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Bioaccumulation of Cadmium and Zinc, and Field Validation of a Histological Biomarker in Terrestrial Isopods

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Since the industrial revolution amounts of biologically available metals in the environment have significantly increased (Hopkin 1989). There is evidence of greater deposition of lead, copper, zinc, nickel and cadmium on ecosystems remote from industries and roads in the present era than was the case either two hundred years ago or in the more distant past (Hughes et al. 1980). There are various anthropogenic sources of metals in the environment. Primary or secondary smelting of metals takes place at various smelters. Metals may also be recovered from scrap by secondary smelting. In modern smelting works efforts have been made to help reduce the amount of metals released into the atmosphere. Unfortunately even the most modern equipment working at efficiencies approaching 100% is unable to completely remove metals (Hopkin 1989).

Multiple toxicants may be simultaneously present under field conditions, producing a complex effect. Only a few conventional endpoints from the classical approach can be assessed as *in situ* experiments or surveys. In recent years there has been an increasing interest in the use of biomarkers in terrestrial invertebrates for the assessment of the potentially adverse effects of chemicals in soil ecosystems. The strength of the biomarker approach is that it deals with the question of bioavailability of chemicals by only reacting to the biologically available fraction of the pollutant. Biomarkers also have the advantage that they can exhibit the effect caused by many toxic compounds present at the same time, and they are applicable under laboratory and field conditions (Scott-Fordsmand and Weeks 1998).

The aims of the present study were first, to determine the accumulation of cadmium and zinc in isopods occurring at an uncontaminated- and contaminated site in the Western Cape, South Africa, and second, to evaluate a histological biomarker response of the hepatopancreas of isopods under field conditions. For a biomarker to be useful and practical it must be employable in a field situation. This paper contributes towards the field validation of our previous laboratory findings (Odendaal and Reinecke 2003) on the use of histopathological changes as a biomarker.

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MATERIALS AND METHODS

Two field sites were chosen for this study: a relatively uncontaminated site at the botanical gardens of the University of Stellenbosch (33°56'15"S; 18°52'5"E), and a contaminated site (33°56'30"S; 18°41'30"E) at a nearby smelting works. The pH, as determined with a Crison micropH 2001, of the soil changed between 6.91 and 6.99 and that of leaf litter was 6.51 in the botanical garden. At the smelter the soil pH changed between 6.36 and 6.42 and that of the leaf litter between 6.63 and 6.70. Specimens of *Porcellionides pruinosus* were collected by hand from the leaf litter present at both sites. Soil and leaf litter samples were also collected from the same spots.

Fifty of the largest specimens of woodlice (*P. pruinosus*) from each of the two sites were dissected to remove the hepatopancreas. Organs of ten woodlice per site were pooled together for acid digestion and metal analysis, resulting in a sample size of five. Rest of the body samples were also pooled for acid digestion and metal analysis. The samples were weighed and temporarily stored in small vials in a freezer. Hepatopancreas- and rest of the body samples were digested in boiling 55% nitric acid according to the procedure of Odendaal and Reinecke (1999). The samples were filtered through 0.45 µm Millipore filter and made up to 10 ml with distilled water. The analysis for cadmium and zinc was performed with a Varian AA-1275 flame atomic absorption spectrophotometer. The dry mass concentrations of cadmium and zinc were determined with the method of Odendaal and Reinecke (1999). Soil and leaf litter samples were dried in an oven at 60 °C for 48 hours and subsequently ground to a powder. 10 ml 55% nitric acid were added to each sample and left overnight. They were heated to a temperature of 40 °C and then to 120 °C for 3 hours. The samples had a whitish coloured sludge, which had to be removed before filtration. Each sample was made up to 20 ml with distilled water and filtered through Whatman No.1 filter paper, followed by filtration through 0.45 µm Millipore filter paper. A blank digestion was also performed together with each of the sets of digestions. This helped to keep a check on possible contamination. All samples were temporarily stored in a refrigerator in plastic containers (Ebdon 1982) until they were used. Percentage recovery from spiked samples was found to be in the region of 90%.

