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Nickel Uptake and Regulation in a Copper-tolerant Decapod, *Cambarus bartoni* (Fabricius) (Decapoda, Crustacea)

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Large amounts of acid forming sulphur dioxide, and "heavy metals" (specific gravity > 5, Niebor and Richardson 1980), including nickel are continuously being released into the environment by mining and smelting operations at Sudbury, Ontario, Canada (see references in Bagatto and Alikhan 1987). As a consequence, a number of lakes in this region has become acidic and metal stressed.

Earlier work by Bagatto and Alikhan (1987) showed that Orconectes virilis and Cambarus bartoni (Astacidae, Decapoda), from three lakes in the Sudbury region of Ontario (Ramsey Lake in Sudbury, Joe and Nelson Lakes in Chelmsford) were tolerant to copper, cadmium and nickel. Furthermore, tissue concentrations of these three metals in the crayfish were related to the distance of the habitat from the emission site.

In the current study the uptake and accumulation of nickel by various tissues of a copper-tolerant crayfish, Cambarus bartoni (Decapod, Crustacea), was monitored for 4 wk in the laboratory to ascertain the dynamic nature (i.e., the pattern in time) of the response of the crayfish to increased levels of this relatively less metabolically essential but toxic metal in the aquatic environment.

MATERIALS AND METHODS

Intermoult adult Cambarus bartoni males and females were trapped along rocky shores of Joe Lake (46° 44' N 81° 01' E) in Chelmsford, Ontario, 30 km north of Sudbury, Ontario. The decapods were transported to the laboratory

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in water-filled plastic containers and housed in concrete laundry tubs. All animals were acclimated to laboratory conditions for two wk. This acclimation also insured that all crayfish were post moult before they were introduced into experimental exposure chambers. Concentrated $NiSO_4.7H_2O$ (BDH Chemicals, Toronto, Ontario) stock solution, diluted to 0.2, 0.4 and 0.8 mg Ni L^{-1}, was delivered to experimental exposure chambers containing crayfish at a rate of 1 mL min^{-1} through a modified (for details, see Zia 1987) continuous-flow mini-diluter system described by Benoit et al. (1982). The flow rate to each of the 8-L experimental exposure chambers amounted to 50 (± 0.5) mL min^{-1}, with the chamber displacement occurring every 2 h and 40 min.

All experiments were performed under L12:D12 at 18°C (± 2°C) water temperature. Charcoal filtered and well aerated Sudbury tap water (mean pH 6.74, mean nickel concentration = 107 ± 07 µg L^{-1}) was used as the dilution medium. Crayfish were fed HiProMin fish food (containing 13.42 ± 0.89 µg Ni g^{-1} dry wt - Hartz Canada Inc., St. Thomas, Ontario) ad libitum.

Six animals (three males and three females) were removed at the start (day 1, wk 1, expressed as wk 0 in Tables 1 - 7) and the end of exposure wk 1, 2, 3 and 4 for metal analysis.

Each crayfish was thoroughly rinsed, and its wet weight (g) and carapace length (mm) were determined to approximate their age-class (see references in Zia 1987) before dissection. The exoskeleton, gills, hepatopancreas, alimentary canal, abdominal muscles, and remaining viscera (reproductive and excretory organs, nervous system, etc.) were placed in pre-weighed aluminium cups and oven dried at 80°C for 24 h to determine their dry wt. Tissue samples, along with procedural blanks, for analysis by Perkin-Elmer 703 atomic absorption spectrophotometer, were digested in boiling aqua-regia {3 mL concentrated nitric acid : 1 mL concentrated hydrochloric acid (BDH Chemicals, Toronto, Ontario)}, diluted to 20 mL with 1 M nitric acid and analyzed for nickel by the flame method. The sensitivity for nickel amounted to 0.15 µg mL^{-1}.

