Bioaccumulation of copper in the tissues of *Potamonautes warreni* (Calman) (Crustacea, Decapoda, Branchiura), from industrial, mine and sewage-polluted freshwater ecosystems

V.E. Steenkamp*, H.H. du Preez and H.J. Schoonbee

Research Unit for Aquatic and Terrestrial Ecosystems, Department of Zoology, Rand Afrikaans University, P.O. Box 524, Auckland Park, 2006 Republic of South Africa

Received 5 July 1993; accepted 25 January 1994

The copper concentration detected in the water and sediments of the Natalspruit River, Bronkhorstspruit River and Nooitgedacht Dam exceeded certain stated limits for the protection of aquatic life. Despite considerable individual variation, the general ranking of copper concentrations in the various tissues was carapace < muscle < gonads < midgut gland < gills. Seasonal variation was detected in the bioaccumulation of copper in crabs sampled from the Natalspruit River. However, this phenomenon did not occur in crabs from the other two water bodies. A significant increase in copper concentrations was detected with a decrease in size, indicating that the size of the crabs is an important influencing factor in the bioaccumulation of copper. It was also found that more copper accumulated in the ovary than in the testis per unit weight. The bioaccumulation factors (BF) calculated for the different tissues with respect to the water were highest in the gills and midgut gland (785,00 – 1257,50 and 432,00 – 1340,00, respectively). The BF with respect to the copper concentration in the sediments was comparatively low for all the tissues (<0,10 – 2,74). It appears that *P. warreni* is able to regulate the copper concentrations in its various tissues and is therefore not a suitable indicator of the presence of copper in the aquatic environment.

Die koperkonsentrasies wat gevind is in die water en sedimente van die Natalspruitrivier, Bronkhorstspruitrivier en Nooitgedacht Dam het sekere neergelegde riglyne vir die beskerming van akwatiese lewe oorskry. Ten spyte van hoë individuele variasies, was die algemene verhouding in die koperkonsentrasies tussen die verskillende weefsels, karapaks < spier < gonades < spysverteringsklier < kieue. Seisoensvariasie is waargeneem in die bioakkumulering van koper in die organe en weefsels van krappe wat in die Natalspruitrivier versamel is. Geen seisoensvariasie is egter waargeneem by krappe wat in die ander twee akwatiese sisteme versamel is nie. 'n Duidelike toename in koperkonsentrasies is gevind met 'n afname in grootte. Krapgrootte lyk dus na 'n belangrike faktor wat die bioakkumulering van koper beïnvloed. Dit is ook gevind dat meer koper akkumuleer in die ovarium as in die testis per eenheidsmassa. Die bioakkumuleringsfaktore (BF) vir koper wat bepaal is vir die verskillende weefsels ten opsigte van die water, was die hoogste in die kieue en spysverteringsklier (785,00 – 1257,50 en 432,00 – 1340,00, respektiewelik). Die BF ten opsigte van die sedimente was relatief laag vir al die weefsels (<0,10 – 2,74). Dit wil voorkom asof *P. warreni* koperkonsentrasies in die verskeie organe en weefsels kan reguleer en dat dit dus nie 'n geskikte indikatororganisme vir die aanwesigheid van koper in die akwatiese omgewing nie.

* To whom correspondence should be addressed

There are several sources of metal pollution in inland waters, e.g. geological weathering, industrial processing of ores and metals, the use of metals and metal compounds, animal and human excrete, as well as leaching from domestic refuse and solid waste dumps. Some of the industries responsible for copper pollution are mining and metallurgy, paints and dyes, cleaning and duplicating, electroplating and metal finishing, chemical manufacturing, explosives, textiles, electrical components and electronics (Förstner & Prosi 1979).

Copper is widely distributed in nature in its free state, as well as in sulphides, arsenides, chlorides and carbonates. In the aquatic environment, copper can exist in three broad categories: particulate, colloidal and soluble. The dissolved phase could contain both the free ion as well as copper complexed to organic and inorganic ligands. Speciation of copper in natural waters is determined by the physico-chemical, hydrodynamic characteristics and the biological state of the water (Moore & Ramamoorthy 1984).

Like many other metals copper is essential for normal growth and development (Friberg, Nordberg & Vouk 1986). Several copper-containing proteins have been identified in biological systems (Moore & Ramamoorthy 1984). Copper is also an essential component of hemocyanin, the oxygen transport blood pigment found in crabs (Engel 1987; Engel & Brouwer 1987; Tulasi & Ramanarao 1988).

When essential metals such as copper are limiting, life processes will not function at their maximum efficiency. Conversely, excess metals usually cause inhibition of the life processes through interference with enzyme activity (George 1982; Bjerregaard & Vislie 1986) and loss of membrane integrity due to the binding of the metals on charged sites of proteins and membranes (Roesijadi 1981). Copper is highly toxic to most freshwater and marine invertebrates with LC_{50}'s generally less than 0,5 mg/l, though they may range from 0,006 to >225,0 mg/l under certain conditions (Moore & Ramamoorthy 1984). The toxicity of copper is thought to be largely attributable to the Cu^{2+} ion. Toxicity is generally greater in freshwater than in marine waters, reflecting the relative proportion of the toxic free copper ion in solution (Hellawell 1986).

The present study was aimed at determining the copper concentrations in the water, sediments and the different tissues and organs of *P. warreni* from three polluted aquatic

environments. Seasonal variation, as well as possible size and sex dependency in the bioaccumulation of copper were also examined.

Materials and Methods

Water, sediment and specimens of *P. warreni* for metal analysis were collected every second month (November 1989 – January 1991) at the Natalspruit River, Bronkhorstspruit River and Nooitgedacht Dam (Figure 1). Six localities with varying metal inputs were chosen in the Natalspruit River wetland ecosystem for the sampling of *P. warreni*. Surface water (at 10-cm depth) and sediment (to 5-cm depth) were taken by using hard pre-washed plastic bottles. The crabs were transported in polyethylene buckets to the laboratory, where samples of the carapace, gills, gonads, midgut gland and muscle were removed. Prior to dissection, carapace width (mm) and sex were recorded for each crab. The crabs were grouped into four size-classes according to the carapace width: 10–30 mm (Group 1), 30–40 mm (Group 2), 40–60 mm (Group 3) and 60–80 mm (Group 4). Of these, Groups 1 and 2 both represented the immature developmental stages of *P. warreni*. Due to small sample weight, it was necessary to pool some of the tissues of specimens of Groups 1 and 2 to obtain the required one gram wet-tissue sample.

