Acta Metallomica – MEEMB, 2014, Tome XI, No.1, 65-71

INTERACTIONS AMONG HEAVY METALS (CU, CD, ZN, PB) AND METALLIC MACROELEMENTS (K, CA, NA, MG) IN ROMAN SNAIL (*HELIX POMATIA*) SOFT TISSUES

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Terrestrial gastropods are known to accumulate high Cu, Zn, Cd, and Pb in their soft tissues. Here, we examine the interactions between these heavy metals and metallic macroelement level (Ca, Mg, Na, K) in snail foot and hepatopancreas. Assessing the pattern of these interactions may reveal new information concerning the mechanisms by which land snails are able to accumulate, store, and concentrate heavy metals in their body. Tissue samples were collected from eight snail population inhabiting areas with different levels of pollution. Our results highlighted the importance of snail hepatopancreas as the main place for heavy metal detoxification. Cd and Cu displayed the strongest effect on macroelement concentrations in the snail hepatopancreas. In addition, Mg is showed to have the most effective response to heavy metal levels in the snail soft tissues. Further research are necessary to elucidate the relationships between mechanisms underlying heavy metal regulation in the snail body and the level of metallic macroelements in the snail soft tissues.

Key words: metallomics, snail, hepatopancreas, detoxification.

INTRODUCTION

Land snails have been shown to accumulate, store, and concentrate high amounts of Cd, Zn, Cu, and Pb in their body, especially in the hepatopancreas (Dalliger, 1993; Nica et al., 2012). Metal uptake through the food (e.g., litter, fungi, dead plants and animals, soil) is regarded as the main route of contamination in terrestrial invertebrates (Nica et al., in press). Metal uptake via epithelium cannot be ignored because terrestrial pulmonates spend their entire lives on or in the upper soil horizons (Nica et al., 2012a), and therefore, the snail tegument comes frequently in direct contact with polluted substrates (Dalliger, 1993). However, in natural environments, metal uptake is a cumulative process that occurs via mixed air, soil, and food exposures, i.e. field exposure (Gomot de Vaufleury et al., 2006). It was found that most ingested metals are metabolically regulated in the snail body either by cellular compartmentalization or by complexation to specific metallothioneins (Berger et al., 1997; Dallinger et al., 2000). Such processes of bioaccumulation in organisms may be associated with significant interactions between these HMs and metalic macroelements (K, Ca, Na, Mg), which can be explained by intensive and prevailing access of toxic metals in toxic reactions (Kamiński et al., 2009).

Although land snails are extensively used as bioindicator organisms of metal pollution (Nica et al. 2012a), little information exists concerning the influence of heavy metal uptake on metallic macroelements in the snail soft tissues. A recent study has investigated if the affiliation of Pb with Ca is mediated by Mg. The results showed that Mg uptake via food is not important for regulating external Pb uptake, although the latter element is efficiently regulated within the snail soft tissues (Beeby and Richmond, 2010). Ca is essential to snail reproduction, shell development, and other physiological needs (Hotopp, 2002), whereas both Na and K are key players for normal cell functioning (Fijałek et al., 2003). In this context, the present study aimed at determining the influence of heavy metal accumulation in the snail soft tissues on the metabolism of selected metallic macroelements.

MATERIALS AND METHODS

Detailed information concerning the investigated sites, sampling methods, and the level to which HMs accumulated in the snail body are presented in our previous work (Nica et al., 2012a). Fig. 1 shows the location of snail sampling points.

Fig. 1. Map showing the locations from which the snail were sampled.

Chemical analysis

Measurements of HM (Cu, Zn, Cd, Pb) and metallic macroelement (K, Ca, Na, Mg) concentrations in *H. pomatia* soft tissues (foot, hepatopancreas) were carried out in our laboratory (Environmental Research Test Laboratory, Banat's University of Agricultural Sciences and Veterinary Medicine, Timişoara, Romania). All samples were weighted on an analytical balance (model TP-214, Denver Instrument Gmbh, Göttingen, Germany) to the nearest 0.01 mg. The digestion solutions (HNO_3 0.5N) were prepared from Merck`Suprapur` nitric acid (65%, $\rho = 1.39$ g/cm^3, Merck KGaA, Darmstadt, Germany).

