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Chapter 30
Effects of Heavy Metal Contamination

Paweł Migula, Grażyna Wilczek, and Agnieszka Babczyńska

30.1 Introduction

Heavy metals are naturally occurring constituents of each environment. Most of them are essential to spiders (such as Ca, Fe, Mg, Mn, Zn or Cu), some only in trace levels (Co, Mo, Ni, V) and just a few are recognised as biologically nonessential xenobiotics (As, Cd, Hg, Pb or Sr). Xenobiotic metals are always harmful, while negative effects of essential metals are dose dependent. Spiders, as carnivores, have been recognised as potent biological indicators of heavy metal contamination (Maelfait 1996). Due to a specific form of feeding, even biogenic metals may reach their body at too high concentrations. If the regulatory mechanisms supporting adequate internal metal levels and their proper cellular distribution are insufficient, spiders may suffer from various health alterations easily seen at the cellular level (Wilczek 2005; Wilczek et al. 2008). At the organism level, negative effects appear later as metabolic disorders, inhibition of growth or changes in life history traits (Babczyńska et al. 2011a; Chen et al. 2011; Eraly et al. 2011). At the population level, heavy metals act negatively on population dynamics, density, survival or reproduction (Hendrickx et al. 2003a; Chen et al. 2011; Eraly et al. 2011). Heavy metals may affect species richness and the guild structure (Ferreira 2010).

In nature, spiders may use two main mechanisms to cope with excessive heavy metal burdens: (1) tolerance through phenotypic, morphological, biochemical or behavioural plasticity or (2) resistance. If adaptations are heritable, adapted organisms may have lower fitness than non-adapted ones (Morgan et al. 2007). Under metal pollution conditions only those spider populations are able to survive which developed various tolerance mechanisms to stressors. Genetic adaptations of spiders to heavy metals were not proven yet. Increased tolerance could be reached through physiological adaptations involving trade-off mechanisms leading to

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different life history traits (Wilczek et al. 2004; Eraly et al. 2010). In this chapter we review the recent knowledge concerning the effects of heavy metals on spiders at various levels of biological organisation. Factors deciding on their distribution in spiders and the potential of spiders to cope with excess of metals will be discussed.

30.2 Factors Determining the Uptake of Heavy Metals

Spiders take up heavy metals mostly from their food which is restricted mainly to the soft tissues of arthropods. Prey types and numbers hunted by spiders at a given location depend on the environmental, climatic and prey population dynamics at a given site. Heavy metal levels might change between years and sites, hunting behaviour and activity (Wilczek and Babczyńska 2000; Ramirez et al. 2011). A high metal body burden of spiders is rather a result of the specificity of metal excretion and storage than the quality of consumed prey (Hopkin 1989; Wilczek and Babczyńska 2000).

Differences between species in accumulation of heavy metals result from physiological and ecological variability between species. In a comparative study on *Pirata piraticus* (Lycosidae) and *Clubiona phragmitis* (Clubionidae), Tojal et al. (2002) showed that both species differ in foraging behaviour and prey composition. The first species feeds on the soil-dwelling insects, while the second forages on flying insects that have lower concentrations of metals.

Studies at the species level have shown differences in the accumulation of heavy metals between juveniles and adults, due to differences in their physiology or temporal differences in their exposure to metals by ingestion (Maelfait 1996; Hendrickx et al. 2003a). In nature, body burdens of metals are often higher in males, irrespective of species (Table 30.1). They have a shorter lifespan and invest less energy to cope with toxic substances. The longer-living females are characterised by a slow rejection of metals, and they should invest more energy into an efficient regulation of metal levels, being responsible for egg production and nursing of the spiderlings (Wilczek et al. 2004). Some laboratory feeding experiments on spiders sampled from polluted areas, however, showed that body concentrations were gender independent, as in the cases of *Pardosa lugubris* (Lycosidae) or *Agelena labyrinthica* (Agelenidae) (Table 30.2).

At specific areas, such as intertidal sites, metal bioavailability for spiders depends on the cation exchange capacity of the sediments. *P. piraticus* often occurs in freshwater and brackish water banks of the rivers and feeds on organisms which also live in close contact with the intertidal sediments. Du Laing et al. (2002) showed that contents of Cd, Cu and Zn in their body were higher than in the sediment in sites situated closer to the river mouth and were characterised by a higher salinity. Chloride content in sediments correlated well with the Cd, Cu and Zn contents in the spiders' body.

