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Heavy metals in alpine terrestrial invertebrates

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Introduction

Invertebrates are in close contact with heavy metal contaminants in the soil. As they are an important link in the food chains, many birds and mammals become exposed to the accumulated contaminants they carry. The toxicity, bioaccumulation and physio-logical adaptations of different invertebrate groups to heavy metals have been targetted by many research studies. This review study presents a concise overview of these topics including suggestions on the use of invertebrates as indicators of heavy metal pollution in high mountain environments.

Heavy metal pollution and invertebrates

Atmospheric pollution with heavy metals is the oldest known long-range pollution. Great concern has arisen only in recent times because of large amounts of lead emitted from leaded gasoline in combustion engines. The lead polution started to become an environmental issue in the 1970´ s - 1980´ s. After the introduction of unleaded gasoline, the level of lead in the atmosphere declined.The highest pollution levels in the sediments – about 60 mg Pb kg^{-1} – were measured at a sediment depth which was 10–30 yr old, i.e., they were deposited between 1960 and 1980. Longe-range lead transport resulted in enhanced lead concentrations in mountain areas (Hagner 2002). However heavy metal pollution is a much older issue. Since 1870 the lead concentrations have increased significantly above the geological background value (Hagner 2002). Lake sediments from the Pyrenees and Alps have revealed heavy metal accumulation during intensive mining and smelting yet in the old Greek and Roman times (Catalan *et al.* 2006). Heavy metals are known to have negative impact on fitness for herbivorous insects (Butler and Trumble 2008). Reductions in weight, growth, survival, fecundity and eclosion success in insects have been associated with exposure to heavy metals (Van Straalen and Van Wensem 1986, Vandecasteele *et al.* 2004, Hobbelen *et al.* 2006, Van Ooik *et al.* 2007). Heavy metal exposure was associated with decreased synthesis of heat shock proteins in grasshoppers, indicating that Hsp70 does not have a protective function against heavy metals (Warchalowska-Siwa *et al.* 2005). Heavy metal particulates which land on plant surfaces can damage photosynthetic systems and result in more toxic plant chemistries with decreased nutritional value (Jensen and Trumble 2003). Odendaal and Reinecke (1999) observed that *Porcellio laevis* (Isopoda) have the ability to discriminate between different Cd concentrations in food and are also able to avoid contaminated leaf. It was proven that some herbivorous insects, such as grasshoppers (*Chorthippus* sp.) are able to select qualitatively their diet, by e.g. reduced consumption of leaves with higher metal content (Migula and Binkowska 1993). Such findings were made for insects pre-exposed in natural conditions to excessive amounts of such metals as cadmium or lead. Insects are not equipped with chaemosensilla sensitive to heavy metals and selection of the quality of eaten material is probably due to recognition of other changes in taste of leaves (Augustyniak and Migula 2000). Weissenburg and Zimmer (2003) studied the Isopod *P. scaber* and noted that the isopods turned significantly more often toward aqueous extracts of copper-poor leaf litter than of copper contaminated leaf litter. The springtail species *Mesaphorura macrochaeta* is probably able to search for and use the detoxifying properties of mineral particles, which have been retrieved in high amount from its gut contents in metal polluted environment (Garnier and Ponge 2004). It has been demonstrated that heavy metals are unevenly distributed within tissues. High amounts are typical for the intestine, as these epithelial cells are highly exposed to metals. In earthworms Fugére *et al.* (1996) showed that the exposure to Hg, Cd and Zn affects the viability of coelomocytes in the gastrointestinal tract of *Lumbricus terrestris*, as well as phagocytotic activity. Pb was relatively well tolerated by the coelomocytes. Adult earthworms are able to accumulate more Cd, Zn and Pb per mass unit than subadults (Ma 1982). Insects are able to reduce the excess of heavy metals by eliminating the degenerated midgut cells loaded with metals, protecting other parts of the body (Rabitsch 1995). Such mechanism may be used only to a certain extent. At higher concentrations metals may reach other organs, also gonads, transported with external body fluids (Dallinger and Rainbow 1993, Heikens *et al.* 2001). Grasshoppers from heavily polluted sites have shown to have bioconcentration index for cadmium two to ten times lower as compared with insects from less polluted sites. A similar trend was also observed for other metals, but with less visible differences. This means that also at the level of a population grasshoppers have different abilities to accumulate metals, with higher tolerance to metals of insects from a heavily polluted site (Augustyniak and Migula 2000). Aquatic insect larvae are known to sequester high levels of metals, particularly Pb, in the cuticle (Hare 1992). Gastropods are known for their ability to retain and inactivate toxic metals, either through intracellular compartmentalisation (confinement within granules and vesicles) and excretion, or through protein binding, including metallothioneins, which store some heavy metals such as cadmium for long periods (Dallinger 1996, Cortet *et al.* 1999). For most trace metals, the main site of accumulation in the gastropod´ s body is the midgut gland (Dallinger 1993). Other important tissues for accumulation of certain trace elements include the foot sole, the mantle and the intestine (Wieser *et al.* 1977).

