Research Article

Acute toxicity of nickel to fresh water prawns

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Abstract: In the present study, the LC_{50} of nickel and its impact on the behaviour of 2 species of freshwater prawns, *Macrobrachium lamarrei* (H. Milne Edwards) and *Macrobrachium dayanum* (Henderson) was evaluated. An inverse relationship between LC_{50} values and exposure duration was obtained. Nickel was found to be 6.33 times more toxic to *M. lamarrei* than to *M. dayanum*. Nickel exposure increased aggression and loss of balance in both species of prawns in a concentration-dependent manner with both parameters being higher in *M. dayanum* (416.47 mg/L) than in *M. lamarrei* (65.77 mg/L). All behavioural parameters decreased with increase in exposure duration in both prawn species, with the exception of grasping behaviour and the number of individuals showing loss of balance.

Key words: Heavy metals, behaviour, aquatic toxicity, crustacean, bioindicator

Introduction

Heavy metals are one of the most important pollutants of surface water (Mason, 1996; Sanders, 1997). Of these, nickel, cobalt, copper, etc. are essential for the growth and maintenance of organisms in small quantities but are highly toxic in higher quantities (Muyssen et al., 2004 and references therein). The potential to be toxic increases many time due to their high solubility, bioaccumulation, and biomagnification (Guthrie et al., 1979; Goodyear and McNeill, 1999; Larter et al., 2010; Cui et al., 2011), thereby affecting various physiological, biochemical, and cellular processes of aquatic organisms.

Nickel, a grey-listed metal (Mason, 1996), is a minor essential element for several animal species (Barceloux, 1999; Phipps et al., 2002), and when present in excess, it affects the behaviour, survival, growth, and reproduction of aquatic animals (Wong et al., 1993). It also causes a number of disorders in human beings (Sreedevi et al., 1992), although its effects owing to bioaccumulation in higher trophic levels in the terrestrial ecosystem are considered unlikely.

While the toxic effects of nickel were extensively assessed in fish (Pane et al., 2003a, 2003b, 2004a, 2004b, 2005; Brix et al., 2004), information on aquatic invertebrates is quite limited (Sreedevi et al., 1992; Martinez-Tabche et al., 1999; Rathore and Khangarot, 2002). Of the many aquatic invertebrates, crustaceans have an important role in aquatic food chains, and these organisms can be used as bioindicators of pollution in aquatic bodies (Vijayraman and Geraldine, 1996). However, their toxicological responses to different heavy metals are less studied (Kabila et al., 1999; Hunt et al., 2002; Pane et al., 2003b).

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Thus, the present study aimed to evaluate the LC_{50} of nickel and the effect of this heavy metal on the behaviour of 2 fresh water prawn species, *Macrobrachium lamarrei* (H. Milne Edwards) and *Macrobrachium dayanum* (Henderson). The 2 species were selected in view of their economical, medicinal, ecological, and academic importance.

Materials and methods

Animal collection and maintenance

Fresh water prawns, *M. lamarrei* and *M. dayanum*, were collected from River Gomti in Lucknow, India, and brought to the laboratory and maintained in glass aquaria (20 L) containing dechlorinated water with the following physicochemical characteristics: pH, 7.3 ± 0.2; dissolved oxygen, 7.0 ± 0.1 mg/L; total hardness, 268.0 ± 2.5 mg/L; and temperature, 26.0 ± 2.0 °C.

Test chemical

A stock solution of nickel chloride ($NiCl_2.6H_2O$; analytical reagent grade; molecular weight, 237.71; Sarabhai Chemicals, Baroda, India) was prepared by dissolving 100 mg of the compound in 100 mL of double distilled water.

Bioassay

Static bioassay tests were carried out according to standard methods (APHA et al., 1998) on animals that had been acclimated to the laboratory for 5-7 days. Two different sets of 5 concentrations of nickel (toxic range earlier determined by exploratory tests to be 50, 65, 70, 75, and 80 mg/L for *M. lamarrei* and 250, 350, 450, and 500 mg/L for *M. dayanum*) were prepared in 10 L of dechlorinated water for both the test species. Ten healthy animals of both species (*M. lamarrei* and *M. dayanum*) of intermolt stage and of average size (4.88 ± 0.54 cm and 5.64 ± 0.42 cm, respectively) and weight (1.11 ± 0.27 g and 3.26 ± 0.68 g, respectively) were carefully introduced in experimental as well as control aquaria from the stock with the help of a hand net. Feeding was suspended throughout the experiment, which lasted 96 h. Continuous air supply was provided by air pumps to all aquaria.

