

Effusion cytology was studied from 18 malignancy associated and 34 non malignancy associated ascitic fluid samples.

The smears collected were submitted to the image analysis. The morphometric differences between benign and malignant serous effusions, as diagnosed by standard cytologic criteria in 58 cases (34 benign and 18 malignant), were studied using the LUCIA Net semi-automated image analysis system, which calculates various parameters from tracings of cellular and nuclear outlines.

The procedure included extraction of multiple descriptors of the nuclear profile: perimeter length, area and distribution table of the nuclear area. Morphometric measurements of cellular and nuclear area, nuclear perimeter, nuclear/cytoplasmic ratio (N/C) and mean nuclear diameter of mesothelial cells were performed on single cells in peritoneal effusions from 18 patients with effusions caused by different types of carcinoma. The results were compared with corresponding measurements on mesothelial cells in peritoneal effusions from 34 patients without malignant disease.

Measurements were performed on the 50 cells in each specimen, excluding multinucleation and polymorphonuclear leucocytes.

RESULTS AND DISCUSSIONS

Effusions associated with malignancy cantained tumor cells and ocasionally small fragments of neoplastic tissue in wich may be recognized the relations of the tumor cells to their stroma. Fluids containing tissue fragments or groups of tumor cells offer little difficulty and ordinarily permit a positive diagnosis of malignancy. The cells of these clumps usually are larger than the other non-neoplastic cells of the sediment nd because of their deeper staining give the slide a mottled appearance even under the low powers of the microscope.

The differentiation of neoplastic cells from mesothelial cells in such sediments is based on marked variation in size and shape, or the presence of large numbers of unusual-appearing cells, as well as on cytological factors such as multinucleation, giant nuclei and the presence of mitotic fitures. Vacuolization of the cytoplasm is more common in tumor cells, but is also seen in the mesothelial cells of long standing effusions.

Some different cytologic findings, including smaller cell and nuclear size, larger relative nuclear area, fewer cells with lrge discrete cytoplasmic vacuoles and macronucleoli, more frequent multinucleation and larger closely packed clusters of cells, were shown.

Cytologic features.

Benign mesothelial cells - when mesothelial cells are aspirated by a needle from the surface of the abdominal cavity, they appear as sheets of polygonal cells, about 15-30 µm in diameter (1.5-2 times the size of neutrophils, but they may vary significantly and my range up to 50 µm in diameter), that are usually separated from each other by clear „windows”. The cells have a delicate, yet sharply demarcated, cyanophilic or eosinophilic cytoplasm and round or oval nuclei. The nuclei, generally located in the center of the cell, are of even size (about 10 µm in dimeter), sharply demarcated, slightly granular and contain one or two, centrally located, readily visible nucleoli (Fig.1).

Reactive mesothelial cells - benign mesothelial cells may proliferate and display a spectrum of reactive changes. These range from minimal simple reactive changes to highly atypical reactive changes that enter into the differential diagnosis for malignancy. The mesothelial cells may maintain the morphologic features af normal mesothelial cells (round to oval nuclei with smooth nuclear contours and fine chromatin) (Fig.2).

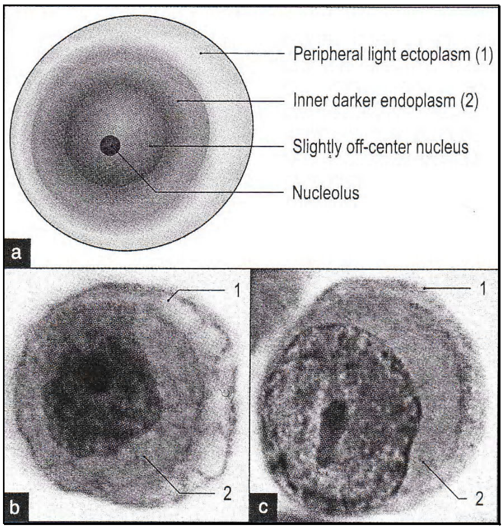

Fig.1. Benign mesothelial cells. The nucleus is usually central or near central (b), but may be eccentric(c). Papanicolaou stained, 100X zoomed.

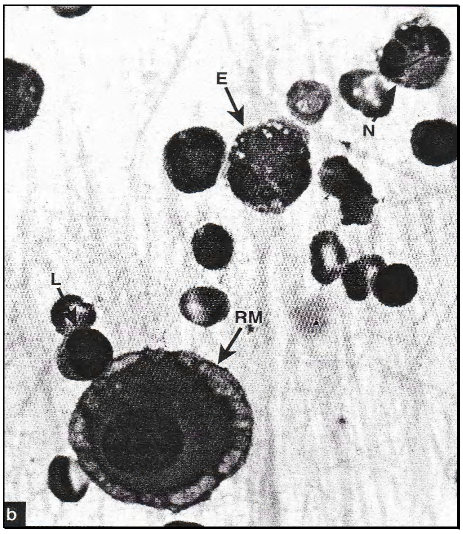

Fig.2. Reactive mesothelial cells (RM) with inflamatory cells (E- eosinophil, L- lymphocyte, N- neutrophil), 100x zoomed.