Water, sediment and tissue samples were digested according to methods described by Van Loon (1980), using 55% nitric acid and 70% perchloric acid. The acid digestion was performed on a hotplate (200–250°C) for at least 5 h, during which clearing of the sample was achieved. The copper concentration in the various tissues, water and sediment was measured by means of flame atomic absorption spectrophotometry (Varian SpectrAA-10). A detailed description of the methods and techniques used during this study are given in Steenkamp, Du Preez, Schoonbee, Wiid & Bester (1993). In order to be able to facilitate the comparison of dry mass data in the literature with the data obtained in this study, the dry matter percentages were determined in each case. The tissues of eight crabs were dissected and dried at 60°C for 48 h.

A bioaccumulation factor (*BF*) which reflects the difference in concentration of metal in the water and tissue was calculated using the formula of Wiener & Giesy (1979):

$BF = Co / Cw$ where Co = wet weight concentration of a given metal in the crab tissue and Cw the average total concentration of the metal in the water. The *BF* with respect to the sediment was determined applying the formula of Kovacs, Nayary & Toth (1984):

BF = [metal] in the tissue / [metal] in the sediment

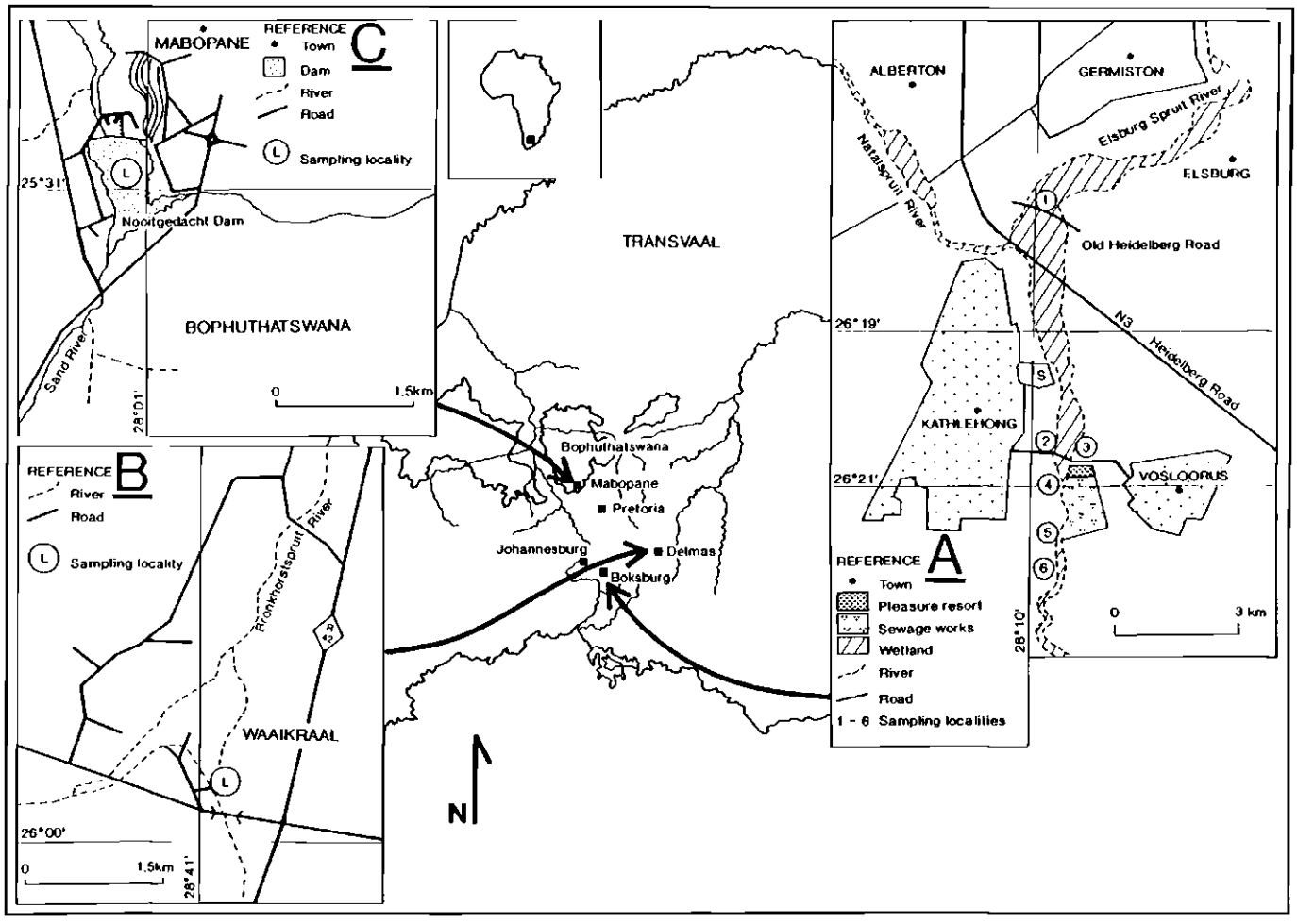

Figure 1 Sampling localities on the Natalspruit River wetland (A), the Bronkhorstspruit River (B) and the Nooitgedacht Dam (C).

Statistical analysis was performed on the data with the aid of the BMDP2V program. If statistically significant differences were found after analyses of variance (ANOVA), Scheffe's test for paired comparison was performed on the data. Student's *t*-test was used to indicate the extent of significant differences which may possibly exist between male and female *P. warreni* in the bioaccumulation of copper.

Results

Water and sediment copper concentrations

The mean copper concentration in the Bronkhorstspruit River (0,04 ± 0,01 mg/l) was only slightly lower than those in the Natalspruit River (0,05 ± 0,02 mg/l) and Nooitgedacht Dam (0,05 ± 0,03 mg/l). The copper concentration was very high in the sediments of the Natalspruit River (mean: 102,9 ± 111,4 µg/g) in comparison with its mean concentration in the Bronkhorstspruit River (45,8 ± 32,3 µg/g) and Nooitgedacht Dam (12,9 ± 2,1 µg/g) (Table 1).

Tissue variation in the bioaccumulation of copper

Each of the individual tissues showed considerable variation in the bioaccumulation of copper. In some tissues, notably the gills and the midgut gland, the mean copper concentration was very high, being 48,8 ± 31,8 µg/g wet weight and 32,0 ± 87,1 µg/g wet weight, respectively. The carapace had the lowest mean copper concentration of 8,4 ± 7,1 µg/g wet weight. There were significant differences ($P < 0,05$) in the copper concentration between all the tissues, with the exception of the copper concentration between the gonads (19,3 ± 12,3 µg/g wet weight) and the muscle (18,7 ± 11,4 µg/g wet weight).

Differences between localities

The bioaccumulation of copper in the carapace of crabs sampled from Localities 4, 5 and 6 was significantly higher ($P < 0,05$) than those from Locality 2 and the Nooitgedacht Dam. The bioaccumulation of copper in the carapace of organisms sampled from Locality 4 was also significantly higher than the carapace copper concentration in crabs sampled at Localities 1 and 3 (Table 2). The concentration of copper in the gills of *P. warreni* sampled at Locality 1 was significantly higher than those from Locality 2 and the Nooitgedacht Dam.

