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HEAVY METALS IN THE GONADS AND HEPATOPANCREAS OF SPIDERS (ARANEAE) FROM VARIOUSLY POLLUTED AREAS

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Abstract

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Spiders, as secondary consumers, ingest considerable amounts of various xenobiotics, including heavy metals. The amounts of ingested metals depends on hunting activity of the spider and on the body composition of their prey. On the other hand, specificity of metal excretion and storage in intracellular granules, rather than the quality of food, is responsible for their high metal body burden. The aim of this study was to determine whether heavy metal levels in the gonads and hepatopancreas of selected spiders species would reflect their adaptability to environmental pollutants in relation to their physiological and behavioural specificity. Contents of CD, Pb, Cu and Zn were measured by means of flameless and flame AAS in females of 6 species: *Araneus diadematus*, *Araneus marmoreus* (Araneidae), *Metellina segmentata* (Metidae), *Linyphia triangularis* (Linyphiidae), *Pardosa amentata* (Lycosidae) and *Agelena labyrinthica* (Agelenidae). The material was collected at two sites which differ in the level of industrial pollutants, including heavy metals: Łosień (near Katowice steelworks – heavily polluted environment) and Brenna-Bukowa in Beskid Śląski Mountains (reference site).

Heavy metal levels in the analysed organs did not reflect the metal content in the predators' biotopes. Nevertheless, Cd, Pb and Zn levels in the hepatopancreas were always higher than in the gonads, irrespective of the site from which the spiders had been collected. This may suggest that midgut glands of the predators are an efficient barrier for these elements, protecting other organs, including gonads, against the toxicity of heavy metals.

Heavy metal concentrations in spiders' tissues appeared, however, species-dependent, and this might be influenced by both behavioural and physiological methods of inactivation of heavy metals in different tissues. Among the web building spiders, *L. triangularis* seems to be the most efficient regulator of heavy metals in the tissues as was indicated by a significantly lower concentration of these xenobiotics in comparison with the other species in both of the tissues investigated. On the other hand, the wandering *P. amentata* and the web-building *M. segmentata* probably store all the heavy metals ingested with their food as mineral concentrations, mainly in cells of the hepatopancreas.

Introduction

The domination of some spider species in industrial areas may indicate that spiders use some specific adaptation mechanisms which enable them to tolerate an excess of xenobiotics, including heavy metals, entering their organs mainly through the alimentary tract. The levels of heavy metals in the tissues of predatory invertebrates are determined, among other things, by hunting strategy influencing the kind of victim, the assimilation of a given metal and behavioural and physiological modes of inactivation of the xenobiotics in tissues (HOPKIN, 1989; TYLER et al., 1989; WILCZEK, MIGULA, 1996). In response to the increased level of heavy metals in food, specific structures appear in spiders' midgut cells. They are called 'temporal spherites' (LUDWIG, ALBERTI, 1988) or mineral granules (BROWN, 1982; HOPKIN, 1988), where heavy metals are stored. Some spider species may also regulate the excess of some heavy metals in food by efficient release in faeces (CLAUSEN, 1991). Thus, interspecific differences in ability tolerate numerous xenobiotics, including heavy metals, may result from functional possibilities of the alimentary tract, especially the hepatopancreas and guts. The efficiency of the alimentary tract as a barrier is verified by the levels of heavy metals in other organs, above all in gonads, which should protect reproductive functions of organisms.

We found it interesting to evaluate whether levels of heavy metals in the above organs differ in relation to the biological properties of various species and the levels of pollutants in analysed sites.

Material and methods

Adult females of the chosen spider species were collected in forest and meadow ecosystems at two sites that differed in the kind and level of industrial pollutants; Łosień near Katowice Steelworks and Brenna-Bukowa in Beskid Śląski Mountains as a reference site. The characteristics of local pollutants are shown in Table 1.

