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Australian Freshwater Crayfish *Cherax destructor* Accumulates and Depurates Nickel

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In recent years contamination of the environment by metal ions has received significant attention with respect to effects on aquatic ecosystems and human health. Metal pollution in Australia is increasing due to mining and industrial activities and environmental contamination by Ni occurs from weathering of minerals, rocks and anthropogenic sources (Hart 1982). The highest Ni concentration of 34,000 µg/L found in Finniss River (Northern Territory) of Australia, was reported by Jeffree and Williams (1980). Nickel is essential for several animal species (Barceloux, 1999), however it may become toxic when present in high enough concentrations in the environment. Nickel as an environmental pollutant can affect survival, growth, and reproduction of aquatic animals (Besinger and Christensen, 1972; Wong *et al.,* 1993). Nickel can also alter carbohydrate and protein metabolism in several aquatic species (Sreedevi *et al.,* 1992; Ghazaly, 1992). Consumption of nickel-contaminated fish by humans can cause a number of disorders in them. (Sreedevi *et al.*, 1992).

The Australian crayfish *Cherax destructor* commonly known as 'the yabby' is an important aquacultured edible species found in eastern and middle regions of Australia. It has been previously reported that *C. destructor* when exposed to Cd and Pb accumulates these metals rapidly in its body tissues (Nugegoda *et al.,* 2000). Due to the common and widespread distribution of *C. destructor* and its consumption by humans and aquatic animals, metal accumulation by this species could affect the health of both humans and aquatic animals.

Several investigators have reported on trace metal acccumulation in aquatic species including crayfish (Rainbow and White, 1989; Anderson *et al.,* 1997). However there is little work on the depuration of Ni in freshwater crayfish. In this study we examine the accumulation and depuration of Ni in different body tissues of *C. destructor* and evaluate the feasibility of using this species as a biomonitor of Ni pollution in Australian freshwaters.

MATERIALS AND METHODS

Adult inter-moult .*C. destructor* measuring 6-10 cm in length were obtained from the Central Yabby Farm in Heathcote, near Melbourne. Animals were

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acclimated in the laboratory for 14 days at 23-25^{0}C in 300-L tanks and fed a daily diet of trout pellets. A total of 120 animals were used in the experiment, any injured animal was discarded and only active animals used. Crayfish were exposed to a series of dissolved Ni concentrations in the laboratory. Twelve yabbies were exposed to each concentration of Ni in replicate 15-L experimental plastic aquaria with control. To avoid cannibalism each yabby was placed individually in a container of 9-cm length and 6-cm diameter with plastic mesh at the bottom. Prior to experiments the test containers and plastic aquaria were filled with the respective solution of Ni for 24-h for initial adsorption of Ni onto inner tank surfaces. After 24-h the solution was discarded and the experiment begun with fresh nickel solution.

Metal salt used for the preparation of stock solution was $NiSO_4.7H_2O$ (analytical grade). Nickel salt was dissolved in distilled water at 37^{0}C to prepare the stock solution of 50,000 mg/L. The required volume of stock solution was added to the respective experimental plastic aquarium to achieve the desired concentration of 10, 25, 50, and 100 mg/L. Replicate tanks without added Ni were used as control. All tanks were constantly aerated, at a temperature of 23 ± 2^{0}C and a 14h light: 10h dark photoperiod utilised for the duration of the experiment. This was a static renewal- bioassay with water freshly changed every second day to maintain Ni levels at desired concentrations and to remove the waste released by the animal during experiment. Animals were fed trout pellets during the experiment. Feeding was conducted every second day in Ni free water for 20 min. Moults and deaths were recorded daily during the experiment. Dead animals and moults were removed immediately from the experimental tanks and stored at -20^{0}C.

After 21-days six crayfish from each tank were sacrificed by instantaneous freezing while rest of the animals were left in clean water for 2-weeks to depurate. Water was changed every second day during depuration period. Later crayfish were dissected into hepatopancreas, gills, white muscle and exoskeleton for analysis of accumulated Ni. Organs were weighed, freeze dried and weighed again for dry weight measurements. Tissues were then acid digested on dry block heaters as described by White and Rainbow (1982).

