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CHEMICAL COMPOSITION OF SHELLS OF *CEPAEA VINDOBONENSIS* (FÉRUSSAC, 1821) (GASTROPODA: PULMONATA: HELICIDAE) FROM LOCALITIES WITH DIFFERENT SUBSTRATA

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ABSTRACT: Chemical composition of shells of *Cepaea vindobonensis* (Férussac) from sites with carbonate and carbonate-silica bedrock in central and south-eastern Poland and from alluvial deposits of the Vistula River indicates a relation between the chemism of the environment in which the species occurs and the chemical composition of the shells. The ability to accumulate heavy metals in the shell of *C. vindobonensis* makes this species a potential bioindicator.

KEY WORDS: *Cepaea vindobonensis*, shell, heavy metals, chemical composition, carbonate rocks, alluvial deposits

INTRODUCTION

Localities of *Cepaea vindobonensis* (Férrussac, 1821) within its continuous distribution range in Poland are associated with carbonate outcrops: chalk, limestones and dolomites, where the species is most often encountered on sunny, xerothermic slopes. On the other hand, many localities, isolated to various extent, are scattered along the valleys of large rivers, on floodplains with alluvial deposits. Such sites offer radically different habitat conditions (for detailed distribution, map, habitats and references see MIERZWA 2009). It is commonly assumed that the kind of substratum is reflected in the elemental composition of the shell (e.g. GÄRDENFORS et al. 1996, JORDAENS et al. 2006). The ecological distribution of *C. vindobonensis* in Poland offered an opportunity to test this assumption. This paper deals with chemical composition of shells of *C. vindobonensis* from two geochemically different groups of localities.

MATERIAL

The shells were collected in 2005 from two groups of localities: in July and August from sites with carbonate and carbonate-silica bedrock (Podzamcze Nature Reserve in Bychawa, Kazimierski Landscape Park in Nasiłów, Wietrznia Nature Reserve in Kielce), in August and September – from alluvial localities in the floodplains of the Vistula River (village of Skurcza, Świder Nature Reserve on the Świder outlet into the Vistula, district Saska Kępa in Warsaw, Zakroczym).

METHODS

Microchemical analyses of the shells were carried out with the use of a scanning electron microscope HITACHI S-3400N provided with an X-ray microanalyser EDS, at the Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw. The analyses included 28 shells (four shells per site). In each shell six microsites were analysed: three at 20 kV and 250× magnification, and three at 25 kV and 500× magnification. In all, 168 microsites were analysed.

RESULTS AND DISCUSSION

The results of microchemical analyses of the shells of *C. vindobonensis* are presented in Table 1. The mean content of Be, B, Na, Mg, Al, C, O, Si, Ca, Fe, Sr and Cu exceeded 0.1% in all the sites. Some elements (P, S, Sn, Ba) exceeded 0.1% only in the carbonate sites, while the content of Ni, Zn, Se, Rb, Mo, Cd, Sn, Ba and Hg was higher than 0.1% only in the alluvial sites. The content of B, Na, Cl, K, Sc, Ti, Cr, Mn, Co, Ba, Os,

Table 1. Mean content of the studied elements in shells of *C. vindobonensis* from the studied sites. Values higher on one kind of substratum are bolded

