Bull. Environ. Contam. Toxicol. (1990) 45:320–328

Bulletin of Environmental Contamination and Toxicology

Uptake of Cadmium and Nickel in Banana Prawn (*Penaeus merguiensis* de Man)

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Biological indicator organisms for monitoring heavy metal pollution in the coastal environment are more important than analyzing seawater or sediments (Phillips 1980). This is because marine organisms usually accumulate metals in their tissues and as a consequence, it is important for public health reasons. There are many reports in the literature on metal accumulation in crustacean tissue. Accumulation usually differs between tissues and species. For example, in *Homarus americanus* cadmium accumulated mainly in the digestive gland, followed by the gills (Thurberg *et al.* 1977), but in *Callinectes sapidus* cadmium accumulated mostly in the gills (Brouwer *et al.* 1984). According to Ray *et al.* (1981), more than 90 % of the body burden of cadmium accumulated in the hepatopancreas in *Homarus americanus*. In shrimp, *Palaemon elegans*, the accumulation of cadmium in the tissues increased significantly with longer exposure times (Skwarzec *et al.* 1984).

This study measured the pattern of cadmium and nickel accumulation in juvenile banana prawns. These metals were chosen because they exhibit different toxicity patterns and because of their potential industrial significance in the related area, as well as in other parts of the world.

MATERIALS AND METHODS

Juvenile banana prawns were captured at low tide from Three Mile Creek, near Townsville, Australia (19°12'42"S;146° 46'30"E), using a 1-cm beach sieve and quickly placed into plastic bins filled with seawater in which they were transported to the laboratory. Those selected for testing ranged in body length (post orbital margin to telson tip) from 45-70 mm and weighed 1.0-2.5 g.

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All tests were conducted in high density polyethylene tanks (56x36x40 cm) containing 50 liters of seawater. The tanks were covered with polyethylene tops and were artificially aerated to maintain oxygen levels above 90% saturation at all times.

The temperature and salinity adopted for testing were 25 $\pm$ 1°C and 36 $\pm$ 1°/oo, respectively, which were considered to be representative of the optimum conditions likely to be encountered in tropical coastal waters.

Stock solutions (100 g/L of cadmium and nickel were prepared by dissolving analytical grade salts (Ajax Chemicals, Australia) of either cadmium chloride ($CdCl_2.2H_2O$) or nickel chloride ($NiCl_2.6H_2O$) in glass-distilled water. These were further diluted with distilled water as required and checked against standards using atomic absorption spectroscopy. The test concentrations of 0, 0.1, 0.3 and 0.5 mg Cd /L and 0, 0.4, 1.2 and 2.0 mg Ni /L were selected on the basis of acute toxicity data previously reported (Denton and Burdon-Jones 1982).

Forty prawns were placed at random in each of the test tanks and were allowed to acclimate to the appropriate experimental conditions for five days prior to treatment. The required toxicant concentrations were pipetted into each tank and were rapidly dispersed by water currents generated by the aerators.

The tests were continued for a period of 30 days with 10 prawns being removed from each tank for metal analysis after 0, 5, 15 and 30 days exposure. The prawns were fed throughout the investigation with finely chopped, blanched squid. This was added to each tank on a twice daily basis with residual amounts and fecal material removed up to 3 hours later.

The test water was changed every 3 days by slowly siphoning the water from each tank simultaneously to within 2-3 cm of the bottom. After flushing with clean water, the tanks were refilled and redosed with metal toxicant. Water was collected from each tank for metal analysis immediately prior to changing and was consistently found to be within 95% of the required concentrations for each metal.

The sampled prawns were dissected into muscle, hepatopancreas and gills and kept at - 20°C until metal analysis. Tissue samples were thawed at room temperature and weighed into 100-ml Erlenmeyer flasks (Pyrex), loosely capped with teflon stoppers and digested with 5 ml of silica-distilled nitric acid on a hotplate at 135° C for 24 hours. The solutions were

evaporated to dryness, redissolved in 2 N nitric acid and analyzed by atomic absorption spectrophotometry. Blanks were treated similarly. Standards were made up in 10 % nitric acid and correction for non-atomic absorption was made simultaneously by the instrument. All glassware was cleaned by refluxing with hot nitric acid and thoroughly rinsed with double-distilled water before use.

