

# UPTAKE AND DISTRIBUTION OF LEAD (Pb) AND CADMIUM (Cd) IN THE FRESHWATER CRAB, *POTAMONAUTES PERLATUS* (CRUSTACEA) IN THE EERSTE RIVER, SOUTH AFRICA

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**Abstract.** The uptake and distribution of lead (Pb) and cadmium (Cd) in the freshwater crab, *Potamonautes perlatus* in the Eerste River, South Africa were studied seasonally over two years by comparing concentrations in crabs, water and sediment in two localities, one upstream and one downstream from the town of Stellenbosch. Lead and cadmium concentrations in whole crabs of different size classes as well as concentrations in various organs and tissues were determined by atomic absorption spectrometry. Data on physical, chemical and bacteriological features are also presented. The mean Zn concentration in the sediment of the downstream locality ( $77.5 \pm 38.4 \mu\text{g g}^{-1}$ ) was significantly higher than in the less polluted upstream locality ( $44.7 \pm 32.8 \mu\text{g g}^{-1}$ ) whilst the manganese concentration of the sediment was significantly higher in the upstream locality. The gonads (mean Pb 23.4, range 0.1–125.0  $\mu\text{g g}^{-1}$ ; mean Cd 5.3, range 0.1–22.2  $\mu\text{g g}^{-1}$ ) and carapace (mean Pb 23.4, range 0.7–327.6  $\mu\text{g g}^{-1}$ ; mean Cd 4.0, range 0.4–18.5  $\mu\text{g g}^{-1}$ ) contained the highest concentrations of both metals irrespective of season or locality. For both Pb and Cd significant differences ( $p < 0.05$ , Student's t-test) were found between the metal content of different organs. The gonads had the highest and the digestive glands the lowest concentrations of both heavy metals. Results indicated that anthropogenic activities did not influence sediment and water concentrations significantly. Smaller crabs accumulated more lead and cadmium than larger crabs with all crabs having significantly higher concentrations than both water and sediment. No significant differences in mean concentrations of lead and cadmium in whole crabs or organs were found between the two localities. Although body loads for both metals did not reflect prevailing environmental levels of these metals reliably, the crabs may still be used to monitor bioavailability over time.

**Keywords:** cadmium, distribution, freshwater crabs, lead, *Potamonautes perlatus*, uptake

## 1. Introduction

Freshwater ecosystems in southern Africa are under increasing threat due to a rapidly expanding and shifting human population. Rivers are very vulnerable since waste and other contaminants from industries, homes and farms reach them either directly through the air, runoff (Schulz *et al.*, 2001; Snyman *et al.*, 2002) or through groundwater seepage. Heavy metals can enter the food chain through air, water, soil and biota and are derived from industrial effluent, agricultural runoff, mining and mineral processing, storm water runoff, natural erosion of bedrock and atmospheric transport.



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Concern has been raised about the environmental levels of Pb in the vicinity of the Eerste River which runs through the town of Stellenbosch in the Western Cape. Relatively high levels of Pb have been determined in earthworms and shrews living close to the river (Reinecke *et al.*, 2000). This raises questions about contamination levels of heavy metals in this river and its biota and possible ecological threats. Several studies have shown that Pb and Cd can accumulate in freshwater decapods (Anderson and Brower, 1978; France, 1987; Van Eeden and Schoonbee, 1991; Du Preez *et al.*, 1993). Most studies have so far focussed on the freshwater crab *Potamonautes warreni* and not on the crab *P. perlatus*. The latter species occurs in rivers in the south-western region of the Western Cape (Barnard, 1950) where it plays an important role in the processing of organic material (Hill and O'Keeffe, 1992). Since *P. warreni* has been advocated as a possible biomonitoring species, the question arises whether *P. perlatus* could fulfill a similar role as biomonitor of heavy metal pollution to indicate exposure levels and bioavailability of these metals (Snyman *et al.*, 2002).

The ecotoxicological relevance of this study is further enhanced by the fact that *P. perlatus* serves as a source of food for various predators including the Cape clawless otter (*Aonyx capensis*), the water mongoose (*Atilax paludinosus*) and the giant kingfisher (*Megaceryle maxima*) (Arkel, 1979; Purves *et al.*, 1994) as well as a number of fish species.

The aims of this study were to determine the heavy metal contamination of the Eerste River and to establish whether and to what extent *P. perlatus* accumulates Pb and Cd from the river since they are not considered to be essential elements that can be regulated by normal physiological processes in contrast to Mn, Zn and Cu. Also to investigate the distribution of these metals in various tissues and organs of the crab's body. By selecting sampling locations in the river both upstream and downstream from the town, the possible influence of human activities on contamination levels in water, sediment and crabs could also be taken into account. Data on physical, chemical and bacteriological features were also obtained in order to interpret differences in conditions prevailing at the two localities, which may influence the bioavailability of Pb and Cd.