Table 30.1 Maximum body burdens of four heavy metals in unpolluted and polluted sites in selected species of spiders

	Unpolluted sites (μg/g body weight)				Polluted sites (percentage of unpolluted site)				
Species	Cd	Pb	Zn	Cu	Cd	Pb	Zn	Cu	Reference
Araneus diadematus	8.2	10	613	62	256	290	309	152	1, 3, 5
Linyphia triangularis	7.1	5.2	694	43	296	519	157	137	1, 3, 5
Agelena labyrinthica midgut glands	31.5	16.8	824	36	240	89	285	183	1
Agelena labyrinthica females	17	2.7	817	129	16	163	73	100	2
Agelena labyrinthica males	2.2	0.4	749	92	2,273	1,600	106	168	2
Metellina segmentata	5.4	7.1	462	41.7	407	239	204	216	3, 5
Pardosa palustris	6	5	512	54	367	240	186	576	3
Pardosa sp.	0.4	0.3	128	14.8	325	200	154	88	4
Pirata piraticus	10.9	30	444	157	41	90	89	64	6

References refer to (1) Babczyńska et al. (2011b), (2) Wilczek et al. (2003), (3) Wilczek and Migula (1996), (4) Van Straalen et al. (2001), (5) Wilczek et al. (1997), (6) Tojal et al. (2002).

Table 30.2 Cd concentrations (mean ± standard deviation) measured in experimentally exposed and control offspring originating from different populations

		Cd concentration (μg/g)	
Species	Material	Not Cd exposed	Cd exposed
Pardosa saltans (1)	Reference site	0.39 ± 0.37	61.1 ± 13.3
	Polluted site	0.45 ± 0.53	57.7 ± 14.5
Pardosa lugubris (2)	Reference site		
	Females	5.9 ± 2.2	141.0 ± 25.0
	Males	6.7 ± 2.6	116.0 ± 58.0
Agelena labyrinthica (3)	Reference site		
	Juveniles	8.2 ± 1.6	339.0 ± 56.3
	Females	1.8 ± 1.7	44.2 ± 29.3
	Males	2.8 ± 0.5	176.6 ± 67.8
	Polluted site		
	Juveniles	28.0 ± 4,3	228.2 ± 25.7
	Females	21.9 ± 10.4	276.7 ± 46.9
	Males	26.5 ± 6.8	278.5 ± 28.7

Data sources: (1) Eraly et al. (2010), (2) Babczyńska and Migula (2002), (3) Babczyńska et al. (2011a), source 1 refers to wet weight; sources 2–3 to dry weight.

30.3 Bioconcentration and Bioaccumulation of Heavy Metals

Metals reach the body through the alimentary tract mostly as sucked liquid digest or mashed pulp of the captured prey (Hopkin 1989). Contamination of spider webs with heavy metals may be an additional source of metal toxicity for orb-web species that regularly consume old webs (Hose et al. 2002).

Ground-dwelling wolf spiders better reflect the pollution level of their biotope than web-building spiders (Peterson et al. 2003; Wilczek et al. 2005; Eraly et al. 2010). Internal Pb, Cd and Cu concentrations increased with the soil concentration for most spiders (as well as for most other taxonomic groups) in the order Pb > Cd > Cu. Body concentrations of Zn were quite constant over a large range of soil concentrations (Heikens et al. 2001), but Jung and Lee (2012) showed different results for *Pardosa astrigera*. The metal content in the soil was in the order of Zn > Pb > Cu > Cd, while in spiders, the order of Zn > Cu > Cd > Pb was identified. The bioconcentration factors (BCF: metal body content/metal soil content) were 28.1, 0.14, 2.7 and 6.3 for Cd, Pb, Zn and Cu, respectively. Based on the ratio of assimilated to eliminated amounts of metals, spiders in general are regarded as macro-concentrators of heavy metals, but they can better regulate biogenic metals. High accumulation of metals (Ni, Pb, Zn) characterised spiders from moderately contaminated lowland floodplain along the river Rhine (Schipper et al. 2008). Their body burdens reached up to ten times higher levels than coleopterans.