There was no significant difference in the copper concentration between the gonad samples of *P. warreni* sampled at the different localities ($P > 0,05$). Crabs from Locality 1 had a significantly higher concentration ($P < 0,05$) of copper in the midgut gland than those sampled at Locality 2 on the Natalspruit River, the Nooitgedacht Dam and the Bronkhorstspruit River.

The muscle copper concentration of *P. warreni* sampled at Localities 4 and 6 was significantly higher ($P < 0,05$) than the copper concentration in the muscles of crabs sampled at Localities 1, 2 and 3, as well as those at the Nooitgedacht Dam and Bronkhorstspruit River. Crabs sampled at Locality 5 had a significantly higher ($P < 0,05$) copper concentration in the muscle than those sampled at Locality 2 and the Nooitgedacht Dam.

Table 1 Concentrations of copper in the water (mg/l) and sediments (µg/g) of the Natalspruit River (Localities 1–6). Bronkhorstspruit River and Nooitgedacht Dam

	Natalspruit River						Bronkhorst-spruit River	Nooitgedacht Dam
	1	2	3	4	5	6		
Water								
November 1989	0,05	0,04	0,10	0,04	0,07	0,05	*	*
January 1990	0,03	0,03	0,03	0,02	0,06	0,03	0,03	0,03
March 1990	0,05	0,03	0,02	0,02	*	0,04	0,04	0,05
May 1990	0,04	0,03	0,05	0,03	*	0,04	0,02	0,05
July 1990	0,03	0,04	0,03	0,02	*	0,05	0,05	0,04
September 1990	0,05	0,05	0,06	0,05	*	0,04	0,03	0,03
November 1990	0,07	0,08	0,11	0,11	*	0,02	0,04	0,11
January 1991	0,06	0,04	0,09	0,02	0,05	0,03	0,04	0,06
X ± SD	0,05 ± 0,01	0,04 ± 0,02	0,06 ± 0,03	0,04 ± 0,03	0,06 ± 0,01	0,04 ± 0,01	0,04 ± 0,01	0,05 ± 0,03
Sediment								
November 1989	*	*	*	*	*	*	*	*
January 1990	98,0	22,0	104,0	184,0	125,5	700,0	83,0	11,0
March 1990	132,0	24,7	116,0	59,0	*	186,0	40,0	14,7
May 1990	106,5	27,0	148,0	16,0	*	19,0	15,0	10,0
July 1990	120,0	34,5	85,5	74,5	*	69,0	53,0	15,6
September 1990	23,0	28,0	96,0	86,0	*	108,5	93,5	12,0
November 1990	120,0	28,0	144,5	124,5	*	86,7	16,0	13,0
January 1991	94,5	25,6	95,0	62,0	89,0	148,5	20,0	14,5
X ± SD	99,1 ± 36,1	17,1 ± 3,9	112,7 ± 24,8	86,6 ± 53,9	107,3 ± 25,8	188,2 ± 232,0	45,8 ± 32,3	12,9 ± 2,1

* No data available

Table 2 The mean concentration of copper (μg/g weight) in *Potamonautes warreni* sampled from the Natalspruit River (Localities 1–6), Bronkhorstspruit River and Nooitgedacht Dam. The bioaccumulation factor (*BF*) for the different tissues calculated with respect to the water and the sediment are also given

Localities		Carapace	Gills	Gonads	Midgut gland	Muscle
Natalspruit River						
1	*n*	63	57	49	56	62
	X ± *SD*	9,6 ± 4,8	61,2 ± 56,3	20,1 ± 15,0	67,0 ± 205,5	16,5 ± 6,4
	BF (H_2O)	192,00	1224,00	402,00	1340,00	330,00
	BF (Sediment)	0,10	0,62	0,20	0,68	0,17
2	*n*	128	129	68	120	121
	X ± *SD*	8,8 ± 4,6	38,5 ± 16,6	19,1 ± 13,0	22,8 ± 21,2	16,0 ± 7,7
	BF (H_2O)	220,00	962,50	477,50	570,00	400,00
	BF (Sediment)	0,32	1,42	0,70	0,84	0,59
3	*n*	65	65	47	61	63
	X ± *SD*	8,9 ± 4,6	55,2 ± 18,1	23,7 ± 15,2	27,2 ± 42,8	19,6 ± 9,8
	BF (H_2O)	148,33	920,00	395,00	453,33	326,67
	BF (Sediment)	0,08	0,49	0,21	0,24	0,17
4	*n*	110	110	33	103	105
	X ± *SD*	14,0 ± 8,2	50,3 ± 24,1	21,9 ± 14,3	32,4 ± 20,6	28,9 ± 14,8
	BF (H_2O)	350,00	1257,50	547,50	810,00	722,50
	BF (Sediment)	0,16	0,58	0,25	0,37	0,33
5	*n*	25	26	6	17	22
	X ± *SD*	14,9 ± 6,8	47,1 ± 22,6	20,0 ± 8,2	37,3 ± 24,2	25,8 ± 23,7
	BF (H_2O)	248,33	785,00	333,33	621,67	430,00
	BF (Sediment)	0,14	0,44	0,19	0,35	0,24
6	*n*	84	84	38	84	80
	X ± *SD*	13,2 ± 8,7	44,2 ± 15,7	18,4 ± 9,4	31,4 ± 14,5	26,7 ± 16,6
	BF (H_2O)	330,00	1105,00	460,00	785,00	667,5
	BF (Sediment)	0,07	0,23	0,10	0,17	0,14
Bronkhorstspruit River						
	n	133	110	43	91	104
	X ± *SD*	10,7 ± 4,7	42,9 ± 74,2	19,1 ± 10,1	21,6 ± 11,9	17,4 ± 7,6
	BF (H_2O)	214,00	858,00	382,00	432,00	348,00
	BF (Sediment)	0,23	0,94	0,42	0,47	0,38
Nooitgedacht Dam						
	n	144	142	81	142	140
	X ± *SD*	9,1 ± 9,2	35,3 ± 25,1	15,9 ± 9,7	20,6 ± 18,0	13,2 ± 4,7
	BF (H_2O)	227,5	882,5	397,5	515	330,00
	BF (Sediment)	0,71	2,74	1,23	1,00	1,02

The bioaccumulation factor (*BF*), calculated for copper using total mean values measured in the water from which the crabs were sampled, was generally the lowest for the carapace, ranging from 148,33 to 350,00 with respect to the water and from less than 0,10 to 0,71 with respect to the sediment (Table 2).

