Geology, Geophysics & Environment 2015, vol. 41 (4): 299–309

Comparative analysis of *Helix pomatia* L. shells found in soils with varying degrees of contamination (southern Poland)

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Received: 2 January 2016; accepted: 4 February 2016

Abstract: Soil samples and snails – *Helix pomatia* Linnaeus, 1758 specimens were collected from the region of Małopolska. Research based on soil from this area showed a differentiated degree of environmental contamination, which influenced the composition of elements that build mollusc shells. Concentrations of Pb, Mn, Cd, Fe, Zn and Cu in the snail shells and soil varied between localities. The highest of these concentrations were found in the soil in the city of Cracow. They were higher than those measured in the regions outside the city, 7 times in the case of Cd, and up to 123 times higher for Cu. Positive association between soil pollution and shell contamination can be observed ($r_s \approx 0.9$). A high concentration of metals in the environment and their bioavailability causes long-term accumulation of these elements in *Helix pomatia* shells.

Keywords: Helix pomatia, shell, metals, bioaccumulation, soil contamination

INTRODUCTION

For the last few decades, increasing attention has been devoted to impact of heavy metals on organisms living in soil and aquatic environments (Gimbert et al. 2006, Aleksander-Kwaterczak et al. 2009, Wardas et al. 2010, Wilk-Woźniak et al. 2011, Sardar et al. 2013). Due to their bioaccumulative nature, potential toxicity and persistence, they constitute a serious environmental problem. These elements accumulate in the food chain and induce various toxic effects in living organisms (Gupta & Singh 2011). The estimation of metal bioavailability in soils and its effects on organisms is an essential problem in the environmental management and ecotoxicology (Gimbert et al. 2006).

The bioaccessibility, bioavailability, and bioaccumulation properties of inorganic metals in soil, sediments and aquatic systems depend on complex factors. Metal that is environmentally available is not sequestered in an environmental matrix and represents the total amount of metal which is available to organisms (McGeer et al. 2004). It is suggested that the bioavailability and the fluxes entering an organism are more relevant in the explanation of metal toxicity than their internal concentrations (Rainbow 2002). The final metal concentration found in animals is the result of complex ecological factors (e.g. life history) and physiological processes, such as essential metal needs or toxic metal kinetics (Zöodl & Wittmann 2003).

Invertebrates are being used as biomarkers of the exposure and of the adverse effects of various environmental pollutants. The potential of invertebrates as biomarkers include DNA alteration,

http://dx.doi.org/10.7494/geol.2015.41.4.299 ISSN 2299-8004 | e-ISSN 2353-0790 | Wydawnictwa AGH

lyposomal integrity, metal-binding proteins, immunological responses and histopathological changes (Kammenga et al. 2000, Scott-Fordsmand & Weeks 2000, Šuteková & Hofman 2011). In pollution studies with urban organisms, invertebrates are usually used as passive, indirect bioindicators or accumulation indicators, because of their short life cycle and relatively simple collection (Zöodl & Wittmann 2003). Furthermore, invertebrates represent the majority of all animal species living in soil and are often present in high population densities (Adis & Junk 2002, Oliver et al. 2011). Therefore, samples taken for analyses do not significantly affect population dynamics or ethical and legal limitations (Kammenga et al. 2000). Among soil invertebrates, snails are frequently described as pertinent bioindicators for evaluating the pollution of terrestrial ecosystems caused by metallic elements. However, there is little research showing the extent to which metals are deposited in the shells of snails or which factors can affect this process (Jurkiewicz-Karnkowska 2004, Mierzwa 2011).

Terrestrial snails have been used by humans as safe food to eat food since prehistoric times (Lubell 2004). Human impact, i.e. deforestation and farming, causes environmental changes. For example, original settlement was connected with the occurrence of species such as *Helix pomatia*, which prefers forests, bushes and also ruderal habitats (Alexandrowicz S.W. & Alexandrowicz W.P. 2010). It has been collected as a source of food since the Middle Ages. The shell of this species is whitish-brown, creamy and usually marked by darker spiral bands. It is large (the width and height can reach 55 mm) and roundish, with convex whorls. The umbilicus of the shell is almost entirely covered (Riedel 1988).