Element	Element content [% weight] in each site						
	Carbonate, carbonate-silica sites			Alluvial deposits of floodplains			
	Podzamcze	Kazimierski L. P.	Wietrznia	Skurcza	Świder	Warsaw	Zakroczym
Be	0.41	0.37	0.42	**0.78**	**0.83**	**0.9**	**0.76**
B	0.9	0.86	0.91	0.92	0.84	0.97	0.89
Na	0.13	0.1	0.12	0.14	0.11	0.12	0.13
Mg	**0.38**	**0.41**	**0.39**	0.12	0.1	0.13	0.14
Al	**0.47**	**0.56**	**0.51**	0.19	0.23	0.24	0.2
P	**0.14**	**0.15**	**0.13**	0.06	0.06	0.08	0.07
S	**0.1**	**0.11**	**0.1**	0.05	0.05	0.06	0.07
Cl	0.07	0.06	0.08	0.08	0.06	0.06	0.07
K	0.09	0.07	0.09	0.08	0.09	0.06	0.07
Sc	0	0	0	0	0	0	0
Ti	0.03	0.05	0.04	0.05	0.03	0.04	0.05
Cr	0.02	0.03	0.02	0.06	0.08	0.08	0.06
Mn	0.08	0.09	0.09	0.02	0.02	0.03	0.02
Fe	**0.41**	**0.47**	**0.45**	0.1	0.12	0.36	0.32
Co	0.05	0.03	0.04	0.06	0.08	0.07	0.07
Ni	0.04	0.04	0.03	**0.12**	**0.10**	**0.11**	**0.12**
Zn	0.04	0.03	0.04	**0.1**	**0.11**	**0.12**	**0.1**
Ga	**0.07**	**0.08**	**0.07**	0.03	0.05	0.04	0.03
Se	0.06	0.05	0.05	**0.11**	**0.1**	**0.12**	**0.1**
Rb	0	0	0.01	0	0.1	0	0
Sr	0.21	0.25	0.2	**0.52**	**0.71**	**0.82**	**0.54**
Cu	0.20	0.19	0.2	**0.26**	**0.26**	**0.28**	**0.27**
Mo	0.07	0.06	0.08	**0.12**	**0.13**	**0.17**	**0.15**
Cd	0.08	0.06	0.07	**0.15**	**0.16**	**0.18**	**0.17**
Sn	0.1	0.09	0.07	**0.16**	**0.18**	**0.17**	**0.18**
Ba	0.06	0.08	0.18	0.11	0.1	0.12	0.11
Os	0.07	0.06	0.07	0.08	0.07	0.06	0.05
Hg	0.07	0.09	0.08	**0.29**	**0.27**	**0.28**	**0.25**
Si	**1.47**	**1.33**	**1.22**	0.23	0.18	0.15	0.27
C	9.85	11.3	10.1	10.1	10.3	9.82	10.4
O	25.1	23.9	26.6	27.1	25.2	28.4	26.9
Ca	59.23	59.03	57.54	57.9	59.4	55.96	57.44

C, O and Ca did not differ between the two groups of sites. Shells from the carbonate localities showed a greater content of Mg, Al, P, S, Fe, Ga (only slightly higher than in the alluvial sites) and Si, whereas those from the alluvial sites contained more Be, Ni, Zn, Se, Sr, Cu, Mo, Cd, Sn and Hg.

Most of the elements analysed here are absorbed by gastropods from the substratum and plant food in the form of hydrated simple mineral compounds (GÄRDENFORS et al. 1996). Elements with the mean content approximating or higher than 0.1% are believed to play an essential role in the snail's physiology (GÄRDENFORS et al. 1996). In the case of snails the most obvious structural component of the shell is calcium. Its mean content in *C. vindobonensis* from a carbonate site in Podzamcze was 50–60% weight (MIERZWA 2008). The corresponding values from the other carbonate sites (see Table 1) were within this range, but – surprisingly – such values for the floodplain sites were very similar (LIS & PASIECZNA 1996). Only in one case (Saska Kępa in Warsaw) was the calcium content lower than in the shells from the carbonate sites. The shells from that site showed a slightly higher content of strontium than those from the remaining floodplain localities, all of which contained significantly more strontium than the shells from the carbonate sites (cf. Table 1).