All data were calculated on ug/g wet weight basis. Linear regression analysis was used to calculate the rate of uptake in each case. Where an obvious curvilinear relationship between tissue concentration and time was apparent, the slope (b) was calculated from the initial linear portion of the graph. To determine the relationship between rates of uptake and water concentrations of each metal, the slope values (b) were plotted against the corresponding levels of exposure.

RESULTS AND DISCUSSION

At all experimental levels of exposure, cadmium was most concentrated by the hepatopancreas and least concentrated by muscle over the 30-day exposure period. Rates of uptake between these tissues varied by factors of 137 and 31 at the highest and lowest levels of exposure. In the hepatopancreas the time required to reach equilibrium decreased with increased level of exposure and ranged from approximately 5 days at the highest level to greater than 30 days at the lowest level. Interestingly, the opposite situation was portrayed by muscle tissue, while in the gills equilibrium was not approached in any of the test concetrations (Figures 1, 2 and 3).

Nickel was accumulated to the greatest extent by the hepatopancreas followed by the gills and then the muscle. The maximum rate of uptake in the hepatopancreas was approximately 17 and 66 times higher than muscle tissue in the highest and lowest levels of exposure, respectively. In both gills and hepatopancreas the time taken to reach equilibrium was less than 5 days at all levels of exposure, whereas in the muscle it took approximately 15 days (Figures 4, 5 and 6).

Inspection of Figure 7 reveals that uptake of cadmium in muscle tissue is directly proportional to the external concentration. However, in the hepatopancreas the relationship appeared to be greater than one of direct proportionality while the reverse was shown by the gills (Figure 8).

Like cadmium, the rates of nickel uptake in all tissues were linearly related to the concentration in the seawater. However, in this instance the relationship in all tissues closely approximated direct proportionality (Figures 7 and 9).

A review of the available literature revealed a number of studies in which crustacean species have been exposed to artificially elevated metal levels. These are briefly discussed with particular reference to findings reported here.

The accumulation rate of cadmium in the muscle, gills and hepatopancreas of *P. merguiensis* in this study increased with increased concentration in the surrounding water. Others working with crustaceans have noted similar findings. For example, Nimmo *et al.* (1977) exposed *Penaeus durarum* to a range of low, sublethal cadmium concentrations and found that uptake in the muscle, gills, hepatopancreas and serum increased with increasing water concentration at $20^{o}/oo$ salinity. Likewise, White and Rainbow (1982) observed a similar effect with the shrimp *Palaemon elegans*.

Cadmium was most concentrated in the hepatopancreas of *P. merguensis* during this study. Similarly, Nimmo *et al.* (1977) noted the greatest accumulation of cadmium in the hepatopancreas of the pink shrimp *Penaeus durarum* exposed to a concentration of 1 mg/L . However, in the crab *Carcinus maenas* exposed to 100-1000 ug/L cadmium, the highest levels were found in the cuticle (54%), followed by the hepatopancreas (22%) (Rainbow 1985). In the crustacean *Daphnia magna* autoradiographic evidence identified the exoskeleton as the major sink for cadmium taken up from solution (Carney *et al.* 1986). Interestingly, levels of nickel in whole tissue of *Daphnia magna* were found to increase with increasing dissolved nickel concentration (Watras *et al.* 1985), which is similar to the findings presented here.