Helix pomatia currently collected in Poland are exported to West European breeding farms, and sooner or later, this may lead to a reduction in the population of *Helix pomatia* in Poland (Alexandrowicz S.W. & Alexandrowicz W.P. 2010). The regulation of the Polish Minister of Environment on species protection contains strict instructions for the proper collection of *Helix pomatia* (*Rozporządzenie Ministra Środowiska…* 2011). The snails should be larger than 3 cm and collected between April 20th and May 30th. This is exactly when the snails come out of their winter shelters and start to breed (Stępczak 2011).

The aim of this work was to determine the long-term heavy metal accumulation in *Helix pomatia* shells, depending on soil contamination levels. An analysis of the degree of metals assimilation by snails was performed, as well as studies on the deposition capability of these elements in shells found in different environments.

METHODS

Sampling area

Samples of *Helix pomatia* snails and soil were collected from five plots, including Cracow, Krzeszowice and Wadowice area, all located in the southern part of Poland. Three samples of soil containing living specimens of *Helix pomatia* were chosen from the polluted areas in Cracow (Fig. 1). The first sample was taken from a lawn near the Mickiewicza Av. (point 1), the second one comes from the former Solvay Soda Factory (point 2), and the third sample represents the Pychowice district (point 3).

In Cracow, both the local and transit traffic go through the city centre. Air monitoring systems (*System monitoringu jakości powietrza. Małopolskie* 2015) have shown a high level of air pollution, not only along the main streets, but also in the whole of the city. The region of Mickiewicza Av., lying in the centre of Cracow, is an area of intensive traffic (the flow of traffic is 3000–4000 at rush hour). According to the results collected, the air in this area is the most contaminated in the city centre with carbon oxide, nitrogen dioxide, dust and lead all found in the dust (*System monitoringu jakości powietrza. Małopolskie* 2015). Also, here the grass is regularly moved around, fertilized and irrigated.

The area of the former Solvay Soda Factory lies in the southern part of Cracow. Construction of the factory started in 1901 and has been under liquidation since 1989. The factory produced waste, which was stored in reservoir buildings at the beginning of the 1930's. The lack of an insulating layer in the reservoir's substrate resulted in the infiltration of rainwater, rich in chloride. Ultimately, this contributed to the salinity of the Wilga and Vistula rivers (Alexandrowicz S.W. 1990, Krzak 2005). Species of *Helix pomatia* found in the area of the factory were collected from the grassland growing in the flood terrace, on the left bank of the Wilga River, close to the dump slope.