Metallothioneins

Metallothioneins are known to be multifunctional proteins, playing an important role in metabolizing essential and non-essential trace elements such as copper, zinc and cadmium (Dallinger 1996). The primary structure or sequence of metallothioneins and metallothionein genes is known only for few terrestrial invertebrates (Dallinger 1996). Terrestrial gastropods possess the copper – rich respiration protein hemocyanin, which is responsible for the carrying of oxygen in the soft parts of the body. At the same time they are extremely tolerant against highly increased concentrations of cadmium in the environment and in their tissues (Wieser *et al.* 1977, Viard *et al.* 2004). In order to keep a balance between the essential copper and toxic cadmium, snails have developed metal-specific metabolic pathways. In Roman snails, copper is bound mainly in the mantle, whereas cadmium in the midgut gland. Dallinger (1996) suggests that it is not necessary to have structurally different metallothionein isoforms in order to perform different functions in the trace element metabolism of an organism. Metallothioneins in the midgut gland and in the mantle may exhibit different fucntions simply because of the fact that they are expressed in different tissues which preferentially accumulate different metals. The synthesis of some metallothionein isoforms in terrestrial invertebrates seems to be inducible by exposure to cadmium, copper, zinc or some organic chemicals. The concentration of cadmium binding metallothionein in cadmium-fed Roman snails rapidly increases even after a short-term exposure to cadmium. After the termination of cadmium treatment, metallothionein concentrations remained on an elevated steady-state level over prolonged periods of time. Cadmium is stored permanently bound to metallothioneins in midgut gland of snails. The expression of metalothioneins may depend on animal condition and growth, developmental stages, organic chemicals, oxidative stress, radioactivity, temperature and food availability (Brady 1991, Templeton and Cherian 1991, Dallinger 1996). The complex function of metallothioneins in the indicator species has to be known before using them as biomarkers for risk assessment in environmental monitoring. In different species, metallothioneins may perform additional functions, that are not related to metal detoxification. Dragonfly larvae (Odonata) were found to be more tolerant of increased concentrations of Pb and Cd than Cu. Cu is known to be preferentially bound more readily by metallothioneins than Pb and Cd, leading to greater bioaccumulation. The presence of metallothioneins in dragonfly larvae has not yet been explored (Tollett *et al.* 2009). Dallinger *et al.* (2001) argues that some metals such as zinc and lead, which are accumulated in cellular granules along with calcium, may be less available to predatory animals than cadmium, which is mainly bound to chelating proteins such as metallothioneins.

Cadmium (Cd)

Cadmium has a strong genotoxic potential in earthworms, inducing DNA damage to coelomocytes at all concentrations, as confirmed by comet assays (Li *et al.* 2009). According to Heikens *et al.* (2001), accumulation of cadmium may or may not depend on soil concentration in different arthropod groups. Generally, internal Cd concentrations are high in Isopoda and low in Coleoptera and Chilopoda. Lumbricidae contain relatively high Cd concentrations in comparison with other heavy metals. Ernst *et al.* (2008) found that tissue Cd concentrations in anecic earthworms were highly correlated to the Cd concentrations in soils (HNO_3 and Lakanen-extractable) and roots. In addition, Cd concentrations in above-ground litter showed a weak but positive relationship with tissue Cd concentrations in epigeic and anecic earthworms. The accumulation of cadmium was found to decrease in different invertebrate groups in the following order (Heikens *et al.* 2001):

