Heavy metal contents (Cd, Cu, Zn) in spiders (Pirata piraticus) living in intertidal sediments of the river Scheldt estuary (Belgium) as affected by substrate characteristics

Gijs Du Laing*,a, Nicolas Bogaerta, Filip M.G. Tacka, Marc G. Verlooa, Frederik Hendrickxb

*a Laboratory for Analytical Chemistry and Applied Ecochemistry, Department of Applied Analytical and Physical Chemistry, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

bLaboratory of Animal Ecology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium

Received 7 June 2001; accepted 28 August 2001

Abstract

Metals are transferred into the food web by ground-dwelling organisms, among others. This study aimed to identify the most important factors that determine the bioavailability of heavy metals to the spider Pirata piraticus living in the intertidal sediments of the Scheldt estuary (Flanders, Belgium). At five locations, which represent a varying degree of metal contamination and salinity, the superficial layer of sediments was characterised for physico-chemical properties and heavy metal (Cd, Cu, Zn) content and extractability. Spiders were sampled at the same locations and analysed for Cd, Cu and Zn. Higher Cd, Cu and Zn contents were found in spiders on sites with lower total metal contents in the sediment. These sites were closest to the river mouth and were characterised by a higher salinity. Significant, positive correlations were found between the chloride content of the sediments and the Cd, Cu and Zn content of P. piraticus. Similarly, a strong relationship was observed between the ratio of exchangeable Cd and Zn to the total cation exchange capacity and the contents of these elements in P. piraticus. These field data indicated that salinity, cation exchange capacity and exchangeable metal contents were of most importance in determining bioavailability of heavy metals in these intertidal sediments. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Bioavailability; Chloride content; Cation exchange capacity (CEC); Heavy metals; Intertidal sediments; Pirata piraticus; Salinity; Scheldt estuary

* Corresponding author. Tel.: +32-9-2645995; fax: +32-9-2646232.
E-mail address: gijs.dulaing@rug.ac.be (G. Du Laing).

URL: http://allserv.rug.ac.be/~ftack/anafs/

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PII: S0048-9697(01)01025-7
1. Introduction

The bioavailability and the ecotoxicological behaviour of metals in sediments are determined by their specific physicochemical form, rather than by their total content (Tack and Verloo, 1995). The degree of toxic metal uptake, translocation and eventual detoxification within an organism depends on metal speciation, but also differs strongly between organisms (Bengtsson and Tranvik, 1989; Hopkin, 1989; Janssen et al., 1993; Mukherjee and Nuorteva, 1994; Doyle and Otte, 1997).

The accumulation of metals in different fractions of sediments is governed by factors such as texture, organic matter content and conditions accompanying the formation of these fractions. In turn, remobilisation of metal reserves in soils or sediments depends both on metal content in the various fractions and alterations of environmental conditions, such as pH and redox (Förstner, 1985). The most bioavailable metal forms are found in the ‘water-soluble fraction’. Adsorbed metal ions can also be easily mobilised, and hence may also be considered as highly available (Gambrell, 1994). Metals which are present in the silicate matrix, in contrast, are fixed and are practically not bioavailable in the lifetime of an organism (Tessier et al., 1979). In between, with respect to metal availability to living organisms, are metals adsorbed or occluded in or onto various solid phases, such as iron and manganese oxyhydroxides, organic matter and precipitates of carbonate. The actual availability of metals in these fractions during the lifetime of an organism in general remains very difficult to appraise from chemical extraction techniques (Tack and Verloo, 1995).

Salinity variations may be an important factor governing the bioavailability of heavy metals towards ground-dwelling organisms. Variations in salinity are characteristic for tidal wetland systems and affect the binding of pollutants in soils. Due to increases in chloride concentrations when inland fresh river water mixes with seawater, heavy metals such as Cd may mobilise from the soil environment as soluble chloride complexes (Hahne and Kroontje, 1973). An increase in the salinity is associated with an increase in the concentration of major elements (Na, K, Ca, Mg), which compete with heavy metals for the sorption sites (Tam and Wong, 1999).