Atypical mesothelial cells - cytologic changes may include enlarged nuclei, proeminent nucleoli, frequent mitosis. Nuclear contours may show some variation, but are still generally round to oval with smooth nuclear membranes (fig 3.).

Atypical mesothelial hyperplazia can mimic many of these cytologic and arhitectural features.

Cancer cells in malignant effusions are most ofen seen in separate individual cancer cells, in sheedts and in spherical three-dimensional higly cellular

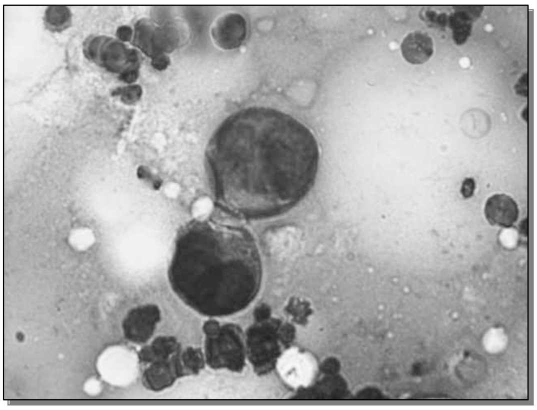

Fig.3. Multinucleation is common in benign conditions. The nuclei in benign multinucleated cells are similar in size, shape , and chromatin patern in contrast to those of malignant giant cells which show marked variation in these features, ob.40X

clusters called morulae. Classic cytologic features af individual cancer cells in malignant peritoneal effusion include enlarged cells with high N:C ratio, coarse cromatin, enlarged and multiple nucleoli and irregular or indented nuclear contours (Fig. 4, 5).

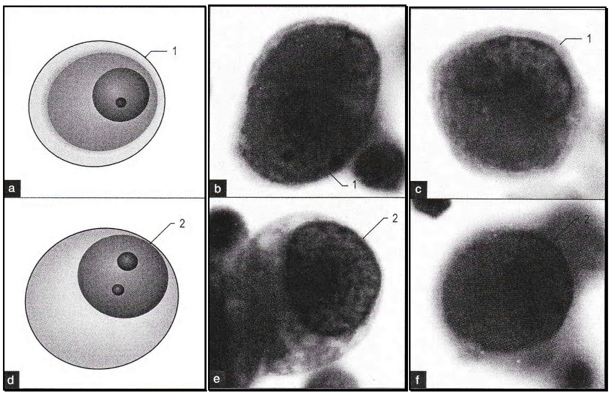

Fig.4. Mesothelial cells (a, b, c) versus adenocarcinoma cells (d, e, f) with eccentric nuclei.

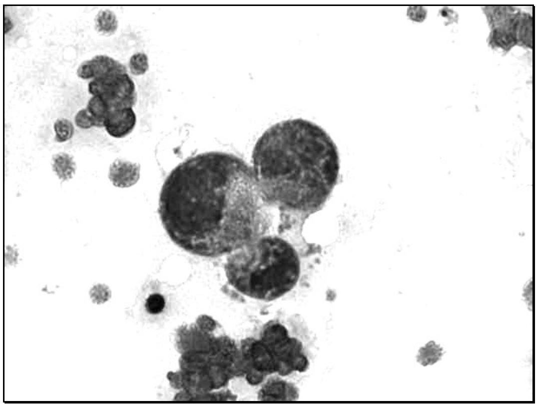

Fig.5. Cancer mesothelial cells are generally larger than normal counterparts with larger nuclei, marked hyperchromasia in well preserved cells suggest malignancy; ob.40X

On cytomorphometry, values for mean nuclear area and mean cytoplasmic area in benign cases were found to be 11.16μm^2 and 60.70μm^2, while for malignant cases were 20.31μm^2 and 68.66 μm^2, respectively (Table 1, Table 2).

Table 1. Results of mean nuclear variables

Nuclear data						
	Mean Area [μm^2]	**Eq. Diameter [μm]**	**Max. Feret [μm]**	**Min.Feret [μm]**	**Elongation**	**Perimeter [μm]**
B	1116	37.70	49.00	33.00	1.48	126.95
R	1585	44.92	58.00	40.00	1.45	151.17
M	2031	50.85	59.00	50.00	1.18	166.08

Global statistics	**Mean**	**StDev**	**Min**	**Max**
Area	1577	457.55	1116	2031
Eq. Diameter	44.49	6.59	37.70	50.85
Max. Feret	55.33	5.51	49.00	59.00
Min. Feret	41.00	8.54	33.00	50.00
Elongation	1.37	0.17	1.18	1.48
Perimeter	148.07	19.75	126.95	166.08

The evaluation of nuclear/nucleolar size was accomplished by the use of a micrometer at X40 magnification using as reference nearby red blood cells, which measured approximately 7 μm.