In areas such as Ni-rich serpentine sites, spiders demonstrated some Ni regulatory possibilities. Concentration of Ni in spiders exceeded 100 μg/g dry weight, and their insect prey also contained Ni in even much higher concentrations than in spiders (Peterson et al. 2003; Boyd 2009). The bioaccumulation factor (metal body burdens of spiders/metal in prey) for two spiders (the thomisid *Misumena vatia* and the pholcid *Pholcus phalangioides*) fed with Ni-rich mirid bugs (*Melanotrichus boydi*) was low and reached the value of 0.54 and 0.60, respectively (Boyd 2009). In this case, Ni was not magnified along the food chain. The wolf spider *P. lugubris* demonstrated also some Cd regulatory possibilities. Spiderlings fed with Cd-contaminated flies until maturation accumulated less Cd than their prey (Table 30.2).

Bioaccumulation rates are metal dependent. For example, *P. astrigera* exposed for 8 weeks reached saturation levels of Pb and Hg within 2 weeks (ca 3–4 μg/spider), while the saturation level for Cd (10 μg/spider) was only reached after 6 weeks (Jung et al. 2005). The excretion rate of Cd is very slow as it was shown experimentally for *Pirata piraticus* fed with Cd-contaminated flies (Hendrickx et al. 2003b).

Although *Agelena labyrinthica* is a web-building species, its ecological niche is similar to wandering spiders, and it accumulates higher amounts of metals than other web-building species (Wilczek and Babczyńska 2000; Wilczek et al. 2005). The bioaccumulation factor of Cd depends on the developmental stage, gender and preadaptation to heavy metals under natural conditions (Table 30.2).

30.4 Internal Metal Distribution and Loss of Heavy Metals

In six wandering and web-building spider species levels of Cd, Pb, Cu and Zn in the midgut glands were higher than in their gonads (Wilczek and Babczyńska 2000). The spiders protect their reproductive functions and thus continue to occur in heavy metal-contaminated sites. Metals are deposed mainly in the digestive cells of the midgut glands in metal-rich mineral granules, which were already described by

Hopkin (1989). Three types (A, B and C) are localised inside and the forth (D) outside the cells. Type A contains Ca, Zn and Pb; type B granules include Cu, Hg, Cd, Zn, Pb or Fe; type C contains Fe; and type D comprises calcium carbonate granules. The structures grow with spider age and with the duration of exposure. Metals from these mineral granules can be transported into the gut lumen and eventually are lost with faeces after every meal. Elimination of Cd with the exuviae is low. In exuviae of Cd-intoxicated *A. labyrinthica,* Cd concentration constituted from 0.2 % in juveniles to 10 % in males of the opisthosomal concentration. Cu losses with the exuviae of this spider are low in males (3 %) but high (36 %) in females (Babczyńska et al. 2011a).

30.5 Subcellular Effects of Heavy Metals

30.5.1 Metallothioneins

As most animals, also spiders are able to synthesise metallothioneins which are small (3–14 kDa) cysteine-rich metal-binding proteins. The level of metallothioneins in web-building spiders reflects the soil pollution with heavy metals. Babczyńska et al. (2011a) showed that in *Araneus diadematus* (Araneidae) from polluted sites, metallothionein synthesis was found to be induced by metals unlike in spiders of this species from the reference site. The response of other examined species [*Linyphia triangularis* (Linyphiidae) and *Agelena labyrinthica*] was less pronounced. The level of metallothioneins is not only species specific but also metal dependent. High metallothionein concentrations follow high concentrations of Cd and Pb, but body concentrations of essential Cu and Zn and metallothionein levels were not correlated. This suggests that metallothioneins play a regulatory role in relation to biogenic metals if their concentrations exceed the tolerable levels. In case of Cd and Pb, the role of metallothioneins shifts rather towards defence than to regulation and storage.

A significant increase of metallothioneins was also indicated in feeding experiments on *A. labyrinthica* fed Cd- or Cu-contaminated fruit flies (Babczyńska et al. 2011b). The protective role of the proteins demonstrated itself by a relative increase of metallothionein-positive cells in the midgut glands and increased total concentration of metallothioneins in this organ. In a majority of cases, the variability of this parameter reflects the degree of pollution in natural habitats of spiders, their developmental stage and gender.

The level of metallothioneins does not increase immediately. Eraly et al. (2010), studying the response of *Pardosa saltans* intoxicated with Cd under laboratory conditions, demonstrated that the synthesis of the proteins is energetically costly and starts only when metals in the spiders' body exceed a certain level. Thus in spiders, metallothioneins as protective molecules in metal intoxication might play rather a complementary role to the other efficient mechanism of metal neutralisation, such as binding of metals and their storage as granular concretions in cells of the midgut glands.