The chemical structure of snail shells can undergo modifications according to the availability of certain elements. Strontium, with its chemical properties similar to those of Ca, is known to replace calcium in the structure of aragonite ($CaCO_3$) (PILKEY & BLACKWELDER 1969). It remains an open question whether strontium, when incorporated into the shell in greater quantities, contributes to the structural anomalies observed in some shells from floodplain localities (MIERZWA 2009). The high Sr content in the shells from Świder and Warsaw can be explained by the considerable concentration of the element on the Vistula floodplains where it is introduced by the polluted Jeziorka River near Konstancin-Jeziorna (BOJAKOWSKA & GLIWICZ 2003).

An effect antagonistic to that of Ca is also exhibited by Ba (0.06–0.18% weight), Be 0.37–0.9% weight) and B (0.84–0.97% weight). Barium (Ba) can not become incorporated into calcium carbonate structures (KABATA-PENDIAS & PENDIAS 1993). Its increased content (0.18% by weight) in the shells from Wietrznia may result from the high content of this element in the hydrothermal veins which are common in the Devonian rocks (POLAŃSKI & SMULIKOWSKI 1969).

The content of some of the analysed elements in the shell depends primarily on the degree of soil pollution and may affect the metabolism of other elements. For example, high content of P and Cd inhibits assimilation of Fe, whereas Be may disturb assimilation of Mg. Deposition of excessive amounts of such elements in shells in the form of low-activity compounds is a defensive mechanism against their adverse effects. However, it is likely that under stress these elements may be reintroduced into the biochemical processes (PERELMAN 1971).

The high Ca content is typical of all groups of terrestrial snails, first of all because of the calcium's shell-building role (PORCEL et al. 1996). Other elements (Fe, Si, P, S, Al) also contribute to the shell structure and growth. Their higher content in the shells from the carbonate sites results from the chemical composition of the bedrock. Mn also plays a role in shell mineralisation – its deficiency may cause anomalies in the shell formation. Higher Al content in the shells from the carbonate sites, compared to those from the alluvial sites, may generate higher Ga concentrations. This happens because the geochemical properties of Ga are similar to those of Al, hence the Ga content is often regulated by Al. According to KABATA-PENDIAS & PENDIAS (1993), the biological role of Ga is unknown.

Shells from the floodplain sites contained a considerable amount of Hg. This results from the considerable chemical activity of this element and its high content in the environment. Exceeding the admissible concentrations of Hg can contribute to development disturbances and inhibit assimilation of Se, which is capable of blocking the toxic effect of excess heavy metals.

Cd is highly toxic, but the mechanism of its toxicity is not well understood. Compared to other heavy metals, inorganic Cd compounds are fairly well soluble in water, which makes them more mobile and bio-accessible. Cd pollution mainly originates from communal wastes, hence its increased content in the shells from the alluvial sites. Snails show a considerable ability to accumulate this element (DALLINGER 1994, DALLINGER et al. 2004) which has an adverse impact on the growth rate and reproduction. Potentially "inaccessible" cadmium ions present in the soil may be bio-accessible to terrestrial gastropods. For example, *Helix aspersa* is capable of accumulating almost 16% from the stable volume of cadmium which is generally inaccessible to organisms (SCHEIFFLER et al. 2003). Gastropods accumulate Cd in the hepatopancreas even in relatively unpolluted areas (KNUTTI et al. 1988). The shell is an additional location for Cd deposit. Cd accumulation in *C. nemoralis* shells in polluted areas is over 10 times greater than in individuals from reference areas. High Cd and Zn concentrations in the shell do not have any adverse impact on its structure and resistance (JORDAENS et al. 2006).

Ni concentration in the shells of *C. vindobonensis* in both kinds of habitats was lower than that of Cd. In terrestrial gastropods Ni is accumulated mainly in tissues, to a smaller degree in shells (NICOLAIDOU & NOTT 1998). In the case of *C. vindobonensis* the increased content of this element in the shells from the alluvial sites seem to result from a greater Ni content in the Vistula alluvial deposits in Warsaw and surrounding areas. NICOLAIDOU & NOTT (1998) analysed the degree of metal accumulation in a marine snail *Cerithium vulgatum*; individuals which lived in the vicinity of the nickel works exhibited concentrations of this element in tissues which were 10 times higher than in snails from reference areas. Ni and its impact on invertebrates has never been broadly discussed, but KABATA-PENDIAS & PENDIAS (1993) state that Ni deficiency in animal organisms may cause growth inhibition and pigmentation anomalies.