Table 2. Results of mean cytoplasmic variables

Cytoplasmic data						
	Area [μm^2]	Eq. Diameter [μm]	Max. Feret [μm]	Min. Feret [μm]	Elongation	Perimeter[μm]
B	6070	87.91	113.00	83.00	1.36	323.05
R	6300	89.56	116.00	74.00	1.57	307.81
M	6866	93.50	121.00	108.00	87.00	310.37

Global statistics	**Mean**	**StDev**	**Min**	**Max**
Area	6412	409.65	6070	6866
Eq. Diameter	90.32	2.87	87.91	93.50
Max. Feret	112.33	4.04	108.00	116.00
Min. Feret	81.33	6.66	74.00	87.00
Elongation	1.39	0.16	1.24	1.57
Perimeter	313.74	8.16	307.81	323.05

Significant differences were found between the mean values from cellular area, nuclear area, and nuclear: cytoplasmatic ratios (N:C) in mesothelial cells from benign effusion versus malignant cells from effusions associated with tumour (Table 3).

Table 3. Results of N/C ratios and standard deviation

	Benign	**Reactiv**	**Malign**
N/C [μm]	1/5	1/4	1/3
Std.dev.	499,0904	708,8335	908,2908

The nuclear size was determined by the assessment and measurement of the predominant nuclear size found throughout the multiple specimens examined in each case. The largest dimension of the nucleus repetitively seen was scored as the nuclear size.

Cytomorphometry hepls in better and quicker sorting for malignant cells. Finally, 34 cases were found to be benign, 22 malignant and 4 in the category of atypical cases.

The results indicated that the range of the values for suspicious cells is closer to the range of the benign cells (Fig.5).