## 2. Material and Methods

### 2.1. STUDY AREA

The Eerste River rises in the Jonkershoek Valley of the Western Cape in South Africa. It flows through the Jonkershoek Forest Reserve, the Assegaaibosch Nature Reserve and several vineyards before reaching the town of Stellenbosch (33°56'10"S; 18°51'34"E). For the rest of its course the river is bordered by agricultural land and small settlements before reaching the sea on the False Bay coast.

One sampling locality was chosen to represent a relatively uncontaminated area upstream of the town and the other downstream from the town where human inter-

vention has taken place. The first locality is situated in the Assegaaibosch Nature Reserve below a small weir (33°58'21"S 18°56'4"E). Apart from crab-eating fish species, the giant kingfisher (*Megaceryle maxima*) was observed frequently during sampling periods. Signs of the Cape clawless otter (*Aonyx capensis*) in the form of scats and eaten crabs were also observed here (Snyman *et al.*, 2002). The water mongoose (*Atilax paludinosus*) is also known to occur in this area (Purves *et al.*, 1994). The second locality, also below a small weir, is situated downstream from the Adam Tas bridge directly behind a large farmers winery (33°56'47"S; 18°50'32"E). An otter latrine indicated that these animals often utilized this area for foraging. The water at this site was often murky and foul-smelling. Runoff from the Plankenburg River and effluent from a winery seemed to affect the quality of the water at this locality.

## 2.2. SAMPLING

Crabs, sediments and water were collected once every season during autumn, winter, spring and summer at both localities from April 1993 to January 1995, amounting to eight sampling occasions. Large (> 40 mm carapace width) and medium-sized individuals (21 to 40 mm carapace width) were caught in baited funnel traps set in the afternoons and left overnight, or until a large enough sample size (>10) was obtained. Small crabs (< 21 mm) were caught infrequently in the traps and numbers often had to be supplemented by hand collecting.

In the laboratory the crabs were, sexed, measured, weighed, frozen and grouped into the three size-classes mentioned above. Representatives of each size-class were used for dissection and for measuring whole-body loads of Pb and Cd. Specimens were dissected to remove the digestive glands, gonads, gills and claw muscles. Whole crabs and carapaces were weighed, dried at 105–110 °C for 24 h, ground and pooled to obtain a large enough sample for each category for analysis. Samples were stored at –10 °C in acid-rinsed bottles. One gram of each pooled sample of the categories whole crab, carapace or thawed tissue, was weighed and digested in a Labcon dual digester, using a 10:1 ratio of 55% nitric acid and 70% perchloric acid (Van Eeden and Schoonbee, 1991). The samples were firstly digested with 10 ml nitric acid for 24 h at room temperature and thereafter at 40–50 °C for 2 h, after which the temperature was increased to 140 °C for one hour. The latter was repeated after adding 1 ml perchloric acid to each sample.

The water samples for Pb and Cd analysis were collected seasonally over the two year period at each locality where the crabs were also sampled in fast-flowing sections of the river, 5 to 10 cm below the surface. Sediment samples were collected every season in the top 10 cm of the river bottom in shallow sections of the river, in close proximity to crab burrows on the river banks.

### 2.3. METAL EXTRACTION AND ANALYSIS

Water samples were acidified with 55% nitric acid and filtered through Whatman 9.0 cm qualitative filter paper as well as a Sartorius Minisart 0.45  $\mu\text{m}$  pore size filter using a needle and syringe. Each sample was diluted with 100 ml distilled water and kept at 6 °C until analysed by atomic absorption spectrometry. Sediment samples were prepared for metal analysis by firstly drying the samples at 100 °C for 24 h, or until the weight stabilized, after which each sample was sieved to obtain a homogenous sample. These were then ground with a pestle and mortar and a subsample of 1 g weighed on a Mettler AE200 balance (Anderson, 1974). The filtered sediment samples were digested using a 1:1 ratio of hydrochloric and nitric acid as described by Anderson (1974). Ten ml 55% nitric acid was added to each sample. After 24 h the temperature was raised to 50 °C for two hours in a Labcon dual digester. The temperature was then increased to 140 °C for one hour after which 10 ml 32% hydrochloric acid was added and the sample reheated for one hour at 140 °C. The samples were allowed to cool after clear solutions were obtained.

Cooled samples were filtered through a Whatman no. 9 qualitative filter paper and a Sartorius Minisart 0.45  $\mu\text{m}$  pore size filter, using a needle and syringe. The filtrate was diluted to 100 ml with distilled water and stored at 6 °C, awaiting analysis. Spiked samples of sediments as well as muscle tissue showed a mean recovery above 78%. The concentrations of Pb and Cd in crabs and Cu, Pb, Zn, Cd and Mn in water and sediments were determined by atomic absorption spectrometry using a Varian AA 250 Plus. Standard solutions from Sigma and de-ionized water were used to prepare the analytical standards. Metal concentrations were expressed as  $\mu\text{m}$  metal per gram of wet mass for all biological samples while the sediment concentrations are expressed as dry mass. Although weighed whole crabs and carapaces were dried before being extracted, concentrations were converted from dry to wet mass for meaningful comparison with other tissues and organs. The bio-concentration factors (BCF) for Pb and Cd (the ratio between the environmental concentration and the body or specific tissue load) (Van Straalen and Verkleij, 1993) was calculated to give an indication of these metals' accumulation from water and sediments to crabs.