By combining the processes of absorption, excretion, detoxification and storage, the higher animals, at least, should potentially be capable of regulating the concentrations of metals in their body despite changes in metal availability in the environment. How well organisms are able to do this can be checked by analyzing the tissues of individuals exposed to different levels of metals in the environment (Bryan 1976), or under test conditions of essential metals such as zinc, copper, and manganese (Bryan 1976). For example, increasing the level of zinc in seawater (0.004-0.2 mg/L) had little effect on tissue concentrations of this metal in the lobster *Homarus americanus*. However, at concentrations above 0.2 mg/L tissue levels of zinc increased, implying that

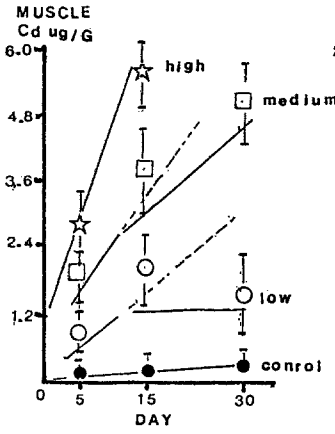
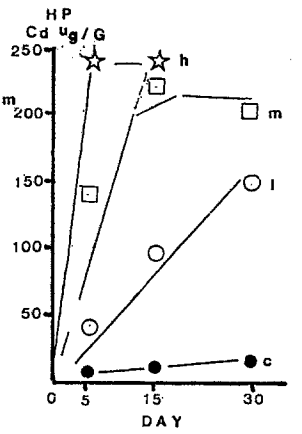


FIGURE 1



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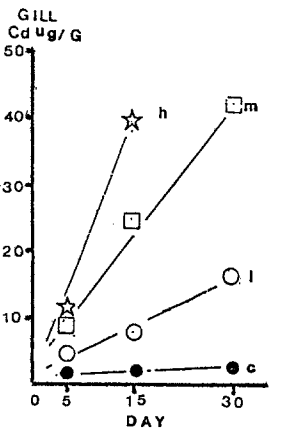


FIGURE 3

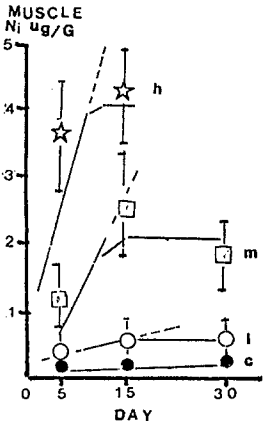
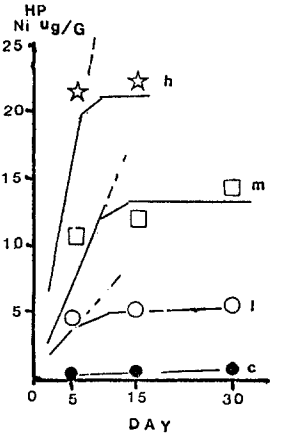


FIGURE 4



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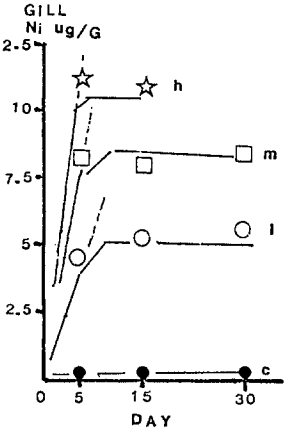

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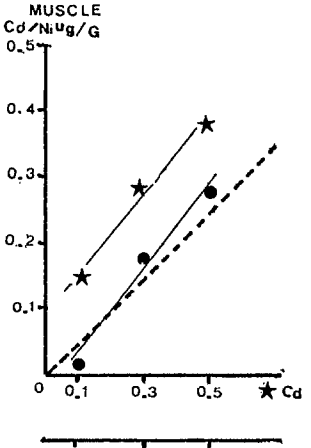
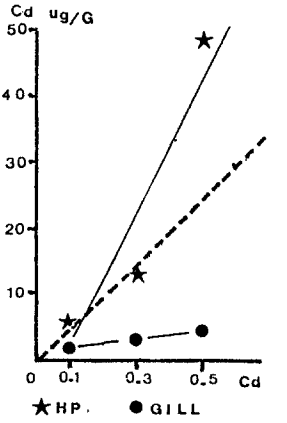


FIGURE 7



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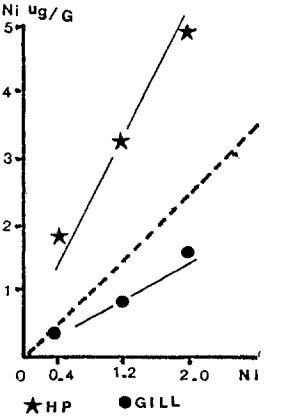