The metal concentrations in the filtrate were determined by flame atomic absorbtion spectrophotometry with high resolution continuum source (Model ContrAA 300, Analytik Jena, Germany), fitted with a specific conditions of particular metal, and they were expressed as micrograms per gram dry weight (ppm d.w.). Mix standard solutions (1000 mg/L) of copper (Cu), zinc (Zn), nickel (Ni), cadmium (Cd), lead (Pb), natrium (Na), potassium (K), calcium (Ca), and magnesium (Mg) – ICP Multielement Standard solution IV CertiPUR, were procured from Merck (Merck KGaA, Darmstadt, Germany). Solutions of varying concentrations were prepared for all metals by diluting suitable volumes of standard solutions. Double distilled water (spectroscopic pure) was used for the preparation of reagents and standards. All chemicals were trace metal grade (Suprapur). All glassware was treated with Pierce solution 20% (v/v), rinsed with cold tap water followed by 20% (v/v) nitric acid and then rinsed with double-distilled water. For quality control purposes all blanks and duplicate samples were analysed during the procedure. NCS Certified Reference Material-DC 85104a and 85105a (China National Analysis Center for Iron&Steel) was analyzed for quality assurance.

Statistical analysis

The foot and the hepatopancreas are the main places of HM accumulation in *H. pomatia* soft tissues (Nica et al., 2012a). These organs were therefore separately investigated when examining the overall relationship existing between HMs and metallic macroelement level in the snail body. The data were standardized before being analyzed by using a Canonical correlation analysis (CCA). This statistic method is commonly used for finding the overall correlations between two sets of multi-dimensional variables. The approach used for interpreting canonical functions have involved examining the overall fit (R), the total redundancy, and the magnitude of canonical loadings (Hair et al., 1998). The HM concentrations were regarded as independent variables, whereas the metalic macroelements were considered as dependent variables. Finaly, the relationships between HMs and metallic macroelements were determing by correlational analysis (Spearman rank correlation matrix).

RESULTS AND DISCUSSIONS

The mean values with standard errors for each investigated metal are shown in Fig. 2. Canonical correlation analysis (CCA) showed that the canonical correlation for the first and second canonical variates were fairly substantial and highly significant, but for the other canonical variates these relationships were non-significant (Table 1). Based on these results, we analyzed the relationship between the first pair of canonical variates, which had the maximum correlation coefficient. The redundancy index for the independent variate, i.e. HMs, was substantial (87.277%). The dependent variate, i.e., metallic macroelements, displayed a lower redundancy index (66.382%), but the difference was not high enough to reveal a significant delineation between the two sets of variables. Table 2 gives the canonical loadings and weights for both sets of variables.

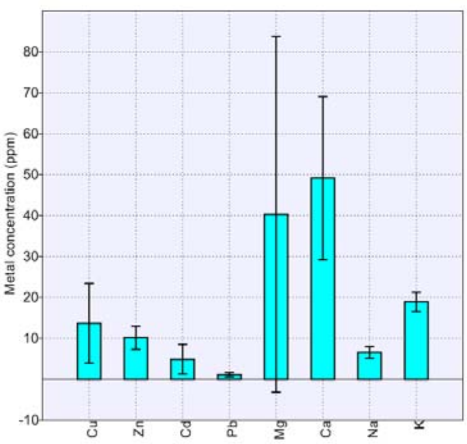

Fig. 2A. Metal levels in the snail hepatopancreas

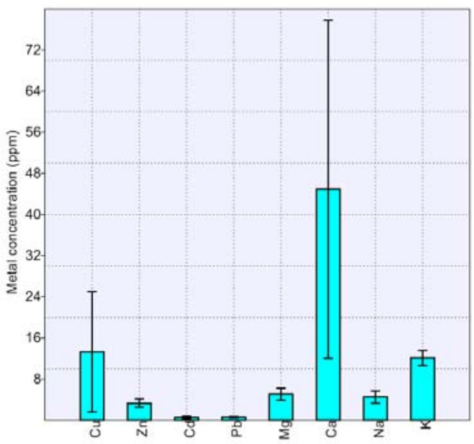

Fig. 2B. Metal levels in the snail foot

Note. To allow a proper graphical representation the levels of certain metals have been reduced by several orders of magnitude: Zn (10^{-1}), Mg (10^{-2}), Ca (10^{-3}), Na (10^{-2}), K (10^{-2}).