> Arthropod groups where Cd accumulation is independent of total soil concentration:
> Isopoda > Formicidae > Chilopoda
>
> Arthropod groups where Cd accumulation depends on the total soil concentration:
> Lumbricidae > Arachnida > Diplura > Diplopoda > Collembola > Coleoptera

Copper (Cu)

Copper is an essential trace metal for the invertebrates. Those invertebrate groups which use hemocyanin as oxygen carrier have developed metabolic pathways to extract Cu from the environment. The accumulation of copper was found to decrease in different invertebrate groups in the following order (Heikens *et al.* 2001) :

> Diplopoda = Isopoda > Collembola > Arachnida > Lumbricidae > Coleoptera = Formicidae

Lead (Pb)

Lead is accumulated nearly exclusively in the humus layer of soils, compounded in metal-organic complexes and reduces sharply in deeper layers. Pb content in natural forest sites in Europe was found to vary between 0.2-280 mg Pb/kg dry soil. In Finland 27– 49 mg Pb/ kg dry soil (Lukkari *et al.* 2004), in Poland 0.4–68 mg Pb/kg dry soil (Rozen 2006) and in South Germany 0.2–280 mg Pb/kg 1 dry soil (Rahtkens and von der Trenck 2006). While lead concentrations of about 150 mg Pb kg^{-1} might damage invertebrates and induce harmful effects on the biochemical soil activity, the soil respiration and microflora are not expected to die until concentrations of more than 500 mg Pb kg^{-1} humus are reached (Tyler 1972).

In comparison to other heavy metals, internal Pb concentrations of many taxonomic groups strongly respond to increasing soil concentration. In general, internal concentrations in Coleoptera and Chilopoda are low, the internal concentrations of Pb are high in Isopoda and Collembola (Heikens *et al.* 2001). Even at very high soil concentrations (± 60,000 μg/g od dry weight), most taxonomic groups were present. The body concentrations of the Collembola increased the most. Terhivuo *et al.* (1994) showed that different species of earthworms have different ability of accumulating Pb in the following order: *Lumbricus castaneus* > *L. rubellus* > *Aporrectodea caliginosa* > *A. rosea*. The epigeic species *Lumbricus castaneus* accumulated Pb at higher rates than other endogeic species in both heavily and slightly polluted soils and in control conditions.

Pb concentration was shown to variate with season in the isopod *Porcellio laevis* (Hussein *et al.* 2006). Pb concentration was significantly higher in summer than in the other seasons. Bioconcentration factors also showed seasonal changes. Ernst *et al.* (2008) studied relationships between heavy metal concentrations in earthworms and chemical soil properties using multivariate analysis. Pb concentrations in soils (HNO_{3-} and Lakanen-extractable) and in leaf litter were the most important parameters determining Pb concentrations in epigeic and anecic earthworms. In endogeic earthworms, there was no such relationship but Pb concentrations were found to have high negative correlations to soil pH and CEC (Ernst *et al.* 2008).

Heavy metals in different invertebrate groups

Most studies on the bioaccumulation of heavy metals in the invertebrates are concerned with cadmium (Cd), copper (Cu), lead (Pb) and Zinc (Zn). Increase in heavy metal concentration in response to soil concentration is metal dependent. The overall pattern for this relation was shown to be Pb > Cd > Cu > Zn (Heikens *et al.* 2001). This may be due to the fact that Pb and Cd are non-essential metals and regulation of these metals is weaker than in Cu and Zn (Dallinger and Rainbow 1993). Seasonal differences in the bioaccumulation of metals have been observed (Ma 1982). Important role plays the exposure route, the regulation capabilities may only partially explain the differences. The exposure route varies for many species, caused by differences in morphology, behaviour, food and habitat preference. Body concentrations of heavy metals in insects usually increase with soil concentrations for Pb, Cd and Cu in most taxonomic groups. Generally high heavy metal concentrations have been found in the Isopoda and low concentrations in the Coleoptera (Heikens *et al.* 2001). Out of several invertebrate groups Van Straalen and Van Wensem (1986) found the highest concentrations of most heavy metals in oribatid mites. Terrestrial Isopoda live in contaminated litter and feed on organic matter. They are known to accumulate heavy metals to certain extent (Hendrickx *et al.* 2004). Coleoptera have less contact with litter and are either herbivores or carnivores. In some cases exposure concentrations cannot explain the differences in internal metal concentrations. E.g. in comparison to Carabid beetles, Arachnids are exposed to similar metal concentrations but internal metal concentrations are much higher in Arachnida. Spiders have been shown to be strong accumulators of metals (Van Hook and Yates 1975, Hendrickx *et al.* 2003). This is most probably due to differences in physiology (Van Straalen and Van Wensem 1986, Dallinger 1993).