Females of six spider species, that differ in hunting strategy (web building or wandering spiders), type and localisation of the web, diet composition and duration of life-cycle were chosen for the analysis: *Pardosa amentata* (CLERCK) (Lycosidae), *Linyphia triangularis* (CLERCK) (Linyphiidae) *Metellina segmentata* (CLERCK) (Metidae), *Araneus diadematus* CLERCK, *Araneus marmoreus* CLERCK (Araneidae), *Agelena labyrinthica* (CLERCK) (Agelenidae). Individuals of the latter species were collected only at the polluted site. The hepatopancreas and gonads were isolated from individuals and prepared for mineralisation. The material was dried at 70°C for about 72 hours. After drying, the samples were weighed and then mineralised at 200°C in a mixture of ultra-pure nitric and perchloric acids in volumetric ratio 4:1. After the mineralisation was completed the samples were diluted in 5 ml of redistilled water. Cadmium, lead, zinc and copper contents were sampled against standard solutions from Merck at initial concentration of 1 g of metal/l of water solution by AAS methods by means of Pye Unicam 939 SP-9 with graphite furnace PU-93090X. The metal content was expressed as $\mu g.g^{-1}$. The metal analyses for the tissues of each species were replicated for 6 samples. The mean dry weight of a single sample, containing the organs of about 10 specimens was 18.8 mg and 5.2 for hepatopancreas and gonads respectively. Statistical analysis of the results obtained was made using one-way ANOVA by means of Statistica v. 4.2. software. Differences between means were tested by analysis of variance, using the LSD test as homogeneity test at $P<0.05$.

T a b l e 1. Annual concentrations of air pollutants in Silesian District in 1996. From: Air pollution in Katowice voivodeship in 1996-1997. Wojewódzka Stacja Sanitarno-Epidemiologiczna Katowice, 1998. Nd-not detectable.

site	**metals fall [mg.m^{-2}.year^{-1}]**				**µg.m^{-3}**	
	Pb	Zn	Cd	Cu	SO_2	NO_2
Łosień	44	159	1.59	6.0	57	72
Brenna	11.75	Nd	0.39	Nd	40	8

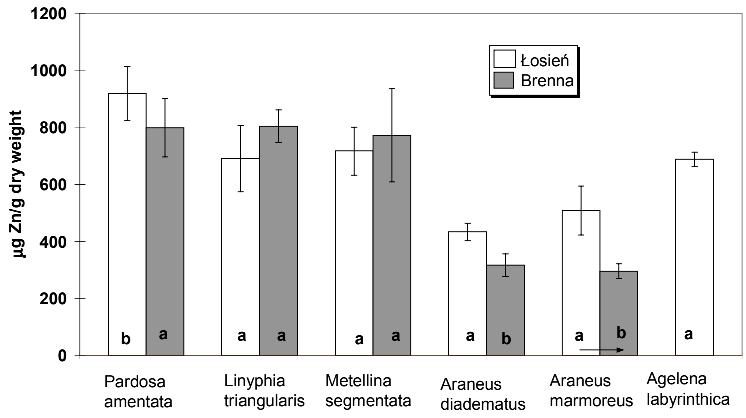

Fig. 1. Cd concentration in the hepatopancreas of six species of spiders from two sites in Southern Poland (given values represent means ± SE). Arrows indicate homogenous groups between sites, different letters indicate interspecies heterogeneity within a site.

Results

Cadmium

Ground spiders *P. amentata* and web-building spiders *L. triangularis* from Beskid ŚląskiMts accumulated significantly more cadmium in the hepatopancreas than individuals of same spider species from “Katowice” Steelworks area. The highest Cd concentration was detected in the hepatopancreas of the web-building *M. segmentata* from Łosień and of the ground-living *P. amentata* from the reference site. However, only in case of *M. segmentata* was the accumulation of cadmium from Silesia twice as high as in spiders from the reference site. The lowest level of the metal was found in the hepatopancreas of *A. labyrinthica* and *L. triangularis* collected in Katowice Steelworks area (Fig. 1).