All other water analysis data, including pH and conductivity, were obtained from the Department of Water Affairs and Forestry who have several sampling stations in the river. Bacteriological data were provided by the Cape Town Municipality and Regional Services Council (Snyman *et al.*, 2002).

### 2.4. STATISTICAL ANALYSIS

Statistical analyses of data were done with Quatro Pro and Statgraphics programs. Student's t-test was used to compare mean values obtained for the metal concentrations in different compartments, localities or seasonal concentration loads. Statistical analysis for purposes of seasonal comparisons of water and sediment

TABLE I

Physical, chemical and bacteriological features of Eerste River water (w) and sediments (s) collected at Assegaibosch Nature Reserve (upstream) and near Distell (formerly Stellenbosch Farmers Winery) (downstream) of the town of Stellenbosch. Determinations based on pooled samples for all seasons during the current study.

	Unit	Upstream	Downstream
pH (w)	pH	6.7	7.1 <sup>a</sup>
Conductivity (w)	mS m <sup>-1</sup>	6.7	18.5 <sup>a</sup>
Temperature (w)	°C	18.3	16.3
Dissolved O <sub>2</sub> (w)	mg l <sup>-1</sup>	10.1	9.0
Susp. Solids (w)	mg l <sup>-1</sup>	7.4	9.1 <sup>a</sup>
Nitrates (w)	mg l <sup>-1</sup>	0.48	0.27
Phosphates (w)	mg l <sup>-1</sup>	0.05	0.06 <sup>a</sup>
Hardness (CaCO <sub>3</sub> ) (w)	mg l <sup>-1</sup>	14.5	45.0
Total coliforms (w)	100 ml	498	128227 <sup>a</sup>
Faecal coliforms (w)	100 ml	105	15347 <sup>a</sup>
Mn (w)	mg l <sup>-1</sup>	0.12	0.13
Cu (w)	mg l <sup>-1</sup>	0.06	0.07
Pb (w)	mg l <sup>-1</sup>	0.03	0.04
Cd (w)	mg l <sup>-1</sup>	0.006	0.006
Zn (w)	mg l <sup>-1</sup>	0.22	0.22
Mn (s)	mg kg <sup>-1</sup>	184.7	55.3 <sup>a</sup>
Cu (s)	mg kg <sup>-1</sup>	7.2	7.4
Pb (s)	mg kg <sup>-1</sup>	5.3	7.0
Cd (s)	mg kg <sup>-1</sup>	0.43	0.45
Zn (s)	mg kg <sup>-1</sup>	44.7	77.5 <sup>a</sup>

(<sup>a</sup> significant difference  $p < 0.05$  between localities)

concentrations were not undertaken due to small sample size. Where data sets were not normally distributed, mean logarithm values were used to calculate t-values. Where normal distribution could not be obtained, the Mann-Whitney test was performed on the original data. In all cases  $p < 0.05$  was accepted as level of significance.

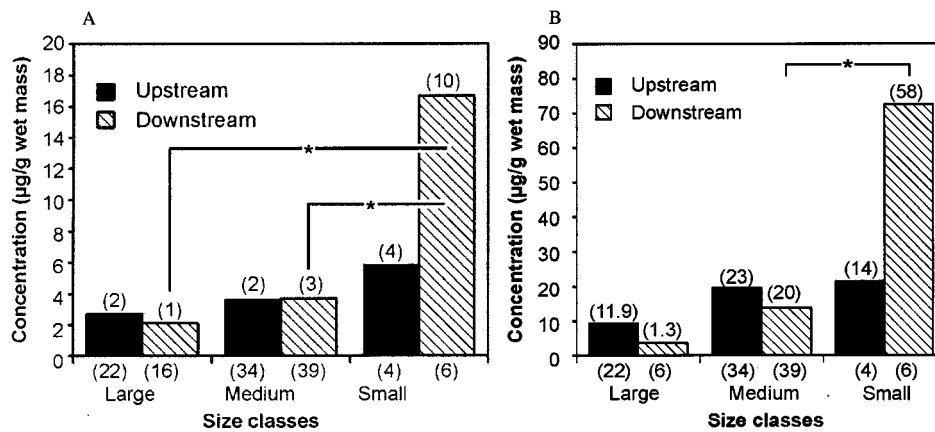


Figure 1. Mean Cd (A) and Pb (B) concentrations (wet weight) in *P. perlatus* whole crabs of various size classes from the localities in the Eerste River upstream and downstream from the town of Stellenbosch. Figures above columns indicate standard deviations and figures in brackets on the x-axis the number of samples analyzed. (\* statistically significantly different,  $p < 0.05$ , Mann-Whitney test).

