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Accumulation and Depuration of Lead and Chromium Using *Nerita lineata*

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Abstract: This study aimed to examine the usefulness of locally found gastropod snail, *Nerita lineata* as suitable biomonitor for metal pollution. The accumulation and elimination of chromium (Cr) and lead (Pb) was examined by exposing *N. Lineata* to different concentrations of Pb (1-4ppm) and Cr (12-15 ppm) under controlled laboratory conditions. The experiments were allowed for four days of accumulation (24-96 hr) and four days of depuration (120-196 hr). The samples were taken out every 24 hour for metal analysis. There was gradual increase with exposure time for Pb indicating higher retention of Pb in tissues of *N. Lineata* and the concentration decreased over time during depuration. However, the accumulation and depuration trends of Cr were not consistent. The findings obtained suggest that *N. Lineata* showed ability to stand high Pb exposure and can be considered as suitable species for biological monitoring in aquatic environments.

Key words: *Nerita lineata* · Lead · Chromium · Accumulation · Depuration

INTRODUCTION

Monitoring heavy metal pollution in aquatic environments has drawn great concern and various biological species are widely utilized for this reason. Heavy metals analysis in aquatic organisms can provide important information on the degree of environmental contamination and its impacts [1]. Gastropods are one example of aquatic organisms that have demonstrated the ability as potential bioindicator and accumulate metals to high concentrations [2]. Heavy metals are persistent and non-biodegradable and may pose high toxicity on the aquatic organisms. Lead and chromium are considered as toxic metals that cause environmental problems and can be very harmful even at low concentration.

N. Lineata or known locally as 'kerikap' or 'tekoyong perempuan' belongs to the class of gastropoda. *N. Lineata* is available in large number in the area of Kampung Goebilt, Muara Tebas and Bako in Sarawak and also in intertidal areas of Peninsular Malaysia. *N. lineata* which is from the family *Neritidea* usually can be found in the estuaries, mangroves and monsoon drains [3]. It can be easily distinguished by its large, thick shell with pronounced spiral cords and commonly inhabits tree trunks on the back-side mangroves.

Various gastropods such as *Turbo intercostalis* [4], *Monodonta turbinate* [5] and *Bembicium nanum* [6] have showed the ability as excellent bioaccumulators of different pollutants. The ability of *Nerita saxtilis* as biological monitor to assess heavy metals pollution was investigated by AbdAllah and Moustafa [7] and this study revealed that *Nerita saxtilis* is capable of bioaccumulating lead and cadmium. Blackmore [8] reported that *Nerita albicilla* is capable of accumulating copper and zinc due to the physiological requirements for these essential elements. In Malaysia, study by Yap and Cheng [9] suggested *Nerita Lineata* as a potential biomonitor of heavy metals and particularly for Pb in the intertidal areas of Peninsular Malaysia.

To the best of our knowledge, *nerita* sp is not well-studied and information on toxicity of heavy metals on *nerita* sp. is scarce. Hence, this study aims to evaluate the accumulation and elimination patterns of lead (Pb) and chromium (Cr) using *N. Lineata* in the laboratory.

MATERIALS AND METHODS

N. Lineata samples and sediments were collected from Kampung Goebilt, Muara Tebas in September and November 2007. Samples with wet weight of 2 to 5 g and shell length of 2 to 3 cm were chosen for this study in order to normalize size of samples for accumulation and depuration tests. *N. Lineata* snails (n=400) were mixed with sediments during the collection and transported to the laboratory.

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Table 1: Experimental Conditions of Water

Conditions	Value
Temperature	20-21°C
pH	6-7
Dissolved Oxygen	4-5 mg/L
Lead(Pb)	3.74 ± 0.59 mg/L
Chromium (Cr)	1.06 ± 0.02 mg/L

Table 2: The Concentration of Metals used in the Experiment

Metals	Control	Conc. 1 (ppm)	Conc. 2 (ppm)	Conc. 3 (ppm)	Conc. 4 (ppm)
Cr	0	12.0	13.0	14.0	15.0
Pb	0	1.0	2.0	3.0	4.0

All experiments were carried out using pre-cleaned (with 10% HNO_3) experimental tanks (17 X 14 X 14 cm^3). The samples of *N. Lineata* were acclimatized in the tanks containing sediments and 1 L of artificial seawater for three days prior to the test and well aerated. The laboratory conditions of water during the acclimatization are shown in Table 1.