Statistical analysis of data was performed with the aid of a DEC-VAX/VMS computer, using $SPSS^X$ software (Statistical Package for Social Sciences, Chicago, Illinois). All data were checked for normality (Kolgomorov-Smirnoff test) and homogeneity of variance (Bartlett-Box F test), and they were log transformed where necessary. As metal accumulation in male and

female crayfish did not show any significant differences ($P > 0.05$), data for the two sexes were pooled prior to analysis. An initial four-way ANOVA evaluated effects on metal levels of exposure time, treatment, sex and tissue. Within exposure time and treatment, tissue metal accumulations were compared using one-way ANOVA with Duncan's Multiple Range Test (applied only when $P < 0.05$). Regression analysis for nickel levels in whole crayfish, as affected by exposure time during each treatment, was performed with the aid of an Apple Macintosh XL computer, using StatView software (Brain Power Inc., Calabasas, California).

RESULTS AND DISCUSSION

The data on the uptake and accumulation of nickel in whole crayfish during the 4-wk exposure period are summarized in Table 1, while those on tissue nickel concentrations as affected by the various treatment over the 4-weeks exposure period are tabulated in Tables 2-7, from which it is evident that nickel was taken up and accumulated in the crayfish tissue, at least during the first week, from various experimental nickel solutions.

Table 1. Mean concentration of nickel (mg L^{-1}) in whole Cambarus bartoni at different exposure times in various treatments.

Exp.time (weeks)	Nickel concentration			
	0.8 mg L^{-1}	0.4 mg L^{-1}	0.2 mg L^{-1}	Control
0	$47.8^{a,1}$* (34.3,66.6)**	$47.8^{a,1}$ (34.3,66.6)	$47.8^{a,1}$ (34.3,66.6)	$47.8^{b,1}$ (34.3,66.6)
1	$71.6^{a,c,1}$ (53.2,96.4)	$52.3^{a,1}$ (43.7,62.5)	$52.5^{a,1}$ (31.3,88.3)	$51.7^{b,1}$ (36.9,72.4)
2	$38.4^{a,1}$ (34.6,42.6)	$47.6^{a,1}$ (26.6,85.3)	$74.5^{a,1}$ (39.2,141.5)	$47.4^{b,1}$ (41.6,54.1)
3	$49.7^{a,b,1}$ (43.3,56.9)	$40.2^{a,2}$ (27.8,58.0)	$46.3^{a,2}$ (36.7,52.0)	$29.0^{a,2}$ (26.1,32.2)
4	$62.2^{b,c,1,2}$ (48.4,80.0)	$65.7^{a,1}$ (51.4,83.9)	$46.6^{a,2}$ (41.8,52.0)	$48.2^{b,2}$ (34.7,66.9)

* Average of six crayfish in each case. Means within each column followed by the same letter, and within each row followed by the same number are not significantly different at 5% level.

** 95% confidence limits.

Table 2. Mean concentration of nickel (mg L^{-1}) in the exoskeleton of Cambarus bartoni at different exposure times in various treatments.

Exp.time (weeks)	Nickel concentrations 0.8 mg L^{-1}	0.4 mg L^{-1}	0.2 mg L^{-1}	Control
0	$84.9^{a,1}$ * (66.0,109.2)**	$84.9^{a,1}$ (66.0,109.2)	$84.9^{a,1}$ (66.0,109.2)	$84.9^{a,1}$ (66.0,109.2)
1	$98.7^{a,1}$ (60.7,98.8)	$98.8^{a,b,1}$ (86.6,113.1)	$91.2^{a,1}$ (66.4,125.1)	$94.1^{a,1}$ (62.8,140.8)
2	$74.1^{a,1}$ (61.0,89.9)	$82.9^{a,1}$ (60.46,113.9)	$131.8^{a,1}$ (45.9,378.5)	$75.5^{a,1}$ (48.6,117.2)
3	$83.4^{a,1}$ (70.8,98.1)	$86.0^{a,1}$ (63.4,117.3)	$88.2^{a,1}$ (64.6,120.5)	$74.2^{a,1}$ (63.7,86.5)
4	$101.0^{a,1,2}$ (83.8,121.8)	$118.5^{b,2}$ (98.1,143.2)	$82.0^{a,1}$ (73.1,91.9)	$80.02^{a,1}$ (59.3,108.0)

*Average of six replicates in each case. Means within each column followed by the same letter, and within each row followed by the same number are not significantly different at 5% level.
**95% confidence limits.

Table 3. Mean nickel concentration (mg L^{-1}) in gills of Cambarus bartoni at different exposure times in various treatments.