30.5.2 Defensive Mechanisms Against Heavy Metals

Metals affect the cellular integrity and functioning of the organism and therefore evoke various defensive mechanisms that repair damages and restore homeostasis. Metals, if not bound to any ligand or sequestered in granules, often attack mitochondria causing disturbances in the respiratory chain and uncoupling of oxidative phosphorylation, thus reducing energy available for defensive processes. Analysis of the cellular effects caused by Cd and Cu indicated a series of alterations in mitochondria of the midgut gland cells of *Agelena labyrinthica* (Babczyńska et al. 2011b). Mitochondria may play a strategic role in cell death initiation, and therefore, a decrease of mitochondrial transmembrane potential in midgut gland cells indicates early stages of apoptosis (Wilczek 2005).

One of the most important negative effects of heavy metals in the organism is to deplete the ATP concentration. The ADP/ATP ratio used as a measure of the energetic status of cells indirectly informs whether the cells proliferate (<0.1), overcome apoptosis (0.1–1) or suffer from necrosis (>1), and this defines the level of ATP depletion. Experiments with *A. labyrinthica*, dietarily exposed to an excess of Cd or Cu, indicated that their energy status was not disturbed. While ATP remained at similar levels in control and intoxicated spiders, the ADP/ATP ratio was two to six times higher for metal-exposed spiders and was age and gender dependent. ATP in females was kept at high levels, and the increased ADP/ATP ratio reflected an increased ADP pool, not a depletion of ATP, suggesting enhanced anabolic processes (Babczyńska et al. 2011b). Such an energy status of females was probably due to activated anaerobic pathways. An enhanced activity of enzymes involved in anaerobic reactions was already documented earlier for females of *A. diadematus*, *Metellina segmentata* (Tetragnathidae), *Linyphia triangularis* and *Pardosa palustris* sampled at heavily polluted sites (Marczyk et al. 1993). The adenylate energy charge index (= ATP + 0.5ADP/ATP + ADP + AMP) used in these spiders to quantify the metabolic energy held in the adenylate pool was kept at a physiologically optimal level (i.e. above 0.75), what can result from the enhancement of anaerobic pathways of energy production (Wilczek 1996).

Heavy metals are responsible for an enhanced production of reactive oxygen species and the inhibition of antioxidant activities. Intracellular accumulation of reactive oxygen species can damage biological macromolecules. The degree of the damage depends on the efficiency of the antioxidative defence which includes nonenzymatic scavengers, such as glutathione or heat-shock proteins, and major antioxidative enzymes: superoxide dismutase, catalase or glutathione peroxidases which are selene dependent or selene independent. Enzymatic antioxidative reactions in spiders living in metal-polluted habitats are species specific. In males of *P. lugubris,* collected from sites along a heavy metal pollution gradient, positive correlations between the activity of glutathione S-transferase and body burdens of Pb and Zn and between both peroxidase types and Cu were found. These spiders also maintained high glutathione concentration. Web-building *A. labyrinthica* collected from the same habitats had lower levels of these parameters (Wilczek et al. 2004).

Similar types of enzymatic relations were identified between catalase and superoxide dismutase and heavy metals in the body of *P. palustris* from metal-contaminated areas. Another examined species, *L. triangularis,* also kept high activities of these enzymes, although the metal level in their body was low (Wilczek and Migula 1996).

The activities of both types of glutathione peroxidases were also higher in *Xerolycosa nemoralis* (Lycosidae) than in *L. triangularis* and *A. labyrinthica* when isolated midgut glands were analysed. Heat-shock proteins (Hsp70) play an important cytoprotective role. In the above-mentioned species from heavily polluted areas, the percentage of Hsp70 positive cells of midgut glands was species, gender and site dependent. They were the highest in *X. nemoralis* and the lowest in *L. triangularis* (Wilczek et al. 2008).

Interspecies differences in detoxifying responses of spiders from heavily polluted habitats were identified studying activity levels of enzymes involved in the neutralisation of xenobiotics during the first and second phase of detoxification. Wolf spiders (*P. palustris*, *P. lugubris*) maintain high hydrolytic processes catalysed by carboxylesterases and the conjugation with glutathione led by glutathione S-transferases. The intensity of these reactions in web-building spiders (*Metellina segmentata, L. triangularis, A. diadematus, A. labyrinthica*) was lower than in wolf spiders (Wilczek and Migula 1996; Wilczek et al. 2003, 2004).