Differences between species from the same taxa (earthworms) in the accumulation of the same metal can be significantly different (Ma 1982, Terhivuo *et al.* 1994, Morgan and Morgan 1999, Ernst *et al.* 2008). In several studies it has been shown that the pH is one of the major factors determining the metal concentration in animal bodies (Van Straalen and Van Wensem 1986). Biovalailable metal concentrations are also dependent on other soil characteristics such as organic carbon content, CEC (cation exchange capactiy) and Ca^{2+} concentration (Ireland and Wooton 1976, Wieser *et al.* 1977, Neuhauser *et al.* 1995). The toxicity of heavy metals is influenced by several variable factors such as soil pH (Van Straalen and Bergema 1995), metal solubility (Neuhauser *et al.* 1995), metal bioaccumulation (Morgan and Morgan 1999). Lead sequestration in earthworms was shown to be affected by calcium-rich chloragosome granules (Tomlin 1992). This may explain why earthworms accumulate Pb to lesser extent than Cd (Van Straalen and Van Wensem 1986). Neuhauser *et al.* (1995) have shown that bioconcentration of heavy metals in earthworms is greater at lower soil concentrations. Significant differences have been found in the concentrations of certain essential and non-essential metals between the earthworm ingesta and bulk soil, suggesting that earthworms are very selective consumers capable of influencing the amount of heavy metals ingested (Morgan and Morgan 1999). Voiding of gut contents can affect the proportion of total ash contributed to earthworm tissue by soil present in the gut (Neuhauser *et al.* 1995). For metals that are bioconcentrated in worm tissue, increasing the voiding period increases the concentration of the metal in the worm-soil complex. Springtails are known to be highly tolerant to metal pollution. This has been in some cases explained by acclimation or adaptation of springtails to elevated metal concentrations (Posthuma and Van Straalen 1993). *Mesaphorura macrochaeta* Rusek is known to change its diet in response to higher concentration of heavy metals (Gillet and Ponge 2003). Springtails exhibit a high capacity to excrete metals. Springtails can store heavy metals in midgut intracellular mineral concretions that are eliminated at each moulting interval by intestinal exfoliation (Posthuma and Van Straalen 1993, Rabitsch 1995, Van Straalen *et al.* 2001). Epigeic collembolans are probably less sensitive to metal pollution than euedaphic species which live in closer contact with the soil (Lock *et al.* 2003). (Van Straalen *et al.* 2001) reported high concentrations of Cd and Pb in ground beetles, but Heikens, Peijnenburg and Hendriks (2001) found that beetles were weak accumulators of heavy metals in comparison to other arthropod groups. Jelaska *et al.* (2007) found that beetles were good indicators of cadmium presence in the environment. Concentrations of Pb and Cd in beetles were corresponding with those in the soil and leaf litter (Rabitsch 1995, Migliorini *et al.* 2004, Jelaska *et al.* 2007).