Field collected woodlice were dissected to remove the hepatopancreas for histological preparation. Hepatopancreas samples were fixed in Bouin's fluid (Preece 1972). Samples were then rinsed in 50% ethanol, dehydrated in a series of ethanol, cleared in xylene, and impregnated with Paraplast wax at 58°C. Finally, they were embedded in Paraplast in metal base moulds, sectioned at a thickness of 6µm and stained using hematoxylin and eosin counterstaining.

The Leica QWin image analysis software package was used for area measurements of the hepatopancreas sections. The area of the lumen was subtracted from the area of the total section to calculate the area covered by the cells in a particular section. The cellular area in a section was expressed as a percentage of the total area of the section, and was termed the Percentage Cellular

Table 1. The mean cadmium and zinc concentrations ($mg.kg^{-1}$; dry mass) (±SD) in soil and leaf litter samples from the uncontaminated- and contaminated sites. Range of concentrations in [brackets]. n=5. ND = not detectable.

	Cadmium ($mg.kg^{-1}$)		Zinc ($mg.kg^{-1}$)	
	Soil	Leaf litter	Soil	Leaf litter
Uncontam. site	ND	ND	79.4 (±4.8) [74.9-84.5]	67.7 (±10.7) [55.5-81.2]
Contam. site	4.2 (±2.9) [2.0-7.5]	6.7 (±1.4) [5.9-8.3]	1292.4 (±354.7) [947.5-1788.4]	1167.2 (±277.9) [857.9-1605.5]

Area (PCA). A total of 40 PCA measurements were made for woodlice from each of the uncontaminated- and contaminated sites. Measurements were made from half way to three quarters of the length of the hepatopancreas lobes (Odendaal and Reinecke 2003).

The data from this field study was analysed by means of the t-test (Jandel Scientific Sigmastat 2.0).

RESULTS AND DISCUSSION

The soil and leaf litter from the smelting works had statistically significantly higher concentrations of cadmium and zinc than those from the botanical gardens ($P<0.05$) (Table 1). The concentrations of cadmium and zinc measured in soil and leaves collected from the unpolluted botanical gardens are typical control values. Hopkin (1990) found 0.72 $mg.kg^{-1}$ Cd and 96.4 $mg.kg^{-1}$ Zn in leaf litter from an uncontaminated site. In a study by Beyer et al. (1982) Cd concentrations between 0.06 and 0.18 $mg.kg^{-1}$ and Zn concentrations between 51 and 56 $mg.kg^{-1}$ were found in uncontaminated soil. Beyer et al. (1982) found concentrations up to 8.2 $mg.kg^{-1}$ Cd and 175 $mg.kg^{-1}$ Zn in soil treated with sewage sludge. At heavily contaminated sites near a zinc smelting works concentrations of Cd and Zn were found at a maximum of 1300 $mg.kg^{-1}$ and 35000 $mg.kg^{-1}$, respectively (Beyer et al. 1984). Leaf litter collected from a metal contaminated site by Hopkin (1990) had Cd concentrations of 26 $mg.kg^{-1}$ and Zn concentrations of 1430 $mg.kg^{-1}$. The site around the smelting works in the present study could be regarded as moderately contaminated with Cd, and heavily contaminated with Zn. Cadmium is ten times more toxic than zinc (Walker et al. 1996) but according to Lock and Janssen (2001) the cadmium-zinc ratio in the field is usually so high that the risk of zinc ecotoxicity for terrestrial invertebrates will usually be greater in comparison with cadmium ecotoxicity. The cadmium-zinc ratio in the leaf litter from the smelting works is Cd:Zn 1:174, implying that zinc, rather than cadmium is more responsible for toxic effects. Given the feeding preferences of woodlice, Cd and Zn concentrations in the leaf litter are more important than those in the soil because it is generally accepted that the main route of metal uptake in woodlice is through the diet (Hopkin and Martin 1984).