FIGURE 9

Figures 1, 2 and 3.
Accumulation of Cd in the muscle, hepatopancreas, and gills, respectively, exposed to 0(c), 0.1 (l), 0.3 (m), and 0.5 (h) mg/L Cd. The accumulation rates (b) in the muscle were 0.01 (c), 0.14** (l), 0.27** (m), and 0.36** (h) ug/g. In the hepatopancreas, 0.14 (c), 4.44** (l), 14.12 * (m), and 49.33** (h) ug/g. In the gills, 0.04 (c), 1.10** (l), 2.63 (m), and 5.51 (h) ug/g. Significance of the regression line *= 0.05>P>0.01, **= P<0.01. The broken line represent the line of linearity.

Figures 4, 5, and 6.
Accumulation of Ni in the muscle, hepatopancreas and gills, respectively, exposed to 0 (c), 0.4 (l), 1.2 (m) and 2.0 (h) mg/L Ni. The accumulation rate (b) in the muscle were 0.01 (c), 0.03** (l), 0.17* (m), and 0.28** (h) ug/g. In the hepatopancreas, 0.12 (c), 1.92** (l), 3.20** (m), and 4.90** (h) ug/g. Significance of the regression line *= 0.05>P>0.01, **= P<0.01. The broken line represents the line of linearity.

Figure 7.
The relationship between rates of accumulation of Cd(★) and Ni(●) in the muscle and the concentration of metals in seawater. Cd, Y=0.95+ 0.54X** , Ni, Y=-0.03+0.16X**. Significance the regression line **= P<0.01. The broken line represents the line of direct proportionality.

Figure 8.
Relationship between rates of accumulation of Cd in the hepatopancreas (★) and gills (●), and concentration of metal in seawater. Hepatopancreas Y=-11.04+112.23X*, and gills Y=-0.22+11.06X**. Sigificance the regression line *= 0.05>P>0.01, **= P<0.01. The broken line represents the line of direct proportionality.

Figure 9.
Relationship between rates of accumulation of nickel in the hepatopancreas (★) and gills (●) and the concentration of Ni in seawater. Hepatopancreas Y=1.12+1.84X**, and gills Y=-0.16+0.94X**. Significance of the regression line **= P<0.01. The broken line represents the line of direct proportionality.

regulatory processes had broken down (Bryan 1976). Non-essential elements such as cadmium appear poorly regulated by crustaceans (Wright and Brewer 1979; Rainbow 1985).

In this study, a linear relationship was found in all tissues between rates of cadmium and nickel uptake and levels of exposure. For nickel, in all tissues the relationship approximates one of direct proportionality, implying little metabolic control over uptake at the levels tested. A similar trend is apparent for cadmium in muscle tissue. The greater than dirrectly proportional realtionship observed for cadmium in the hepatopancreas may be due to the concentration-dependent induction mechanism responsible for the synthesis of new ligands (e.g. metalothionein), while the less proportional relationship in the gills may be attributed to the presence of a number of binding sites for cadmium adsorption. Such a situation is possible, for example, if the uptake of cadmium by gill tissue is surface-area dependent.

In all reported cases where exposure of an organism has been continued for a sufficient period of time, tissue levels of the accumulated metal invariably plateau (Ward 1982; Evtushenko *et al.* 1986; Watras *et al.* 1986). The attainment of such equilibria possibly represents an increased metal absorption, as suggested by Bryan (1984). In the present study equlibrium was attained for both metals in most tissue implying some regulatory processes are functioning albeit at fairly simple levels.

This study indicated that the accumulation of cadmium in all tissues in prawns was higher than for nickel, despite the lower test concentrations used. This suggested that the cell membranes are far more permeable to cadmium than nickel, which is no doubt, accounts for the much greater toxicity of the former element to these organisms (Denton and Burdon-Jones 1982).

