Sensitivity of Freshwater Organisms to Nickel

M. Shuhaimi-Othman, N. Yakub, and N.A. Ramle

***Abstract*—**The sensitivity of freshwater organisms to nickel (Ni) has been determined in a laboratory. Acute toxicity tests were performed on eight different freshwater species, namely *Macrobrachium lanchesteri* (prawn), *Poecilia reticulata* and *Rasbora sumatrana* (fish), *Melanoides tuberculata* (snail), *Stenocypris major* (ostracod), *Chironomus javanus* (midge larvae), *Nais elinguis* (annelid) and *Duttaphrynus melanostictus* (tadpole). The purpose of this study was to determine the acute toxicity and sensitivity of freshwater organisms to Ni. Results showed that 96h-LC_{50} values of Ni for *M. lanchesteri*, *P. reticulata*, *R. sumatrana*, *M. tuberculata*, *S. major*, *C. javanus*, *N. elinguis* and *D. melanostictus* were 8.05, 15.62, 0.83, 8.46, 19.74, 5.32, 0.64 and 8.8 mg/L, respectively. Results indicated that *N. elinguis* was the most sensitive organisms to Ni, and *S. major* was the most resistant.

***Keywords*—**Acute, freshwater, heavy metals, sensitivity, soft water, toxicity.

I. Introduction

LIVING organisms constitute a vast diversity of taxonomy, life history, physiology, morphology, behavior, and geographical distribution. Thus different species have different sensitivities to compounds. Sensitivity is the ability of an organism to respond and react to external stimuli. In the field, a multitude of species can be exposed to numerous toxicants. Therefore, to predict the effects of toxicants and to understand changes in species composition within communities, it is desirable to know how sensitive individual species are to specific toxicants [1]. Aquatic ecosystems consist of diverse networks of interacting species, each with unique characteristics and habits. This biological diversity poses a major challenge in terms of ecological risk assessment, since each species will respond differently to similar levels of exposure to toxic substances. Therefore, in order to predict the negative effects of such substances on these species, it is necessary to gain a greater understanding of the biological and ecological factors underpinning these sensitivities. Data on species sensitivity can be used to derive a value (concentration) which is sufficiently low to be adequately protective of ecosystem structure and function or, in the case of risk assessment, to describe the fraction of the aquatic community which might be impacted by a given exposure concentration [2].

Nickel is a naturally occurring element and an anthropogenic contaminant in aquatic and terrestrial environments. Nickel compounds are important in modern industry. The sources of environmental contamination are the production, processing, and recycling of various Ni products, including stainless steel, electroplating, pigments, and ceramics. Several toxic effects of Ni are known, including allergies, carcinogenesis, and cardiovascular and renal disorders in higher animals, including humans. Nickel causes respiratory disorder, Mg^{2+} antagonism, and kidney lesions in fish and other aquatic animals. However, Ni is essential to aquatic organisms especially for plants and bacteria [3], [4]. Macroinvertebrate and fish as a test organisms in toxicity tests has several valuable characteristics such as its widespread distribution and common occurrence in freshwater, its ecological importance and ease of handling during testing, as well as its rapid growth, short life cycle and sensitivity to contaminants [5], [6]. Therefore, these organisms have the potential to act as a bioindicator of heavy metals pollution in an aquatic environment and as organisms for toxicity testing.

The purpose of this study was to determine the acute toxicity of Ni to eight local freshwater organisms and to compare the sensitivity between them.

II. Materials and Methods

A. Organisms and test chemicals

In this study eight local freshwater organisms were used in toxicity testing, namely a prawn *Macrobrachium lanchesteri*, two fish *Poecilia reticulata* (guppy, family Poecilidae) and *Rasbora sumatrana* (family Cyprinidae), a snail (Gastropoda) *Melanoides tuberculata* (family Thiaridae), an ostracod *Stenocypris major*, a midge larvae *Chironomus javanus* (Diptera, Chironomidae), an annelid *Nais elinguis* and a tadpole *Duttaphrynus melanosticrus*. *M. lanchesteri* and *R. sumatrana* were obtained from local pet stores. *P. reticulata*, *D. melanostictus* and *M. tuberculata* were collected from the field. *N. elinguis*, *S. major* and *C. javanus* were collected from a fish pond filter system. Species chosen are representative of a range of taxa, exhibit a wide distribution, and are abundant and easy to collect. Prior to toxicity testing, the organisms were acclimatized for one week under laboratory conditions (28-30 ^{o}C with 12h light: 12h darkness) in 20-L stocking tanks using dechlorinated tap water (filtered by several layers of sand and activated carbon; T.C. Sediment Filter® (TK Multitrade, Seri Kembangan, Malaysia)) and aerated through an air stone. During acclimation the organisms were fed with commercial fish food Tetramin® (Tetrawerke, Germany). The

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standard stock solution (100 mg/L) of Ni was prepared from analytical grade metallic salts of $NiSO_4.6H_2O$ (Merck, Darmstadt, Germany). The stock solutions were prepared with deionized water in 1-L volumetric.