Exp.time (weeks)	Nickel concentrations 0.8 mg L-1	0.4 mg L-1	0.2 mg L-1	Control
0	$70.1^{a,1}$ * (25.9,189.8)**	$70.1^{a,1}$ (25.9,189.8)	$70.1^{a,1}$ (25.9,189.8)	$70.1^{a,1}$ (25.9,189.8)
1	$223.4^{a,1}$ (120.5,414.2)	$143.9^{a,b,1}$ (52.0,398.3)	$167.3^{a,1}$ (63.5,440.9)	$120.6^{b,1}$ (73.5,197.9)
2	$69.8^{a,1}$ (19.6,248.8)	$114.4^{a,1}$ (72.6,180.2)	$148.4^{a,1}$ (100.0,220.1)	$149.2^{b,1}$ (89.0,250.2)
3	$183.6^{a,1}$ (109.5,307.8)	$102.8^{a,1}$ (49.0,215.4)	$97.7^{a,1}$ (9.6,99.7)	$37.1^{a,1}$ (6.5,213.2)
4	$185.9^{a,1,2}$ (96.8,356.9)	$329.5^{b,2}$ (202.8,535.4)	$97.88^{a,3}$ (79.2,120.9)	$124.9^{b,1}$ (78.2,199.8)

* Average of 6 tissues in each case. Means within each column followed by the same letter, and within each row followed by the same number are not significantly different at 5% level.
**95% limits.

Table 4. Mean nickel concentration (mg L^{-1}) in hepatopancreas of Cambarus bartoni at different exposure times in various treatments.

Exp. Time (weeks)	Nickel concentrations 0.8 mg L^{-1}	0.4 mg L^{-1}	0.2 mg L^{-1}	Control
0	$21.0^{a,1}$*	$1.0^{a,1}$	$21.0^{a,1}$	$21.0^{a,1}$
	(5.4,81.6)**	(5.4,81.6)	(5.4,81.6)	(5.4,81.6)
1	$335.7^{b,1}$	$141.8^{b,1,2}$	$126.5^{b,1,2}$	$38.0^{a,2}$
	(85.0,1325.9)	(28.2,712.7)	(40.4,396.2)	(6.6,100.9)
2	$92.8^{b,1,2}$	$40.3^{a,b,1,2}$	$143.5^{b,1}$	$25.2^{a,2}$
	(43.7,197.0)	(9.7,167.5)	(53.2,387.2)	(6.3,32.0)
3	$79.7^{a,b,1}$	$25.9^{a,b,1}$	$16.8^{a,b,1}$	$6.10^{a,1}$
	(11.1,573.7)	(2.3,293.2)	(4.4,63.7)	(1.2,32.0)
4	$188.9^{b,1}$	$159.3^{b,1}$	$108.1^{b,1}$	$95.2^{a,1}$
	(48.2,731.0)	(43.7,581.6)	(27.1,432.2)	(19.1,475.3)

* Average of six replicates in each case. Means within each column followed by the same letter, and within each row followed by the same number are not significantly different at 5% level.
** 95% confidence limits.

Table 5. Mean Ni concentration (mg L^{-1}) in digestive gut of Cambarus bartoni at different exposure times in various treatments.

Exp.Time (weeks)	Nickel concentrations 0.8 mg L^{-1}	0.4 mg L^{-1}	0.2 mg L^{-1}	Control
0	$59.4^{a,1}$*	$59.4^{a,1}$	$59.4^{a,1}$	$59.4^{a,1}$
	(25.0,140.9)**	(25.0,140.9)	(25.0,140.9)	(25.0,140.9)
1	$147.8^{a,b,1}$	$107.6^{a,1}$	$127.8^{a,1}$	$67.6^{a,1}$
	(88.6,246.8)	(67.9,170.5)	(75.9,215.1)	(8.3,550.4)
2	$58.7^{a,1}$	$79.0^{a,1}$	$135.6^{a,1}$	$50.3^{a,1}$
	(19.8,174.3)	(60.0,104.1)	(77.7,236.4)	(17.2,144.8)
3	$244.3^{b,1}$	$55.7^{a,1}$	$74.6^{a,1}$	$81.9^{a,1}$
	(127.3,468.9)	(4.1,751.3)	(10.1,554.0)	(43.4,154.5)
4	$229.9^{b,1}$	$205.1^{a,1}$	$120.1^{a,2}$	$141.2^{a,2}$
	(117.3,450.5)	(160.1,262.7)	(95.0,151.88)	(102.9,193.7)

* Average of six tissues in each case. Means within each column followed by the same letter, and within each row followed by the same number are not significantly different at 5% level.
** 95% confidence limits.