Nickel in samples was determined by Flame Atomic Absorption Spectrophotometry (AAS). The standards used to make calibration curve were 1, 5, 10, 15 and 20 mg/L. A three-way analysis of variance was used to compare Ni concentration among replicates, nickel treatments and tissues. As metal accumulation in replicate tanks was not significantly different from each other, samples were pooled and two way Anova was performed followed by Duncan's Multiple range test as post hoc test. Groups were considered significantly different from each other if $p< 0.05$. All analyses were performed using the statistical package, Statistica 6.0 (Statsoft).

RESULTS AND DISCUSSION

Water parameters during the experiment remained constant at dissolved oxygen 6.5-7.0 mg/L, temp 23 ± 2^0C, pH 6.9-7.3 and total hardness 30 mg/L. The percentage of individuals surviving and moulting activity during the 21 days exposure period is shown in Fig-1.
Highest survival was recorded in the control group while 62% animals survived even in the highest Ni treatment of 100 mg/L. Half of the animals died in the 25 mg/L Ni treatment which is probably a result of an accidental shut down of oxygen supply to these treatments on the tenth day.

The mean Ni concentration in body tissues (gill, hepatopancreas and exoskeleton) of *C. destrutor* after 21 days exposure is shown in Fig-2. Ni was accumulated by different body tissues of *C. destructor* during the three-week exposure period. The quantity of Ni adsorbed by the gill tissues was greater than the amount of Ni accumulated in the hepatopancreas and exoskeleton.

There was no detectable Ni accumulation in white muscle after three-weeks of exposure to elevated dissolved Ni concentrations. Mean Ni concentrations in tissues of all exposed groups were significantly different from that of the control, whereas no significant difference was found among Ni in the sample tissues of crayfish from different treatments except in the 10 mg/L exposure (Fig 2), demonstrating no dose-response relationship in Ni accumulation in the tissues.

Duncan's post hoc test showed a significant difference between gill and hepatopancreas ($P< 0.05$), gill and exoskeleton but not between exoskeleton and hepatopancreas. The absence of Ni accumulation in white muscle is not surprising as many studies have shown that white muscle accumulates low concentrations of most metals (AliKhan and Zia, 1989; Anderson *et al.,* 1997 and Naqvi *et al.,* 1998). Whether this is a result of the absence of binding molecules for metal storage in white muscle tissue, remains to be elucidated.

Previous authors have also demonstrated that adsorption of metals by gills is higher as compared to other tissues. Similar results showing gills with the highest Cd and Pb concentrations were reported by Pastor *et al.,* (1988); Meyer *et al.,* (1991) and Anderson *et al.*, (1997). The gills of aquatic animals are in direct contact with the water and play an important role in gas exchange and are therefore the point of entry of dissolved metal into the body of crayfish. In addition the crayfish has an impermeable exoskeleton. Gills have been shown to be the first organ, which exhibits the symptoms of sublethal exposure of heavy metals (Torreblanca *et al.*, 1989; Naqvi *et al.*, 1998).

The hepatopancreas is considered to be storage organ for food, and the detoxification and storage of heavy metals in this organ has been reported by Icey and Nott, (1992). It is presumed that high Ni concentrations in the exoskeleton is due to adsorption since no difference in accumulation in any treatment after the 21 days metal exposure was observed. It could be explained as Ni epithelial cell

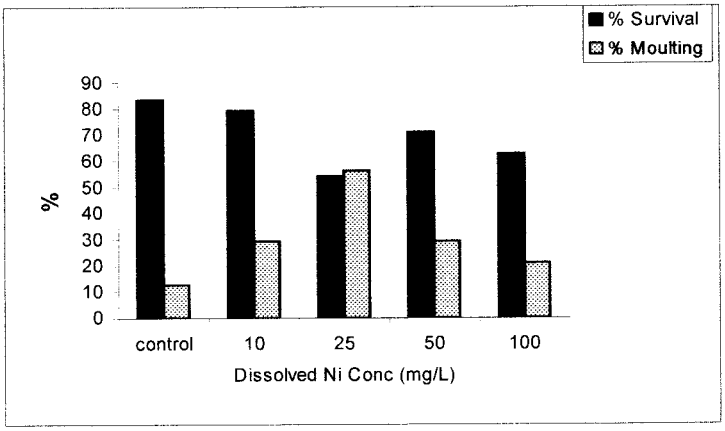

Figure-1. Percentage survival and moulting during the 21-days exposure to Ni in *C. destructor* at 23 ± 2^{0}C.