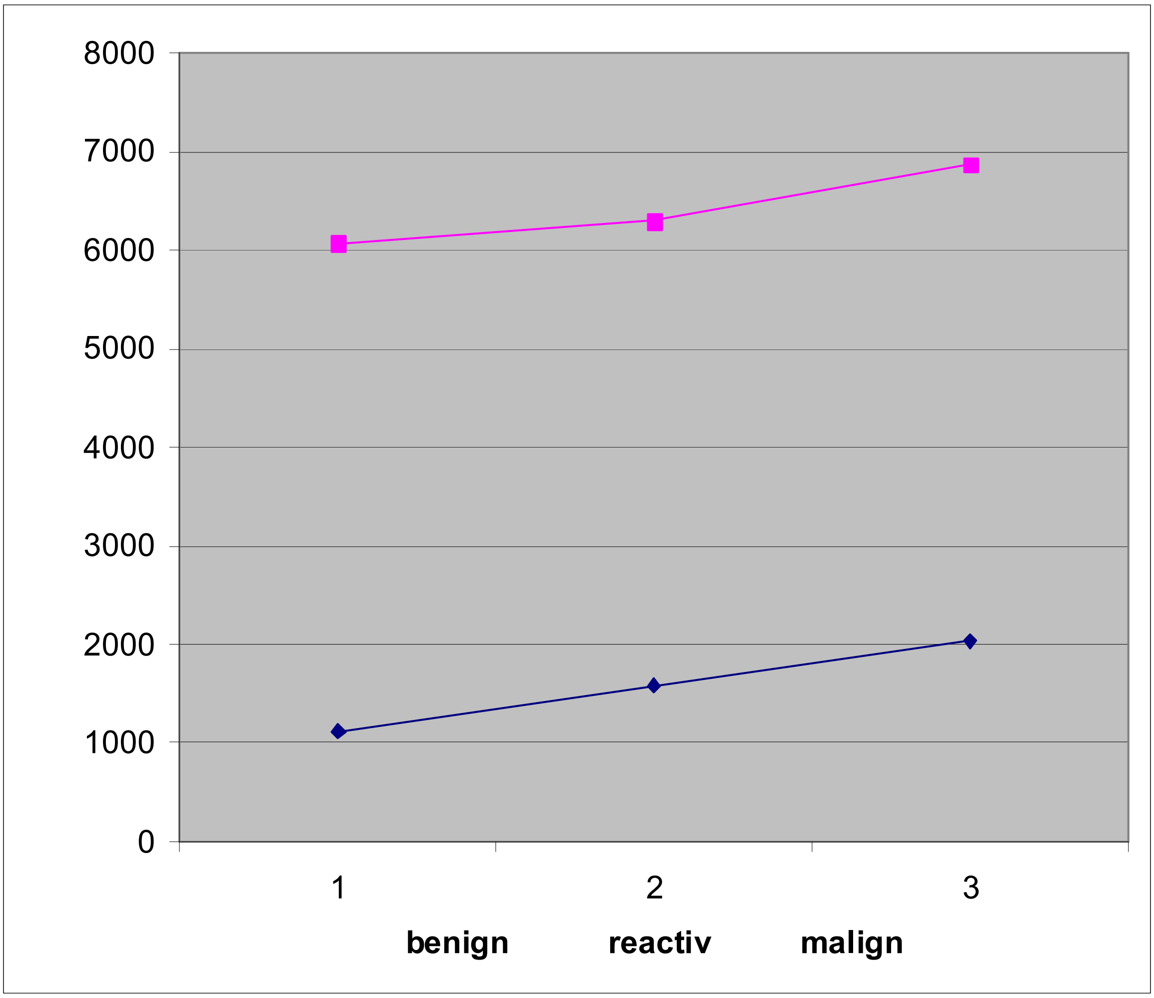

Fig.5. Mean nuclear and cytoplasmic area of mesothelial cells

CONCLUSIONS

1. The previous study showed that existed significant differences between morphometric variables derived from nuclear and cytoplasmic outlines in benign and malignant effusions.In conclusion, these results confirm data from literature and indicate that cytometric analysis of nuclear parameters represent a useful marker for identification of malignant cells in equivocal effusions and can be used to increase the cytological sensitivity in doubtful mesothelial proliferations.

2. Morphometric measurements of peritoneal fluids can be a valuable adjunct to routine light microscopy of cytology specimens and may provide important information for the differentiation of atypical atypical mesothelial cells from malignant adenocarcinoma cells. Increasing the sample size may improve test performance. Morphometry is of value in several areas of diagnostic histopathology. Its application to cytology would be of considerable benefit both in terms of diagnostic accuracy and as the basis of an automated screening programme.

3. Ascitic fluid cytology is a simple and useful procedure and should be routinely requested. In a set up like ours where definite involvement of peritoneal liquid cytology still can detect malignant cells. As presence or absence of malignant cells is useful in predicting the prognosis of patients and sometimes it can be the only clue to presence of malignancy, ascitic fluid cytology should be routinely requested.

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AMBROSIA MOWING IN NOVI SAD AND THE NUMBER OF CHILDREN WITH ALLERGIC DISEASES REGISTERED IN PRIMARY CARE

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ABSTRACT

Purpose was to find out the difference in the number of outpatient registered allergic children, after Ambrosia mowing in 2007, comparing to the year 2006, considering the third(III), fourth(IV) quarter and the whole year. Method. Ambrosia Eradication Headquarters conducted Ambrosia mowin in 2006 and in 2007. We collected the data on all allergic diseases in 2006 and 2007 from the Health Center official records, before and after Ambrosia mowing and compared them. Results. The number of the outpatient registered allergic small children (aged 0-6years) showed statistically significant differences in the III quarter of the year 2006 to the same period of 2007 (p=0.000) and the number of the outpatient registered school children with all allergic diseases showed statistically significant differences (p=0.000) in III and in IV quarters of each year and in the whole year of 2007 (p=0.000). Conclusion: There was significantly smaller number of outpatient registered small children with allergic diseases only in III quartal of the year 2007, and significantly lower number of school children with allergic diseases in III, IV quartal and the whole year of 2007. Total number of outpatient registered children was significantly smaller in the year 2007.

Key words: Ambrosia, mowing, allergic diseases, children

INTRODUCTION

Ambrosia artemisifolia L. is one year, foreign, invasive ragweed which was brought from North America, at the end of the XIX century. It spreads from the west to the east part of Europe and it came to Serbia from Hungary, mainly along the main roads.

Ambrosia pollen is harmful for human health and the main thing is the number of pollen grains in the air per cubic meter. It is known that 30 grains harm respiratory system in sensitized persons causing, itchy, running, sneeze, stuffy nose i.e. allergic rhinitis and wheezing, tight chest, lack of air, i.e. bronchial asthma. But, the whole plant can also provoke eczema or atopic dermatitis (1). As avoidance of allergen is the first step of therapy to relieve symptoms and Ambrosia eradication before its flowering period, is of the main significance.

„Metal Elements in Environment, Medicine and Biology", Tome VIIII, pp. 285-288, Publishing House "Eurobit" Timişoara, 2008

Proc. of 8th Int. Symp. of Roumanian Academy - Branch Cluj-Napoca and Branch Timişoara, 2008, December 5-6, Timişoara, Roumania

Purpose was to find out the difference in the number of outpatient registered allergic children, after Ambrosia mowing in 2007, comparing to the year 2006, considering the third, fourth quarter and the all twelve months of the each year.