Responses of spiders living in habitats chronically polluted by heavy metals to chemical stress are often gender dependent. Maintaining a high glutathione level is an important element in the defence process of males against high metal loads and their pro-oxidative activity. The antioxidative reactions in females are species specific and depend mainly on high glutathione peroxidase and catalase activities, although the levels of these enzymes are species specific (Wilczek et al. 2008). It cannot be excluded that under an increased impact of pollutants, female spiders are able to utilise various detoxifying mechanisms and strategies, even if energetic costs are high, to protect their genetic material. The main direction in the use of the described parameters by different spider species is schematically presented in Fig. 30.1.

30.6 Effects of Heavy Metals on Spider Populations and Communities

30.6.1 Heavy Metals and Spider Populations

Often a cascade of ecological effects can be seen under heavy metal pollution. In the case of spiders, this could be measured as reduced secondary productivity due to restrictions in the energy budget caused by such factors as reduced prey availability or increased predation risk leading to reduced fitness. This, in turn, affects both reproductive output and survivorship.

The relationship between density and richness of ground-dwelling spider species along a heavy metal pollution gradient was documented by Koponen and Koneva

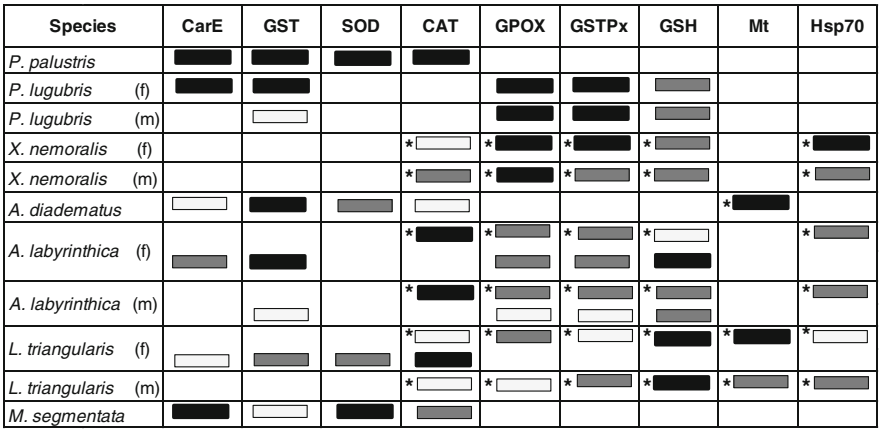


Fig. 30.1 Relative levels of *CarE* carboxylesterase, *GST* glutathione S-transferase, *SOD* superoxide dismutase, *CAT* catalase, *GPOX* and *GSTPx* glutathione peroxidases, *GSH* glutathione contents, *Mt* metallothioneins and *Hsp70* heat-shock proteins in the body of actively hunting and web-building spiders from heavily polluted environments. *Black shaded* high, *grey shaded* medium, *white shaded* low, *asterisk* measured in midgut glands, *f* females, *m* males

(2005) for the areas around the Severonickel smelter (Kola Peninsula). In the nearest belt, 2.5 km from the smelter, the spider density was low (6 individuals/m^2) with a diversity of only two species. Also at a distance of 5 km, only 3 individuals/m^2 and up to 10 km (heavily polluted and afforested zone) 8 individuals/m^2 were collected. At a distance of 30 km, the density reached nearly 60 individuals/m^2. In another Ni-contaminated area the density of spiders representing 18 species was 20 individuals/m^2, while in only slightly polluted sites it was six times higher and represented by 58 species. Linyphiid spiders were most abundant in slightly polluted sites (64 % of all species and 60 % of the individuals). At heavily polluted sites their contribution was reduced to 23 % and 38 %, respectively, but they still were the most abundant family among identified ten families of spiders (Koponen 2011).

Changes in growth, reproduction and survival are sensitive factors, indicating sublethal effects caused by the metal pollution at population level. The overall effects on reproductive traits depend on trade-off mechanisms of energy investments between inactivation or elimination of metals and reproduction. Pollutants are limiting the availability of energy which could be allocated for reproduction. In such conditions, less but larger offspring is favoured. Such a strategy is used by the wolf spider *Pirata piraticus*. Hendrickx et al. (2003a) studied populations from four polluted sites located on the banks of the river Schelde and one unpolluted inland reference site. Reproductive output and fecundity of spiders from populations with high metal loads was strongly reduced as they allocated energy to detoxification processes. The size of the females was positively correlated with metal levels and negatively with fecundity and reproductive output, but they produced larger eggs.