In spiders, the variations in the nature and quantity of heavy metals accumulated was found to be dependent on the spider species (Marc *et al.* 1999). A significant site × species interaction for the bioaccumulation of heavy metals in spiders was found (Hendrickx *et al.* 2004), which means that different spiders not only accumulate trace metals to different equilibrium concentrations in their tissues (Dallinger and Rainbow 1993), but differential accumulation patterns can be found at different localities. Spiders are generalist predators which obtain most of their heavy metal burden from their prey, therefore the composition of prey species may strongly influence heavy metal levels in spiders, which may not be directly related to heavy metal content of the soil. Larsen *et al.* (1994) found out that the extent of bioaccumulation of Cd, Cu, Pb and Zn correlates with the amount of heavy metals in the soil. Ground spiders contained significantly higher levels of Cd and Cu than web spiders, mainly due to there rather immobile ground-dwelling prey (Marc *et al.* 1999, Hendrickx *et al.* 2003). Rabitsch (1995) has found that spiders demonstrate no obvious differences in their metal levels on the species, genus and even family level at the same sampling site. High potential for cadmium accumulation was found in spiders even at less polluted sites. The comparison of heavy metal concentrations in different invertebrate groups is shown in Table 1-4.

Use of invertebrates as bioindicators of heavy metals in alpine habitats

Mountain landscapes represent steep gradients for a wide range of environmental parameters, particularly temperature, precipitation, UV radiation, atmospheric gas concentrations and so on. The combination of relatively specialized species and naturally harsh conditions makes animal communities at high altitude especially vulnerable to environmental disturbance, particularly anthropogenic pollution and

Species	**Fe**	**Zn**	**Cu**	**Pb**	**Cd**
Earthworms					
Lumbricus castaneus	909 ± 771	336 ± 84	19.5 ± 18.0	<0.5	23.6 ± 33.4
Lumbricus rubellus	825 ± 723	385 ± 95	11.0 ± 4.6	8.18 ± 10.8	8.04 ± 6.84
Lumbricus terrestris	3309 ± 4291	385 ± 169	9.3 ± 6.6	12.5 ± 4.0	6.2 ± 2.8
Aporrectodea caliginosa	390 ± 58	480 ± 212	10.2 ± 5.1	1.41 ± 2.82	11.1 ± 8.3
Aporrectodea rosea	374 ± 145	355 ± 167	15.5 ± 6.6	126 ± 72	26.9 ± 13.6
Springtails					
Orchesella cincta	699 ± 210	79.3 ± 67.6	6.22 ± 2.94	1.54 ± 0.01	0.13 ± 0.03
Orchesella flavescens	329 ± 180	58.7 ± 3.2	4.38 ± 0.21	0.75 ± 0.30	0.08 ± 0.05
Tomocerus sp.	560 ± 39	71.9 ± 38.1	6.95 ± 0.28	1.79 ± 0.61	0.15 ± 0.09
T. flavescens	490 ± 390	50.3 ± 7.7	5.33 ± 1.83	1.12 ± 0.64	0.12 ± 0.04
Carabid beetles					
Agonum assimile	59.6 ± 45.8	140 ± 59	32.4 ± 7.8	59.6 ± 45.8	9.16 ± 5.11
Agonum obscurum	438 ± 620	78.8 ± 77.2	5.80 ± 8.20	35.2 ± 48.5	2.32 ± 3.28
Pterostichus niger	160 ± 19	126 ± 14	13.1 ± 1.5	12.4 ± 4.5	5.16 ± 1.23
P. oblongopunctatus	210 ± 23	170 ± 29	15.6 ± 2.7	41.2 ± 32.9	7.37 ± 3.19
Isopods					
Hyloniscus riparius	732 ± 955	25.3 ± 9.2	2.96 ± 0.60	2.50 ± 2.34	1.55 ± 0.52
Spiders					
Pardosa sp.	272 ± 68	197 ± 39	13.1 ± 1.3	0.61 ± 0.10	1.28 ± 0.25
Centipedes					
Unidentified	582 ± 314	182 ± 52	5.51 ± 2.47	0.99 ± 1.32	0.44 ± 0.55
Oribatid mites					
Various species*	2638 ± 1208	545 ± 43	37.4 ± 11.3	185 ± 120	3.75 ± 0.77

Table 1. Comparison of heavy metal concentrations in different arthropod groups from the same polluted site (Van Straalen *et al.* 2001).