### 3. Results

#### 3.1. METALS IN WATER AND SEDIMENT

The mean water concentrations of Zn, Mn, Cu, Pb and Cd from the upstream locality did not differ significantly from the downstream locality (Table I) ( $p > 0.05$ , Student's t-test) ( $n = 8$ ). Samples were pooled for all seasons to compare the two localities because small sample size precluded meaningful comparison of seasonal differences of water and sediment concentrations between the two localities. Since only one water and one sediment sample were analysed per locality per season, they only represented a snapshot indication of concentrations at the time and could not be seen as representative of a particular season since runoff events could change concentrations quickly. The mean Zn concentration in the sediment of the downstream locality ( $77.5 \pm 38.4 \mu\text{g g}^{-1}$ ) was significantly higher than in the less polluted upstream locality ( $44.7 \pm 2.8 \mu\text{g g}^{-1}$ ) ( $p < 0.05$ , Student's t-test) whilst the manganese concentration of the sediment was significantly higher in the upstream locality ( $p < 0.05$ , Student's t-test). The mean concentrations of all the other metals did not differ significantly ( $p > 0.05$ ) between the sediments of the two localities (Table I). The mean Pb contents of the water from the two localities were  $0.03$  and  $0.04 \mu\text{g g}^{-1}$  respectively and  $0.006 \mu\text{g g}^{-1}$  for Cd at both localities.

The mean Pb concentrations in the sediments were  $5.3$  and  $7.0 \mu\text{g g}^{-1}$  in the upstream and downstream localities respectively and  $0.4 \mu\text{g g}^{-1}$  in both localities for Cd. The conductivity, suspended solids, phosphates and coliform counts were significantly higher in the downstream locality (Table I).

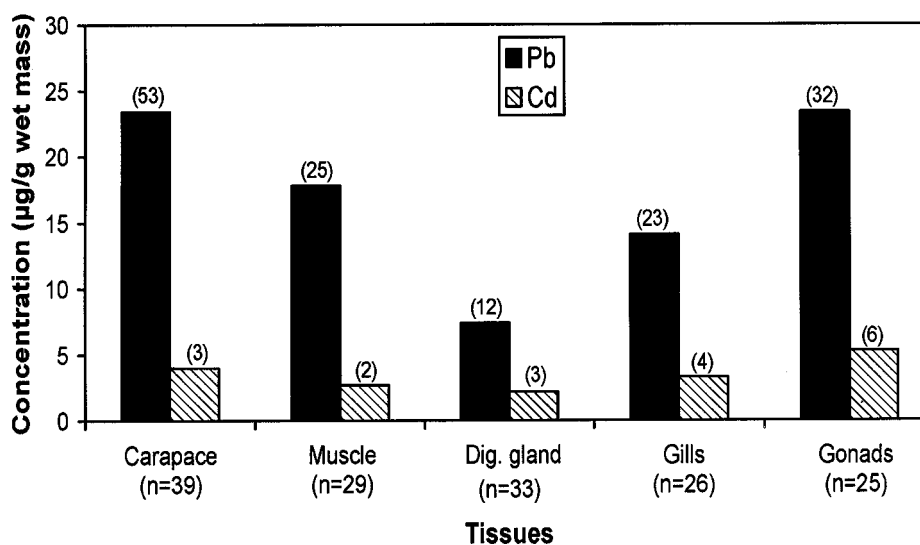


Figure 2. Mean Pb and Cd concentrations (wet weight) in the different tissues of *P. perlatus* pooled data of the two localities in the Eerste River, upstream and downstream of Stellenbosch). Figures above columns indicate standard deviation (n = number of samples analyzed).

### 3.2. METALS IN CRABS

The whole crab concentrations of Pb and Cd in crabs from both localities are presented in Figure 1 according to size class distribution. The crabs showed a large individual variation in whole body, tissue and carapace concentrations (Figure 2) for both Pb and Cd. The pooled data per size class of all whole crabs from both localities were used to determine the relationship between the body size of crabs and the metal concentrations. A negative correlation ( $r = -0.47$ ) was obtained for Pb as well as for Cd ( $r = -0.52$ ). Comparison of the Pb concentrations in the various size classes per locality, using a non parametric Mann-Whitney test, showed significant differences ( $p < 0.05$ ) in Pb concentrations only between medium sized ( $n = 39$ ) and small crabs ( $n = 6$ ) at the more polluted downstream locality and also for Cd between large ( $n = 6$ ) and small ( $n = 6$ ) and medium ( $n = 39$ ) and small crabs ( $n = 6$ ). The smallest size class had the highest mean Pb as well as Cd concentrations (wet mass) of  $72.6 \pm 59$ , ( $n = 6$ , range 1.95–164.7) and  $16.7 \pm 9.9 \mu\text{g g}^{-1}$  ( $n = 6$ , range 4.8–33.7) respectively while large crabs showed the lowest ( $3.6 \pm 1.3$  for Pb and  $2.1 \pm 0.8 \mu\text{g g}^{-1}$  for Cd, wet mass).