The *BF* for the gills and the midgut gland was relatively high. The *BF* for the gills varied between 785,00 and 1257,50 with respect to the water, and 0,23 and 2,74 with respect to the sediment, while the *BF* for the midgut gland ranged from 432,0 to 1340,0 and 0,17 to 1,0 with respect to the water and sediment, respectively (Table 2).

There were significant differences ($P < 0,05$) in the copper concentration in the carapace, gills, gonads and muscle between the crabs sampled from the Natalspruit River (pooled localities) and the Nooitgedacht Dam. There were also statistically significant differences ($P < 0,05$) in the muscle copper concentration between crabs from the Natalspruit River and the Bronkhorstspruit River, as well as between crabs from the Nooitgedacht Dam and the Bronkhorstspruit River (Figure 2).

Seasonal variation

In crabs sampled from the Natalspruit River, significant differences (ANOVA: $P < 0,05$) were found in the bioaccumulation of copper between the carapace, gills, gonads and muscle. *Potamonautes warreni* sampled during January 1990 had a significantly higher ($P < 0,05$) carapace copper concentration than crabs sampled during March, July, September and November 1990. Crabs sampled during September 1990 had a significantly lower ($P < 0,05$) carapace copper concentration than crabs sampled during May and

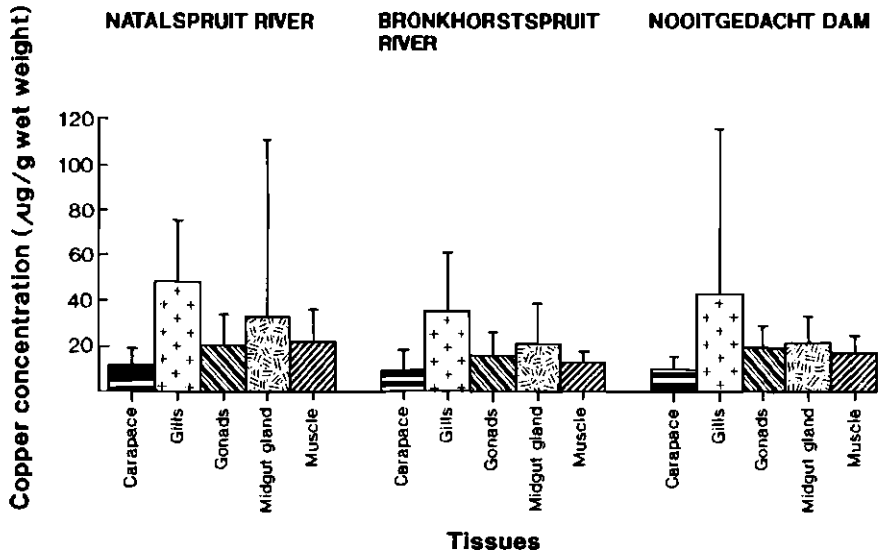

Figure 2 The mean concentrations ($X \pm SD$) of copper (µg/g wet weight) in the selected tissues and organs of *Potamonautes warreni* sampled from the Natalspruit River (pooled localities), Bronkhorstspruit River and Nooitgedacht Dam.

November 1990, and during January 1991 (Table 3).

The gill copper concentration of crabs sampled during January and March 1990 was found to be significantly higher ($P < 0,05$) than the copper concentration in the gills of crabs sampled during November 1989, and during May and November 1990. Crabs sampled during January and March 1990 had a significantly higher ($P < 0,05$) copper concentration in the gonads than crabs sampled during September and November 1990, and January 1991.

The mean concentration of copper in the midgut gland of crabs sampled during January and March 1990 was higher than that sampled during November 1989 and May 1990 to January 1991. These differences were not significant, however, probably because of the high variability of the data. Crabs sampled during March 1990 had a significantly higher copper concentration ($P < 0,05$) in the muscle than crabs sampled during May 1990 to January 1991. There was a marked decrease in the bioaccumulation of copper in *P. warreni* over this period. In general, the concentration of copper was high during January 1990 and March 1990. A degree of seasonal variation was observed with respect to the copper concentration in the carapace and gills, the concentration being lower during the colder winter months.

Crabs sampled from the Bronkhorstspruit River and Nooitgedacht Dam showed significant differences only in the bioaccumulation of copper in the muscles (ANOVA: $P < 0,05$) during the various months. Crabs sampled from the Bronkhorstspruit River during May 1990 had a significantly lower ($P < 0,05$) copper concentration in the muscles than crabs sampled during March and September 1990 (Table 3). Crabs collected from the Nooitgedacht Dam during July 1990 had a significantly higher ($P < 0,05$) copper concentration in the muscles than crabs collected during May and November 1990 (Table 3). Although significant rises in the copper concentration occurred for some periods, no seasonal variation could be observed in the bioaccumulation of copper in the various organs and tissues of *P. warreni* sampled from the Bronkhorstspruit River and the Nooitgedacht Dam.

Sex and size-related variation

There were significant differences ($P < 0,05$) only between the copper concentration in the gonads of mature male and female *P. warreni* sampled at the Natalspruit River and Bronkhorstspruit River. There were, however, no significant differences in the copper concentrations of male and female crabs from the Nooitgedacht Dam. The bioconcentration of copper does not appear to be related to the gender of the animal, except in the case of the gonads of the mature animals (Groups 3 & 4).

There were significant differences (ANOVA: $P < 0,05$) in the copper concentrations in the carapace, gills and muscle between the four size groups of crabs sampled at the Natalspruit River. The Sheffè test for paired comparison of the four size groups showed that all the size groups differed significantly ($P < 0,05$) with respect to the copper concentration in the carapace. There was a distinct increase in carapace copper concentration with a decline in carapace width.

The copper concentration in the gills of *P. warreni* from Group 4 was significantly higher ($P < 0,05$) than the copper concentration in crabs from Groups 2 and 3. The mean copper concentration in the gills of crabs from Group 1 was significantly higher ($P < 0,05$) than that of Group 4. There was also a significantly higher ($P < 0,05$) copper concentration in the muscle of crabs from Group 1 than that in crabs from Group 4.