MATERIALS AND METHODS

Ambrosia Eradication Center Headquarters was conducting Ambrosia mowing from the end of June till the beginning of November in 2006 and in 2007. In the year of 2006, mowing of ragweed was conducted on 500 ha and in the year of 2007. on the 600 ha (urban area). Mowing was conducted mechanically and chemically (herbicide glyphosate in the rate of 1200-2400 g a.i/ha was applied). During 2007 only, on the territory of the City of Novi Sad ragweed mechanical control was performed at 367 030 m2, and at suburban areas herbicide treatments were applied on about 325 000 m2 (2). We considered allergic diseases classified in International Statistical Classification on Diseases and Related Health Problems as: L10-L99, where are ciphers for allergic skin diseases implicated, as Dermatitis atopica (L20), Dermatitis contacta allergica (L23) and Urticaria (L50), and for allergic respiratory diseases as Morbus pulmonis obstructivus chronicus alius (J44), Asthma bronchiale (J45) and Rhinitis vasomotoria et rhinitis allergica (J30) (3). We collected the data on all allergic diseases from the Health Center official records, before and after Ambrosia mowing and compared them to the data of the same period (III, IV quarters and the whole year) of the year before. The source of number of children was last census from 2002 (Population: Census, 2002) (4). Statistical method: t test.

RESULTS AND DISCUSSIONS

Regweed have flowering period from July to October each year.The number of pollen grains depends on several factors. The gratest number is in mid August and in early September (peak days), in the morning of dry, hot, mild windy day. High pollen loads in Vienna occur with winds coming from Hungary (south-eastern directions) (5,6). Although Ambrosia spp. mainly inhabits disturbed soil, such as agroecosystems, no significant difference has been observed between regweed airborne pollen characteristics in rural and urban areas (7). During the period of 2000-2004 both the annual sums of daily averages and count of peak days changed with fluctuations. Diurnal ragweed pollen counts were over 30 pollen grains for 6-57 days of its 2-3 months long season, which means severe pollen release of the air (8). During the period of 2005-2007 ragweed pollen mean concentrations were increased considering the year 2005 (9). In the year 2007 a larger Ambrosia area eradication was conducted than it was in 2006 so were expecting to find a lower number of outhospital registered allergic children in 2007.

Table 1 The number of small and school outhospital registered allergic children in the years 2006 and 2007 and quarter distribution

Quater	I	II	III	IV	I- XII mo	I- XII mo 0-19years
2006 small	2821	1339	2002	1806	7968	
2006 school	1761	1431	2270	1736	7198	15166
2007 small	2812	2086	1663	1764	8325	
2007 school	1750	2213	776	1119	5858	14183

Small=small children
School=school children

In the III quarter of 2006 (Table 1), there were more registered allergic small children (n=2002) and school children (n=2270) than in the same period of 2007 (n=1663, n=776, respectively). The same situation is in the IV quarter. Considering the number of registered allergic children for the whole year (I-XII) there were more small allergic children in 2007 than in 2006, but not school allergic children. In total (0-19 years), in 2007 there were less allergic children than in 2006.

Table 2. Comparation of the third and fourth quarters of the years 2006. and 2007.- outhospital registered small allergic children

Quarters	m1(2006)	n1	m2(2007)	n2	t	P
III	2002	20300	1663	20300	5.871	0.000
IV	1806	20300	1764	20300	0.736	0.462
I-XIImo	7968	20300	8325	20300	-3.615	0.000

M1= number of small allergic children (0-6years) in the III quarter of the year 2006
N1, N2= Number of small children (0-6years) in Novi Sad (Census 2002)
M2= number of allergic small children (0-6 years) in the IV quarter of the year 2007

Matching the number of the outpatient registered children with allergic disease (Table 2) (aged 0-6years) very high statistically significant differences were seen in the III quarter of the year 2006 to the same period of 2007 in the benefit of the year 2007 (p=0.000), while comparing to the number in the IV quarters of each year there was no statistically significant difference (p=0.462). Comparing the total number of the small allergic children in the whole year (I-XII) of 2006 to the whole year 2007, there were significantly smaller number in the year of 2006 (p=0.000).

Table 3 Comparation of the third and fourth quarters of the years 2006. and 2007.- school and total number of allergic children (0-19 years)

Quarters	M1	N1	M2	N2	t	P
III	2270	46373	776	46373	27.526	0.000
IV	1736	46373	1119	46373	11.729	0.000
I-XIImo	7198	46373	5858	46373	12.652	0.000
Wh.yr0-19	15166	66673	14183	66673	6.497	0.000

M1= number of allergic school children (7-19years) in the III quarter of the year 2006
N1, N2= Number of school children (7-19 years) in Novi Sad (Census 2002)
M2= number of allergic school children (7-19 years) in the IV quarter of the year 2007

There were (Table 3) significant differences in the number of outhospitaly registered allergic school children in the third and in the fourth quarter of the year 2006 to the same period in the year 2007, in the benefit of the year 2007 (p=0.000). In the whole year of 2007 there were statisticaly significantly less number of allergic school children (7-19 years) and of the total number of children (0-19 years) (p=0.000) comparing to the same period of the year 2006.