Ecophysiological group	**Species**	**CF soil-earthworms**		
		Pb	**Cd**	**Hg**
Epigeic	*Lumbricus rubellus* (n = 16)	0.2 ± 0.1	26.9 ± 26.6	1.1 ± 0.6
	Dendrodrilus rubidus (n = 8)	4.7 ± 3.5	37.8 ± 43.4	4.1 ± 3.3
Endogeic	*Aporrectodea caliginosa* (n = 5)	0.8 ± 1.5	11.2 ± 19.6	2.9 ± 2.5
	Aporrectodea rosea (n = 5)	4.5 ± 9.8	120.6 ± 179.9	15.2 ± 15.3
	Octolasion tyrtaeum (n = 7)	0.3 ± 1.5	28.1 ± 16.9	7.6 ± 4.5
	Octolasion cyaneum (n = 9)	1.2 ± 2.4	47.9 ± 70.1	14.7 ± 14.3
Anecic	*Lumbricus terrestris* (n = 4)	0.3 ± 0.2	26.5 ± 15.8	1.5 ± 0.8
	Aporrectodea longa (n = 6)	0.7 ± 0.5	57.4 ± 86.8	10.7 ± 11.3

Table 2. Means and standard deviacions of species-specific concentration factors (CF) soil-earthworms (earthworm tissue concentrations divided by HNO_{3-} extractable HM soil concentrations) (Ernst *et al.* 2008).

Seasons		Cd			Pb			Cu			Zn		
		Animal	Soil	Litter	Animal	Soil	Litter	Animal	Soil	Litter	Animal	Soil	Litter
A: Summer	Mean	2.32	0.39	0.57	40.6	10.5	13.7	189	11.3	11.8	212	68.5	20.2
	n	7	7	7	7	7	7	7	7	7	7	7	7
	SD	0.39	0.1	0.2	13.1	1.09	2.25	28.4	3.12	1.2	27.2	10.2	6.42
	LSD	d*	d**	NS	b,c,d**	b**	c*, d**	NS	b**	b**	b,c,d**	b*	b*
B: Autumn	Mean	2.2	0.42	0.58	31.4	9.05	14.4	188	9.44	13.8	182	64.7	23.9
	n	7	7	7	7	7	7	7	7	7	7	7	7
	SD	0.28	0.11	0.29	6.32	3.49	2.49	49.4	1.47	4.26	26.4	7.98	11.4
	LSD	NS	NS	NS	a**	a,d**	d**	NS	a**	a,c,d**	a**	a*,c,d**	a*,c,d**
C: Winter	Mean	2.23	0.39	0.58	31.6	9.9	14.8	177	10.5	11.7	179	71.3	18.4
	n	7	7	7	7	7	7	7	7	7	7	7	7
	SD	0.34	0.08	0.37	3.7	1.08	1.9	52.6	2.13	3.25	27.8	11.9	4.5
	LSD	NS	d**	NS	a**	NS	a*,d**	NS	NS	NS	a**	b**	b**
D: Spring	Mean	2.18	0.44	0.57	32.8	10.6	12.4	178	10.7	10.6	176	70	17.7
	n	7	7	7	7	7	7	7	7	7	7	7	7
	SD	0.23	0.08	0.37	4.88	1.93	3.07	48.6	1.9	1.99	15.2	8.8	3.28
	LSD	a*	a,c**	NS	a**	b**	a,b,c**	NS	NS	NS	a**	b**	B**

*The mean difference is significant at the 0.05 level **The mean difference is significant at the 0.01 level NS, The mean difference is not significant

Table 3. Seasonal fluctuations of heavy metals (mean ± standard deviation) of heavy metals concentration (μg/g dry weight) in Porcellio laevis (animal), soil, and litter at different seasons by (Hussein *et al.* 2006).