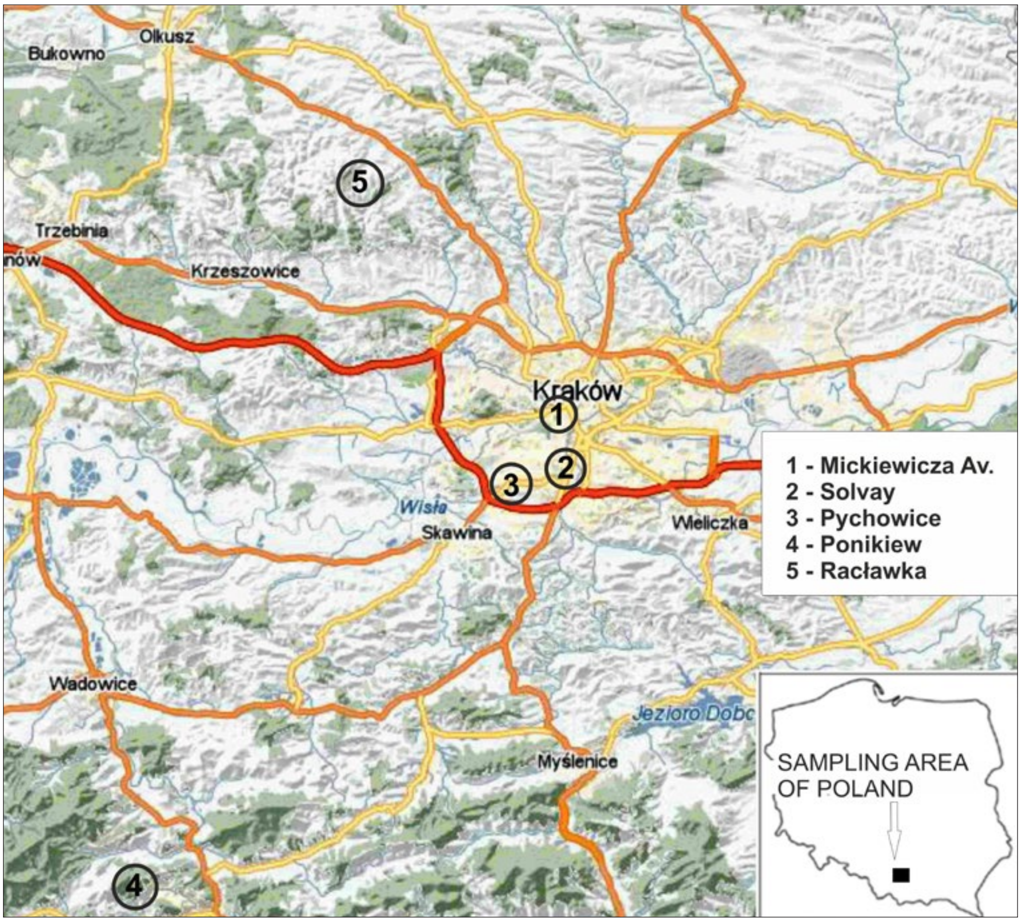

Fig. 1. *Localization of sampling places*

Pychowice is the region of Cracow localized about 5 km away from the centre of the city, on the right bank of the Vistula River. It is characterized by numerous environmental values. This area is overgrown by meadows and forests, among which single-family homes were built.

Locations in Wadowice and Krzeszowice were used as unpolluted reference areas (Alexandrowicz S.W. & Szulc 1984, *System monitoringu jakości powietrza. Małopolskie* 2015). The small village called Ponikiew (point 4 – Fig. 1) lies in the Wadowice community, in the east part of the Beskid Mały Mts, situated on the Ponikiewka stream valley. *Helix pomatia* snails were found in the grassland, at an altitude of about 380 m above sea level. This area lies in a foothill valley, surrounded by forests and mountains. Environmental pollution is relatively low in this village (*System monitoringu jakości powietrza. Małopolskie* 2015). The fifth sample was taken from the bank of the Racławka River, which crosses the Nature Reserve of the Racławka Valley in the Krzeszowice area. The bank of the river is built of a 9 m high calcareous tufa, formed during the Holocene. During the accumulation of the tufa, no visible impact of human activity on this area was recorded. The shell of *Helix pomatia* found in the mentioned calcareous tufa represents the late part of the Atlantic Phase or the beginning of the Subboreal Phase of the Holocene (5000–4500 years BP) (Alexandrowicz S.W. & Szulc 1984, Alexandrowicz W.P. 2004). This period, is represented by a shade environment, with light deciduous or mixed forests and relative warmth and humidity (Alexandrowicz W.P. 2004).

Biochemical analysis

From a surface area of 1 m^2, five samples containing empty shells of *Helix pomatia* were collected by hand-searches. All shells were washed before malacological analysis. The collected remains of malacofauna were measured using a calliper. The whole analyzed material comprised of 30 specimens of *Helix pomatia* snails. In total, samples were represented by the number of taxa, which ranged from 1 to 12 per sampling location. Moreover, samples of shells were taken for chemical analysis, carried out on the largest specimens selected from each sampling place. Before the analysis of metals' concentration, shells were cleaned carefully and left in sterile water for 24 hours. Then, they were dried, minced and digested in a mixture of 10 cm^3 of 65% HNO_3 and 2 cm^3 of 30% H_2O_2, using microwave digestion techniques. The concentrations of metals (Cd, Cu, Fe, Mn, Pb and Zn) were determined using a flame AAS spectrometer (AAS SOLAAR). Metal analyses were obtained according to the standard certified analytical quality control procedure (PN-EN ISO 17294-1:2007).

Biometric analysis

For *Helix pomatia* shells, biometric analysis was performed, for which the following parameters were measured (Fig. 2): mean height (*H*, *h*) and width (*W*, *w*). The analysis was carried out to compare biometric features of the founded population of *Helix pomatia* with those described previously.

