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Short Communications

Depuration of Gut Contents in the Intertidal Snail *Nerita lineata* is Not Necessary for the Study of Heavy Metal Contamination and Bioavailability: A Laboratory Study

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ABSTRACT

Some of the scientific papers in the literature regarding heavy metal concentrations in the soft tissues of molluscs are always rejected because there is no depuration of metals before the molluscs samples are analyzed for heavy metal accumulation, although the acceptance of a paper in a journal is assessed based on many other factors. The depuration of gut contents of molluscs has been the initial step before the metal analysis on the soft tissues of molluscs by many researchers. The depuration process in some molluscs involves holding the animals in clean water or clean sediment for a suitable period (8–24 hrs) to purge their guts after exposing them to contaminated conditions, and before they are analyzed for whole-body contaminant burden (Neumann *et al*., 1999; Gillis *et al*., 2004). The depuration ensures that metal-contaminated particles in the animal's gut do not lead to overestimation of metal bioavailability. Undoubtedly, clearing the gut contents is theoretically a laboratory technique in order to get an accurate estimate of heavy metal concentrations accumulated in the soft tissues of molluscs. In addition, the suggestion on the use of molluscs as biomonitors of metal bioavailability becomes invalid because their soft tissues were not depurated. Consequently, validity on the data of metal concentrations could not be achieved. Therefore, in order to determine if a particular species could be used as biomonitor for metals, depuration of soft tissues of the molluscs is imperative to effectively determine the availability of metal (Riba *et al*., 2005; Wang *et al*., 2005; Szefer *et al*., 1999; Cravo *et al*., 2004; Baldwin and Maher, 1997; Nicholson and Szefer, 2003). In standard protocols (ASTM, 2003), although it is not a standard practice to clear the gut of organisms before analyzing their tissues for whole-body metal accumulation, some investigators do transfer animals to clean water conditions in order to purge their guts after they have been collected from the field. In this study, the snail known as *Nerita lineata* of the *Neritidae* family, were collected to study if there was any significant difference in the concentrations of Cu and Zn in the soft tissues, before and after four weeks of depuration.

Keywords : Depuration, gut contents, *Nerita lineata*, mollluscs

MATERIALS AND METHODS

The snails, *N. lineata* in their adult stage, were collected from Sg. Janggut, in Selangor (N 04º 8'10''; E 101º 22'31'') where water irrigation activities were observed. Aquaria were cleaned and acid-washed before the experiment was carried out. For this purpose, thirty snails of almost similar size and shell length (14.00-32.22 cm) were selected from the collected samples. Meanwhile, fifteen snails were placed in one laboratory aquarium without feeding for 4 weeks, and fifteen other snails were analysed

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for the initial concentrations of Cu and Zn (Yap *et al*., 2003: 2006). After 4 weeks, the depurated snails were analyzed for Cu and Zn concentrations using the established methods (Yap *et al*., 2003: 2006). They were dried in n oven for 72 hours at 105°C to constant dry weights. The dried tissues of the snails were digested in concentrated nitric acid (AnalaR grade, BDH 69%) by placing them in a hot-block digester; first at low temperature for one hour and they were then fully digested at high temperature (140°C) for at least three hours. After that, the digested samples were diluted to 40 mL with double distilled water. After filtration, the samples were determined for their Cu and Zn concentrations using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The data were presented in μg/g dry weight basis. The metal levels found in the soft tissues of *N. lineata*, before and after 4 weeks of depuration, were statistically analyzed to check for any significant difference using t-test in the SPSS software version 15.0 for Windows.

There was no significant ($P>0.05$) difference in the concentrations of Cu and Zn, before and after 4 weeks of depuration in the intertidal snails *N. lineata* (Table 1). This indicated that the depuration of metals in *N. lineata* can be neglected. Although Cu and Zn are essential metals for the molluscs, since they are incorporated in the enzymes of the organisms, there is regulative mechanism for the essential metals in gastropods (Phillips and Rainbow, 1993). Therefore, these two metals are preferentially found in the soft tissues of the snails (Cravo *et al*., 2004). Moreover, Cu and Zn are likely to be detoxified and this involves metallothioneins and depurated if present in excess (Ng *et al*., 2007). Therefore, the choice for the two metals in this study is reasonable.

There are four important points which can be highlighted to make reasonable arguments to reject the necessity of depuration of gut contents in *N. lineata* besides the significant finding from this laboratory study. First, the depuration process may easily cause metal contamination (Rainbow *et al*., 2004). For this pragmatic reason, the snails should not be allowed to depurate their gut contents (Rainbow *et al*., 1989). This contamination will certainly increase the actual metal concentrations and metal bioavailability. Many studies (e.g. Gunther *et al*., 1999; Rainbow and Blackmore, 2001; Rainbow *et al*., 2004; Sidoumou *et al*., 2006; Espana *et al*., 2007) on molluscs did not carry out depuration prior to the analysis of heavy metals in soft tissues. Nonetheless, these papers are still accepted for publication in international journals. This could be due to the fact the depuration process would reduce the validity of data collected because of errors and

TABLE 1

A comparison of the concentrations (µg/g dry weight) of Cu and Zn in the soft tissues of *Nerita lineata* collected from Sg. Janggut snails between, before, and after weeks of depuration. N= 3 based on 15 individuals of snails

	Before			After 4 weeks			Significance level
Cu shell	2.23	±	0.29	3.54	±	0.01	p>0.05
Cu operculum	5.62	±	0.71	6.04	±	0.22	p>0.05
Cu soft tissues	14.31	±	2.13	11.89	±	0.10	p>0.05
Zn shell	8.07	±	0.64	7.29	±	0.16	p>0.05
Zn operculum	27.85	±	5.54	33.56	±	0.59	p>0.05
Zn soft tissues	91.69	±	1.50	96.12	±	2.88	p>0.05

external contamination. Second, *Nerita* snails are generally strong accumulators of heavy metals. Therefore, snails could potentially contain gut content, but these are considered to represent only a small proportion of the total body metal content, given the characteristic of molluscs (*Nerita*) which are particularly strong heavy metal accumulators (Rainbow, 1987: 1998).

Third, any variability introduced into the results is minimized and accounted for by the pooling technique used during the metal analysis in the laboratory and/or the statistical treatment of data (Rainbow and Blackmore, 2001). Since *Nerita* snails are small in size, the soft tissues must be pooled in order to get enough tissues for the metal analysis, and these pooled tissues would certainly reduce the variability due to gut contents.

Fourth, according to Rainbow and Phillips (1993), deposit-feeding species living in soft muds may exhibit different total body concentrations of metals, before and after gut depuration. Therefore, the depuration of the gut contents of deposit feeders is needed. In contrast, the need for depuration of the gut content is not necessary for all the molluscs, especially for the small intertidal snails and non-deposit filter feeders such as *N. lineata*. The problem of bioavailability overestimation of heavy metals in *N. lineata* without depuration is almost non-existing.

In conclusion, the above four points, plus the results of the present laboratory study, suggest that the determination of the bioavailability and accumulation of metals using the total soft tissues of *N. lineata* without depuration, can still be interpreted with high validity. Therefore, the time consuming depuration of gut contents in the intertidal snail *N. lineata* is not necessary for the study of heavy metal contamination and bioavailability.

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