Group-species	Habitat	HM	Soil or food	Organism	CF
Trichocera annulata	Grassland	Cu	15.1	25.9	1.71
Trichocera annulata	Grassland		543.0	85.4	0.15
Trichocera annulata	Grassland		11000.0	210.0	0.02
Tiulidae	Various forests	Cu		35.0	
Limonidae	Various forests	Cu		9.0	
Dolichopodidae	Various forests	Cu		48.0	
Lauxanidae	Various forests	Cu		37	
Muscidae	Various forests	Cu		11.0	
Sciaridae	Meadow	Cu	5.0[a]	31.0	
Sciaridae	Meadow NPK[b]	Cu	5.0[a]	67.0	
Trichocera annulata	Grassland	Cd	0.8	2.2	2.75
Trichocera annulata	Grassland	Cd	6.9	6.9	1.0
Trichocera annulata	Grassland	Cd	15.4	24.8	1.61
Tiulidae	Various forests	Mn		27.0	
Limonidae	Various forests	Mn		16.0	
Dolichopodidae	Various forests	Mn		134.0	
Lauxanidae	Various forests	Mn		305.0	
Muscidae	Various forests	Mn		66.0	
Sciaridae	Meadow	Mn	140.0[c]	50.0	0.36
Sciaridae	Meadow NPK[b]	Mn	195.0[c]	67.0	0.34
Sciaridae	Spruce forest	Pb	48.0	3.1	0.06
Cecidomyiidae	Spruce forest	Pb	48.0	4.7	0.10
Tipula scripta	Spruce forest	Pb	48.0	0.5	0.01
Sciaridae	Meadow	Zn	53.0[c]	14.0	0.26
Sciaridae	Meadow NPK[b]	Zn	54.0[c]	67.0	1.24
Sciaridae	Meadow	Fe	160.0[c]	480.0	3.00
Sciaridae	Meadow NPK[b]	Fe	270.0[c]	670.0	2.48

[a]Grass [b]Fertilization 860kg NPK/ha^{-1} [c]Litter, content of HM (Czerwiński *et al.* 1978).

Table 4. Content of heavy metals in soil dwelling Diptera and in soil or dipteran food in various habitats. HM – heavy metal (Frouz 1999).

changing climate (Hodkinson and Jackson 2005). Before choosing a suitable bioindicator system, it is first necessary to define clearly the objectives and endpoints of the study. This should include a statement of exactly what it is intended to measure, how it will be measured, and why. It needs to take into account the nature of the problem, whether it is a response to a single pollutant at a restricted site or an attempt to compare biodiversity over a broader area (McGeoch 1998). Invertebrates within any given habitat are generally taxonomically diverse and it may be unrealistic to expect the simple bioindicator organism. Community composition is thus more usually used to bioindicate broader aspect of habitat quality (Hodkinson and Jackson 2005). Aquatic invertebrates have been long used in the monitoring and assessment of habitat quality, however the assessment of terrestrial environment using invertebrates as bioindicators is still less developed (Dallinger *et al.* 2001). Springtails, snails and isopod species are known to suffer mortality at known threshold levels heavy metals (Van Straalen 1998, Cortet *et al.* 1999), which could make them suitable as bioindicators of heavy metal pollution in the alpine environments.

Springtails and mites are known to be sensitive to a variety of environmental conditions including pollutants and already have been used as suitable bioindicators in extreme environments (Sinclair and Stevens 2006). Gastropods live even at altitudes over 2,000m a.s.l. in sufficient densities in Central Europe, which makes them also suitable as bioindicators of heavy metal pollution in high mountain environments (Dallinger *et al.* 2001). The strong affinity of terrestrial gastropods for certain trace elements has also been observed in uncontaminated habitats (Knutti *et al.* 1988). Terrestrial gastropods are likely to play an important role in directly transferring certain pollutants to higher trophic leves of terestrial food chains, serving as prey or hosts for a variety of other animals (Thompson *et al.* 1993). Earthworms can be found in soils at higher elevations and could be used as indicators of heavy metals in the alpine environments. Especially epigeic species feeding in the top soil layer are known to strongly accumulate heavy metals (Ernst *et al.* 2008). Few carnivorous invertebrate groups living at high eleaetions apart from spiders are known to be good accumulators of heavy metals. Although beetles have been shown to respond to heavy metal concentrations in the soil, the relationship was not as clear as in spiders (Marc *et al.* 1999, Heikens *et al.* 2001, Hendrickx *et al.* 2004).