Table 6. Mean nickel concentration (mg L^{-1}) in abdominal muscles of Cambarus bartoni at different exposure times in various treatments.

Exp.Time (weeks)	Nickel concentrations 0.8 mg L^{-1}	0.4 mg L^{-1}	0.2 mg L^{-1}	Control
0	$10.9^{a,1}$*	$10.9^{a,1}$	$10.9^{a,1}$	$10.9^{a,1}$
	(3.3,36.7)**	(3.3,36.7)	(3.3,36.7)	(3.3,36.7)
1	$79.4^{b,1}$	$62.8^{b,1}$	$66.9^{b,1}$	$56.1^{c,1}$
	(26.3,239.6)	(28.0,141.2)	(33.0,135.5)	(32.0,98.3)
2	$13.8^{a,1}$	$8.8^{a,1}$	$29.6^{a,b,1}$	$42.9^{c,1}$
	(3.0,63.8)	(2.4,32.6)	(8.5,103.4)	(27.4,67.3)
3	$78.1^{b,1}$	$17.0^{a,1,2}$	$15.6^{a,1,2}$	$3.2^{b,2}$
	(41.2,148.3)	(1.2,235.4)	(1.6,160.4)	(1.5,6.7)
4	$84.3^{b,1}$	$81.6^{b,1}$	$78.2^{b,1}$	$74.2^{c,1}$
	(42.7,166.2)	(50.6,131.8)	(44.2,140.9)	(45.0,122.4)

*Average of six tissues in each case. Means within each column followed by the same letter, and within each row followed by the same number are not significantly different at 5% level.
**95% confidence limits.

Table 7. Mean nickel concentration (mg L^{-1}) in remaining viscera in Cambarus bartoni at different exposure times in various treatments.

Exp.Time (weeks)	Nickel concentrations 0.8 mg L^{-1}	0.4 mg L^{-1}	0.2 mg L^{-1}	Control
0	$40.3^{a,1}$*	$40.3^{a,1}$	$40.3^{a,1}$	$40.3^{a,1}$
	(25.4,63.7)**	(25.4,63.7)	(25.4,63.7)	(25.4,63.7)
1	$54.1^{a,1}$	$32.8^{a,1}$	$24.5^{a,1}$	$37.7^{a,1}$
	(28.3,103.4)	(13.8,43.6)	(2.1,281.9)	(16.9,83.9)
2	$32.6^{a,1}$	$37.7^{a,1}$	$46.8^{a,1}$	$17.9^{a,1}$
	(27.1,39.3)	(15.6,88.9)	(25.6,85.7)	(2.5,127.5)
3	$34.6^{a,1}$	$37.6^{a,1}$	$35.8^{a,1}$	$23.1^{a,1}$
	(24.5,48.8)	(13.3,105.9)	(26.9,47.7)	(18.5,28.7)
4	$38.3^{b,1}$	$41.1^{a,1}$	$31.5^{a,1}$	$35.0^{a,1}$
	(25.7,57.2)	(29.3,53.8)	(24.4,40.8)	(23.2,152.7)

* Average of six tissues in each case. Means within each column followed by the same letter, and within each row followed by the same number are not significantly different at 5% level.
** 95% confidence limits.

In general, Ni concentration was highest in gills and alimentary canal, and lowest in the viscera (Table 2). However, nickel concentration in whole crayfish varied from treatment to treatment and within the exposure period (Table 1).

Nickel concentrations in gills (Table 3), hepatopancreas (Table 4), alimentary canal (Table 5) and abdominal muscles (Table 6), as well as in the whole crayfish (Table 1), showed time related fluctuations in various treatments. Regression analysis of the data on nickel tissue concentrations in whole crayfish as a function of exposure time in 0.4 and 0.8 mg Ni L^{-1} showed a significant third degree polynomial relationship, suggesting that the crayfish undergoes a definite cycle of 2 wk of active nickel uptake and 2 wk of active excretion of amounts which could not be accommodated in various tissues. The existence of such a cycle provides a logical explanation for the great variability in nickel concentrations in individual crayfish forming a single sample.