Terrestrial gastropods accumulate Cu in their soft tissues; copper is necessary for biosynthesis processes (DALLINGER et al. 1993, 1997, VAŠÁK 2005). Its increased content in the shells from the alluvial sites may result from soil pollution with Cu from communal wastes in Warsaw and its environs. The Cu content in shells in all the sites, approximating 0.2% weight, probably results also from the fact that Cu absorption is favoured by Ca. Cu is absorbed more easily in acidic pH environment (KABATA-PENDIAS & PENDIAS 1993). This is the case in the floodplain habitats, where the shells show a higher Cu content, compared to the carbonate habitats.

According to KABATA-PENDIAS & PENDIAS (1993), Cu-Mo and Cu-Zn antagonisms are observed in animals. Hence the difference between the amount of Mo and Zn against Cu. Complete elimination of Mo and Zn is not advantageous, as these elements are essential for proper functioning of the organism at enzymatic level. Moreover, Mo and Rb regulate the concentrations of Cl and K ions in tissues (MENTA & PARISI 2001). Cl, K, Cr, Co and Na play physiological roles. The presence of Zn in gastropod organisms is justified, as Zn may alleviate the toxic effects of Ni (SIDHU et al. 2004). Physiological function of Sn is poorly understood. It is essential for the proper development of the organism (KABATA-PENDIAS & PENDIAS 1993). The higher Sn content in the shells from floodplain localities may result from the soil pollution with this element.

The shells of *C. vindobonensis* contained also Ti and Os. Ti is considered to have no obvious effect on the proper development of the organism. Furthermore, its accumulation poses no toxicological risk to animals. Os has a slight toxic effect, and its higher concentrations may lead to gland damage (KABATA-PENDIAS & PENDIAS 1993).

The size, thickness and mechanical properties of the snail shell are related to its chemical compostion which may be influenced by such environmental factors as heavy metal pollution (JORDAENS et al. 2006). Furthermore, snails from much polluted localities are incapable of reproduction; the inability to lay eggs results from a combination of decreased consumption and an increased energy demand for the accumulation and detoxification of metals (NOTTEN et al. 2006).

In the case of the majority of heavy metals, their increased content results from sorption of the metal occurring on the shell surface. Heavy metals bind with conchiolin but the links are very unstable, so that the increased content of heavy metals in shells is often of an episodic nature. In most molluscs metal accumulation is more permanent and the concentrations are higher in the soft tissues than in the shells. This suggests the direction of further studies: analysis of the chemical composition of soft tissues of *C. vindobonensis*. The ability to accumulate heavy metals in the shell of *C. vindobonensis* makes this species a potential bioindicator, but this would require an interpretation of a number of results of biochemical survey of heavy metals.

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REFERENCES

BOJAKOWSKA I., GLIWICZ T. 2003. Wyniki geochemicznych badań osadów wodnych Polski w latach 2000–2002. Wydawnictwo Naukowe Gabriel Borowski, Warszawa.

DALLINGER R. 1994. Invertebrate organisms as biological indicators of heavy metal pollution. Appl. Biochem. Biotech. 48: 27–31.

DALLINGER R., BERGER B., HUNZIKER P., BIRCHLER N., HAUER C. R., KÄGI J. H. R. 1993. Purification and primary structure of snail metallothionein. Similarity of the N-terminal sequence with histones H4 and H2A. Eur. J. Biochem. 216: 739–746.

DALLINGER R., BERGER B., HUNZIKER P., KÄGI J. H. R. 1997. Metallothionein in snail Cd and Cu metabolism. Nature (London) 388: 237–238.