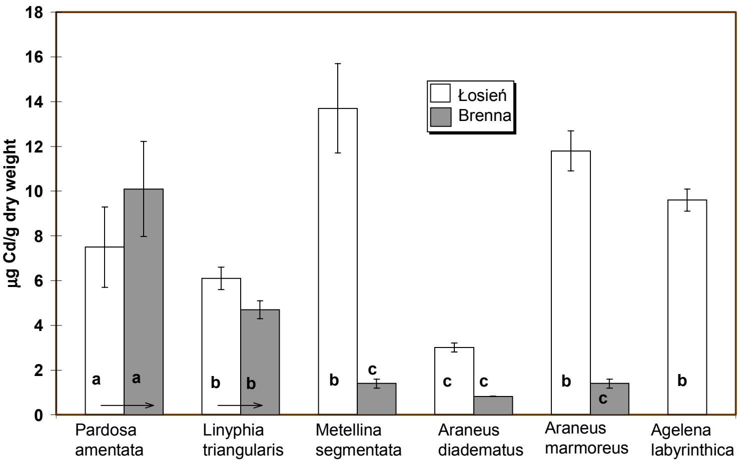

Fig. 2. Cd concentration in the ovaries of six species of spiders from two sites in Southern Poland. For further explanations see the Fig. 1.

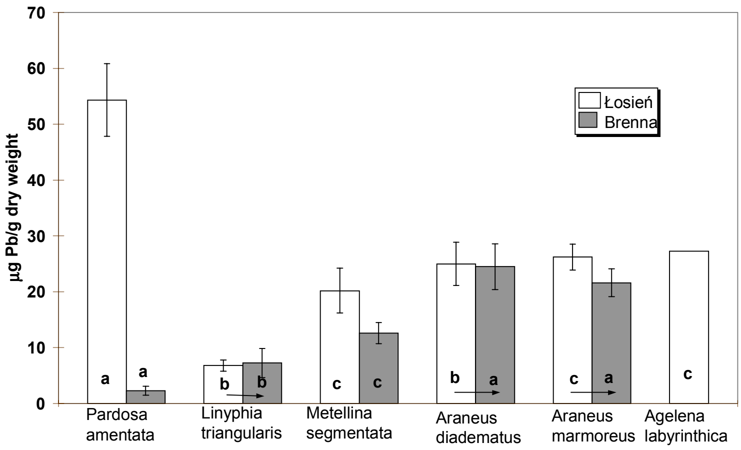

Fig. 3. Pb concentration in the hepatopancreas of six species of spiders from two sites in Southern Poland. For further explanations see the Fig. 1.

The cadmium level in ovaries was up to 85% lower than in the hepatopancreas. Moreover, the specimens of all species tested from Katowice Steelworks area had higher concentration of the metal in comparison to the spiders from the reference site. The highest Cd concentrations were found in ovaries of the web-building *M. segmentata* and *A. marmoreus* from Łosień, in average almost 10 times more than in the gonads of the spiders of both species from the reference site (Fig. 2).

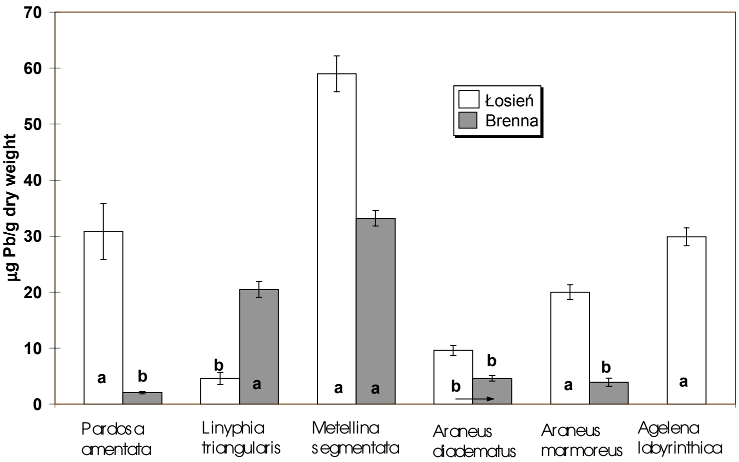

Fig. 4. Pb concentration in the ovaries of six species of spiders from two sites in Southern Poland. For further explanations see the Fig. 1.

Lead

Irrespective of the site, the lowest lead concentration was detected in the web-building *L. triangularis*, where the metal level was almost three times lower than in remaining species. The highest Pb concentration was found in the hepatopancreas of the wandering *P. amentata* from Łosień, and was on average twice as high as in the organs of web-building species: *M. segmentata*, *A. labyrinthica* and in both species of Araneidae and exceeded by 8 times the Pb concentration in the hepatopancreas of Linyphiidae (Fig. 3).