The mean Pb and Cd concentrations in the various selected tissues of crabs (pooled for both localities and all seasons) are depicted in Figure 2. For Pb significant differences ( $p < 0.05$ , Student's t-test) were found between the metal content of the digestive gland and gonads, the digestive gland and carapace, and between the gills and carapace. For Cd significant differences were found between muscle and carapace, digestive glands and gonads, digestive glands and carapace, and

TABLE II

The mean concentration of Pb and Cd in whole crabs (wet mass) from the upstream locality (n = 60) and the downstream locality (n = 51) as well as the water and sediment concentration (n = 8) and the bioconcentration factors (BCF<sub>w</sub> and BCF<sub>s</sub>) for water and sediment

Locality Contaminant	Upstream		Downstream	
	Pb	Cd	Pb	Cd
Whole crab conc. $\mu\text{g g}^{-1}$	15.8 $\pm$ 19.0	3.4 $\pm$ 2.8	19.5 $\pm$ 32	5.0 $\pm$ 6.3
Mean water conc. $\text{mg l}^{-1}$	0.03	0.005	0.04	0.006
Mean sediment conc. $\mu\text{g g}^{-1}$	4.4	0.4	5.8	0.4
BCF <sub>w</sub>	528	686	487	840
BCF <sub>s</sub>	3.6	9.5	3.4	13.3

gills and carapace. The gonads had the highest and the digestive glands the lowest concentrations of both heavy metals.

Comparison of the total Pb and Cd contents (wet mass) of whole crabs between the two localities revealed the highest mean Pb and Cd concentrations ( $19.5 \pm 32.4 \mu\text{g g}^{-1}$ , n = 51, range 1.2–164.7) and  $5.04 \pm 6.2 \mu\text{g g}^{-1}$ , n = 60, range 0.5–33.7) respectively, at the downstream locality although no statistically significant difference could be established between the two localities for both metals. Similarly, no significant differences could be obtained between the two localities for the Pb content of the different size classes, genders and between the carapace and other tissues. For Cd only the smaller sized crabs from the downstream locality (n = 6) had a significantly higher mean whole body concentration than small crabs (n = 6) from the upstream locality (p < 0.05, Student's t-test).

A comparison of seasonal changes in the mean Pb (Table III) and Cd (Table IV) contents of whole crabs for pooled data from the two localities showed that the highest mean concentration (wet mass) for both metals occurred in autumn ( $39.3 \pm 35.9 \mu\text{g g}^{-1}$ , n = 16 and  $5.8 \pm 5.9 \mu\text{g g}^{-1}$ , n = 16 for Pb and Cd respectively) and the lowest ( $10.8 \pm 13.9 \mu\text{g g}^{-1}$ , n = 18 and  $3.5 \pm 3.0 \mu\text{g g}^{-1}$ , n = 49, respectively) in summer. The whole crab concentrations for Pb differed significantly (p < 0.05, Mann-Whitney test) between summer and autumn, winter and autumn and spring and autumn. No significant differences between seasons in whole crab concentrations of Cd were obtained.

Although no significant differences could be found between the Pb concentrations in whole crab, tissues and carapace concentrations, on the one hand and sediment concentrations of Pb on the other, the water concentrations (n = 8) was



TABLE III

Seasonal differences in whole crab lead concentrations ( $\mu\text{g g}^{-1}$  wet mass) (Mann-Whitney test)

	n	Mean	SD	Range	z-value	p-value
Summer	49	10.78	13.97	1.62–66.44		
vs					2.597	< 0.05
Autumn	16	39.25	35.89	1.25–99.61		
Summer	49	10.78	13.97	1.62–66.44		
vs					-1.935	= 0.05
Winter	21	21.1	37.95	0.78–164.7		
Summer	49	10.78	13.97	1.62–66.44		
vs					-1.309	> 0.05
Spring	25	13.82	18.23	1.16–64.83		
Autumn	16	39.25	35.89	1.25–99.61		
vs					-2.652	< 0.05
Winter	21	21.1	37.95	0.78–164.7		
Autumn	16	39.25	35.89	1.25–99.61		
vs					-2.713	< 0.05
Spring	25	13.82	18.23	1.16–64.83		
Winter	21	21.1	37.95	0.78–164.7		
vs					1.434	> 0.05
Spring	25	13.82	18.23	1.16–64.83		

significantly lower ( $p < 0.05$ , Mann-Whitney test) than those in whole crab, tissue and carapace for both localities. The  $\text{BCF}_w$  and  $\text{BCF}_s$  of Pb and Cd (Table II) for whole crabs were very similar for both localities. For Cd the whole body concentrations at both localities were also significantly ( $p < 0.05$ ) higher than the water or sediment concentrations.