Mortality was carefully recorded in all aquaria. Behavioural responses, namely hyperactivity (surfacing and horizontal crawling movements),

scraping movement (movement of cheliped in branchiostegite and over general body region), compact aggregation or schooling (number of animal groups present in an area at a specific time), fighting encounters (animals gripping others by cheliped and pulling), and grasping (catching and holding ability of inserted objects such as a glass rod), were observed. Observations were taken at 3-h intervals between 0700 and 2200 hours with each observation lasting for 5 min. Each of the behaviours was quantified by only one independent investigator, who was blind to the animals' status, to minimise interobserver variations. All experiments were replicated 3 times.

Parameters observed and statistical analysis

Mortality data were subjected to statistical analysis to obtain LC_{50} values and their confidence limits, following the trimmed Spearman-Karber method (Hamilton et al., 1977). The data on behavioural observations were subjected to one-way ANOVA followed by Tukey's comparison of means (Minitab, 2003).

Results and discussion

The LC_{50} values and 95% confidence limits of $NiCl_2$ for 24, 48, 72, and 96 h for *M. lamarrei* and for *M. dayanum* are given in Table 1. On the basis of the 96-h LC_{50} values for both species, nickel chloride is 6.33 times more toxic to *M. lamarrei* than *M. dayanum*. The values for both species were higher than those known for other aquatic organisms with the exception of *Mysidopsis bigelowi* (EPA, 1980; Table 2). The values were found to have an inverse relationship with the exposure duration. The differences in LC_{50} values of nickel chloride among both species of prawns may be attributed to *M. dayanum* being stouter with a thicker cuticle than *M. lamarrei*. In addition, the difference in living habits might be an issue; *M. dayanum* being a relatively deep dweller may reduce its exposure to nickel.

Over the duration of 96 h of exposure to nickel chloride, overall statistically significant changes in almost all behavioural patterns were observed in both *M. lamarrei* and *M. dayanum*, with the exception of aggression in the former and schooling in the latter.

After 24 h of acute exposure to nickel chloride, significantly increased hyperactivity in terms of

Table 1. LC_{50} values and their 95% confidence limits for nickel chloride in fresh water prawns, *Macrobrachium lamarrei* and *Macrobrachium dayanum*.

Experimental animals	Exposure time (h)	LC_{50} (mg/L)	95% confidence limit (mg/L)	
			Lower	Upper
Macrobrachium lamarrei	24	77.46	70.09	85.60
	48	72.46	65.11	80.63
	72	68.26	60.33	77.24
	96	65.77	60.29	71.50
Macrobrachium dayanum	24	487.00	445.21	532.71
	48	474.34	402.91	558.44
	72	446.97	383.36	521.12
	96	416.47	365.33	474.76

Table 2. LC_{50} values for nickel chloride in various aquatic invertebrates, including present report.

S. no.	Animal	Time duration	LC_{50} value	Reference
1	*Ceriodaphnia dubia*		13 mg/L	Schubauer-Berigan et al. (1993)
2	*Ceriodaphnia dubia*	48 h	200 µg/L	Schubauer-Berigan et al. (1993)
3	*Haliotis rufescens*		145.5 µg/L	Hunt et al. (2002)
4	*M. lamarrei*		65.77 mg/L	Present study
5	*M. dayanum*		416.47 mg/L	Present study
6	*Gammarus* sp.		13.0 mg/L	EPA (1980)
7	*Mysidopsis bigelowi*		510-640 mg/L	EPA (1980)
8	*Mysidopsis formosa*	96 h	150 mg/L	EPA (1980)
9	*Hyalella azteca*		890 µg/L	Schubauer-Berigan et al. (1993)
10	*Hyalella azteca*		2.0 mg/L	Schubauer-Berigan et al. (1993)
11	*Atherinops affini*		26,560 µg	Hunt et al. (2002)
12	*Mysidopsis intii*		148.6 µg/L	Hunt et al. (2002)

surfacing and horizontal movements, scraping movements, schooling, and aggression displays were observed in comparison to controls in both prawn species. Scraping of body parts with chelate legs was particularly prominent in the gill region in both species. Aggregation of animals at corners of aquaria was observed in *M. lamarrei*, whereas aggregation was near air diffusers in *M. dayanum*.

Increased aggressive behaviour in the form of fighting encounters following exposure to acute concentrations was also noticed in both species.