An increase in carapace width and therefore crab size is generally followed by a decrease in copper concentration. This phenomenon was very clear in the carapace copper concentration. In crabs sampled from the Bronkhorstspruit River there were significant differences (ANOVA: $P < 0,05$) in the copper concentration in the carapace and gills between the different size groups (Table 4). Crabs from Groups 1 and 2 had a significantly higher ($P < 0,05$) carapace copper concentration than crabs from Groups 3 and

Table 3 The mean concentratians of copper (μg/g wet weight) in the selected tissues of *Potamonautes warreni* sampled over a period of 14 months (November 1989 to January 1991) at the Natalspruit River, Bronkhorstspruit River and Nooitgedacht Dam

Month		Carapace	Gills	Gonads	Midgut gland	Muscle
Natalspruit River						
November 1989	*n*	35	33	–	12	22
	X ± *SD*	14,4 ± 5,2	33,3 ± 19,9		21,0 ± 15,1	24,0 ± 25,7
January 1990	*n*	51	56	30	52	54
	X ± *SD*	15,9 ± 9,9	60,9 ± 20,4	29,6 ± 14,9	40,4 ± 19,0	25,1 ± 7,9
March 1990	*n*	65	59	52	58	63
	X ± *SD*	11,0 ± 6,0	60,6 ± 19,2	29,8 ± 13,8	61,4 ± 200,7	31,4 ± 22,8
May 1990	*n*	41	41	13	41	38
	X ±) *SD*	12,1 ± 10,1	33,3 ± 9,8	20,2 ± 12,0	33,9 ± 21,6	17,9 ± 7,1
July 1990	*n*	35	35	15	33	35
	X ± *SD*	10,2 ± 0,5	39,7 ± 14,4	19,7 ± 8,2	37,9 ± 45,7	19,0 ± 7,3
September 1990	*n*	81	81	62	80	78
	X ± *SD*	7,1 ± 5,0	47,5 ± 46,9	16,3 ± 12,5	18,6 ± 9,2	19,1 ± 8,1
November 1990	*n*	90	90	47	90	88
	X ± *SD*	10,2 ± 5,1	43,1 ± 23.6	14,9 ± 9,7	34,1 ± 39,8	19,1 ± 11,1
January 1991	*n*	77	76	22	75	75
	X ± *SD*	12,6 ± 5,7	52,2 ± 17,6	15,0 ± 6,6	21,8 ± 8,4	20,5 ± 10,3
Bronkhorstspruit River						
January 1990	*n*	14	14	10	14	14
	X ± *SD*	10,6 ± 5,5	9,05 ± 204,4	13,9 ± 5,7	27,3 ± 20,4	14,2 ± 5,0
March 1990	*n*	13	13	7	12	13
	X ± *SD*	9,3 ± 5,5	39,3 ± 12,5	19,0 ± 13,4	20,8 ± 11,9	21,2 ± 7,1
May 1990	*n*	12	12	5	9	12
	X ± *SD*	9,7 ± 3,7	29,3 ± 8,9	14,3 ± 3,9	14,8 ± 7,8	11,0 ± 2,3
July 1990	*n*	20	19	2	20	17
	X ± *SD*	11,0 ± 4,9	38,9 ± 18,9	16,5 ± 1,4	20,4 ± 4,5	18,9 ± 12,6
September 1990	*n*	20	18	13	21	18
	X ± *SD*	12,5 ± 4,7	44,8 ± 14,3	22,2 ± 7,9	20,8 ± 10,3	21,2 ± 6,2
November 1990	*n*	13	13	6	12	12
	X ± *SD*	11,0 ± 5,4	26,6 ± 11,8	25,6 ± 16,1	25,4 ± 11,6	14,2 ± 4,1
January 1991	*n*	21	21	–	3	18
	X ± *SD*	10,3 ± 3,2	33,4 ± 10,7		16,8 ± 6,0	18,0 ± 4,7
Nooitgedacht Dam						
January 1990	*n*	16	15	9	15	14
	X ± *SD*	12,8 ± 8,2	42,1 ± 13,3	15,0 ± 9,4	26,9 ± 26,4	15,0 ± 4,9
March 1990	*n*	10	10	7	10	10
	X ± *SD*	8,3 ± 5,2	41,3 ± 17,0	13,9 ± 8,5	28,7 ± 19,2	12,9 ± 3,8
May 1990	*n*	12	12	9	12	11
	X ± *SD*	7,5 ± 8,4	23,5 ± 8,4	17,7 ± 8,1	14,5 ± 7,5	10,5 ± 4,9
July 1990	*n*	28	28	19	28	28
	X ± *SD*	7,8 ± 2,8	30,5 ± 10,1	19,9 ± 11,5	15,0 ± 5,8	16,2 ± 6,0
September 1990	*n*	22	22	15	22	22
	X ± *SD*	12,1 ± 19,0	33,3 ± 11,9	17,1 ± 9,5	19,8 ± 16,5	14,4 ± 3,9
November 1990	*n*	31	31	9	30	30
	X ± *SD*	8,7 ± 6,8	32,4 ± 11,5	14,4 ± 11,1	19,7 ± 15,5	10,7 ± 3,0
January 1991	*n*	25	24	13	25	25
	X ± *SD*	7,3 ± 3,0	45,7 ± 54,1	10,6 ± 6,0	24,6 ± 25,5	12,3 ± 3,6

4. Crabs from Group 1 also had a significantly higher ($P < 0,05$) gill copper concentration than crabs from Group 3 (Table 4). However, crabs sampled from the Nooitgedacht Dam showed no significant differences ($P > 0,05$) in the tissue copper concentrations between any of the size groups.

Discussion

The high copper concentrations measured in the water and especially in the sediments of the Natalspruit River may be attributed to mining and industrial activities in its headwater regions as well as to effluents from sewage purification

Table 4 The mean concentrations of copper (μg/g wet weight) for the four size groups of *Potamonautes warreni* with respect to the selected tissues and organs of crabs sampled from the Natalspruit River, Bronkhorstspruit River and Nooitgedacht Dam. (Mean dry matter percentages: carapace = 80,9% dry weight; gills = 13,2% dry weight; gonads = 39,3% dry weight; midgut gland = 32,6% dry weight and muscle = 19,6% dry weight)

Size group		Carapace	Gills	Gonads	Midgut gland	Muscle
Natalspruit River						
1	*n*	40	38	–	34	33
	$X \pm SD$	19,9 ± 7,0	40,6 ± 13,5		34,9 ± 15,1	28,5 ± 21,2
2	*n*	51	52	–	47	43
	$X \pm SD$	14,7 ± 7,2	37,3 ± 26,5		30,5 ± 19,8	24,0 ± 13,9
3	*n*	135	132	42	126	129
	$X \pm SD$	11,5 ± 6,1	42,6 ± 18,5	19,4 ± 9,6	27,3 ± 26,0	23,2 ± 15,8
4	*n*	249	249	198	234	248
	$X \pm SD$	9,0 ± 5,9	53,9 ± 31,7	21,3 ± 14,2	37,1 ± 103,9	20,0 ± 10,7
Bronkhorstspruit River						
1	*n*	12	11	–	10	11
	$X \pm SD$	18,0 ± 3,1	103,8 ± 231,1		24,1 ± 7,4	20,3 ± 7,8
2	*n*	15	14	–	16	11
	$X \pm SD$	14,5 ± 4,3	28,7 ± 8,3		22,8 ± 10,2	19,4 ± 5,6
3	*n*	36	36	15	26	32
	$X \pm SD$	9,2 ± 3,8	32,2 ± 13,4	21,4 ± 8,7	18,4 ± 7,8	15,8 ± 5,2
4	*n*	50	49	28	39	50
	$X \pm SD$	8,8 ± 3,1	41,3 ± 14,6	17,8 ± 10,6	22,5 ± 15,1	17,3 ± 9,0
Nooitgedacht Dam						
1	*n*	9	9	–	8	7
	$X \pm SD$	15,9 ± 9,8	29,7 ± 10,3		23,4 ± 9,5	16,6 ± 2,2
2	*n*	12	11	–	12	12
	$X \pm SD$	10,9 ± 3,8	31,4 ± 9,3		29,6 ± 27,1	11,9 ± 3,9
3	*n*	47	47	11	46	47
	$X \pm SD$	9,2 ± 6,7	33,1 ± 12,0	15,8 ± 9,9	20,2 ± 16,7	13,7 ± 4,8
4	*n*	76	75	70	76	74
	$X \pm SD$	8,0 ± 10,6	37,9 ± 32,8	16,0 ± 9,7	19,1 ± 17,7	12,8 ± 4,9