Physicochemical analysis

Soil samples were taken from 1 × 1 m square fields and to a 20 cm depth, in places corresponding to the shell's collection. The average weight of each soil sample was about 0.5 kg. The water content (dry at 105°C until constant mass), loss of ignition (4 h, 550°C) and carbonate content (4 h, 925°C) were measured. Both the active (pH_{H_2O}) and the potential (pH_{KCl}) acidity of the soil sample were determined. From every sample the silt-clay fraction (0.063 mm) was wet-separated for metal concentration analysis. The following heavy metals were analyzed: Cu, Cd, Pb, Zn, Mn and Fe. They were brought into the solution in Teflon bombs, using microwave digestion techniques (10 cm^3 of 65% HNO_3 and 2 cm^3 of 30% H_2O_2). The concentrations of metals were determined using a flame AAS spectrometer (AAS SOLAAR), according to the standard certified analytical quality control procedure.

RESULTS

Biometric analysis

Helix pomatia appeared at the studied areas in varying amounts. The height and width of shells and apertures were measured (Fig. 2). The largest shell size was determined for a specimen taken from the Racławka Valley. The smallest molluscs lived in Pychowice. Most of the shells were 2.5–4.5 cm wide and 3.0–5.0 cm high, with the aperture's heights between 2.0 cm and 3.0 cm, and widths between 1.5 cm and 2.5 cm. The results of these measurements were in accordance with the maximum sizes given by Wiktor (2004). The broad ranges of *Helix pomatia* specimen size might be explained by the effect of the varying degrees of contamination or different ages of the molluscs. However, only one shell representing Mickiewicza Av. and Racławka samples made the biometric statistical analysis impossible. The number of specimen whorls (4.5 or 5 in each location) was in accordance with measurements given by Stępczak & Bogucki (1983). Almost all of the mollusc shells were faded. The best preserved snail's shell was found in the Racławka Valley.

Soils characteristic

Soil from the Racławka Valley Reserve came from areas not exposed to significant sources of pollution and conducive to the development of *Helix pomatia* species.

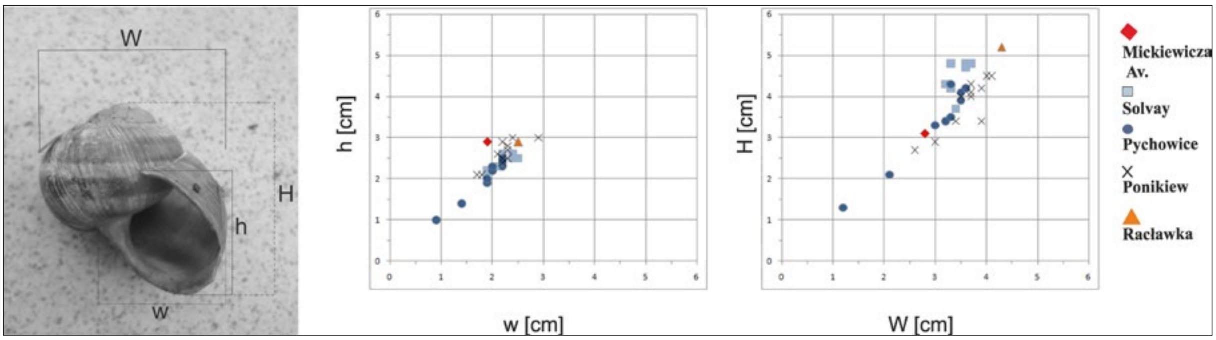

Fig. 2. *Measured dimensions of Helix pomatia (L.): H – height of the shell, h – height of the aperture, W – width of the shell, w – width of the aperture*

Table 1
Soil characterization

Localization	Sampling point	Water content	Organic matter content (LOI)	Carbonates content	Silty-clay fraction content	Density	pH (H_2O)	pH (KCl)
		[%]				[g/cm³]		
Mickiewicza Av.	1	1.52	10.82	2.69	8.16	1.14	6.88	6.50
Solvay	2	6.95	6.19	2.95	8.67	1.66	7.66	7.50
Pychowice	3	3.77	1.94	0.95	10.06	1.62	7.43	7.32
Ponikiew	4	9.96	11.75	2.92	14.37	1.04	4.43	3.93
Racławka	5	6.43	1.18	75.35	14.51	1.28	8.54	8.27

Their pH and carbonate content (Tab. 1) were specific and typical for carbonated material. Specific soil samples were also taken from the area of Ponikiew, due to their low pH value and relatively high content of organic matter (LOI). In these two locations, the highest silty-clay grain fraction was noted. Soils from the other three locations (in Cracow) were characterized by a pH-neutral and a lower content of fine fraction. The samples from the Solvay and Pychowice areas exhibited higher density and lower organic matter content than those taken near the Mickiewicza Av.