B. Acute toxicity test

Acute Ni toxicity experiments were performed for a four-day period (96-h) using adult animals (prawn, fish, ostracod, worm, and snail) or larvae (fourth instar midge larvae and tadpole). Following a range finding test, five Ni concentrations were chosen. Metal solutions were prepared through the dilution of a stock solution with dechlorinated tap water. A control with dechlorinated tap water only was also used. The tests were carried out under static conditions with renewal of the solution every two days. Control and metal treated groups each consisted of two to four replicates of five randomly allocated organisms. No significant stress was observed for the organisms in the solution indicated by 95-100% survival for the organism in the control water until the end of the study. For each species, a total of 10 to 20 animals per treatment (concentrations) were used in the experiment. Samples of water for metal analysis taken before and immediately after each solution renewal were acidified to 1% with ARISTAR® nitric acid (65%) (BDH Inc, VWR International Ltd., England) before metal analysis by flame or furnace Atomic Absorption Spectrophotometer (AAS – Perkin Elmer (Massachusetts, USA), model Analyst 800) depending on the concentrations. To avoid possible contamination, all glassware and equipment used was acid-washed (20% HNO_3; Dongbu Hitek Co. Ltd., Seoul, Korea, 68%) and the accuracy of the analyses were checked against blanks. Procedural blanks and quality control samples made from standard solution for Ni (Spectrosol, BDH, England) were analyzed in every ten samples in order to assess the accuracy of samples. Percentage recoveries for metals analyses were between 90-105%.

During the toxicity test, organisms were not fed. The experiments were performed at room temperature of 28-30 ^oC with photoperiod 12h light : 12h darkness, using fluorescent lights (334-376 lux). Water quality parameters (pH, conductivity, and dissolved oxygen) were measured every two days using portable meters (model Hydrolab Quanta®, Hach, Loveland, USA) and water hardness samples were fixed with ARISTAR® nitric acid and measured using a flame atomic absorption spectrophotometer (AAS– Perkin Elmer Analyst 800). Mortality was recorded every 3 to 4 hours for the first two days and then at 12 to 24 hour intervals throughout the test period. Any dead animals were removed immediately.

Median lethal concentrations (LC_{50}) for the animals exposed to Pb were calculated using measured metal concentrations. FORTRAN programs based on the methods of Litchfield [7] and Litchfield and Wilcoxon [8] were used to compute the LC_{50}.

III. Results And Discussion

The mean water quality parameters measured during the test were pH 6.68 ± 0.2, conductivity 180.3 ± 4.6 µS/cm, dissolved oxygen 6.25 ± 0.3 mg/L and total hardness (Mg^{2+} and Ca^{2+}) 18.72 ± 1.72 mg/L as $CaCO_3$. Table 1 summarizes acute data for eight macroinvertebrate and fish species to Ni.

This study showed that the worm, *N. elinguis* and fish, *R. sumatrana* were the most susceptible species to Ni (Fig. 1). Although it is in fact known that the worm to be relatively tolerant to organic pollution and was reported to be the dominant worm in the activated sludge tank [9] and sewage filter beds [10]. Chapman et al. [11] suggested that metal tolerances in aquatic oligochaetes were species-specific and worm tolerance to Cd and Hg were the reverse of sewage sludge tolerances. Similar results were also reported by William et al. [12] with mayfly, *Baetis rhodani* which was relatively tolerant to organic pollution, but proved to be the species most sensitive to cadmium, phenol, ammonia and lindane. Among the two fish species tested, *R. sumatrana* was found to be much more sensitive than guppy *P. reticulata* to Ni with a difference in the 96h-LC_{50} of approximately 19 folds (Table 1). The results of the present study were in agreement with a study conducted by Zakaria-Ismail and Fatimah [13] on the tolerance levels of common freshwater fish in Peninsular Malaysia. The study showed that *P. reticulata* has a tolerance level of 4.0, higher than that of *R. sumatrana*, which has a tolerance level of 2.5 (value ranged from 0.5, being the most sensitive, to 4.5, being the most tolerant species). This difference seen for trace metals might be explained by metallothionein (MT) synthesis, which is believed to play a protective role against toxic metals in aquatic animals such as fish [14], [15]. The availability of toxic metals is known to be reduced by binding to MT. Verbost et al. [16] concluded that cells with a limited capacity to synthesise MT and a low cellular turnover rate are expected to develop severe disturbances in their Ca homeostasis when exposed to Cd. Other studies also provided evidence that the hypothalamic-pituitary–adrenocortical (HPA) axis, crucial in vertebrates coping with stressors, is one of the metal targets in several animal species, including teleost fish [17], [18].