If the reproductive output is neglected, such a strategy indicates adaptive trade-off mechanisms between egg size and number of produced eggs.

Such a strategy, as described above, is not common in spiders. Effects of heavy metals on both growth and reproduction could be negative and are species dependent. For example, in laboratory conditions, the total number of eggs laid by females of Pb and Zn-stressed *Pardosa astigera* was sharply reduced (Chen et al. 2011). In another wolf spider (*Pardosa saltans*) experimentally exposed to Cd (100 μg/g wet body weight) also resulted in prolonged development, reduced growth and reproduction (Eraly et al. 2010). The authors concluded that the main defence mechanism against heavy metals is the up-regulated expression of metallothionein-like proteins (MTLP). Further field studies on this species indicated that metals affected life history and physiological traits in four out of six *P. saltans* populations (Eraly et al. 2011). Cd concentrations correlated negatively with the adult size and positively with the egg mass. Reproduction and the reproductive output (fecundity, egg mass, cocoon weight) were the lowest or even postponed in population with the highest Cd and Zn body burdens. At the most polluted site the highest number of females carrying cocoons appeared later in the season than in other sites. These changes were not related with an increase of MTLP, and the authors stated that under field conditions, physiological defence plays a lower role than indirect and synergistic effects of heavy metal pollution.

Fluctuating asymmetry (random differences in the development of both sides of a bilateral symmetrical character) is often used as an indicator of environmental and/or genetic stress. In *P. piraticus* Maelfait and Hendrickx (1998) recorded a significant positive correlation between the distance between two dorsal spines on tibia IV and internal concentrations of Cd. Further studies on the same species showed that in populations adapted to metal stress fluctuating asymmetry was negatively correlated with clutch mass but positively with the egg size. Selection obviously eliminated suffering spiders and favours females producing larger eggs. Thus, diminished clutch masses are compensated by a production of larger eggs (Hendrickx et al. 2003c). A stronger relationship between fluctuating asymmetry and fitness demonstrated possible selection against developmentally unstable individuals with the evolution of adaptive responses to environmental stress and the resources devoted to reproduction.

30.6.2 Heavy Metals and Spider Communities

Species composition in areas around the contamination sources is directly and indirectly influenced not only by heavy metals but also by many other factors, such as alterations of vegetation structure, properties of soils and their acidification or deacidification by liming (Maelfait 1996; Ormerod and Rundle 1998).

The composition of spider species may differ distinctly between highly polluted and unpolluted sites. Read et al. (1998) analysed the relations between spider species and a suite of environmental variables in woodlands. They showed that

the heavy metal pollution near the main pollution source affected the complete invertebrate community, causing a lack of lycosid spiders in highly polluted woods. This vacant niche was filled by large agelenid species which seem to be more adaptable to such pollution levels.

More difficult is to demonstrate differences in spider communities between moderately polluted and unpolluted sites. Jung et al. (2008), using a Shannon diversity index, showed that a moderate contamination with heavy metals did not affect communities of ground-dwelling spiders, although the diversity index was higher in the unpolluted site, and species diversity was negatively correlated with Pb levels in soil. Using discrimination techniques, the authors stated that among the dominant spider families, only the composition and structure of Linyphiidae separated unpolluted and moderately polluted sites. Moreover, some lycosid species (*Pardosa laura*, *P. astrigera*) were highly abundant at moderately polluted sites and could even be used as indicators of metal bioaccumulation.

The use of guilds (groups of organisms that explore the same resource, e.g. generalist feeders of arthropods that differ in hunting behaviour) is one of the functional ways of community structure analysis. Guilds may reflect functional diversity and alterations of the integrity of biological communities. Ferreira (2010) assessed the impact of Cu and Zn in surface soils on the abundance and community structure of ground-dwelling spider communities in an area of 2 km around a copper mine. The abundance of spiders increased with increased distance from the contamination source, but Cu in the body was not correlated with Cu in soils. The author stated that the abundance of the ground hunter guild responded better to soil contamination, while specialist web-building spiders responded better to atmospheric deposition.

30.7 Conclusions

The results presented above indicate that under strong environmental pressure as a consequence of high concentrations of heavy metals in soils and prey, spiders are able to protect themselves and their genetic material using various, efficient strategies. The strategies differ between species. Hunting spiders in general intensify the activity of detoxifying enzymes, including antioxidative defence, whereas web-building species with their moderate levels of protective mechanisms seem better adapted to the unfavourable conditions in postindustrial or ore-bearing areas.

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