Several of the discussed terrestrial invertebrate groups have the potential to be used as bioindicators of heavy metal pollution in alpine environments. The availability of the bioindicator species in the studied environment and its significance in the food chain will have to be considered before selecting a suitable species. All suggested groups including earthworms, gastropods, spiders, springtails and isopods can be found in locations with sufficient soil cover. Rocky areas in limestone formations can still be rich in gastropods and spiders, but springtails would be scarce and earthworms and isopods nearly absent. Acidic conditions of granite mountains are unfavourable for gastropods, whereas earthworms, spiders and springtails can be abundant here. It can be debated how is the acidity of the soil going to affect the accumulation of heavy metals in invertebrate groups which process some components of the soil or plant material such as earthworms, gastropods and springtails in the alpine environment. The comparison of heavy metal accumulation in detritivorous (earthworms, isopods, springtails), herbivorous (gastropods) and carnivorous (spiders, harvestmen) invertebrate groups in relation to the acidity of the ground could be an interested subject of study. Although the relationship between heavy metal concentration in the environment and body concentration in diptera is not quite clear (Frouz 1999), larvae of some tipulid species which feed on plant roots constitute an important food source for insectivorous birds and small mammals in the alpine environments and therefore offer yet unexplored possibilities for study. Harvestmen have not been used as bioindicators in the lower elevation zones perhaps because of their comparatively low abundance. However, in the alpine zone, harvestmen are important predators and food source for higher levels of the food chain and could be potentially used as indicators of heavy metal pollution. Therefore the choice of a suitable invertebrate indicator species for heavy metal pollution will depend on both local conditions and the main aim of the study. An overview of methods of the sampling and analysis of heavy metals in several invertebrate groups is provided.

Preparation of invertebrates for heavy metal analysis by AAS

A micromethod for the analysis of metals in small invertebrates (small beetles, isopods, spiders, collembolans, centipedes) was described by (Van Straalen and Van Wensem 1986).

Isopoda

Isopods have to be cultured for one day in the laboratory on clean substrate to void their gut and then they can be preserved in 70% ethanol or frozen. Isopods are then washed in deionized water, oven dried at 80° C and weighed. Weighted samples are boiled with concentrated (65%) HNO_3 to digest organic matter. Samples are then dried at 135° C and redisslolved in 0,1 M HNO_3. Concentrations of heavy metals are measured using AAS.

Coleoptera

Carabid beetles have to be washed with deionized water, dried at 105° C, weighted and dry-ashed at 450° C in a muffle furnace and digested to form 10 mL 0,5% nitric acid solution before AAS analysis (Jelaska *et al.* 2007).

Araneae according to Hendrickx *et al.* (2004)

Selected species were captured by hand as pitfall trapping is impossible because formaldehyde solution, and perhaps other killing and preservative solutions used to preserve the specimens, influences the dry weight of the organism and consequently affect the metal concentration in the body. Animals were transported alive to the laboratory and killed by freezing at -10° C. All specimens were weighed and analysed individually, except for species too small to obtain accurate individual metal and weight measurements. In such cases pooled samples of five individuals were used for this species. Before digestion, all specimens were dried for 48 h at 70° C and weighed afterwards. All species were digested using concentrated HNO_3 and H_2O_2 according to a method described in Tack *et al.* (2000). Animals were immersed in 5 ml ultra-pure 65% HNO_3 solution at 130° C. After 1 h, 2 ml of 20% H_2O_2 was added to the solution. After 30 min another 2 ml of 20% H_2O_2 was added to complete digestion. The sample was finally diluted with 1% HNO_3 to 10 ml. Metal concentrations were determined by means of flame atomic absorption spectrometry (AAS) on a graphite furnace AAS equipped with Zeeman background correction for Cd. All metal concentrations are expressed as mg metal/g dry weight.

Lumbricida

Earthworms can be collected from approximately 0,25 m^2 plots by handsorting (Edwards and Bohlen 1996) or an extraction with 0,3-0,5% formaldehyde solution (Pelosi *et al.* 2009). Earthworms are collected, washed with deionised water to remove formaldehyde. Adult individuals should be starved for 3 days at 15° C until their gut contents are completely egested. Earthworms are then frozen in liquid nitrogen, freeze-dried and ground to a fine powder using a swing mill.

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