At 48 h of exposure, surfacing as well as horizontal movements decreased in both species. In *M. lamarrei*, other behaviours increased significantly, accompanied by increased whitening of abdominal muscles and mucous depositions on the gills and
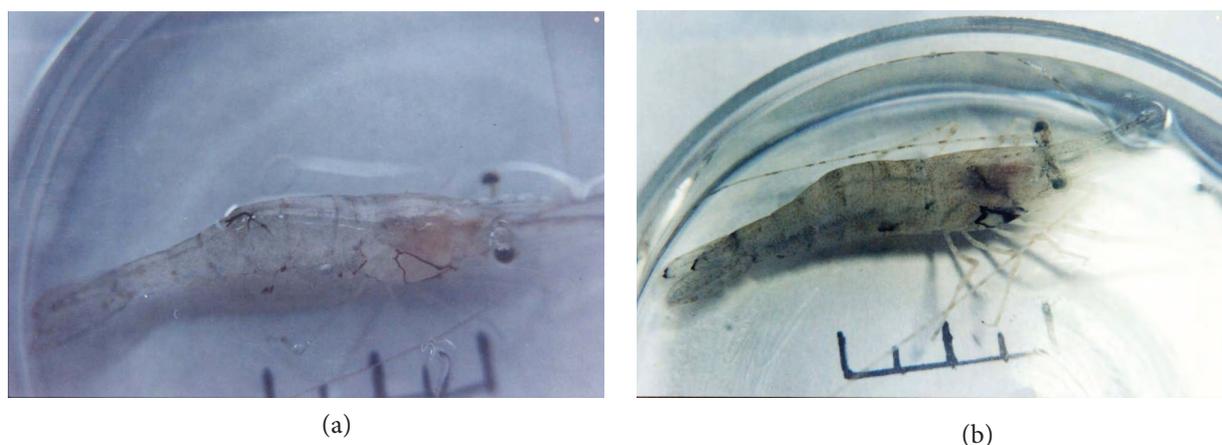
(a)

(b)

Figure 1. Photomicrographs showing blackening in (a) *M. lamarrei* and (b) *M. dayanum* after 96 h of acute exposure (65.77 mg/L and 416.47 mg/L, respectively) to nickel chloride.

abdomen, and they became bottom dwellers. Observed aggregations were less compact in *M. lamarrei*. In *M. dayanum* aggression, body balance and grasping increased, albeit not significantly, in comparison to that at 24 h.

After 72 h of exposure, both species showed decreased surfacing and horizontal movements, schooling, aggression, and increased grasping movements, and more individuals showed loss of body balance. Damaged II chelate legs (due to initial aggression), rostrum, and sensory feelers and enhanced mucous secretion and black demarcations on serrated margins of uropods were observed in *M. lamarrei*. In *M. dayanum*, jerky movements, further reduced grasping power, and slight black depositions on rostral teeth were noticed.

During the last phase (96 h) of the experiment, all body activities were nearly ceased. Complete loss of body balance during walking and increased blackening over gills and other parts of the body (Figures 1a and 1b), along with enhanced mucous secretion and feeble response to gentle paddling, were found in individuals of both species, while softening of the exoskeleton and enhanced whitening in the abdominal muscle was noticed in *M. lamarrei*.

Behavioural alterations in an individual indicate the presence of environmental stressors (Clotfelter et al., 2004; Zala and Penn, 2004). Crustacean behaviour in relation to metal toxicity has not been investigated. The initial increased hyperactivity in terms of both

surfacing and horizontal movements in both prawn species may be a form of avoidance behaviour to the toxicant (Tables 3 and 4). The other probable reasons could be narcotic effects (Svecevičius, 2005), change in the sensitivity of chemoreceptors (Sutlerlin, 1974; Hoarau, 1976), and/or the blocking effect of the receptor membrane or enzymatic component (Devi and Fingerman, 1995).

Such behavioural avoidance of irritating substances is well recorded in fish for heavy metals such as lead, nickel, zinc, and mercury (Black and Binge, 1989; Santhakumar and Balaji, 2000; Svecevičius, 2001, 2005; Vincent et al., 2002; Prashanth et al., 2005) and in crustaceans (Spear, 1981; Brown, 1982; Verma et al., 2005). Sornaraj et al. (1995) reported similar behavioural responses in spotted snakehead, *Channa punctatus*, after chromium, nickel, and zinc exposure and in Asian stinging catfish, *Heteropneustes fossilis*, after mercury exposure (Sindal et al., 2004). Crustaceans have also shown behavioural alterations in similar patterns as in fish (Murti and Shukla, 1984; Sharma and Shukla, 1990).