This study aimed to identify the most important factors determining the bioavailability of heavy metals towards organisms which are at the base of the food web. For this purpose, the ground-dwelling spider Pirata piraticus was studied. Ground-dwelling spiders are considered to reflect the pollution level of their biotope more clearly than web-building spiders. This organism can easily be sampled and occurs both on the freshwater and brackish-water banks of the river Scheldt (Flanders, Belgium). For a description of the life cycle of these spiders, we refer to Toft (1979). Not much is known about their feeding pattern, but it is assumed that these spiders feed on organisms which also live in close contact with the intertidal sediments. They do not build a web, but actively chase and attack their prey. They consume the prey, while they alternately inject digestion fluid and suck out the dissolved parts.

Sediment properties were determined at several locations along the river Scheldt to examine whether metal contents of ground-dwelling biota may be estimated on the basis of physico-chemical characteristics and metal contents of the intertidal sediments. These included: total heavy metal content; chloride content; CEC; texture; pH; conductivity; organic matter content; carbonate content; exchangeable metals; EDTA-extractable metals; and metal fractions as determined by the European Community Bureau of Reference (BCR) sequential extraction procedure (Rauret et al., 1999).

2. Materials and methods

2.1. Study area, sampling and sample preparation

Four sites along the river Scheldt, at Saeftinghe, Galgenschoor, Notelaar and Konkelschoor, were sampled (Fig. 1). The sites are listed from most saline — i.e. closest to the river mouth — to not saline. The sampling sites were selected between Ghent and Saeftinghe, according to ex-
expected differences in salinity and pollution level of the sediments. Konkelschoor and Notelaar are situated in the freshwater part of the Scheldt estuary. A steep decrease in salinity was expected downstream from Antwerp. As the salinity varies between 3 and 10 mg l⁻¹ in Saeftinghe and Galgenschoor, both sites are considered to be brackish. However, Galgenschoor is situated in the middle of the harbour of Antwerp, whereas Saeftinghe is a natural reserve. The Damvallei site near Ghent, but not situated along the river Scheldt, was considered the reference site.

In the summer of 1999, four samples of the upper 1 cm of the sediments were taken at each of these locations. The sediments sampled are essentially oxic. They are inundated during spring tides only and the upper 1 cm of the sediments sampled was always in an oxic state at the time of sampling. The sampling and sample preparation therefore essentially preserved the states. At each location, the samples were taken randomly at a few m from each other, taking into account the site heterogeneity at the sampling time. Four cylindrical PVC tubes (height 150 mm, diameter 200 mm) were hammered in the ground, dug up, placed on a PVC plate and transported to the laboratory. The upper layer (1 cm) of the sediments was removed and collected there. These samples were air-dried for 3 weeks and then ground in a hammer-cross beater mill (Gladiator BO 3567).

At each of the five sites, spiders (*Pirata piraticus*) were sampled by the Laboratory of Animal Ecology (Ghent University) in the summer of 1997. The common sampling method using a pitfall could not be used because of tidal flooding. Therefore, the spiders were caught by hand and frozen in the laboratory. Only adult females sampled at the same time were subjected to analyses because of the probable interaction of the metal content with sex and growth status (Hopkin, 1989; Rabitsch, 1995). A total of 15 spiders were analysed from each site. They were washed with 1% HNO₃ before destruction.

![Map of the sampling sites](image)


Fig. 1. Situation of the sampling sites Saeftinghe, Galgenschoor, Notelaar and Konkelschoor along the river Scheldt at 53, 61, 94 and 131 km, respectively, from the river mouth (adapted from Zwolsman and van Eck, 1999).
2.2. Sediment properties