The limited ability of prawns to control either metal against changes in external concentrations indicates that they have potential as bio-indicators of pollution by this elements. It would be of interest, therefore, to determine whether the relationships observed here between rates of metal uptake and ambient levels exist at lower, more realistic concentration gradients.

Acknowledgment. The author is greatful to Prof. R.S.F. Campbell, Dr.G.R.W. Denton and the administration at the Tropical Veterinary Science and Biological Sciences, James Cook University, for their advice and cooperation. This study was supported by James Cook

University-Balitvet colaborative project, sponsored by The Australian International Development Assistance Bureau.

REFERENCES

Brouwer M, Brouwer-Haexum T, and Engel DW (1984) Cadmium acumulation by the blue crab, *Calinectes sapidus*: Involvment of haemocyanin and characterization of cadmium-binding proteins. Mar Environ Res 14:71-88

Bryan GW (1976) Some aspects of heavy metal tolerance in aquatic organisms. In: Lockwood AP(ed) Effects of Pollutants on Aquatic Organisms, Cambridge Press London.

Bryan GW (1984) Pollution due to heavy metals and their compounds. In: Bryan GW (ed) Marine Ecology. Vol 5, Part 3. John Wiley & Son Ltd. London.

Carney GC, Shore P, and Chandra H (1986) The uptake of cadmium from a dietary and soluble source by crustacean *Daphnia magna*. Environ Res 39:290-298

Denton GRW and Burdon-Jones C (1982) The influence of temperature and salinity upon the acute toxicity of heavy metals to the banana prawns (*Penaeus merguiensis* de Mann). Chem Ecol 1:131-143.

Evtushenko ZS, Belcheva NN, and Lukyanova ON (1986) Cadmium accumulation in organs of the scallop *Mizuhopecten yessonensis*-II. Subcellular distribution of metals and metal-binding proteins. Comp Biochem Physiol 83C(2):377-383

Nimmo DWR, Lightner DV and Bahner LH (1977) Effects of cadmium on the shrimps, *Penaeus durorum, Palaemonetes pugio* and *Palaemonetes vulgaris*. In:Vernberg FJ, Calabrese A, Thurberg FP, and Vernberg WB (ed) Physiological Response of Marine Biota to Pollutants. Academic Press Inc London, 131-183

Phillips DJH (1980) Biological indicators: a retrospective summary. in: Quantitative Aquatic Biological Indicators. A pollution monitoring series. Applied Sc Publishers Ltd London.

Rainbow PS (1985) Accumulation of zinc, copper and cadmium by crabs and barnacles. Estuar coast shelf sc 21:669-689

Ray S, Mc Leese DW and Buridge LE (1981) Cadmium and tissues of lobster captured near a lead smelter. Mar Poll Bull 12:383-386

Skwarzec B, Kentzer-Baczeweska A, Styczynska-Jurewicz E and Nevgebauer E (1984) Influence of accumulation of cadmium on the content of other microelements of two species of Blacksea Decapods. Bull Environ Contamin Toxicol 32:93-101

Thurberg FP, Calabrese A, Gould E, Greig RA, Dawson MA, and Tucker RK (1977) Respons of the lobster *Homarus americanus* to sublethal levels of cadmium and mercury. in:Vernberg FJ, Calabrese A, Thurberg FP, and Vernberg WB (ed) Physiological Responses of Marine Biota to Pollutants. Academic Press London. 185-197

Ward TJ (1982) Laboratory study of the accumulation and distribution of cadmium in the Sydney rock oyster *Saccostrea commercialis* (I&R). Aust J Mar Freshw Res 33(1):33-34

Watras CJ Mac Farlane J and Morel FMM (1985) Nickel accumulation by "Scnedesmus" and "Daphnia": Food chain transport and geochemical implications. Can J Fish Aquatic Sc 42:724-730

White SL and Rainbow PS (1982) Regulation and accumulation of copper, zinc and cadmium by the shrimp *Palaemon elegans*. Mar Ecol Prog Series 8:95-101

Wright DA and Brewer CC (1979) Cadmium turnover in the shore crab *Carcinus maenas*. Mar Biol 50:151-156

Received April 25, 1989; accepted October 10, 1989.