Absorption of nickel appears to be an active process in the ciliate Paramecium caudatum (Andrivon 1974) and the embryo of the sea urchin (Timourian and Watchmaker 1972). According to Fuhrmann & Rothstein (1968), nickel, cobalt and zinc are absorbed in the yeast by a system that transports both magnesium and manganese. In higher organisms, movement of nickel may be mediated by a carrier system used primarily for calcium and magnesium (Timourian and Watchmaker 1972). In terrestrial isopods, nickel acts as an activator for body tissue alkaline phosphatase (Saleem and Alikhan 1974a), but inhibits hepatopancreatic acid phosphatase (Saleem and Alikhan 1974b).

According to Mierle (1981), nickel concentration in cells is not a true intracellular uptake, but probably represents amounts bound at the cell surface. Since gills, hepatopancreas and alimentary canal cells have large, but variable lengths of luminal surfaces, they would hold variable amount of nickel at the cell surface; this would explain the large fluctuations in nickel concentrations observed in the present study in these tissues.

One noticeable observation made during the present study was that crayfish in the control showed substantial, although relatively lower nickel concentrations than those detected in individuals exposed to three nickel treatments. This was probably a reflection of nickel concentration (107 ± 0.7 µg L^{-1}) of the Sudbury tap water.

According to Hall (1978), nickel absorption is a result of flux-equilibrium conditions established by a balance between uptake and loss. It may be suggested that flux occurs when no more incoming metal can be accommodated by the tissue and this period, in the present study, appears to take approximately 2 wk. Nickel concentrations in whole crayfish exposed to 0.2 mg Ni L^{-1}, however, did not show any specific trend. This may have been due to the non synchronization in the nickel uptake and excretion behavior of individual crayfish comprising the experimental population. Fluctuations observed in nickel concentrations also indicated that exposure of crayfish to various nickel concentrations did not induce metallothionein production, since if such had been the case, a steady rise in nickel concentrations in the hepatopancreatic tissue (considered to be the site for metallothionein production) would have been observed.

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REFERENCES

Andrivon C (1974) La perméabilite´ `a l'ion nickel chez Paramecium:ses ropport avec le reneversement ciliaire. Protistologica, 10:175-183

Bagatto G, Alikhan MA (1987a) Copper, cadmium, and nickel accumulation in crayfish populations near copper-nickel smelters at Sudbury, Ontario, Canada. Bull Environ Contam Toxicol 38:540-545

Benoit DA, Mattson VR, Olson DL (1982) A continuous-flow mini-diluter system for toxicity testing. Water Res 16:457-464

Furhmann G, Rothstein A (1968) Transport of $zinc^{2+}$, Co^{2+} and Ni^{2+} into yeast cells. Biochem Biophys Acta 163:1151-1155

Hall T (1978) Nickel uptake retention and loss in Daphnia magna. M.Sc.thesis,Department of Botany, University of Toronto, Ontario, Canada

Mierle GM (1981) Uptake of copper and other heavy metals by a green alga, Scenedes mus, and the relation of metal accumulation to toxicity. Ph D thesis Department of Botany,University of Toronto, Ontario, Canada

Nieboer E, Richardson DHS (1980) The replacement of the non-descriptive term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environ Pollut 1B:3-26
Saleem M, Alikhan MA (1974a) Characteristics of alkaline phosphatases from the terrestrial isopod, Porcellio laevis Latreille (Porcellionidae, Isopoda). Comp Biochem Physiol 47B:307-316
Saleem M, Alikhan MA (1974b) Characteristics of acid phosphatase in Porcellio laevis Latreille (Porcellionidae, Isopoda). Comp Biochem Physiol 49B:129-137
Timourian H, Watchmaker G (1972) Nickel uptake by sea urchin embryos and their subsequent development. J Exp Zool 182:379-88
Zia S (1987) A laboratory study of the relationship between levels of copper and nickel in water, and their uptake and accumulation by various body tissues in Cambarus bartoni (Fab.) (Decapoda-Crustacea). M Sc thesis Laurentian University, Sudbury, Ontario, Canada
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