#### 4. Discussion

Setting of water quality criteria for heavy metals is notoriously difficult due to the fact that so many variables are involved which may influence exposure, bioavailability and uptake (Griscom *et al.*, 2000; Peijnenburg *et al.*, 1997). Since we found no significant differences ( $p > 0.05$ ,  $n = 8$ ) in water concentrations of Pb and Cd between the locality upstream and downstream of the town of Stellenbosch, this may indicate that household, industrial or other human related activities did not have a major influence on Pb and Cd concentrations in the river water at the time,

TABLE IV  
 Seasonal differences in the whole crab cadmium concentrations ( $\mu\text{g g}^{-1}$ ) (expressed as to wet mass) (Mann-Whitney test)

	n	Mean	SD	Range	z-value	p-value
Summer	49	3.45	3.09	0.49–14.6		
vs					1.728	> 0.05
Autumn	16	5.77	5.92	1.1–21.34		
Summer	49	3.45	3.09	0.49–14.6		
vs					-1.0	> 0.05
Winter	21	5.24	7.72	0.62–33.67		
Summer	49	3.45	3.09	0.49–14.6		
vs					0.731	> 0.05
Spring	25	3.67	2.98	1.0–11.2		
Autumn	16	5.77	5.92	1.1–21.34		
vs					-1.794	> 0.05
Winter	21	5.24	7.72	0.62–33.67		
Autumn	16	5.77	5.92	1.1–21.34		
vs					-0.842	> 0.05
Spring	25	3.67	2.98	1.0–11.2		
Winter	21	5.24	7.72	0.62–33.67		
Vs					1.5	> 0.05
Spring	25	3.67	2.98	1.0–11.2		

or could not be detected due to small sample size and the transient nature of diffuse input. However, the significant differences between other parameters of the two localities (pH, conductivity, suspended solids, phosphates and coliforms) were a clear indication that runoff or effluents from various sources in and around the town influenced water quality in other respects.

The sediment concentrations of Pb and Cd at both localities were very similar but significantly higher than the concentrations in the water. The mean levels in the sediments were lower than those in the whole crabs. Little is known about the mobility of Pb and Cd associated with these sediments and the factors influencing them. Many other parameters (Table I) did however differ between the two localities which in turn could affect bioavailability and consequently, uptake and toxicity of Pb and Cd in biota (Lewis and McIntosh, 1986). Comparison of our data with those of previous studies in the Eerste River (King, 1981) indicated that, with the exception of an increase in nitrates, probably resulting from agricultural activities, most other parameters remained fairly constant over time. The Pb concentrations

in the sediments from both localities were very similar to those cited for other relatively unpolluted wetlands (Watling and Watling, 1984).

The mean Pb concentrations (wet mass) in whole crabs of respectively 15.8 (n = 60) and 19.5  $\mu\text{g g}^{-1}$  (n = 51) for the two localities compare favourably with the concentrations found in *P. warreni* (2.0 to 13.9  $\mu\text{g g}^{-1}$ ) for a mine-polluted wetland (Van Eeden and Schoonbee, 1991). Individual variation was however much greater in *P. perlatus* and included individuals with relatively high concentrations of up to 164.7  $\mu\text{g g}^{-1}$  compared to the highest concentration of 13.9  $\mu\text{g g}^{-1}$  obtained by the latter authors for *P. warreni*.

The relatively high BCF values exhibited by crabs at both localities for both Cd and Pb (Table II) indicate that Pb and Cd were accumulated from the environment by *P. perlatus*. Although the environmental concentrations of Pb were higher than those of Cd, relatively more of the latter was accumulated. The high concentration factors probably illustrate an inability of the crabs to regulate these non-essential metals and highlights the problems associated with Pb and Cd pollution in relatively unpolluted aquatic environments (Kempster *et al.*, 1980; Snyman *et al.*, 2002) such as the Eerste River. Body burdens of Pb and Cd can reach serious 'threshold' levels should environmental levels change only moderately or should factors governing bioavailability and uptake change.

Comparison of our results for Pb with those of Du Preez *et al.* (1993) for *P. warreni* from polluted freshwater ecosystems showed some differences. These authors found  $86.6 \pm 73.4 \mu\text{g g}^{-1}$  (wet mass) in the carapace which was much higher than the values we obtained for *P. perlatus* (23.4  $\mu\text{g g}^{-1}$ ). Their value of  $3.0 \pm 1.4 \mu\text{g g}^{-1}$  in the muscle tissue of *P. warreni*, was much lower than our value for the same tissue in *P. perlatus* (range 14.2, 22.4  $\mu\text{g g}^{-1}$ ). This could be attributed to species differences, however, many other factors differed, making a meaningful comparison impossible.

Our study indicated that the carapace and gonads contained the highest concentrations of Pb despite seasonal variation (Figure 2). This is in agreement with the findings of Du Preez *et al.*, (1993) for carapace concentrations in *P. warreni* and may provide further support for the idea that Pb is incorporated mainly into the exoskeleton which serves as a storage or immobilisation site (Anderson and Brower, 1978). Periodic moulting could provide a mechanism by which Pb is eliminated, limiting long term accumulation. Moulting processes frequently recur during adult life in many crustaceans, depending on food supply and environmental conditions.