Metal concentration

The soil from the examined sites was treated as an environmental component subjected to long-lasting pollution exposure, and also as a source of pollutants absorbed by snails (contaminated food and water). The composition of metals varied significantly between soil samples, i.e. between sampling environments (Tab. 2). The lowest values for total amount of heavy metals were detected in the Racławka Valley and Ponikiew, while soil from Solvay was even more polluted than samples from Pychowice and Mickiewicza Av.

Of the six metals analyzed, iron was the most abundant metal in soil samples with the highest concentration in soil near the Mickiewicza Av. The most polluted soil with Pb, Cd, Zn and Cu was found in Solvay, while the highest manganese concentration was present in the Pychowice sample. Generally, in all soil samples, there was a significant relationship between pairs of metals: Cd, Pb, Zn and Cu (correlation coefficient r_s, between 0.98 and 0.99).

Table 2
Metal concentration in soil samples

Localization	Sampling point	Cd	Cu	Pb	Zn	Mn	Fe
		[mg/kg]					
Mickiewicza Av.	1	2.19 ±0.17	227.30 ±1.75	145.90 ±0.31	626.10 ±0.98	323.70 ±1.68	**18 201.00** ±2.36
Solvay	2	**4.24** ±0.18	**737.60** ±3.56	**424.90** ±0.23	**1 584.00** ±1.12	332.70 ±1.56	14 200.00 ±1.96
Pychowice	3	1.38 ±0.18	21.75 ±1.35	54.32 ±0.21	140.20 ±0.60	**486.40** ±2.13	12 478.00 ±2.05
Ponikiew	4	1.09 ±0.13	12.27 ±1.12	69.53 ±0.25	113.80 ±0.23	284.50 ±1.89	10 225.00 ±1.98
Racławka	5	0.59 ±0.10	6.00 ±0.95	8.74 ±0.18	97.06 ±0.52	131.10 ±1.23	3 457.00 ±1.12

Table 3
Metal concentration in shells of Helix pomatia

Localization	Sampling point	Cd	Cu	Pb	Zn	Mn	Fe
		[mg/kg]					
Mickiewicza Av.	1	**0.34** ±0.06	2.30 ±0.78	**1.99** ±0.18	**14.08** ±0.23	2.40 ±0.86	64.90 ±0.18
Solvay	2	0.15 ±0.01	**3.45** ±0.87	0.10 ±0.03	5.42 ±0.26	1.65 ±0.32	38.67 ±0.29
Pychowice	3	0.21 ±0.06	0.58 ±0.43	0.39 ±0.12	4.28 ±0.21	**4.62** ±1.12	47.77 ±0.35
Ponikiew	4	0.17 ±0.03	0.16 ±0.55	0.52 ±0.15	8.69 ±0.28	3.67 ±0.95	32.17 ±0.35
Racławka	5	0.16 ±0.02	0.75 ±0.49	0.35 ±0.13	3.57 ±0.25	1.89 ±0.89	**123.30** ±0.25

Only in the soil sample from the Racławka Valley was the concentration of all the analyzed heavy metals below the limit values regulated by the Directive of the Minister of Environment (*Rozporządzenie Ministra Środowiska*… 2002). The values of metal concentration in soil from Ponikiew and Pychowice slightly exceeded standards set for Pb, Cd and Zn contamination. In the sample from Mickiewicza Av. and Solvay, Pb, Cd, Zn and Cu concentrations were both above these standards as well as above PEL values (Probable Effect Level, according to Smith et al. 1996). This set of data corresponds with the air pollution indicators, which were the smallest in the Racławka Valley and the highest in Cracow.

In all shell samples, the cadmium concentration was low and comparable, while the contents of others metals were more differentiated (Tab. 3). The highest amounts of Cd, Pb and Zn were determined in the shells from the Mickiewicza Av. sampling point. Increased copper concentration was detected in molluscs from Solvay and Mickiewicza Av., while an elevated level of manganese occurred in shells from Pychowice. The greatest iron concentration was characteristic for shells from the Racławka Valley.

In the shells, Cd, Pb and Zn concentrations were positively correlated (with r_s between 0.82 and 0.96). Additional analysis of the obtained data revealed that the only metal with a strong soil-concentration dependent deposition in shells is copper, with a correlation coefficient at 0.94. There was also a tendency to accumulate more Mn in shells, where this metal was more frequent in the soil sample ($r_s = 0.67$). A reverse tendency was noticed for Fe concentrations: higher levels of Fe in soil were accompanied by lower levels of Fe in shells ($r_s = -0.64$).