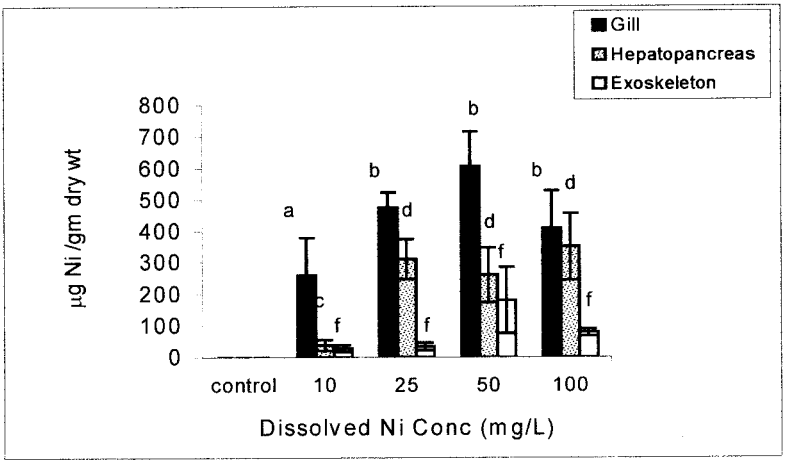

Figure-2. Nickel accumulation in different body tissues of crayfish exposed to different concentrations of nickel for 21days. All values are expressed as the mean ±SEM. Means with superscripts in the same alphabets are not significantly different from each other.

Table 1. Mean concentration (± SE) of Ni (μg/g) in body tissues of *C. destructor* following two weeks depuration (n=7 except 100mg/L where, n=2).

Exposure concentration (mg/L)	Gill		Hepatopancreas		Exoskeleton	
	Ni mg/g dry wt	* % *change*	Ni mg/g dry wt	* % *change*	Ni mg/g dry wt	* % *change*
10	nd♣	*0*	nd♣	*0*	0	*0*
25	6.42 (6.43)	*98.6*	13.44 (12.74)	*95.7*	20.16 (10.24)	*40.86*
50	13.72 (15.12)	*97.7*	7.8 (5.01)	*97.16*	10.46 (5.57)	*94.19*
100	nd♣	*0*	nd♣	*0*	14.35 (14.12)	*81.6*

♣ non detectable.
*Compared with 3 week accumulation shown in *fig-1*

receptors onto the surface of exoskeleton are completely blocked and no more Ni could be transferred to the exoskeleton.

Similar results regarding Pb concentration in the exoskeleton of the American crayfish *P. clarkii* was shown by Anderson *et al.*, (1997). The results do not demonstrate a dose dependent bioaccumulation of Ni in the different body tissues studied in *C. destructor*. One reason of this could be flux-equilibrium conditions in which the rate of accumulation balances the rate of excretion; resulting in no net accumulation of Ni when exposed to the concentrations used. Alternatively the Ni already accumulated in body tissues may have saturated all Ni binding substances in the body tissues and there are no more sites left for further accumulation of Ni. Results also suggest that Ni excretion is not at its maximum in *C. destructor* even in the high concentrations used in this experiment. If this was not the case it could be expected that at least the highest concentration of Ni would become toxic to the crayfish in 21-days. However no increase in mortality was observed in the highest concentration.

Results from the depuration study demonstrates that *C. destructor* has the ability to depurate Ni. The study demonstrates that a significant decrease in tissue Ni concentration occurs when crayfish were transferred from nickel contaminated water to clean water and held for 2-weeks (Table 1). The effective depuration of Ni from the body tissues of *C. destructor* shows that it might have obtained far more Ni than what is actually required and therefore a mechanism for its removal may have been developed. The rapid removal of Ni from tissues of *C. destructor* also explains its temporary adsorption and storage in the tissues. There was 100% survival of crayfish in all treatment for the 2-week depuration period except for those that had been treated with 100 mg/L nickel of which 26% survived. This may be a result of increased stress on the physiology of the crayfish associated

with ionic regulation in the higher Ni treatments.

This study clearly demonstrates that the Australian freshwater crayfish *C. destructor* has the ability to take Ni from surrounding medium in its body tissues. Gill and hepatopancreatic tissue from yabbies could be used to detect the presence of Ni in contaminated waters. However because of the rapid depuration of Ni by the yabby, *C. destructor* should not be used as an organism for the routine biomonitoring of nickel pollution in Australian freshwaters. In addition the lack of nickel accumulation in the white muscle demonstrates that *C. destructor* collected from nickel contaminated waters could be safe for human consumption provided only the white muscle is utilized.

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