The pH was measured in a 1:5 sediment/distilled water suspension after equilibration for 18 h (Van Ranst et al., 1999). The conductivity was measured in the filtrate of a 1:5 sediment/distilled water suspension, shaken for 30 min (Van Ranst et al., 1999). The organic matter content was determined by measuring the weight loss after incineration of oven-dried samples (2 h at 450°C). The carbonate content was determined by back-titration (with 0.5 M NaOH) of an excess 0.25 M H₂SO₄ added to 1 g of sediment (Nelson, 1982). Texture analyses were performed using the pipette method of Kohn (Gee and Bauder, 1986). To determine chloride content, 10 g of sediment was suspended in 50 ml of 0.15 M HNO₃ and shaken for 30 min. The filtrate was titrated with 0.05 M AgNO₃ using potentiometric end-point detection (Van Ranst et al., 1999). The cation exchange capacity was measured by percolation of 150 ml of 1 M NH₄OAc through a percolation tube filled with a mixture of 5 g of sediment and 35 g of quartz sand, followed by washing through the excess with 300 ml of denatured ethanol. The exchangeable ammonium ions were then eluted with 500 ml of 1 M KCl and analysed in the percolate by means of steam distillation (Tecator Kjeltec System 1002 distilling unit). The exchangeable cations were also analysed in this percolate using flame atomic absorption spectrometry (Varian AA-1475, Varian, Palo Alto, CA) (Van Ranst et al., 1999).

Pseudo-total Cd, Cu and Zn contents were determined by aqua regia extraction (Ure, 1990). The metal content in the extracts was analysed using flame atomic absorption spectrometry (Varian AA-1475). CRM 277 (estuarine sediment) was also analysed using the same method. Results varied from 92.5 to 101.7% of the certified values. A sequential extraction was performed according to a BCR procedure (Rauert et al., 1999). EDTA-extractable Cd, Cu and Zn contents were analysed in the supernatant solution after centrifugation (10 min at 3000 rev./min) of a suspension of 5 g of sediment and 50 ml of 0.05 M EDTA-ammonium salt solution (shaken for 1 h). The metal content was analysed using flame atomic absorption spectrometry (Varian AA-1475).

2.3. Determination of heavy metal content in spiders

Total metal content of individual oven-dried
spiders (70°C for 48 h) was determined. After the addition of 5 ml of 65% HNO₃, the whole was refluxed for 2 h at 150°C. Then 4 ml of 30% H₂O₂ was added in portions of 0.5 ml. The whole was then heated again for 15 min (150°C). After dilution to 25 ml, Cd, Cu and Zn contents were measured in the extract using flame (Varian AA-1475) or graphite furnace atomic absorption (Varian SpectraAA-800/GTA-100).

3. Results

Total Cd, Cu and Zn contents in the oxic superficial layer of the intertidal sediments are presented in Fig. 2. Total metal contents were markedly lower on the brackish sites (Saeftinghe and Galgenschoor) than on the freshwater sites upstream. Metal contents in sediments of the river Scheldt were higher than these found in the reference site at Damvallei.

Metal contents in *P. piraticus* sampled on the various sites were characterised by large standard deviations, especially for low values of the Cd content (Table 1). Cadmium and copper contents on the brackish sites were rather high compared to contents in other ground-dwelling spiders in an unpolluted forest in Germany (Scharenberg and Ebeling, 1996), near a lead–zinc smelter in Austria (Rabitsch, 1995) and polluted industrial areas in Poland (Wilcek and Migula, 1996). In contrast, zinc contents were rather low.

Remarkably, Cd, Cu and Zn contents of *P. piraticus* in Saeftinghe, Galgenschoor, Notelaar and Konkelschoor are inversely correlated with the total metal contents of the superficial sedi-

![Graphs showing metal content measurements](image)

**Fig. 3.** Cd, Cu and Zn contents of *Pirata piraticus* plotted to the total Cd, Cu and Zn contents of the superficial layer of the intertidal Scheldt sediments (SA, GS, NO, KO). The reference site Damvallei (DA) is also included (mean ± standard deviation; SA, Saeftinghe; GS, Galgenschoor; NO, Notelaar; KO, Konkelschoor; DA, Damvallei).

### Table 1

Cd, Cu and Zn contents of *Pirata piraticus* (mean ± standard deviation; *n* = 15) in June 1997

<table>
<thead>
<tr>
<th>Location (1)</th>
<th>Cd (mg kg⁻¹ DW)</th>
<th>Cu (mg kg⁻¹ DW)</th>
<th>Zn (mg kg⁻¹ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saeftinghe</td>
<td>78 ± 18</td>
<td>378 ± 82</td>
<td>990 ± 120</td>
</tr>
<tr>
<td>Galgenschoor</td>
<td>97 ± 22</td>
<td>534 ± 139</td>
<td>817 ± 143</td>
</tr>
<tr>
<td>Notelaar</td>
<td>19 ± 8</td>
<td>222 ± 29</td>
<td>606 ± 135</td>
</tr>
<tr>
<td>Konkelschoor</td>
<td>24 ± 8</td>
<td>214 ± 51</td>
<td>702 ± 93</td>
</tr>
<tr>
<td>Damvallei</td>
<td>8 ± 3</td>
<td>167 ± 62</td>
<td>500 ± 94</td>
</tr>
</tbody>
</table>

Metal extractability/fractionation data could explain the total metal contents in *P. piraticus* observed at different locations.