Table 1

Chi-square tests with successive roots removed (snail hepatopancreas)

Root removed	Canonical R	Canonical R-sqr	Chi-sqr.	p	Lambda
0	0.995	0.983	100.258	0.000	0.000
1	0.976	0.952	11.162	0.264	0.011
2	0.852	0.727	3.544	0.471	0.242
3	0.334	0.111	0.296	0.586	0.888

Table 2

Canonical loadings for the first canonical variates (snail hepatopancreas)

Independent variate		Dependent variate	
Cu	-0.335	Mg	-0.518
Zn	-0.091	Ca	-0.266
Cd	0.488	Na	-0.659
Pb	0.144	K	-0.388

The highest loadings among HMs were observed to occur for Cu and Cd; therefore, one can conclude that these metals accounted for most of the significant canonical correlation between the two sets of variables (as shown in Table 2). Several author studies showed that both Cu and Cd can be sequestred in *H. pomatia* hepatopanreas by complexation to specific metallothioniens (Berger et al., 1997; Dallinger et al., 2000). However, these two HMs have different functions in the snail body. Being a component of the chromoprotein hemocyanin, Cu is particularly important for snail respiration, although at high levels it can act as a toxic element as well (Nica et al., in press). Cd, by contrast, has no known physiological function in terrestrial gastropods (Nica et al., 2012b), but it was shown to easily being accumulated in the snail hepatopancreas (Dallinger et al., 2000). This information may explain not only the magnitude, but the also the sign of canonical loadings reported for these two HMs. Pb displayed a lower influence on metallic macroelements than either Cu and Cd. This may be related to the fact that unlike Cd, Cu, and Zn, this HM does not induce a specific metallothionein (Beeby and Richmond, 2010). In addition, Pb was demonstrated to be 5-6 times less toxic than any of the aforementioned HMs (Laskowski and Hopkin, 1996).

Investigating the metallic macroelements, Ca levels in the hepatopancreas was found to be the least influenced by HM uptake. This may be associated with essential role that this metal in the snail metabolism (Hotopp, 2002). Correlational analysis revealed that Cu and Zn burdens were positively correlated with Mg concentration in hepatopancreas (Cu-Mg: $r_s = 0.761$, $p < 0.05$; Zn-Mg: $r_s = 0.738$, $p < 0.05$). Close association of Mg and Ca metabolism (Vormann, 2003), may explain these relationships. Although Pb correlated highly with K ($r_s = 0.714$, $p < 0.05$), no significant relationships was found among the other variables ($p > 0.05$).

Table 3

Chi-square tests with successive roots removed (snail foot)

Root removed	Canonical R	Canonical R-sqr	Chi-sqr.	p	Lambda
0	0.889	0.783	10.705	0.498	0.125
1	0.869	0.756	5.528	0.786	0.209
2	0.739	0.546	1.999	0.735	0.449
3	0.095	0.009	0.023	0.879	0.990

Interestingly, we have found no canonical correlation between HM levels and metallic macroelements in the snail foot (as shown in Tabel 3). There were significantly positive relationships between either Na or K and Cu (Cu-Na: $r_s = 0.928$, $p < 0.05$; Cu-K: $r_s = 0.904$, $p < 0.05$). A similar relationship was observed between Zn and Na ($r_s = 0.787$, $p < 0.05$). However, no other relationships among HMs and metallic macroelements in snail foot attained statistical significance ($p > 0.05$). These results highlight the importance of hepatopancreas in regulation of HMs in the snail soft tissues. In addition, signficant relationships between Mg and either Cu or Zn suggest that this macroelement might be more deeply involved in Cu and Zn homeostasis in terrestrial snails that we actually know. Future studies should therefore investigate the potential implications of Mn in metal uptake, regulation, and detoxification in the snail body.

CONCLUSIONS

The present study reveals that HM uptake induces different effects in snail hepatopancreas and foot in terms of metallic macroelement level. In hepatopancreas, Ca, K, Mg, and Na levels acted synergically in response to external HM influx, with Cd being the most important inductive factor and Na and Mg the most sensitive respondents. Although there was no relevant impact of HM uptake on metallic macroelement level in snail foot, Mg appears to be the most important factor in regulating HM concentrations in foot.

ACKNOWLEDGMENTS. All authors have equal rights and have contributed evenly to this paper. The present work was funded by project "Post-doctoral School of Agriculture and Veterinary Medicine Posdru/89/1.5/S/62371", co-financed by the European Social Fund through the Sectoral Operational Programme for the Human Resources Development 2007–2013.

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