DISCUSSION

Uncontrolled inputs of heavy metals are not desirable. This is because they can accumulate in the particulates. This contributes to their elimination from air and water, but it causes heavy contamination of aquatic sediments and soils (Aleksander-Kwaterczak & Ciszewski 2012, Aleksander-Kwaterczak & Rajca 2015).

We found that only Mn showed a tendency to be more easily accumulated in shells if this metal is strongly represented in the soil. Only Cu is deposited in shells from soil in a concentration-dependent manner, which is statistically significant. Other metals did not follow that pattern. The content of Cu was the largest in Solvay soil and shells. Concentration of this metal was below the standard levels in Ponikiew, the Racławka Valley and Pychowice. Copper enters the soil from municipal and industrial wastewater and pesticides. The occurrence of organic matter and acidic pH environments is favorable for snails' copper absorption (Kabata-Pendias & Pendias 1993, Murray & Hendershot 2000, Mierzwa 2011). In the opinion of Dallinger & Wieser (1984), *Helix pomatia* is an effective accumulator of Cu and Zn, relative to an experimental diet with highly soluble and potentially available metals. Of these four metals, Cu has the most rapid turnover in the soft tissues. However, copper concentration measured in *Helix aspersa* (Müller) can only serve as a short-term indicator of pollution, because it is rapidly lost from soft tissues (Beeby & Richmond 2002). Our research may indicate that a large portion of this metal is also deposited in the shells.

Many species of terrestrial gastropods have an effective mechanism of trace element assimilation and show the ability to store them mainly in soft tissues. According to this mechanism, the final concentration of metal assimilated into the body is the result of accumulation processes including collection, holding, storage and elimination of the metal (Dallinger et al. 2001). However, studies of the chemical composition and structure of *Cepaea vindobonensis* (Fér.) shells showed that the soil ingredients can affect not only the abundance of snail populations (Mierzwa 2008), but also the shells' elemental composition (Digby 1968, Jordaens et al. 2007). The shell of *Cepaea vindobonensis* (Fér.) is mainly built of the $CaCO_3$ aragonite form and possesses only 2% of Fe, Mg, Mn, Al, Na and K. Analysis of these shells using an electronic dispersive spectrometer showed dark zones containing Fe and Mn (Mierzwa 2008). The latest research carried out by Mierzwa (2011) showed a different concentration of heavy metals depending on the chemical composition of the bedrock. Shells from carbonate sediments had a higher Mn and Fe concentration than from alluvial sites, which contained more Zn, Cu and Cd. Snails absorbed these elements from the soil surface and plants (Gärdenfors et al. 1996).

Undoubtedly, the most important role in metal accumulation in organisms is played by the bioavailability of metals, dependent on factors like pH of the soil or organic matter content. However, there is no universal method for evaluating metal bioavailability for the snails (Pauget et al. 2012). In our investigation, metals other than copper were absorbed in the shells, irrespective of their concentration in the soil samples. The highest lead and cadmium contamination in soils was detected in Solvay (Tab. 2), where the concentrations of these metals were the lowest. Such situations may occur, due to the alkaline pH of the soil, and thus, low bioavailability of metals. This area is also characterized by lower emissions and lower metal concentrations in the air, than in the centre of Cracow, near the very busy roads. In soil located close to such areas, as in the case of Mickiewicza Av., metal concentrations exceed the value of the highest legal concentration several times over. This mainly concerns metals such as lead, zinc and cadmium, which was confirmed by the high amount of these compounds in the shell samples. It should be noted, that soils near the busy Mickiewicza Av. had a slightly acidic pH and a relatively high content of organic matter, but only Fe concentration was higher here, when compared with soil from Solvay. Metals from polluted air are first adsorbed directly into acidic soil, and then are accumulated by plants and finally eaten by snails. Snails breathe through their mantle cavity (Wiktor 2004) and adsorb metals directly from the air (Regoli et al. 2006). Land molluscs feed on 270 species of plants (Frömming 1954), but also breathe polluted air. Most likely because of this, the highest Cd, Pb and Zn metal content is observed in shells from Mickiewicza Av.