The pattern of Pb accumulation in ovaries of the analysed species was similar as stated for the hepatopancreas. The highest concentration of the metal was detected in the ovaries of web-building *A. labyrinthica* and wandering spiders *P. amentata* from Katowice Steelworks area. Only in *L. triangularis* was the level of Pb higher in specimens from the reference site (Fig. 4).

Copper

L. triangularis was the only species where the concentration of copper in the hepatopancreas of spiders from Brenna-Bukowa exceeded significantly the concentrations noted for the individuals of the species collected in Katowice Steelworks area. In other species no significant differences were detected. Only in the reference site were statistically significant interspecific differences in Cu accumulation in this organ recorded (Fig. 5).

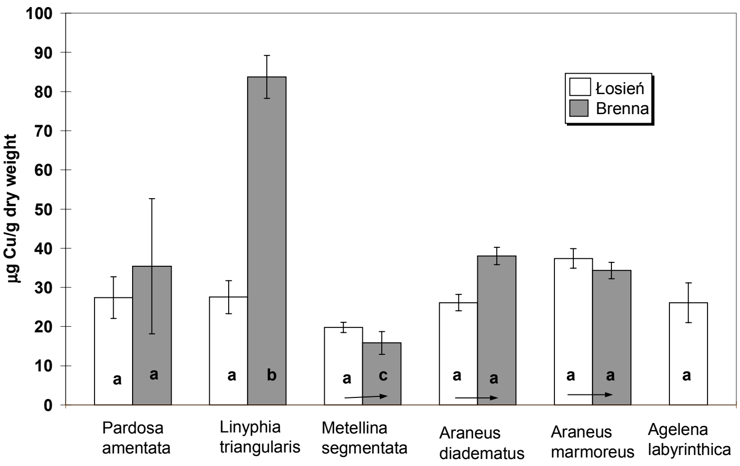

Fig. 5. Cu concentration in the hepatopancreas of six species of spiders from two sites in Southern Poland. For further explanations see the Fig. 1.

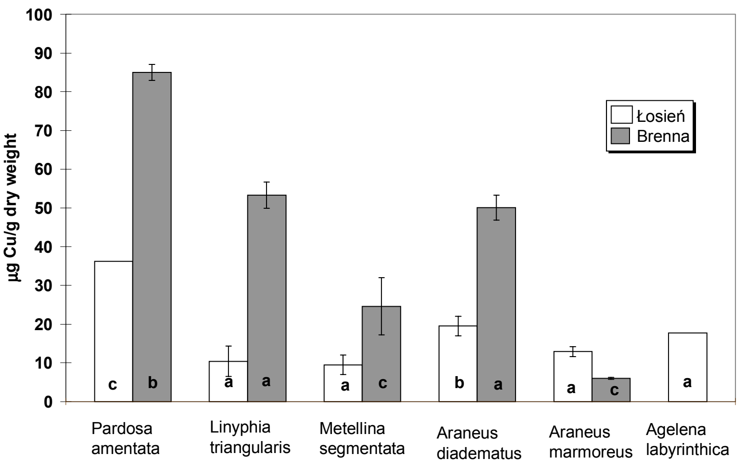

Fig. 6. Cu concentration in the ovaries of six species of spiders from two sites in Southern Poland. For further explanations see the Fig. 1.

In *A*. *marmoreus* Cu accumulation in ovaries was higher in Łosień than at the reference site but in other species the concentration in the ovaries was greater at the reference site. The highest concentration of copper was detected in females *P*. *amentata* from Brenna-Bukowa (Fig. 6).