Increased scraping of body parts, particularly the gill region by I and II chelate legs, may be a reaction to remove the deposited toxicant and irritation caused by toxicants on body surface (Murti et al., 1983, Murti and Shukla, 1984; Black and Binge, 1989; Sharma and Shukla, 1990; Sornaraj et al., 1995). The scraping of body parts to clean the "aquatic dirt" deposited on the body surface, a common behavioural pattern

Table 3. Alteration in behavioural responses after acute exposure (65.77 mg/L) to nickel chloride in freshwater prawn, *Macrobrachium lamarrei*.

| Group | Exposure duration (h) | Hyperactivity | | Scraping (movements/ min) | Schooling (individuals/ school) | Aggression (movements/ min) | Loss of balance (no. of individuals) | Grasping (movements/ min) |
		Surfacing (movements/ min)	Horizontal (movements/ min)					
Control	24	2.80 ± 0.37	13.80 ± 1.77	0.40 ± 0.24	0.40 ± 0.24	0.80 ± 0.37	-	-
	48	1.40 ± 0.2	9.80 ± 0.66	0.80 ± 0.20	0.60 ± 0.24	1.00 ± 0.32	-	-
	72	1.40 ± 0.24	11.00 ± 1.30	2.00 ± 0.55	1.00 ± 0.32	1.40 ± 0.24	-	-
	96	2.60 ± 0.40	12.40 ± 1.36	2.20 ± 0.66	2.20 ± 0.58	0.80 ± 0.20	-	-
F-value		**5.46***	**NS**	**NS**	**4.64****	**NS**	-	-
Experimental	24	7.00 ± 0.71	64.40 ± 3.85	10.20 ± 0.66	1.80 ± 0.37	1.60 ± 0.51	-	-
	48	4.80 ± 0.86	41.00 ± 3.07	16.80 ± 1.46	3.20 ± 0.37	1.80 ± 0.37	-	-
	72	1.60 ± 0.24	15.60 ± 1.99	14.40 ± 1.17	1.20 ± 0.37	1.40 ± 0.51	0.20 ± 0.20	1.20 ± 0.37
	96	1.00 ± 0.32	4.60 ± 1.08	2.20 ± 0.58	1.60 ± 0.40	1.20 ± 0.37	4.60 ± 0.60	4.40 ± 0.51
F-value		**22.63***	**97.53***	**38.39***	**5.22****	**NS**	**51.47***	**43.20***

*, **, and *** indicate F-values to be statistically significant at P < 0.05, P < 0.01, and P < 0.001, respectively.
NS indicates F-values to be nonsignificant (P ≥ 0.05).

Table 4. Alteration in behavioural responses after acute exposure (416.47 mg/L) to nickel chloride in freshwater prawn, *Macrobrachium dayanum*.

| Group | Exposure duration (h) | Hyperactivity | | Scraping (movements/ min) | Schooling (individuals/ school) | Aggression (movements/ min) | Loss of balance (no. of individuals) | Grasping (movements/ min) |
		Surfacing (movements/ min)	Horizontal (movements/ min)					
Control	24	2.00 ± 0.46	11.60 ± 1.36	0.20 ± 0.20	0.60 ± 0.24	0.60 ± 0.40	-	-
	48	1.40 ± 0.24	9.80 ± 0.66	1.20 ± 0.37	1.20 ± 0.37	0.80 ± 0.37	-	-
	72	1.00 ± 0.32	9.00 ± 0.89	1.40 ± 0.24	0.80 ± 0.37	1.20 ± 0.20	-	-
	96	0.60 ± 0.24	6.40 ± 0.93	1.20 ± 0.37	1.20 ± 0.49	1.00 ± 0.32	-	-
F-value		**3.40***	**4.71****	**3.09***	**NS**	**NS**	**NS**	**NS**
Experimental	24	5.80 ± 0.80	42.40 ± 2.50	10.60 ± 0.87	2.20 ± 0.58	2.40 ± 0.24	-	-
	48	3.80 ± 0.66	22.00 ± 2.19	15.60 ± 1.08	3.20 ± 0.37	2.80 ± 0.37	0.40 ± 0.37	0.40 ± 0.20
	72	1.00 ± 0.32	9.80 ± 0.66	10.80 ± 1.07	2.00 ± 0.55	1.20 ± 0.37	2.00 ± 0.71	2.00 ± 0.55
	96	0.60 ± 0.24	2.00 ± 0.71	3.00 ± 0.54	3.00 ± 0.45	0.40 ± 0.24	6.20 ± 0.66	5.80 ± 0.49
F-value		**19.44***	**103.35***	**33.28***	**NS**	**12.13***	**32.15***	**49.84***