DALLINGER R., LAGG B., EGG M., SCHIPFLINGER R., CHABICOVSKY M. 2004. Cd accumulation and Cd-metallothionein as a biomarker in *Cepaea hortensis* (Helicidae, Pulmonata) from laboratory exposure and metal-polluted habitats. Ecotoxicology 13: 757–772.

GÄRDENFORS U., BIGNERT A., CARELL B., FORBERG S., MUTVEI H., WESTERMARK T. 1996. Elemental composition of same snail shells (Mollusca, Gastropoda) and observations of environmental interest. Abstracts, International Symposium on Biomineralization N°7, Monaco, Monaco, 17 November 1993: 432.

JORDAENS K., DE WOLF H., VANDECASTEELE B., BLUST R., BACKELJAU T. 2006. Associations between shell strength, shell morphology and heavy metals in the land snail *Cepaea nemoralis* (Gastropoda: Helicidae). Sci. Total Environ. 363: 285–293.

KABATA-PENDIAS A., PENDIAS H. 1993. Biogeochemia pierwiastków śladowych. PWN, Warszawa.

KNUTTI R., BUCHER P., STENGL M., STOLZ M., TREMP J., ULRICH M., SCHLATTER C. 1998. Cadmium in the invertebrate fauna of an unpolluted forest in Switzerland. Environ. Tox. Ser. 2: 171–191.

LIS J., PASIECZNA A. 1996. Geochemical atlas of Poland. Polish Geological Institute, Warsaw.

MENTA C., PARISI V. 2001. Metal concentration in *Helix pomatia*, *Helix aspersa* and *Arion rufus*: a comparative study. Environ. Poll. 115: 205–208.

MIERZWA D. 2008. Chemical composition and structure of the shell of *Cepaea vindobonensis* (Férussac, 1821) (Gastropoda: Pulmonata: Helicidae). Folia Malacol. 16: 17–20.

MIERZWA D. 2009. *Cepaea vindobonensis* (Férussac, 1821) (Gastropoda: Pulmonata: Helicidae) in central, northwestern and western Poland. Folia Malacol. 17: 185–198.

NICOLAIDOU A., NOTT J. A. 1998. Metals in sediment, seagrass and gastropods near nickel smelter in Greece: possible interactions. Mar. Poll. Bull. 36: 360–365.

NOTTEN M., OOSTHOEK A., ROZEMA J., AERTS R. 2006. Heavy metal pollution affects consumption and reproduction of the landsnail *Cepaea nemoralis* fed on naturally polluted *Urtica dioica* leaves. Ecotoxicology 15: 295–304.

PERELMAN A. J. 1971. Geochemia krajobrazu. PWN, Warszawa.

PILKEY O. H., BLACKWELDER B. W. 1969. Comparison of the Sr/Ca ratio of fossil and recent mollusc shells. Nature 224: 1223–1224.

POLAŃSKI A., SMULIKOWSKI K. 1969. Geochemia. Wydawnictwa Geologiczne, Warszawa.

PORCEL D., BUENO J. D., ALMENDROS A. 1996. Alterations in the digestive gland and shell of the snail *Helix aspersa* Müller (Gastropoda, Pulmonata) after prolonged starvation. Comp. Biochem. Physiol. 115A: 11–17.

SCHEIFFLER R., DE-VAUFLEURY A., SCHWARTZ C., ECHEVARRIA G., MOREL J. L. 2003. "Non-available" soil cadmium is bioavailable to snails: evidence from isotopic dilution experiments. Environ. Sci. Tech. 37: 81–86.

SIDHU P., GARG M. L., DHAVAN D. K. 2004. Protective role of zinc in nickel inducted hepatotoxicity in rats. Chem.-Biol. Interact. 150: 199–209.

VAŠÁK M. 2005. Advances in metallothionein structure and functions. J. Trace Elem. Med. Biol. 19: 13–17.

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