Table 2. The mean cadmium and zinc concentrations ($mg.kg^{-1}$; dry mass) (±SD) in hepatopancreas and rest of the body samples of *P. pruinosus* collected from an uncontaminated site and contaminated site. n=5. Range of concentrations in [brackets]. ND = not detectable.

		Hepatopancreas	Rest of the body
Cd	Uncontam. Site	ND	ND
	Contam. Site	89.9 (±7.6) [81.6-98.4]	2.9 (±0.57) [2.2-3.6]
Zn	Uncontam. Site	313.7 (±61.8) [243.9-411.7]	14.7 (±2.0) [11.7-17.1]
	Contam. Site	10537.5 (±4035.5) [6408.2-15913.0]	37.0 (±7.8) [28.8-46.2]

Cadmium concentrations in hepatopancreas- and rest of the body samples were significantly higher in woodlice collected from the smelting works than those from the botanical gardens (P=0.008) (Table 2). Zinc concentrations in hepatopancreas- (P=0.008) and rest of the body samples (P=0.016) were also statistically significantly higher in woodlice collected from the smelting works than those from the botanical gardens (Table 2). Metal analysis of the hepatopancreas and rest of the body samples of *P. pruinosus* collected from the two field sites (Table 2) showed that at least 95% of the cadmium and zinc compartmentalized in the hepatopancreas, making the hepatopancreas of *P. pruinosus* an important target organ for cadmium and zinc in the field. The variability in metal concentrations in hepatopancreas samples, especially zinc at the smelting works, may be due to genetic variability, feeding behaviour, physiological- and reproductive status (Depledge 1990). In a study by Beyer and Storm (1995) it was found that woodlice survival decreased with increasing metal concentration in the leaf litter. Zinc was determined to be the toxic factor in terms of woodlice mortality (Beyer and Storm 1995). A review of literature on the accumulation of metals in terrestrial invertebrates by Heikens et al. (2001) revealed that isopods accumulate zinc in their bodies at higher concentrations than other tested taxa. In the present study it was shown that *P. pruinosus* accumulated zinc at levels above 10000 $mg.kg^{-1}$ in the hepatopancreas. If an animal that accumulates metals at high levels, such as *P. pruinosus,* is preyed upon, the predator population may suffer more serious effects than the prey (Hopkin 1994).

The high concentrations of cadmium and especially zinc recorded in *P. pruinosus* at the smelting works are not only hazardous for the woodlice but also for their predators. Woodlice have a large range of predators (Avery 1966; Sunderland and Sutton 1980; Avery et al. 1983), which could be at risk of being contaminated with high levels of cadmium and zinc.