*, **, and *** indicate F-values to be statistically significant at P < 0.05, P < 0.01, and P < 0.001, respectively.
NS indicates F-values to be nonsignificant (P ≥ 0.05).

commonly known as "grooming behaviour", is widely studied in crustaceans (Felgenhauer and Schram, 1979; Schram, 1986).

The increased aggression observed in the present study may be due to alteration in synaptic transmission. Studies have shown that heavy metals such as mercury, cadmium, lead, aluminium, nickel, and tin affect chemical synaptic transmission in the brain and peripheral systems (Annau and Cuomo, 1988; Casdorph, 1995). They have also been found to disrupt brain and cellular calcium levels, thereby significantly affecting many body functions in 2 ways: by affecting cognitive development and degenerative CNS diseases, and by resulting in depressed levels of serotonin, norepinephrine, and acetylcholine, causing mood and motivational changes (Smith et al., 1989; Devinsky et al., 1992; Goyer, 1997). Low serotonin levels, abnormal glucose levels tolerance (hypoglycaemia), low foliate levels, and low chromium have been found to be associated with affective disorders, impulsiveness, and violent behaviour in aquatic organisms (Salzer, 1966; Master et al., 1998; Walsh, 2000). Serotonin systems are also implicated as key physiological mechanisms in the control of agnostic behaviour and social dominance in species ranging from ants (Kostowski et al., 1975) to humans (Lesch and Merschdrof, 2000). Serotonin is also known to play an important role in controlling aggression in clawed crustacean species (Antonsen and Paul, 1997; Doernberg et al., 2001; Panksepp et al., 2003). Heavy metals are known to alter serotonin levels as well as cellular permeability (Almeida et al., 2003). Thus, these factors could be responsible for the aggressive displays seen particularly in *M. dayanum* upon exposure to nickel, since such behaviour was less prominently displayed in *M. lamarrei*.

The loss of balance in swimming and walking observed in both prawn species with increasing metal concentration may be due to impairments either in nervous tissues or in muscle fibres (Ellgaard et al., 1995; Santhakumar and Balaji, 2000; Verma et al., 2005). Alterations in enzyme levels like acetylcholinesterase after metal exposure have been observed in crustaceans and could be a reason for improper impulse transmission resulting in loss of coordination (Devi and Fingerman, 1995). Changes

in blood cortisol and glucose levels (Scherer, 1992; Svecevičius, 2005) owing to heavy metal intake may also result in improper coordination.

Mucous secretion by the gills, carapace, and whole body surface as observed in the present study is a common response to toxicants or irritants and may be a protective measure for inhibiting uptake of chemicals. Heavy metals are usually trapped in the mucous layer, which is made up of barrier glycoproteins (Plonka and Neff, 1969; McKone et al., 1971; Coombs et al., 1972; Lock and Van Overbeek, 1981). Excessive mucous may, however, result in death due to suffocation as a result of coagulation-precipitation with metals on gill surface.

The metal ions have toxic effects on the epidermal tissues of gills and the general body surface. Damage to these tissues may result in a route of entry for chitinoclastic bacteria, which are an opportunistic pathogen of prawn causing cell death in gill tissue, which in turn results in "shell disease" (Cook and Lofton, 1973; Young and Pearce, 1975). Metallic action might also be a reason for gill blackening in crustaceans (Nimmo et al., 1977), while Ghate (1984) opined that melanin deposition causes gill blackening in prawns. The possibility of gill blackening in crustaceans due to metallic sulphides also cannot be ruled out.

The results of this study clearly indicate that such behavioural parameters can be used as effective biomarkers to assess the worsening of aquatic bodies as well as the health status of these economically important animals, as they are sensitive parameters employed by the organisms as the first line of evasive action and defence against any toxicant. Furthermore, the study clearly indicates the effect of increased duration of exposure to nickel, indicating its possible long-term consequences. However, the precise mechanism of such behaviour needs much confirmation and precise neurophysiological research.

Acknowledgement

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