CONCLUSIONS

1. Matching the III and IV quarters of the years 2006 and 2007, during and after Ambrosia mowing, there was significantly lower number of outpatient registered small children with allergic diseases in the III quarter of 2007 and not significantly lower in the IV quarter of 2007.
2. The total number of outpatient registered small children in the all twelve months in the year 2007 was significantly higher than in 2006.
3. The number of outpatient registered school children with allergic diseases in the both quarters of the year 2007 was significantly lower than in 2006.
4. Total number of outpatient registered school children was significantly lower in all twelve months of the year 2007 comparing to the same period of the year 2006.
5. Total number of all outpatient registered allergic children (0-19years) was lower in all twelve months of 2007 comparing to all twelve months of 2006.

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RETROSPECTIVES

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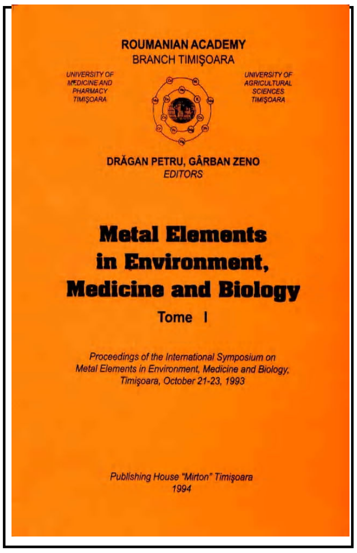

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Metal Elements
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Tome I

Proceedings of the International Symposium on
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Timişoara, October 21-23, 1993

Publishing House "Mirton" Timişoara
1994

1."Metal Elements in Environment, Medicine and Biology" – Tome I (312 p)
(Editors Drăgan Petru, Gârban Zeno)

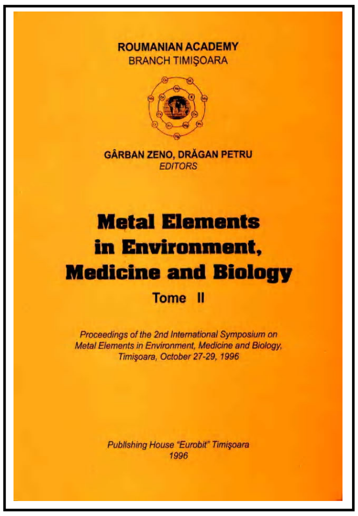
Foreword by Prof. Mihai Şerban, PhD – corresponding member of Roumanian Academy, president of Comission of Biochemistry of Roumanian Academy Bucureşti

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)

ISBN 973 – 578 – 045 - 3



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Metal Elements in Environment, Medicine and Biology,
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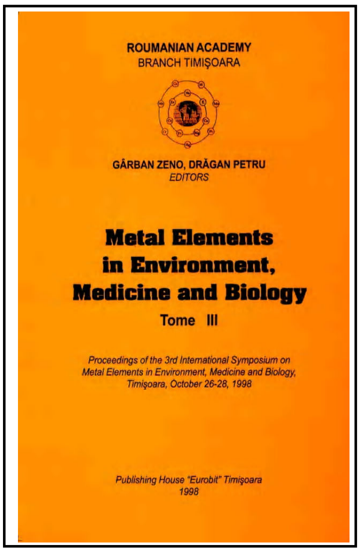
Proceedings of 2nd International Symposium,
October 27-29, 1996

Organized under the auspices of Roumanian Academy – Branch Timişoara and Comission of Biochemistry of Roumanian Academy Bucureşti

Publishing House "Eurobit" Timişoara (First Edition - 1996, Second Edition - 1997)

ISBN 973 - 9336 -15 - 9



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Publishing House "Eurobit" Timişoara
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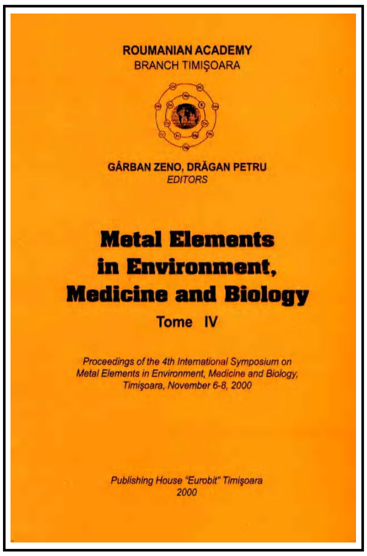
Proceedings of 3rd International Symposium, October 26-28, 1998

Organized under the auspices of Roumanian Academy – Branch Timişoara and Comission of Biochemistry of Roumanian Academy Bucureşti

Publishing House “Eurobit” Timişoara (1998)

ISBN 973 – 9336 – 19 – 1
ISSN 1583 – 4204



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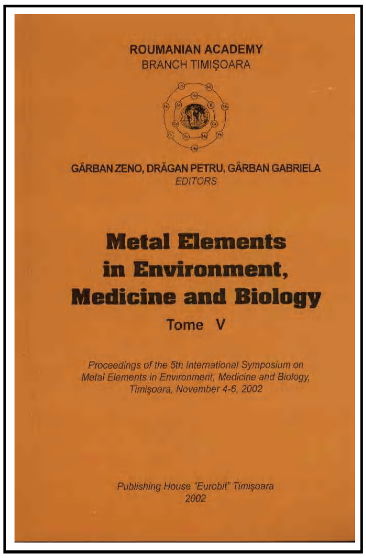
Proceedings of 4th International Symposium, November 6-8, 2000