Physico-chemical sediment properties and metal extractability data are presented in Table 2. As expected, chloride content and conductivity were significantly higher on the brackish sites at Saeftinghe and Galgenschoor. All Scheldt river
sediments were characterised by a neutral pH. At the more saline sampling sites, pH was slightly but significantly higher than at the freshwater sites. This coincided with markedly higher carbonate contents in the saline sampling sites. The Damvallei site was characterised by a pH just below 6, but percent levels of carbonates were still present. Carbonate determination by back titration of added acid is not highly selective, however, and it may be suspected that the high levels of organic matter, and not carbonates, accounted for the acid neutralisation capacity observed for that sediment. Organic matter contents were lowest on the most saline locations. Sand content decreased inland, while clay content increased. Cation exchange capacity varied according to the levels observed in clay and organic carbon. It was lowest at the brackish locations. At Damvallei, the CEC is much higher compared to the sites near the Scheldt because of the elevated organic matter compared to the other sites. On the riverbanks, higher levels of exchangeable and EDTA-extractable Cd, Cu and Zn were found than at the reference site of Damvallei. A maximum for the EDTA-extractable content of Cd, Cu and Zn was observed in Notelaar. Exchangeable Cu was significantly higher in Notelaar and Konkelschoor compared to Saeftinghe and Galgenschoor. Exchangeable Cd was much lower and exchangeable Zn was much higher in Konkelschoor, compared to the brackish locations, but also to Notelaar.

Metal partitioning showed that Cd was mostly present in the acid-extractable and reducible fraction in the intertidal sediments of the Scheldt estuary (Fig. 4). This is often found for a broad spectrum of soil types (Miller and McFee, 1983; Szakova et al., 1998). In Saeftinghe and Galgenschoor sediments, acid-extractable Cd was significantly higher than in Notelaar, Konkelschoor and Damvallei sediments. At each location, Cu is mainly found in the oxidisable fraction. The strong association of Cu with the oxidisable fraction is also a feature that is commonly reported (Harison, 1981; Miller and McFee, 1983; Szakova et al., 1998) and it is attributed to strong binding of this metal with organic matter. Zinc is primarily found in the reducible fraction. Because of the high

