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Immunotoxic effects of nickel in the mud crab *Scylla serrata*

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Abstract The presence of xenobiotic contaminants especially metals in coastal waters is a major concern as they are immunotoxic to aquatic animals even at low concentrations. In our present study, mud crab *Scylla serrata* was exposed to three sublethal concentrations (0.4, 0.6 and 0.8 mg/L) of nickel for 30 days under laboratory conditions and the alterations of hematological parameters like haemocyte count, clotting time, haemocyte viability, protein content and immunomodulatory components like phenoloxidase, phagocytosis and superoxide anion generation were measured. In addition, the accumulation patterns of nickel were measured in gills, hepatopancreas and ovary. The accumulation was more in gills when compared to hepatopancreas and ovary of crabs exposed to nickel and was not detected in the control crabs. The results revealed a significant ($P < 0.05$) induction of superoxide anion generation and phagocytosis activity in the haemolymph of the crabs exposed to nickel when compared to control. On the contrary, the rest of the parameters were significantly ($P < 0.05$) reduced in the experimental groups when compared to the control. All the studied parameters exhibited a concentration dependent response.

Introduction

During the last decade, interest has increased regarding the use of biomarkers in the field of aquatic ecotoxicology. The utility of the immunological biomarker approach is based on the fact that sublethal toxicant levels cause immune responses within individual organisms before those effects are observed at higher levels of biological organization. Immunological biomarkers of exposure, effect or susceptibility are complementary and important to understand the overall health impact of toxicants. Xenobiotic contaminants present in the coastal waters influence the increase of disease incidence by adversely affecting immunity, thereby enhancing susceptibility to infection and stress [1].

Estuarine ecosystems are known to contain high concentrations of various metal contaminants which are derived from land-based anthropogenic activities. Nickel is often found in the coastal environment as a result of industrial

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discharges from electroplating, smelting, mining and refining operations, and other industrial emissions [2]. Relative to other divalent metals, nickel has not been well studied in terms of toxicity to different species and mode of action.

Mud crabs are common sentinels used in invertebrate ecotoxicity studies; however, studies are scanty for deriving immunotoxicological biomarkers particularly in this species. Among the mud crabs, *Scylla serrata* serves as common sentinels in invertebrate ecotoxicology studies due to the following reasons: (1) their wide distribution in the estuarine environment which they rely on as a nursery area for feeding, growth and development, (2) they are semipelagic in nature, (3) sensitive to pollutants, (4) abundantly available all year round minimizing the difficulties involved in locating and sampling in the field, (5) can be acclimatized to laboratory conditions easily, and (6) have a significant role as a commercial fishery due their nutritive value [3].

In animals, phagocytosis (PC) plays an important role in defense mechanisms. As the first line of internal defense against invaders, any modulation of the activity of phagocytic cells by a toxicant can result in an impairment of the animal's ability to cope with pathogenic infections [4]. Phenoloxidase (PO) is the terminal enzyme of the prophenoloxidase cascade which uses oxygen as a proton acceptor for converting phenols into a variety of end products, including quinones that spontaneously rearrange into the end product melanin. Phenoloxidase is activated in situ by an endogenous serine protease and its activity is controlled by proteinase inhibitors [5]. Although oxygen is an essential element for aerobic cells, it also causes potential cytotoxic problems due to the generation of highly reactive oxygen species O_2^- as a result of xenobiotic metabolism and toxic manifestations produced by harmful microorganisms [6]. Immunomodulation studies can thus be used as potential signatures of environmental stressors integrating a whole battery of markers, thereby providing early warning signals [7].

In crustaceans, haemocytes circulating in the haemolymph represent an important component of the immune system [8]. These cells are capable of executing several immune functions like phagocytosis, protection against cytotoxicity exerted by reactive oxygen intermediates etc. Apart from these, they serve as a vehicle during vitellogenesis by transporting the yolk protein (vitellogenin) to the ovary [9]. As far as crabs are concerned, haemocyte function may represent a useful biological marker to study immunomodulatory effects of aquatic contaminants.

It is well documented that the immune system of aquatic animals is quite susceptible to metal pollutants [10—13]. Though many studies are available regarding the toxic effect of nickel on the survival of crustaceans, there is a paucity of information regarding the immunotoxicity of nickel in the mud crab *S. serrata*. Therefore, the present investigation deals with the effect of nickel on the immunotoxicological biomarkers of the estuarine edible crab *S. serrata*.

Materials and methods

Animals

Intermolt female *S. serrata* weighing 85 ± 10 g were collected from the Muttukadu brackish water regions, Kovalam, near Chennai, Tamil Nadu, India. Crabs were acclimated to laboratory conditions with temperature 28 ± 1 °C, seawater pH 8.0 and a photoperiod of 12 L:12 D for one week.

Chemicals

Nickel (II) chloride hexahydrate (purity 97%) was obtained from Fisher Scientific (Pittsburgh, PA, USA) and all other chemicals used for the biochemical studies were of analytical grade procured from the local vendors.

Nickel bioassay test

Acute toxicity (96 h) study was carried out to determine the lethal (LC_{100}), median lethal (LC_{50}) and sublethal (LC_0) levels of nickel to *S. serrata* by the static renewal method. The physico-chemical parameters of the test water were measured by following the protocols described in Ref. [14] and are presented in Table 1. Stock solution of nickel was prepared at 1 part per thousand (PPT) (nickel as nickel (II) chloride hexahydrate) using ultra pure Milli Q water. From this stock solution, the following concentrations viz. 0.4, 0.8, 1.2, 1.6, 2, 2.4, 2.8, 3.2, 3.6 and 4 mg/L were prepared to test the toxic effect of nickel on crabs. The concentration of the nickel stock solution was measured in an atomic absorption spectrophotometer

Table 1 Physico-chemical parameters of the test medium.

Environmental variables	Values
Temperature (°C)	28.0 ± 1.0
pH	8.3 ± 0.07
Oxygen	6.2 ± 0.08
Alkalinity as '$CaCO_3$'	142
Calcium hardness as 'Ca'	1984
Magnesium hardness as 'Mg'	845
Sodium as 'Na'	816
Potassium as 'K'	364
Iron total as 'Fe'	0.15
Manganese as 'Mn'	Nil
Free ammonia as 'NH_3'	6.78
Nitrite as 'NO_2'	0.81
Nitrate as 'NO_3'	8.24
Chloride as 'Cl'	32,148
Fluoride as 'F'	0.38
Sulphate as 'SO_4'	2742
Phosphate as 'PO_4'	2.18
Silica as 'SiO_2'	3.98
Chemical oxygen demand	59.14
Biological oxygen demand	20
Copper as 'Cu'	BDL
Mercury as 'Hg'	BDL
Nickel as 'Ni'	BDL
Total 'PHC'	BDL

Results are expressed as mg/L for the parameters except for temperature and pH.
BDL: Below detectable limit. Only temperature, pH and DO were measured daily and the mean standard deviation ($n = 30$) is presented. Other variables were measured at the onset of the experiment.