From Figure 1 it can be seen that the hepatopancreas of *P. pruinosus*

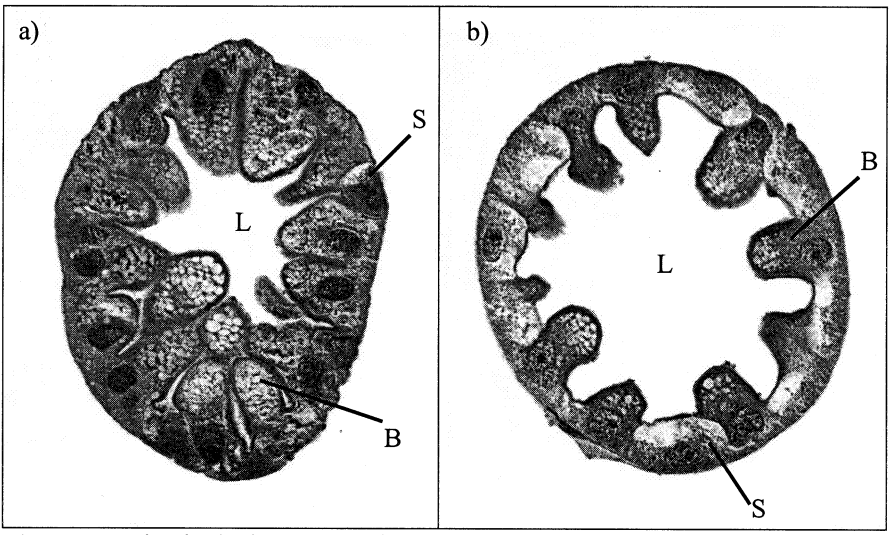

Figure 1. Histological sections of the hepatopancreas of *P. pruinosus* collected from the uncontaminated- (a) and contaminated site (b). Magnification = X250. (L = lumen; B = B cells; S = S cells).

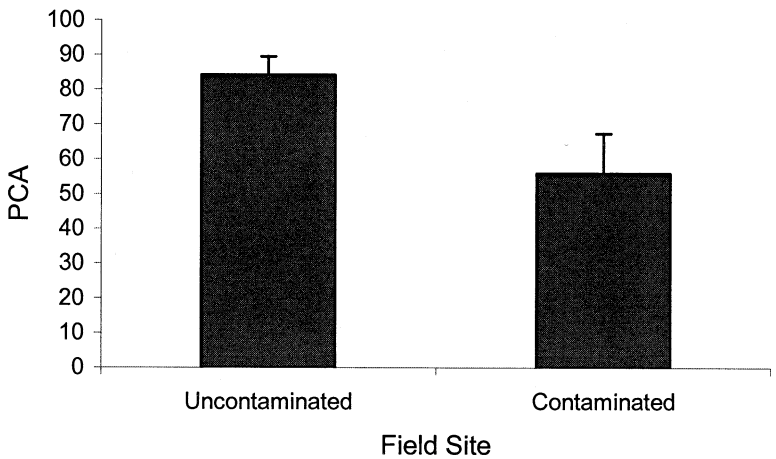

Figure 2. The mean percentage cellular area (PCA) (±SD) of the hepatopancreas of *P. pruinosus* collected from the uncontaminated- and contaminated sites. n=40. P=<0.001.

from the smelting works differed substantially from those from the botanical gardens. Histopathological damage to digestive glands of molluscs due to cadmium exposure was demonstrated by other authors. Cells in the digestive glands of *Littorina littorea*, *Mytilus sp.* and antarctic limpets were negatively affected by cadmium exposure (Marigomez et al. 1990; Da Ros et al. 1995; Najle

et al. 2000). Qualitative damage to the hepatopancreas of the marine isopod *Idotea baltica* after cadmium exposure was reported by De Nicola et al. (1993). Very few studies have been conducted in the past to assess the impact of zinc on the digestive glands of aquatic or terrestrial invertebrates. Qualitative damage in digestive gland (hepatopancreas) cells of the grey garden slug, *Deroceras reticulatum* (Triebskorn and Köhler 1996) and *Porcellio scaber* (Drobne and Strus 1996) were observed after zinc exposure. Statistical analysis showed that there was a highly significant difference between the uncontaminated site [84.2 (±5.3)] and contaminated site [55.9 (±11.4)], concerning PCA values in the hepatopancreas ($P=<0.001$) (Fig. 2). The considerable variability, as evident in the range (33.2-74.9), in PCA values in the woodlice from the smelting works may be due to genetic variability of individuals and differential feeding of contaminated leaf litter (Depledge 1990). The reduction in size of the B-cells could have negative implications for the maintenance of mass and growth of the woodlice, since the B-cells are involved in the digestive and absorptive processess (Storch 1984) and their impairment is likely to influence the growth of woodlice negatively.

On the basis of these findings it is concluded that it may be possible to use PCA-values of the hepatopancreas as a biomarker to measure metal induced stress in woodlice in a real field situation. An important proviso is that the necessary controls must be in place to ensure that the observed differences in the PCA-values can be causally linked to the metal contamination and not to stresses resulting from other environmental factors.

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