| Table 2 | Properties of the superficial sediment layer in Saeftinghe, Galgenschoor, Notelaar, Konkelschoor and Damvallei (mean ± standard deviation, n = 4; for texture and carbonate content, mixed sample, n = 1) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Distance from river mouth (km) | Damvallei | Saeftinghe | Galgenschoor | Notelaar | Konkelschoor |
| Chloride (mg g⁻¹ DW)           | 0.10 ± 0.08    | 3.84 ± 0.51    | 3.38 ± 0.66    | 0.53 ± 0.14    | 0.08 ± 0.02    |
| Conductivity (mS cm⁻¹)         | 0.73 ± 0.32    | 3.57 ± 0.32    | 3.10 ± 0.43    | 0.75 ± 0.08    | 0.58 ± 0.09    |
| Texture (%)                    |                |                |                |                |                |
| Clay                            | 38             | 23             | 24             | 38             | 41             |
| Loam                           | 20             | 25             | 46             | 54             | 53             |
| Sand                           | 42             | 52             | 30             | 8              | 6              |
| Calcium carbonate (%)          | 1.7            | 10.9           | 11.4           | 7.3            | 5.8            |
| CEC (cmol(+) kg⁻¹)             | 51.1 ± 11.1    | 14.2 ± 1.5     | 15.2 ± 0.7     | 29.2 ± 0.5     | 25.6 ± 1.5     |
| pH                             | 5.94 ± 0.45    | 7.80 ± 0.08    | 7.75 ± 0.13    | 7.38 ± 0.06    | 7.28 ± 0.10    |
| Organic matter (g kg⁻¹ DW)     | 346 ± 100      | 75 ± 13        | 86 ± 13        | 122 ± 12       | 90 ± 5         |
| Exchangeable                   |                |                |                |                |                |
| Cd (mg kg⁻¹ DW)                | 0.26 ± 0.07    | 1.22 ± 0.09    | 1.47 ± 0.10    | 1.48 ± 0.15    | 0.85 ± 0.13    |
| Cu (mg kg⁻¹ DW)                | 0.58 ± 0.15    | 1.72 ± 0.46    | 1.28 ± 0.14    | 2.40 ± 0.16    | 2.61 ± 0.26    |
| Zn (mg kg⁻¹ DW)                | 9.6 ± 3.9      | 29.4 ± 2.5     | 27.8 ± 4.5     | 28.0 ± 2.4     | 35.9 ± 3.3     |
| EDTA-extractable               |                |                |                |                |                |
| Cd (mg kg⁻¹ DW)                | 1.22 ± 0.10    | 3.03 ± 0.37    | 3.06 ± 0.73    | 4.92 ± 0.63    | 2.56 ± 0.17    |
| Cu (mg kg⁻¹ DW)                | 14.2 ± 1.1     | 27.5 ± 1.5     | 26.4 ± 5.8     | 53.5 ± 3.1     | 42.3 ± 1.0     |
| Zn (mg kg⁻¹ DW)                | 134 ± 22       | 185 ± 13       | 200 ± 44       | 370 ± 35       | 301 ± 5
stability constants of Zn-oxides, many soils show a similar fractionation type (Kuo et al., 1983). Lead strongly complexes with natural organic matter and is considered as not very bioavailable in most of the sediments (Scokart et al., 1987). Hence, Pb is high in the oxidisable fraction and lower in the acid-extractable fraction. In the intertidal Scheldt sediments, a high presence of lead in the reducible fraction is also noted.

4. Discussion

The majority of the parameters and metal content measured in the sediments did not significantly correlate with the Cd, Cu and Zn contents of *Pirata piraticus*. It is, however, of interest to observe the changes in the ratio between Zn and Cd in the soil and the organism. Based on total contents in the substrate, the ratio between Zn and Cd ranged from 118 to 163 in the sediments. In contrast, the ratio between total Zn and total Cd in the organisms decreased to between 8 and 66. This would suggest that the organism *P. piraticus* accumulated Cd more strongly than Zn. It has been established that conclusions with respect to availability of metals should not be based on total contents. Indeed, when increasingly more ‘labile’ metal fractions in the sediment are considered, the ratio between Zn and Cd decreased to 61–118 based on EDTA-extractable contents, and to 19–42 based on exchangeable (replaced by neutral 1 M NH₄OAc) contents (Fig. 5). The latter ratios do actually correspond to the ratios found in *P. piraticus*, and hence it is shown, based on readily available contents, that on a relative basis, Cd and Zn were accumulated from the substrate into the organism to an equal extent. Nevertheless, low correlation coefficients indicate that exchangeable contents do not unequivocally relate to metal contents in the organisms. Other factors need to be included to assess more accurate relationships with contents in organisms. Considering the ratio between exchangeable metal and CEC of the substrate, good correlations were obtained.

Fig. 4. Results of the European Community Bureau of Reference (BCR) sequential extraction of the superficial layer of the sediments in Damvallei (DA), Saeftinghe (SA), Galgenschoor (GS), Notelaar (NO) and Konkelschoor (KO).
Following arguments may support and explain this observation.