Among the metals studied in all analyzed shell samples, iron was found in large quantities, with the highest iron level in the sample of soil form Mickiewicza Av. However, the iron concentration in shells was average there. Other investigations showed that the high content of cadmium inhibits the assimilation of iron (Mierzwa 2011), which was not observed in our study. Interestingly, the highest concentration of Fe was found in shells from the potentially clean habitats in Racławka, where soil concentration of Fe was the lowest. Thus, iron might have been assimilated by *Helix pomatia* from additional sources, such as the water, which washed out the Fe from the soil. Calcareous tufa from the Racławka Valley had the least amount of organic matter, but the highest content of carbonates and pH value from all of the soil samples. These conditions could also affect Fe uptake by molluscs and its effective accumulation in the shells. Research carried out by Jurkiewicz-Karnkowska (2004) showed that Fe concentration increases with the age of shells. Therefore, the individual snails picked up from Racławka lived a little longer than the snails in others samples (the biggest shell, Fig. 2).

Concentrations of metals may show positive or negative correlations with weight (van Straalen & van Wensem 1986) and age (Sohal & Lamb 1979, Williamson 1979) of the snail. They may also increase with higher trophic levels (Grodzinska et al. 1987). Moreover, even in the same environment, different species showed different concentrations of heavy metals. It is therefore necessary to take these parameters into account when conducting biological monitoring studies.

The growth toxicity test of *Helix aspersa* (Müller) made by Gomot-de Vaufleury (2000) showed that a high content of Cu (above 1 μg/kg), Zn (4 μg/kg) and Cd (0.14 μg/kg) in soil inhibited the growth of the snails, while Pb had no toxic effects. The major route of contamination in terrestrial organisms is pollutant absorption through food (Hopkin 1989). However, metals in their stable forms showed extremely low bioavailability, which reduced the plants uptake (Murray & Hendershot 2000). It is therefore reasonable to assume that the absolute ratio of metal concentration in the shell to the concentration in the soil was lower than 4% in most cases (Fig. 3). However, varying percentages of environmentally accessible metals can be deposited in the shells. Since we analyzed soils of different features, there was a possibility to see if soil quality affects metal adsorption efficiency. Cadmium was always accumulated more efficiently than other metals in different soil samples. Molluscs have the ability to absorb cadmium even from a less polluted environment (Knutti et al. 1988). However, neither Cd nor Zn affects shell structure (Jordaens et al. 2006). Only in Racławka, the ratio of Cu content in the shell compared to the content in the soil was high (27%). Other elements should be classified as micro-concentrated.