works located alongside this stream. The Bronkhorstspruit River and Nooitgedacht Dam were subjected to limited domestic, agricultural and industrial effluents. The concentration of copper obtained during this study in the water of the Natalspruit River was consistently higher than the concentrations obtained by Van Eeden (1990) for the same localities, while no marked differences could be observed in the metal concentration in the sediments. It should be remembered, however, that measurements were only taken once every two months, and that an accurate assessment of the copper concentrations in the environment could therefore not be obtained. There are also several factors, including rainfall, which may have influenced the concentration of this metal at the different sampling sites.

The copper concentrations detected in the water of the Natalspruit River, Bronkhorstspruit River and Nooitgedacht Dam fell within the limits for the protection of aquatic life as stated by Kempster, Hattingh & Van Vliet (1980) but exceeded the stated limits by Gardiner & Zabel (1989), Environment Canada (1987) and Hart (1974). This indicates that copper could pose a potential threat to these aquatic ecosystems. It should be remembered, however, that the metal species, rather than the total concentration of the metal in the water, is the key to its effective bioaccumulation by the aquatic biota. The physico-chemical conditions in receiving waters may lead to a change in the speciation and toxicity of the metals. The Natalspruit River was subjected to a greater degree of organic and mineral pollution than the other two aquatic systems (Steenkamp *et al.* 1993). This phenomenon, however, could facilitate a reduction in the toxic effect of metals. This effect is usually attributed to the complexing of the toxic cations and the low toxicity of the resulting complexes (Zitko & Carson 1976). However, the parameters measured (temperature, pH, conductivity, total hardness, oxygen, ammonia, nitrate, nitrite, sulphate and phosphate) were within the limits for the protection of aquatic life for all three ecosystems.

There was a degree of variation in the copper concentration between individuals of *P. warreni*. The tissues also differed from one another in accumulating copper. These intraspecific variations can be related to various influencing factors, which include changes in salinity, water hardness,

dissolved oxygen, the presence of other metals or of complexing agents, change in temperature and pH, size differences, starvation, age, stage in life history, sex and moulting (Bryan 1976). The rate at which accumulation occurs in an organism depends not only on these biological, chemical and environmental factors, but, the ultimate level which is reached is governed by the ability of the organism to excrete the pollutant or, alternatively, to store it (Bryan 1979).

There is evidence that the ability to regulate body concentrations of essential metals such as copper, is a feature of decapod crustaceans, but non-essential metals (e.g. Pb, Hg) seem to be less well regulated. This ability is not extended to other crustacean taxa (Rainbow 1985; Rainbow & White 1989). At higher ambient copper concentrations this regulatory mechanism breaks down, resulting in an increase in copper levels in all the internal organs (Bryan 1976; White & Rainbow 1984).

Potamonautes warreni is able to bioaccumulate comparatively high levels of copper. Van Eeden & Schoonbee (1991) detected copper concentrations as high as 99,3 μg/g dry weight in whole crabs. In this study the copper levels in the different tissues and organs investigated revealed high individual variations, with the general ratio of the copper concentration between various organs and tissues being carapace < muscle < gonads < midgut gland < gills. Thus the lowest copper concentration (8,4 ± 7,1 μg/g wet weight) was found in the carapace. The variable amounts of copper found in the carapace could also be a result of the stage of moult cycle, as was suggested by Arumugam & Ravindranath (1983) for the lagoon crab *Scylla serrata*. However, the relationship between carapace copper concentrations of crabs of different moult stages and the concentrations in the surrounding water or food being ingested, still needs investigation. Nevertheless, since the carapace is shed periodically, one may speculate that this could represent a route of excretion in decapods.

Several studies indicate that the most significant variation of toxicant levels is to be found in tissues involved with regulatory processes, such as the midgut gland and the gills. This phenomenon was also found in this study, the concentrations in the midgut gland and gills being 32,0 ± 87,1 μg/g and 48,8 ± 31,8 μg/g wet weight, respectively. Almost all studies on the distribution of metals in crustacean tissues showed that the midgut gland is by far the most important storage organ for copper (Bryan 1976; Baggato & Alikhan 1987). Variations are often found in the copper concentration of the midgut gland among animals of the same species because the midgut gland seems to act as a store when excess copper is absorbed from the food or from the surrounding water (Bjerregaard & Vislie 1986). Copper concentrations as high as 2725,0 μg/g dry weight were found in the midgut gland of the crayfish *Cambarus bartoni* (Alikhan, Bagatto & Zia 1990).

The gills appeared to be the main site for the absorption and loss of copper on the body surface. High copper concentrations in the gills can be attributed partially to the presence of hemolymph in these tissues. Lethal and sub-lethal effects of copper in crustaceans have been ascribed tentatively to the disruption of gill function (Bjerregaard & Vislie 1986; Boitel & Truchot 1989; 1990).

Since hemocyanin has a high affinity for copper-ions, these metals are absorbed by the hemolymph and then transferred to the midgut gland, keeping the copper level in the hemolymph and other internal organs constant. Variation in the midgut gland copper concentration among the different animals in the same species can be substantial, as is the case with *P. warreni*. Bryan (1968) found that concentrations in the midgut gland of *Homarus vulgaris* varied from 68 to 1150 μg/g ash and in *Austropotamobius pallipes pallipes* from 21 to 690 μg/g ash. Metallothioneins, which occur in the hemolymph and midgut gland of decapods, are low molecular-weight cytosolic proteins with a high affinity for copper and other metals. These proteins probably play a role in the regulation of essential metals (Klerks & Levinton 1989). The ability of different individuals within a population to detoxify metals by binding them with metallothioneins is very variable. This may underlie some of the differences observed in metal tolerance between individuals. The tissue copper concentration of metallothioneins is normally very low, but increases after exposure to metals.