Considering the differences in body concentrations we found between small and large crabs, one would expect larger, presumably older, crabs which moult less frequently, to have higher carapace burdens of metals than smaller, presumably younger, crabs. No distinction could however be made in this regard in our study since carapace concentrations were determined for pooled samples which did not take size class of crabs into account. Whole crab analysis did however take size class into account and showed, contrary to expectations, that smaller crabs had higher mean body loads of both metals than larger crabs (Figure 1).

Various explanations can be offered for this phenomenon. Faster growth rates in younger animals could have influenced the bioconcentration positively. The higher metabolic rate of smaller/younger crabs could play a role in this regard. Hill and O' Keeffe (1991) found significant differences in food preference between large and small individuals of this species. This provides a further possible explanation since smaller crabs tended to prey more on aquatic invertebrates whilst the larger crabs preferred plant material. Since various studies have shown that aquatic invertebrates tend to accumulate heavy metals, while plants will do so to a limited extent, transfer and accumulation along the invertebrate food chain could provide a plausible explanation for the observed higher metal concentrations in smaller crabs. This interpretation must be taken with the necessary caveat that we are still far from understanding the behaviour of metals in the environment (Janczur *et al.*, 2000). Also our general assumption that smaller crabs are in all instances younger than larger ones may in some instances be incorrect, making our distinctions between size classes, and equating them to age differences, less reliable. Our study seems to show that size (and therefore probably also age) could influence uptake and distribution of Pb and Cd in *P. perlatus*. This finding for Pb is in agreement with that of Du Preez *et al.* (1993) for *P. warreni*. Snyman *et al.* (2002) also found that smaller crabs in the Eerste River accumulated more Cu than larger crabs.

Van Straalen and Van Wensem (1986) suggested that the levels of heavy metals in animals may not be determined by trophic level or body weight but rather by the physiological properties of the organism. This may differ substantially between young and old animals. The dynamic model of Janczur *et al.*, (2000) for heavy metal accumulation indicated that the toxicant concentration in food affects the growth rate and the adult size. Their model showed that growth is slower when it is optimal to allocate energy to immobilization and/or removal of toxicants. Without these allocations for high food contamination, growth rate may be the same as that for the non-toxic food, and dwarf adult body size may result, attributed to the accelerated maturation. Slower growth, as a consequence of keeping the body clean, may eventually lead to a larger body size because of later maturation and longer life-span. This is expected to occur under low external mortality and low or moderate toxicant concentration in food. The applicability of this approach to our data will require more information on the relationship between age/size and maturation time in the crab population in this river, but could help in explaining the higher concentrations of heavy metals found in smaller crabs.

The relatively low levels of Pb and Cd found in the digestive glands of *P. perlatus* indicated that this organ may not be an important storage site for these metals. This is in contrast to the findings of Snyman *et al.* (2002) for Cu, which is an essential nutrient that tends to increase predominantly in the digestive gland. Roldan and Shivers (1987) exposed crayfish to low doses of lead acetate, which resulted in electron dense particles accumulating in the R-cells of the digestive gland. They thought this to be an effective detoxification mechanism. Our study

did not render support for the existence of something similar in crabs, although the possibility is still not excluded.

It is concluded from this study that although the Eerste River is relatively unpolluted by Pb and Cd, the freshwater crab *P. perlatus* accumulates these metals from the environment and could therefore play a role in transferring these metals along the food chain to predators. It is well known that crabs serve as food for a variety of predators on the banks of the Eerste River and these species are considered to be economically and aesthetically valuable wildlife. The crabs could therefore constitute an important pathway for entry of Pb and Cd into the food chain of these animals. The risk will depend on rates of consumption, levels of contamination as well as the toxicological effects of the metals on these animals.

Whether the crabs can serve as effective monitors of Pb and Cd contamination in the river is still unclear, since no evidence of good correlations between body loads of these metals and environmental concentrations could be established. The transient nature of environmental levels of contaminants and their mobility often preclude the use of organisms as biomonitors for the accurate reflection of currently prevailing environmental concentrations. The levels of these metals in the crabs however provided an indication that they were bioavailable and present in readily measurable quantities. The crabs can therefore still serve to monitor bioavailability over time in spite of the fact that these concentrations do not, and probably never can, directly reflect existing environmental levels reliably. The influence of seasonality (which includes differences in flow volume of the river which may affect concentrations) and changes in uptake and excretion rates (related to life-cycle stages and feeding patterns) may account for the high variation in observed body burdens of individual crabs and should be duly considered in order to obtain a more complete picture.