Preliminary range finding tests were conducted to arrive at the five exposure concentrations of metal. Standard experimental protocols were adopted from Sathyanathan [10] with necessary modifications. Stock solutions were freshly prepared using lead chloride ($PbCl_2$) and chromium chloride ($CrCl_2$). About 40 specimens of *N. Lineata* were exposed to different concentrations of Pb (1, 2, 3 and 4 ppm) for 96 hr. For Cr, higher acclimation concentrations were used (12, 13, 14 and 15 ppm) as pre-exposure of 10 ppm of Cr did not show any mortality. This concentration chosen is above the FAO limit (5 mg/kg) [11]. Control tests were also carried out without metals addition in the test tanks. The experiments were allowed for four days of metal uptake and four days of depuration. All the test tanks were aerated throughout the experiments. During the experiments, the test organisms were monitored daily for behavior and mortality. Sampling of *N. Lineata* samples from each tank were done every 24 hr for four days.

For depuration test, *N. Lineata* was taken out from the accumulation tanks. The sediment and water were changed with metal-free sediment and water. After that, the samples of *N. Lineata* were transferred into the tanks. In this test, the samplings of *N. Lineata* (four samples) from each tank were also done every 24 hr for subsequent four days.

After exposure and depuration, *N. Lineata* samples were removed and washed with distilled water. Mortar was used to crack open the shells. The tissues were removed from the shell and washed with deionized water. Then, the tissues were oven-dried at 70°C for 48 hours and ground in a mortar to 60 mesh size. All the powdered tissues were kept in desiccators prior to further chemical analysis. The tissues samples were weighed accurately to approximately 0.5 g into 100 ml beaker and digested with 5 ml of concentrated HNO_3 and 5 ml of H_2O_2 (30%). The beaker was covered with a watch glass and left aside until the initial vigorous reaction subsided. Then, the samples were heated on a hot plate for about two hours to reduce the volume to 3-4 ml. After that, the samples were allowed to cool, filtered and diluted to 25 ml in volumetric flask with deionized water [12].

All samples were analyzed for lead (Pb) and chromium (Cr) by using Flame Atomic Absorption Spectrophotometer (FAAS) (Perkin-Elmer Model 3110). All metal concentrations are expressed in terms of dry weight (mg/kg). The accuracy of the analysis was checked against blanks and with the standard addition testing procedure. For metals recovery analysis, samples were spiked with known amount of metals. The recoveries obtained for Cr and Pb ranged between89-100% and 80-95% respectively.

Statistical Analysis: One-Way Analysis of Variance (ANOVA) was performed to determine if there is any significant difference between accumulation and depuration of metals between different treatments.

RESULTS AND DISCUSSION

Accumulation and Depuration of Lead and Chromium in Tissues: Accumulation and depuration of Pb in tissue samples versus time for four different spiked concentrations is depicted in Figure 1. Generally, the concentration of Pb increased during accumulation period from 24 hr upto 96 hr and decreased during depuration period (from 120 upto 192 hr). The mean concentrations of Pb in control, 1 ppm, 2 ppm, 3 ppm and 4 ppm were 50.91 mg/kg, 158.59 mg/kg, 175.03 mg/kg, 208.66 mg/kg and 203.81 mg/kg respectively. Pb in control was significantly lower than other treatments. However, there was no significant difference ($P > 0.05$) in the concentrations of Pb in four other treatments.

The concentration of Pb in tissues displayed progressive increase from 24 hr upto 96 hr (Figure 1). The increasing trend showed that *N. Lineata* has the ability to withstand high concentration of Pb and able to accumulate Pb in the body tissues. While for depuration test, the concentration of Pb decreased upto 120 hr but