It is reasonable to assume that the metals found in the soil solution and in the exchangeable fraction are the most bioavailable, and that the ratio between a heavy metal and other exchangeable ions remains more or less constant within a certain period. When organisms that live in close contact with the sediments do not selectively take up ions, they should accumulate heavy metals and other elements in their tissues in roughly the same proportion as they occur in the exchangeable fraction. The total intake or adsorption of metals by spiders in a fixed proportion to their body weight is thus a major assumption. To support this argument, the content of exchangeable Cd ions was divided by the CEC of the sediment and the metal content of the spiders was plotted against this ratio (Fig. 6). For Cd, a high correlation was found ($r = 0.949, P = 0.014$). Similarly, Zn uptake is correlated with the ratio of exchangeable Zn and the CEC of the sediments ($r = 0.957, P = 0.011$). For Cu, this correlation is much weaker ($r = 0.457, P = 0.439$). This is explained by the fact that Cd and Zn are mostly present in the exchangeable fraction, while Cu is mainly present in the reducible and oxidisable fractions (Fig. 4). It can therefore be presumed that Cu is less readily available, and because Cu is an essential element, active uptake may be more important in determining Cu content in spiders.

The distance of the sampling location from the river mouth was clearly reflected in the chloride content of the sediments (Table 2). Besides lower contents of clay and organic carbon (Tack et al., 1997), increased salinity may be a factor that explains the lower total contents in the sediments closer to the river mouth. At the same time, the increased salinity will tend to increase the bioavailability of the heavy metals present (Norrström and Jacks, 1998). The presence of chlorides may result in desorption of metals which are more soluble as complexes with chloride (Doner, 1978). In a laboratory experiment involving contaminated dredged materials, dissolved concentrations of several elements increased when the salinity was increased from 2 to 10 g l$^{-1}$ NaCl, which corresponds to approximately 3 and 15 mS cm$^{-1}$ (Gambrell et al., 1991). Cadmium, in particular, is known to readily form complexes with chlorides (Doner, 1978; Comans and Van Dijk,

![Diagram](image_url)

Fig. 5. Zn/Cd ratio in *Pirata piraticus* plotted against the Zn/Cd ratio based on exchangeable, EDTA-extractable or total contents in the sediments of Damvallei, Saeftinghe, Galgenschoor, Notelaar and Konkelschoor.
Fig. 6, \( r = 0.457 \) for Cu and 0.957 for Zn), copper in the spiders strongly correlates with the chloride content of the sediments \((r = 0.889, P = 0.043)\), but for zinc, this correlation was weaker \((r = 0.889, P = 0.044)\). This can probably be attributed to the relative absence of Cu in the exchangeable fraction on the one hand (Table 2), and the possibility of chlorides to form complexes with less readily available elements, such as Cu, on the other hand (Fig. 4). In brackish marsh soils, Gambrell et al. (1991) found increasing soluble concentrations with increasing salinity for Cd, Cr, and Cu, which corresponds with our observations.

Fig. 7. Cd, Cu and Zn contents of Pirata piraticus plotted against the chloride content of the superficial layer of the sediments (mean ± standard deviation).
The linear regression analysis revealed a relatively small intercept and a high slope when Cd content was plotted against the ratio of exchangeable Cd/CEC or the chloride content. Zinc and Cu content varied less as a function of these parameters and an intercept significantly different from zero was observed. This can be explained by Cd being non-essential and Cu and Zn being essential for biota. Even in conditions of low available trace elements, an organism will need to accumulate a minimum amount of essential elements in order to survive.

5. Conclusion

Higher contents of Cd, Cu and Zn in *Pirata piraticus* were found at locations with lower total sediment contents of these metals. High correlations were observed between the chloride content of the intertidal sediments and the Cd, Cu and Zn contents of *P. piraticus*. The correlations differed, depending on the ability of the metals to form complexes with chlorides. Also, the ratio between exchangeable Cd or Zn and the CEC of the sediments correlated strongly with the Cd or Zn content of *P. piraticus*. The magnitude of these correlations was related to the percentage of the total metal content present in the exchangeable fraction. These correlations should still be validated using supplementary analyses of *P. piraticus*. However, it is obvious that salinity, CEC and exchangeable metal contents cannot be neglected when studying bioavailability and toxicology of heavy metals in intertidal sediments.

Acknowledgements

This work was supported by the Research Fund of Ghent University as a part of the research programme ‘Transfer van zware metalen in terrestrische ecosystemen: speciatie, bioaccumulatie en effecten op populatieniveau’ (BOF 01110498). The authors would also like to thank the Laboratory of Animal Ecology (Ghent University) for the sampling of the spiders.

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