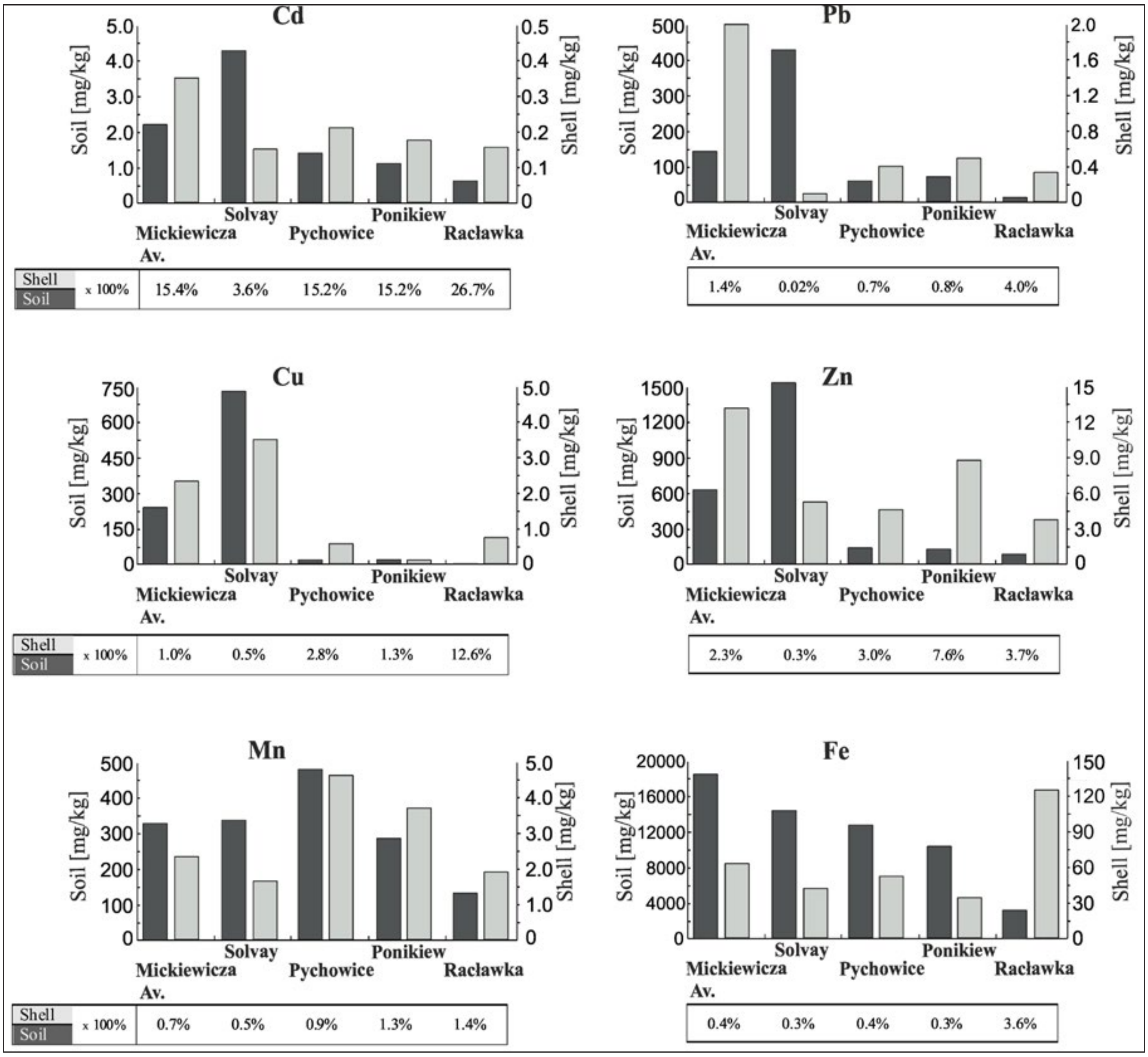

***Fig. 3.** The ratio of metal concentration in molluscs' shells to metal concentration in soil*

Studies conducted by Jurkiewicz-Karnkowska (2004) showed that the level of heavy metal accumulation in aquatic molluscs' shells reflects the concentration of these elements in the environment in a very specific way. Accumulation of Mn and Fe were greater in the shells than in tissues, while Cu, Zn, Pb and Cd accumulated mainly in tissues. Interestingly, the concentration of zinc and iron in the older shells increased, but copper, lead and cadmium were less abundant than in living specimens. It can be concluded, that even a low content of highly toxic metals in the shell means higher content of these elements in the tissue, and indicates environmental contamination. Due to the loss of Cu, Pb and Cd from the shell structure through time, old shells should not be regarded as an indicator of environmental pollution. Proportional presence of Pb in shells and tissues makes it possible to calculate the degree of environmental pollution. Complex studies are needed to fully understand the correlation between shell, tissue and environmental metal concentration.

CONCLUSION

Bioavailable heavy metals can easily move from the polluted soils to the snail's body, causing passage of toxic elements into the food chain. A visible effect of urban soil contamination with metal on the elemental composition of the shells of *Helix pomatia* was determined. Higher Fe concentration in shells collected from deeper layers of uncontaminated carbonate soil in Racławka was measured. This may be the result of secondary pollution due to groundwater level fluctuations. Shells taken from the contaminated surface layer of soil contained more heavy metals (Zn, Cu, Pb and Cd). Doubtless to say, it is the bioavailability of metals that play the most important role in metal accumulation in organisms. Due to the alkaline pH of the soil, despite the high level of contamination, the concentrations of these elements in shells were rather low. In such situations where, even in the same region, shells of one species show different concentrations of heavy metals, it becomes necessary to take the soil parameters into account when conducting biological monitoring studies. It can be concluded, that in the living snails, even a low content of highly toxic metals in the shell means a higher content of these elements in the tissue and indicates environmental contamination. Due to different parameters, such as long term accumulation or loss of metals from the shell structure, old shells should not be regarded as an indicator of environmental pollution. Sensitive bio-monitoring of terrestrial snails can be improved by the independent use of the shell, instead of the whole snail.

This work was supported by the Polish Ministry of Science and High Education, Grant No. 11.11.140.199.

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