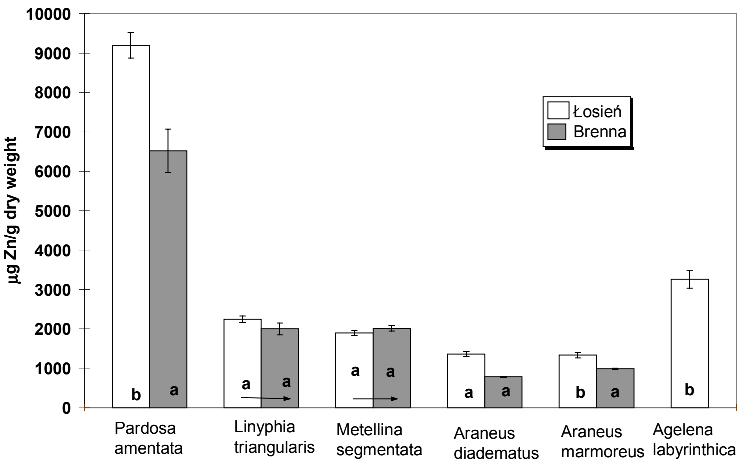

Fig. 7. Zn concentration in the hepatopancreas of six species of spiders from two sites in Southern Poland. For further explanations see the Fig. 1.

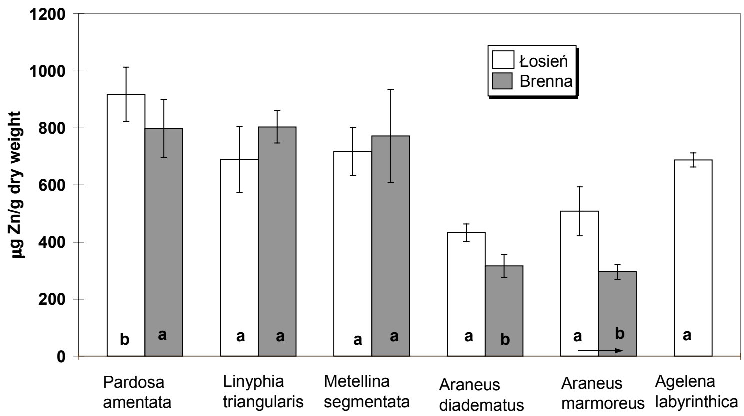

Fig. 8. Zn concentration in the ovaries of six species of spiders from two sites in Southern Poland. For further explanations see the Fig. 1.

Zinc

Irrespective of the site the highest concentration of zinc was noted in the hepatopancreas of *P. amentata*, and individuals of the species from Łosień accumulated significantly more zinc than the ones from Brenna-Bukowa (Fig. 7). The level of zinc in the gonads of the wandering spiders was almost ten times lower than in the hepatopancreas and in the web-building species – nearly four times lower. Like in the hepatopancreas, however, the highest level of this element was detected in the ovaries of *P. amentata* from both analysed sites (Fig. 8).

Discussion

Higher concentrations of copper, zinc and cadmium in the hepatopancreas than in the gonads of investigated spiders suggests that midgut glands in these species are a barrier for some heavy metals and may effectively protect the animal against their penetration into other organs. According to our expectations, the organs of wandering spiders from the family Lycosidae were heavily burdened with heavy metals, mainly with cadmium, zinc and lead. These metals were mostly accumulated in the hepatopancreas of the spiders, but also high concentrations of the metals were detected in ovaries of the females. The results are in accordance with those obtained in previous investigations where whole bodies of *Pardosa* sp. assayed for metals showed higher burdens of cadmium, copper and zinc than did web-building spiders (Salo et al., 1991; Marczyk et al., 1993; Wilczek, Migula, 1996). Considerable heavy metal concentrations in spiders from the Lycosidae probably result from high hunting intensity of these spiders and, particularly, in metal burdens in their victims bodies (Nuorteva, 1990; Salo et al., 1991). Detritophages, especially Collembola and Diptera larvae, known to be good metal accumulators, prevail in their diet (Joosse, Verhoef, 1983; Hunter et al., 1987; Hopkin, 1989). In *P. amentata* the slow rate of metabolism of the hepatopancreas accentuates heavy metal accumulation. Half of the total content of a metal is released from the organ only after 100 days, as was confirmed by experimental data (Hopkin, 1989). Probably because individuals of the *Lycosidae* are more exposed to the influence of pollutants, due to their habitats and high metal uptake with food, they are not very numerous in polluted areas and their abundance is lower in industrially changed environments (Łuczak, 1984; Deeleman-Reinhold, 1989). Contrary to the mentioned metals, the level of lead in the hepatopancreas and ovaries of spiders from the reference site was higher in web-building than wandering species. This may be explained by the fact that an additional source of the metal for web-building spiders arises from consumption of their web, which captures dust deposits containing heavy metals (Foelix, 1982; Nuorteva, 1990).