Excess copper appears to be stored as granules in the cells of the midgut gland (Bryan 1971). It is possible that these two detoxification mechanisms are related, e.g. metals could first become bound to metallothionein-like proteins and then be enclosed in a granule (Klerks & Levinton 1989).

Several studies demonstrated that the tissue concentrations of copper alter markedly during the moult cycle (Engel 1987; Engel & Brouwer 1987; Depledge 1989). There is a decrease in the midgut gland copper concentration during the moult cycle due to a decrease in hemocyanin. Variations in the midgut gland copper concentrations could thus also be related partially to the different stages in the moult cycle (Bryan 1968).

The most important mechanism by which both blood and midgut gland may increase in copper content may be via the intake of copper-rich food. Although food is the major source of copper in decapod crustaceans, copper may also be obtained from the surrounding water column. It seems that the midgut gland and the gills are the most sensitive to copper pollution (Bryan 1976).

The copper concentration in the gonads and muscle was relatively low compared with that of the other tissues. The presence of copper in the muscle and gonads is probably due to the presence of hemolymph in this tissue (Arumugam & Ravindranath 1983; Bardeggia & Alikhan 1991). The hemolymph is also very sensitive to copper and this sensitivity may be reflected in these two tissues. Concentrations of copper in muscle tissue are normally quite low. Values of 3,2–9,0 μg/g ash were found in studies done by Bryan (1968).

There was a significant difference in the copper concentration in samples of the carapace and muscle between various localities, the highest concentrations being those found at Localities 4, 5 and 6. This rise in copper concentration can be attributed to effluents from two sewage purification works situated in the catchment area of these three localities.

The capability of most organisms to concentrate certain metals is enhanced by certain feeding and metabolic processes which may lead to enormously high concentration factors. Food is a very important source of heavy metals for

decapods (Bryan 1976). As *P. warreni* live and feed in close proximity to the sediments, this must be regarded as a possible source of metals. The *BF*, determined with respect to the water as well as the sediment, was much lower for the carapace than the *BF* for the gills and the midgut gland of *P. warreni*, indicating a better concentrating ability in the latter two tissues. Since the copper concentration in the various tissues and organs of *P. warreni* seemed to be well regulated and independent of concentrations in the environment, however, the *BF* did not provide an accurate indication of the relative availability of the metal in the different localities.

However, this study indicated that the mean copper concentrations in the different tissues of *P. warreni* sampled from the Natalspruit River alter with seasonal changes. It is known that temperature has an effect on metabolism and it can thus be expected that the organism's sensitivity increases with an increase in temperature. The organism may receive greater amounts of the toxicants because of the increased diffusion or active uptake associated with higher rates of water movement across the gills or other cell membranes. This sensitivity is most obvious in the carapace and gills, the concentration of copper being lowest during the colder months of May, July and September. There is also a decrease in the copper concentration in the midgut gland with a decrease in temperature, but this was not significant, probably because of the high variability of the data. These findings correspond with results obtained by Engel (1987) and Djangmah & Grove (1970) for the crabs *Callinectes sapidus* and *Crangon vulgaris*, respectively. However, no seasonal changes in the bioaccumulation of copper could be observed in the different tissues of *P. warreni* sampled from the Bronkhorstspruit River and Nooitgedacht Dam.

The concentration of copper was essentially the same for both sexes, with the exception of the mature gonads. More copper accumulated in the ovary than in the testis per unit weight. Bagatto & Alikhan (1987), Alikhan *et al.* (1990) and Bardeggia & Alikhan (1991) also found that the two sexes did not differ significantly in their zinc concentrations. Sex could thus not be regarded as a variation factor for copper levels in the carapace, gills, midgut gland and muscle.

Anderson & Brower (1978), as well as Alikhan *et al.* (1990), could not establish significant relationships between tissue copper concentrations and the size of the crayfish *Orconectes virilis* and *Cambarus bartoni*, respectively. In this study, however, younger *P. warreni* were able to bioaccumulate more copper per unit weight than the mature crabs. A significant increase in copper concentration was found in the gills and muscle, with a decrease in carapace width. This can be attributed to the fact that regulatory mechanisms are not yet well developed in these immature animals, as well as to the fact that a higher metabolic rate and growth rates occur in the younger crabs.

Potamonautes warreni are also able to regulate the copper concentration in the various tissues, although not to the same extent as for zinc (Du Preez, Steenkamp & Schoonbee 1993). The ability to adapt to these pollutants is obviously a considerable advantage to these organisms as it provides them with a degree of protection. There is, therefore, no certainty that the copper concentration in the various tissues will accurately reflect the degree of contamination in their aquatic environment. This makes *P. warreni* and other decapod crustaceans less suitable as indicators of the availability of copper in the aquatic environment.

Acknowledgements

The authors wish to thank Ms S. Breytenbach for typing the tables and Ms H. Roets for preparing the figures. Funding was provided by the Rand Afrikaans University, the Foundation for Research Development and the City Council of Germiston.

References

ALIKHAN, M.A., BAGATTO, G. & ZIA, S. 1990. The crayfish as a 'biological indicator' of aquatic contamination by heavy metals. *Wat. Res.* 24(9): 1069–1076.

ANDERSON, R.V. & BROWER, J.E. 1978. Patterns of trace metal accumulation in crayfish populations. *Bull. envir. Contam. Toxic.* 20: 120–127.

ARUMUGAM, M. & RAVINDRANATH, M.H. 1983. Nature and distribution of copper in the green lagoon crab *Scylla serrata* (Forskal). *J. Exp. Mar. Biol.* 70: 271–280.

BAGATTO, G. & ALIKHAN, M.A. 1987. Copper, cadmium and nickel accumulation in crayfish populations near copper-nickel smelters at Sudbury, Ontario, Canada. *Bull. envir. Contam. Toxic.* 38: 540–545.

BARDEGGIA, M. & ALIKHAN, M.A. 1991. The relationship between copper and nickel levels in the diet, and their uptake and accumulation by *Cambarus bartoni* (Fabricius) (Decapoda, Crustacea). *Wat. Res.* 25(10): 1187–1192.

BJERREGAARD, P. & VISLIE, T. 1986. Effect of copper on ion- and osmoregulation in the shore crab *Carcinus maenas*. *Mar. Biol.* 91: 69–76.

BOITEL, F. & TRUCHOT, J-P. 1989. Effects of sublethal and lethal copper levels on hemolymph acid-base balance and ion concentrations in the shore crab *Carcinus maenas* kept in undiluted sea-water. *Mar. Biol.* 103: 495–501.