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### References

- Anderson, J.: 1974, 'A study of the digestion of sediment by the HNO<sub>3</sub>-H<sub>2</sub>SO<sub>4</sub> and the HNO<sub>3</sub>-HCl-procedures', *At. Ab. N.* **13**, 31–32.
- Anderson, R. V. and Brower, J. E.: 1978, 'Patterns of trace metal accumulation in crayfish populations', *Bull. Environ. Contam. Toxicol.* **20**, 120–127.
- Arkel, G. B. F.: 1979, 'Aspects of the feeding and breeding biology of the Giant Kingfisher', *Ostrich* **50**, 176–181.
- Barnard, K. H.: 1950, 'Descriptive catalogue of South African decapod Crustacea', *Ann. S.A. Mus.* **38**, 179–194.

- Du Preez, H. H., Steenkamp, V. E. and Schoonbee, H. J.: 1993, 'Bioaccumulation of zinc and lead in selected tissues and organs of the freshwater crab, *Potamonautes warreni*', *Sci. Tot. Environ. Suppl.*, 469–478.
- France, R. L.: 1987, 'Calcium and trace metal composition of crayfish (*Orconectes virilis*) in relation to experimental lake acidification', *Can. J. Fish. Aquat. Sci.* **44**, 1078–1113.
- Griscom, S. B., Fisher, N. S. and Luoma, S. N.: 2000, 'Geochemical influences on assimilation of sediment-bound metals in clams and mussels', *Environ. Sci. Technol.* **34**, 91–99.
- Hill, M. P. and O'Keeffe, J. H.: 1992, 'Some aspects of the ecology of the freshwater crab (*Potamonautes perlatus* Milne Edwards) in the upper reaches of the Buffalo river eastern Cape Province, South Africa', *S.A. J. Aquat. Sc.* **18**, 42–50.
- Janczur, M., Kozłowski, J. and Laskowski, R.: 2000, 'Optimal Allocation, Life History and Heavy Metal Accumulation: Dynamic Programming Model', in J. Kamenga and R. Laskowski (eds), *Demography in Ecotoxicology*, Wiley, Chichester, UK, pp. 179–197.
- Kempster, P. L., Hattingh, W. H. J. and Van Vliet, H. R.: 1980, 'Summarized water quality criteria', *Dept. Environ. Aff. Tech. Rep.* 108.
- King, J. M.: 1981, 'The distribution of invertebrate communities in a small South African river', *Hydrobiol.* **83**, 43–65.
- Lewis, T. E. and McIntosh, A. W.: 1986, 'Uptake of sediment-bound lead and zinc by the freshwater isopod *Asellus cummunis* at three different pH levels', *Arch. Environ. Contam. Toxicol.* **15**, 495–504.
- Peijnenburg, W. J. G. M., Posthuma, L., Eijssackers, H. J. P. and Allen, H. E.: 1997, 'A conceptual framework for implementation of bioavailability of metals for environmental management purposes', *Ecotoxicol. Environ. Saf.* **37**, 163–172.
- Purves, M. G., Kruuk, H. and Nel, J. A. J.: 1994, 'Crabs, *Potamonautes perlatus* in the diet of otter, *Aonyx capensis* and water mongoose *Atilax paludinosus* in a freshwater habitat in South Africa', *Z. Säugetierk.* **59**, 332–341.
- Reinecke, A. J., Reinecke, S. A., Musibono, D. E. and Chapman, A.: 2000, 'Transfer of lead (Pb) from earthworms to shrews (*Myosorex varius*)', *Archiv. Environ. Contam. Toxicol.* **39**, 392–397.
- Roldan, B. M. and Shivers, R. R.: 1987, 'The uptake and storage of iron and lead in cells of the crayfish (*Orconectes propinquus*) hepatopancreas and antennal gland', *Comp. Biochem. Physiol. C* **86**, 201–214.
- Schulz, R., Peall, S. K. C., Dabrowski, J. M. and Reinecke, A. J.: 2001, 'Current use insecticides, phosphates and suspended solids in the Lourens River, Western Cape, during the first rainfall event of the wet season', *Water S. A.* **27**, 65–70.
- Snyman, R. G., Reinecke, A. J. and Nel, J. A. J.: 2002, 'Uptake and distribution of copper in the freshwater crab, *Potamonautes perlatus* (Crustacea) in the Eerste River, South Africa', *African Zoology* 81–89.
- Van Eeden, P. H. and Schoonbee, H. J.: 1991, 'Bio-accumulation of heavy metals by the freshwater crab *Potamonautes warreni* from a polluted wetland', *S. A. J. Wildl. Res.* **21**, 103–108.
- Van Straalen, N. M. and Van Wensem, J.: 1986, 'Heavy metal content of forest litter arthropods as related to body size and trophic level', *Environ. Poll. A*, **42**, 209–221.
- Van Straalen, N. M. and Verkleij, J. A. C.: 1993, *Leerboek Oecotoxicologie*, V. U. Uitgeverij, Amsterdam, The Netherlands, pp. 423.
- Watling, R. J. and Watling, H. R.: 1983, 'Metal surveys in South African estuaries, VII, Bushmans, Kariega, Kowie and Great Fish Rivers', *Water S. A.* **9**, 66–70.