Under high pollution conditions, some species enhance the regulative processes for heavy metals in their tissues by intensified excretion with faeces. Linyphiidae probably use this strategy. Surprisingly, mainly the levels of cadmium and lead in the hepatopancreas and cadmium, lead and copper contents in the ovaries from the spiders from Katowice Steelworks area were always significantly lower than concentrations in spiders from the reference site. It cannot be excluded that linyphiids under high pollution conditions enhance some regulative processes, decreasing resorption or increasing the rate of removal from the hepatopancreas cells. High hunting intensity and, at the same time, frequency of food uptake, typical for this family, may make the metabolic rate more intensive, and make the holocrynic release of the xenobiotics into the gut lumen easier (Ludwig, Alberti, 1988b).

The fact that in highly polluted environments the niche left by sensitive species is occupied always by *L. triangularis* (Łuczak, 1986) may be explained, partly at least, by its low sensitivity to heavy metals .

In the case of the remaining species of web-building spiders, no distinct relationships between distribution and concentrations of the analysed metals and the habitats were noticed. It seems that the dynamics of bioaccumulation of the metals in organisms is species-specific and depends on physiological properties of the organisms and on properties of the metals themselves. Among orb-web spiders cadmium and zinc reached the highest concentrations both in the hepatopancreas and ovaries of *M. segmentata*, whereas the representatives of the Araneidae showed high copper and lead concentrations in midgut glands, irrespective of the site. Cadmium, copper and zinc levels in the hepatopancreas of funnel web spiders from polluted areas was similar to levels found in this organ in other web spiders. Only lead was accumulated in the ovaries of *A. labyrinthica* in high quantity, and its level was similar to that for wolf spiders, but reached only about half the level noted in *M. segmentata*. No distinct differences in the accumulation of sampled metals were observed amongst spiders of different life cycle, which confirms the assumption that there are species-specific factors that determine the heavy metal levels in spiders' tissues. These factors, according to some researchers, explain 70% of the variety of metal contents in spiders' bodies (Salo et al., 1991).

The accumulation of heavy metals in spiders may also depend on the concentration and characteristics of each element in the spiders' environments. The chemical form of the metals and their interactions with other pollutants, like SO_2 or NO_x, which usually assist the transport of heavy metals into spiders' bodies, also influence the final concentration of the elements (Kabata-Pendias, Pendias, 1993). The high level of SO_2 in Katowice Steelworks area might have caused the highest zinc and lead burdens that were found just in the organs of spiders from that site. However, this does not explain the high concentration of cadmium and copper in the hepatopancreas and copper in the ovaries of the spiders from Beskid ŚląskiMts. Maybe the accumulation of heavy metals was enhanced by the pollutants that are transferred there from Ostrava-Karvina Industrial District or the area of Upper Silesia, which depends on the wind direction. Similarly high level of metals in the tissues of spiders from polluted and reference sites was also noted in other comparative researches, but the origin of the metals and the reasons for the highest levels being in tissues of predators has not been explained (Nuorteva, 1990).

The levels of heavy metals in the tissues of different invertebrates, including spiders, are affected by the time of year when the material was collected (Janssen et al., 1990) Usually the level of copper decreases slightly from spring to autumn. The highest levels of copper and cadmium in Araneidae appear during different periods, which suggests that the accumulations of different metals occurs independently (Hunter et al., 1987). Because the adult females of analysed species were collected from May to September, as the individuals matured, the interspecies diversity of metal concentrations may be explained by seasonal differences in levels of these xenobotics in the environment.

Despite the interspecific differences in the concentrations of the metals assayed in the hepatopancreas of behaviourally different species, it seems that this organ plays the most important role in neutralisation of metals, preventing penetration into other organs, including gonads. Probably it's the strategy of the neutralisation of the xenobiotics in the form of inactivated granules or increased release with faeces that govern the metal burdens of the tissue. The effectiveness of the alimentary tract as a barrier is verified by the level of the metals found in the ovaries, which secure reproductive functions of organisms and thus – their occurrence in industrially degraded areas.

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