The present study showed that the ostracod, *S. major* and guppy *P. reticulata*, proved very resistant to the Ni with 96h-LC_{50} of 19.74 and 15.62 mg/L, respectively (Table 1). The midge, snail, prawn and tadpole were relatively insensitive to Ni. Khangarot and Das [19] also reported higher 48h-LC_{50} of Ni to freshwater ostracod *Cypris subglobosa* with value of 75.8 mg/L. Several studies have reported that the guppy can tolerate highly polluted water and withstand a high degree of salinity [20-22]. According to Von Der Ohe and Liess [1] taxa belonging to Crustacea were similar to one another and to *Daphnia magna* in terms of sensitivity to organics and metals, and that Molluscs have an average sensitivity to metals. Mitchell et al. [23] reported that the snail has a tightly sealing operculum which allows it to withstand desiccation and apparently also increases its tolerance to chemicals. Brix et al. [2] showed that warm water fish, crustaceans other than cladocerans and other invertebrates were consistently of intermediate sensitivity and that insects were the least sensitive taxonomic group evaluated for five

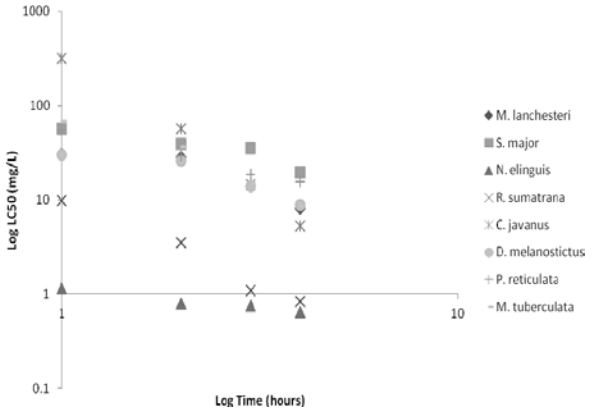

Fig. 1 The relationship between median lethal concentrations (LC_{50}) and exposure times for eight freshwater organisms exposed to Ni

metals (Cd, Cu, Pb, Ni, Zn).Brix et al. [24] also reported that aquatic insects were relatively insensitive to metals when compared with other aquatic organisms especially in short term exposure. This finding was in agreement with the results of present study (Table 1). It is well documented that major differences in sensitivity to environmental contaminants exist among species. Hoekstra et al. [25] predicted the variation in sensitivity of aquatic species (invertebrates and vertebrates) to toxic chemicals, and observed that variation in LC_{50} values was small for narcotic chemicals (e.g. acetone and benzene) and much larger for chemicals with a specific mode of action (e.g. metals).

According to Luoma and Rainbow [26], the rank order of toxicity of metals will vary between organisms, and the factors that affect the rate of uptake of metals affect the toxicity of metal. Metal toxicity results from the accumulation of metals at an undesirable site(s) in the organism and disrupts important molecular function. Toxicity ensues once the threshold of metal availability has been passed, indicating that the rate of uptake exceeds both the rate of excretion and detoxification. Metals can also inhibit the uptake of major ions (Na^+, Ca^{2+}, Mg^{2+}, Cl^-) by freshwater organisms through either competitive or direct inhibition [27]. Metals such as Ni have no binding preference and will form ligands with many functional groups [28]. Pane et al. [29] demonstrated that Ni inhibited Mg^{2+} reabsorption in the kidney of adult rainbow trout.

TABLE I
MEDIAN LETHAL CONCENTRATION (LC_{50}) FOR SIX FRESHWATER ORGANISMS AT DIFFERENT EXPOSURE TIMES FOR LEAD (IN MG/L)

Species	24h	48h	72h	96h
M. lanchesteri	30.79	28.53	13.90	8.05
S. major	57.28	39.18	35.13	19.74
N. elinguis	1.15	0.79	0.75	0.64
R. sumatrana	9.75	3.49	1.10	0.83
C. javanus	316.2	56.82	14.51	5.32
D. melanostictus	30	26	14	8.8
P. reticulata	54.43	29.66	18.72	15.62
M. tuberculata	68.35	36.46	15.04	8.46

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