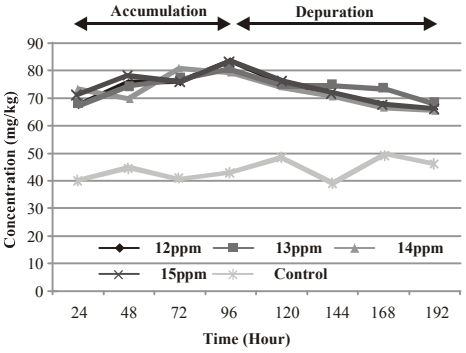

Fig. 1: Pb Concentration in Tissue Samples during Accumulation and Depuration

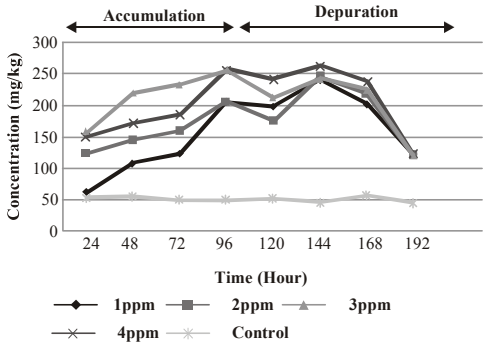

Fig. 2: Cr Concentration in Tissue Samples during Accumulation and Depuration

increased rapidly for all spiked concentrations upto 144 hr and decreases thereafter. The highest mean value of Pb was attained at this hour. The mean concentration of Pb upto 144 hr period for control, 1 ppm, 2 ppm, 3 ppm and 4 ppm were 47.5 mg/kg, 240.50 mg/kg, 246.25 mg/kg, 242.25 mg/kg and 263.00 mg/kg respectively. The concentration of Pb decreased again upto 168 hr and this demonstrates that during depuration, Pb was cleared from tissue. At 96 hr, the concentrations of Pb for 3 ppm and 4 ppm were almost similar and as well as for 1 ppm and 2 ppm. The uptake of Pb increased proportionally with the increasing concentrations of Pb and period of exposure.

The content of Pb found in tissues of *N. Lineata* were much higher than the permissible limits stipulated for Pb by Malaysian Food Act (2.00 mg/kg dry weight) [13] and the Department Of Environment for marine water (0.1 mg/L) [14]. However, Pb did not cause any harmful effects to *N. Lineata.* This indication showed the ability of *N. Lineata* to accumulate Pb and able to survive in highly Pb-contaminated aquatic environments.

Figure 2 shows the accumulation and depuration of Cr in tissue samples versus time for four different spiked concentrations. For chromium, the mean concentrations in control, 12 ppm, 13 ppm, 14 ppm and 15 ppm were 43.93 mg/kg, 71.41 mg/kg, 72.50 mg/kg, 73.44 mg/kg and 72.28 mg/kg respectively. Concentrations of Cr accumulated in tissues did not vary much between the spiked concentrations (12 ppm - 15 ppm). Statistical analysis also showed no significant difference ($P>0.05$) between the treatments for accumulation and depuration period. There were discrepancies in accumulation and depuration trends of Cr. Depuration phase in clean water indicates fluctuating elimination pattern of Cr from *N. Lineata*. As Pb, accumulation values of Cr in all treatments were similar at 96 hr. This might be due to the ability of *N. Lineata* to uptake Cr has reached the maximum concentration i.e. in the range of 78-83 mg/kg.

The control samples contained Pb and Cr ranging from 45-57 mg/kg (Figure 1) and 39-49 mg/kg (Figure 2) respectively and their concentrations varied non-uniformly especially during depuration period. This presumably due to leaching of metals from tanks or metals brought into the water surface by natural upwelling process [15].

Experiments conducted with *N. Lineata* yielded different patterns of accumulation and depuration for Pb and Cr. Accumulation pattern of Pb was more notable than Cr. Tissues of *N. Lineata* could accumulate higher concentration of lead compared to chromium. According to AbdAllah and Moustafa [7], *N. Lineata* can accumulate lead and cadmium up to 50 folds of that of surrounding marine water without showing any signs of hispathological changes. Increasing and decreasing trends during accumulation and depurations respectively over time was also observed by Shuhaimi-Othman and Pascoe [16] and Yap *et al.* [17] in their toxicity studies. This result also corroborate well with the observations by Otitoloju and Don-Pedro[18] whereby the concentrations of Pb, Zn and Cu accumulated in body tissues of an edible periwinkle, *Tympanotonus fuscatus var radula* (L.) increased with times after exposed to the metals for 30 days. The patterns of heavy metals occurrence (Pb > Cr) in tissues of *N. Lineata* were similar to that of soft tissues of sea snail *Rapana venosa* (Pb > Cr) [19]. Throughout the experiments, *N. Lineata* did not exhibit any abnormalities in behavior and LC_{50} could not be calculated as there was no mortality occurred. The main reason for mollusks to withstand the high accumulation of heavy metals has been attributed to the binding ability of metals to proteins called metallothionine [7]. Prosobranch snails have opercula to protect themselves when surrounding water becomes hazardous [20].

CONCLUSION

On the basis of the findings obtained, the following conclusions are drawn: (1) *N. Lineata* tissue concentrations of Pb and Cr increased with exposure duration although some discrepancy was found. (2) *N. Lineata* could accumulate Pb better than Cr in body tissues due to wider range of Pb concentrations than Cr. (3) *N. Lineata* was found to be useful and capable biomonitor for metal specifically Pb.

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