BOITEL, F. & TRUCHOT, J-P. 1990. Comparative study of the effects of copper on the haemolymph ion concentrations and acid-base balance in shore crabs *Carcinus maenas* acclimated to full strength or dilute sea-water. *Comp. Biochem. Physiol.* 95C(2): 307–312.

BRYAN, G.W. 1968. Concentrations of zinc and copper in the tissues of decapod crustaceans. *J. mar. biol. Ass. U.K.* 48: 303–321.

BRYAN, G.W. 1971. The effects of heavy metals (other than mercury) on marine and estuarine organisms. *Proc. Roy. Soc. Lond B.* 177: 389–410.

BRYAN, G.W. 1976. Some aspects of heavy metal tolerance in aquatic organisms. In: Effects of pollutants on aquatic organisms, (ed.) A.P.M. Lockwood. Cambridge University Press, New York.

BRYAN, G.W. 1979. Bioaccumulation of marine pollutants. *Phil. Trans. R. Soc. Lond. B.* 286: 483–505.

DEPLEDGE, M.H. 1989. Re-evaluation of metabolic requirements for copper and zinc in decapod crustaceans. *Mar. Env. Res.* 27: 115–126.

DJANGMAH, J.S. & GROVE, D.J. 1970. Blood and hepatopancreas copper in *Crangon vulgaris* (Fabricius). *Comp. Biochem. Physiol.* 32: 733–745.

DU PREEZ, H.H., STEENKAMP, V.E. & SCHOONBEE, H.J. 1993. Bioaccumulation of zinc and lead in selected tissues and organs of the freshwater crab, *Potamonautes warreni*. *Sci. Tot. Environ.* 134: 469–478.

ENGEL, D.W. 1987. Metal regulation and molting in the blue crab, *Callinectes sapidus* copper, zinc and metallothionein. *Biol. Bull.* 172: 69–82.

ENGEL, D.W. & BROUWER, M. 1987. Metal regulation and molting in the blue crab, *Callinectes sapidus:* metallothionein function in metal metabolism. *Biol. Bull.* 173: 239–251.

ENVIRONMENT CANADA. 1987. Canadian water quality guidelines. Report prepared by the task force on water quality guidelines of the Canadian Council of Resource and Environmental Ministers.

FÖRSTNER, U. & PROSI, F. 1979. Heavy metal pollution in freshwater ecosystems. In: Biological Aspects of Freshwater Pollution, (ed.) O. Ravera. Pergamon Press, Oxford.

FRIBERG, L., NORDBERG, G.F. & VOUK, B.V. 1986. Handbook on the toxicology of metals. Second edn, Vol 11: Specific metals. Elsevier Science Publishers, Amsterdam.

GARDINER, J. & ZABEL, T. 1989. United Kingdom water quality standards arising from European Community directives — an update. Water Research Centre, Report No. PRS 2257–17.

GEORGE, S.G. 1982. Subcellular accumulation and detoxication of metals in aquatic animals. In: Physiological mechanisms of marine pollutant toxicity, (eds.) W.B. Vernberg, A. Calabrese, F.P. Thurberg & F.J. Vernberg. Academic Press, New York.

HART, B.T. 1974. A compilation of Australian water quality criteria. Australian Water Research Council. Technical Paper No. 7.

HELLAWELL, J.M. 1986. Biological indicators of freshwater pollution and environmental management. Elsevier Applied Science Publishers, London.

KEMPSTER, P.L., HATTINGH, W.A.J. & VAN VLIET, H.R. 1980 Summarized Water Quality Criteria. Dept. of Water Affairs, Forestry and Environmental Conservation, Pretoria. Technical Report No. Tr108.

KLERKS, P.L. & LEVINTON, J.S. 1989. Effects of heavy metals in a polluted aquatic ecosystem. In: Ecotoxicology: Problems and Approaches, (eds.) S.A. Levin, M.A. Harwell, J.R. Kelly & K.D. Kimball. Springer-Verlag, New York.

KOVACS, M., NAYARY, I. & TOTH, L. 1984. The microelement content of some submerged and floating aquatic plants. *Acta Bot.* 30: 173–185.

MOORE, J.W. & RAMAMOORTHY, S. 1984. Heavy metals in natural waters. Applied monitoring and impact assessment. Springer-Verlag, New York.

RAINBOW, P.S. 1985. Accumulation of Zn, Cu and Cd by crabs and barnacles. *Estuarine, Coastal and Shelf Science* 21: 669–686.

RAINBOW, P.S. & WHITE, S.L. 1989. Comparative strategies of heavy metal accumulation by crustaceans: zinc, copper and cadmium in a decapod, an amphipod and a barnacle. *Hydrobiologia* 174: 245–262.

ROESIJADI, G. 1981. The significance of low molecular weight, metallothionein-like proteins in marine invertebrates: current status. *Mar. Env. Res.* 4: 167–179.

STEENKAMP, V.E., DU PREEZ, H.H., SCHOONBEE, H.J., WIID, A.J.B. & BESTER, M.M. 1993. Bioaccumulation of iron in the freshwater crab (*Potamonautes warreni*) from three industrial, mine and sewage-polluted freshwater ecosystems in the Transvaal. *Water SA.* 19: 281–290.

TULASI, S.J. & RAMANARAO, J.V. 1988. Effects of lead on copper content of fresh water field crab *Barytelphusa guerini* (H. Miline Edwards). *Indian J. Exp. Biol.* 26: 323–324.

VAN EEDEN, P.H. 1990. Die voorkoms en akkummulering in die rioolbesoedelde, organies-verrykte Elsburgspruit-Natalspruit vlei-ekosisteem. Unpub. M.Sc. thesis. Rand Afrikaans University, P.O. Box 524, Auckland Park, 2006 South Africa.

VAN EEDEN, P.H. & SCHOONBEE, H.J. 1991. Bioaccumulation of heavy metals by the freshwater crab *Potamonautes warreni* from a polluted wetland. *S. Afr. J. Wildl. Res.* 21(4): 103–108.

VAN LOON, J.C. 1980. Analytical Atomic Absorption Spectroscopy. Selected Methods. Academic Press, New York.

WIENER, J.G. & GIESY, J.P. 1979. Concentrations of Cd, Cu, Mn, Pb and Zn in fishes in a highly organic softwater pond. *J. Fish. Res. Board Can.* 35: 270–279.

WHITE, S.L. & RAINBOW, P.S. 1984. Zinc flux in *Palaemon elegans* (Crustacea: Decapoda): molting, individual variation and tissue distribution. *Mar. Ecol. Ser.* 19: 153–166.

ZITKO, V. & CARSON, W.G. 1976. A mechanism of the effects of water hardness on the lethality of heavy metals to fish. *Chemosphere* 5: 299–303.