Organized under the auspices of Roumanian Academy – Branch Timişoara and Comission of Biochemistry of Roumanian Academy Bucureşti

Publishing House “Eurobit” Timişoara (2000)

ISBN 973 – 8181 – 39 – 9
ISSN 1583 – 4204



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Proceedings of the 5th International Symposium on
Metal Elements in Environment, Medicine and Biology,
Timişoara, November 4-6, 2002

Publishing House "Eurobit" Timişoara
2002

5."Metal Elements in Environment, Medicine and Biology"– Tome V (368 p)
(Editors Gârban Zeno, Drăgan Petru, Gârban Gabriela)

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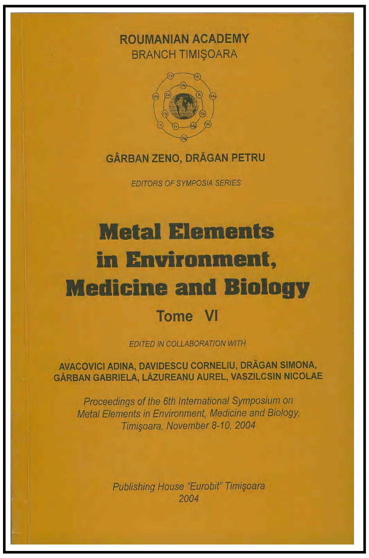
Proceedings of 5th International Symposium, November 4-6, 2002

Organized under the auspices of Roumanian Academy – Branch Timişoara and Comission of Biochemistry of Roumanian Academy Bucureşti

Publishing House "Eurobit" Timişoara (2002)

ISBN 973 – 620– 035– 3
ISSN 1583 – 4204



ROUMANIAN ACADEMY
BRANCH TIMIŞOARA

GÂRBAN ZENO, DRĂGAN PETRU

EDITORS OF SYMPOSIA SERIES

Metal Elements
in Environment,
Medicine and Biology
Tome VI

EDITED IN COLLABORATION WITH

AVACOVICI ADINA, DAVIDESCU CORNELIU, DRĂGAN SIMONA,
GÂRBAN GABRIELA, LĂZUREANU AUREL, VASZILCSIN NICOLAE

Proceedings of the 6th International Symposium on
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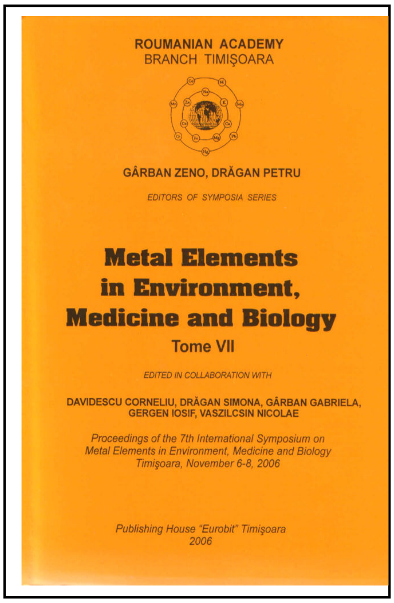

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ISBN (10) 973-620-238-0
ISBN (13) 978-973-620-238-4

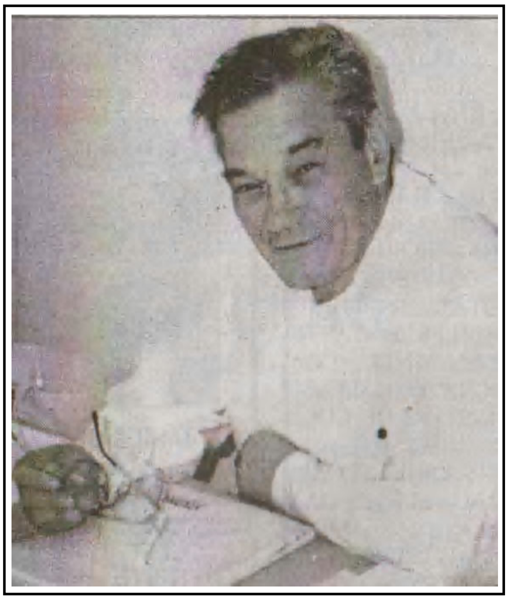
ISSN 1583-4204

IN MEMORIAM

PROF. PETRU DRĂGAN, MD, PhD

Member of the Academy of Medical Sciences

February 2, 1932 - November 12, 2007



On November 12th passed away Prof. Petru Drăgan, MD, PhD. distinguished personality of medical sciences – with remarkable contributions in the domain of urological surgery, general surgery and the application of molecular medicine in urology.

Prof. Petru Drăgan had a prodigious and prestigious professional activity working at the University Clinic of Urology of the Faculty of Medicine – University of Medicine and Pharmacy “Victor Babeş” Timişoara.

Born on February 2, 1932 in town Jimbolia - Timiş county, he started his studies in a school from his native town and continued at the “C.D. Loga” high school in Timişoara (graduated in 1951). His university studies were performed at the Faculty of General Medicine – University of Medicine and Pharmacy Timişoara (graduated in 1957).

At the beginning of his professional career Petru Drăgan, MD worked as a general practitioner physician in town Bocşa Română (1957-1959). Then, after a competition, he occupied a job of assistant professor in the University Clinic of Urology Timişoara – Fculty of Medicine. His doctoral thesis had as subject “Intrarenal circulation in shock”. He achieved, successively, all the university degrees up to university professor (1990).

During his qualification he attended numerous specializations in Romania and abroad. Among them one can mention his specialization in U.S.A. at the University of California Los Angeles (UCLA) – Department of Urology (1969-1970) with a Fulbright scholarship. At UCLA he worked under the direction of Prof. W. Goodwin, MD, PhD and Prof. Kaufmann, MD, PhD.

During the period of specialization in U.S.A. he sustained and graduated all the exams – very rarely in case of Fulbright’ scholars - equalizing the Diploma of physician revealing the high degree of his competence.

Returned in Romania he contributed to the development of the «School of Urology» from Timişoara, dedicating for this purpose a part of his existence and offering it a world-wide prestige.

In this framework there are mentioned some of his essential contributions:

- introducing of over 15 new surgical interventional technics in urology;
- promoting endourology in Timişoara and later on being taken over in Romania
- performing the first renal transplant with kidneys from corpse (1981) – being a pioneer of renal transplant in Romania
- contributing to the development of concepts and applications of Molecular medicine in Urology in Romania (starting from 1980). In this context there are mentioned

contributions on molecular mechanisms of the heterogenous nucleation in uroconcrements biogenesis.

- publishing as first author or editor of over 15 books with monographic character
- author of over 200 scientific papers published in various specialty journals in Romania and abroad
- editor of seven volumes from the Series of International Symposia "Metal Elements in Environment Medicine and Biology" (initiated in 1993)
- coordinator, as chief editor of the journal «Timişoara Medicală» – official periodical of the University of Medicine and Pharmacy "Victor Babeş" Timişoara
- laid the basis of the "Urological Foundation Timişoara" – under its auspices were organized some National Symposia of Urology
- as a real leader of the School of Urology Timişoara he trained over 60 specialists in urology and as a mentor he had the capacity to coordinate, to stimulate and to promote valuable personalities in urology who are working now either in Romania or abroad – in different European states
- as advisor for doctoral thesis in medicine he coordinated the elaboration of numerous doctoral thesis by young physicians, approaching complex surgical, physiological, biochemical, physiopathological, histological problems with application in urology

Recognition of his praiseworthy activity was also proved by his affiliation to various *national scientific societies*, e.g.: Romanian Association of Urology; Romanian Association of Endourology (as founding member); Romanian Society of Biochemistry (by his contributions in Molecular medicine); National Council for Prostate Diseases (founding member); Society of Urologists from Moldavia a.o. and *international scientific societies*, e.g.: European Association of Urology; Britain Association of Urological Surgeons; Society of Urology from South-East Germany; American Association of Urology a.o.

Regarding his professional activity one must mention that he worked as professor at the University of Monrovia – Liberia (1975-1976) being also the private urologist of William Tolbert Jr. – president of the state Liberia.

Merits of Prof. Dr. Petru Drăgan were recognized at national level, too, being rewarded with various state prizes such as : Work Medal Class III (1979); National medal „For Merit" in Great Officer rank (2000).

At local level he was awarded with the titles : Honorary Citizenship of Jimbolia; Honorary Citizenship of Timişoara (2002).

He was titular member of the Academy of Medical Sciences and of Romanian Academy of Scientists.

A wide culture in the domain of history - from Antiquity to Contemporaneity, in the domain of arts, history of culture, economical geography, prove the huge assimilation capacity of a brilliant scientific and cultural mind.

He was appreciated by Acad. Nicolae Cajal, Acad. Pius Brânzeu, Acad. Ionel Haiduc, Acad. Toma Dordea – personalities who honoured by their presence the scientific meetings where Prof. Dr. Drăgan was the chairman.

Many times the scientific preoccupations and, especially, those related to medical practice did not permit him to elaborate manuscripts but each discussion with his students and friends was an intellectual revelation.

Passing into eternity of Prof. Petru Drăgan, MD, PhD – a well known personality, is a great loss not only for the medical sciences but also for the Romanian science and culture.

Zeno Garban

LIST OF PUBLICATIONS
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PROF. PETRU DRĂGAN MD, PhD

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