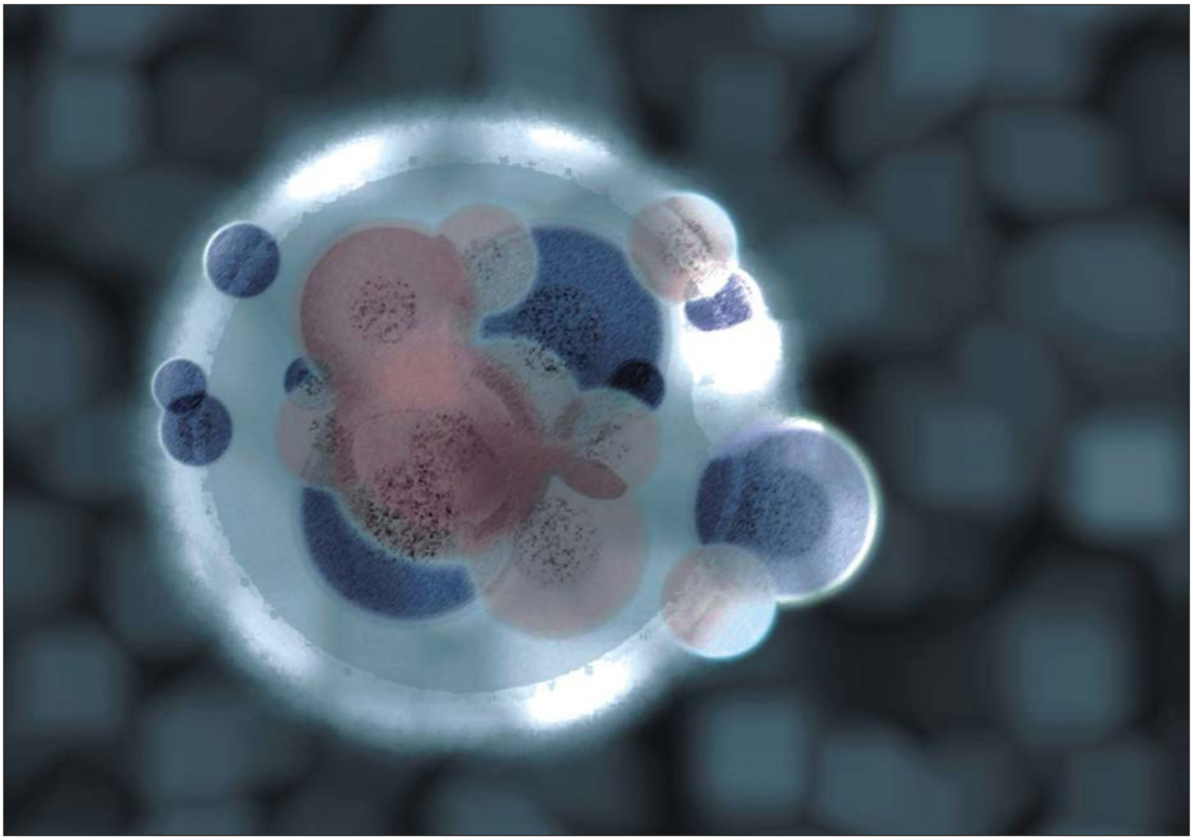

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The Bioaccumulation of Trace Essential Metals by the Freshwater Snail, *Turritella* sp. Found in the Rivers of Borneo East Malaysia

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Abstract: A study on the bioaccumulation behaviour of four trace essential metals (Cu, Fe, Mn and Zn) by the freshwater snails, *Turritella* sp. collected from three rivers in Danum Valley Forest Conservation area and Sarawak was carried out. The concentrations (μg g^{-1}) of Cu, Fe, Mn and Zn determined in the whole body of the snails was 31.6-63.1, 368.7-741.4, 107.6-926.7 and 119.9-716.4, respectively. This study has shown that under natural environment, *Turritella* sp. is capable of bioaccumulates trace essential metals, especially Zn and Cu. The results have shown that *Turritella* sp. can accumulate Cu and Zn up to 1-2 times higher than that determined in sediments. The accumulation behaviour was strongly dependent on the amount of trace metals in the sediment and this indicated that ingestion may be the main route to the uptake of these essential metals. Most of the trace essential metals were accumulated in the viscera, followed by soft tissue and snail shell.

Key words: Freshwater snail, *Turritella* sp., trace essential metals, sediment, bioaccumulation, Danum Valley, Borneo

INTRODUCTION

In aquatic environment, both biotic and abiotic accumulation of chemicals may occur. Abiotic accumulation of chemicals takes place within two or more physical components in the aquatic environment, e.g. between water and sediment. Biotic accumulation, or bioaccumulation involves the accumulation of chemicals in living organisms. Bioaccumulation becomes an environmental problem when chemicals accumulated are toxic, where this will lead to an elevated amount in the organisms' body. Toxicity may occur along the food chain when the contaminated species is consumed.

Bioaccumulation of chemicals is an important factor in the assessment of environmental hazard. It has been accepted as a trigger factor for decisions of administrative relevance[1]. The term bioconcentration, has in the past tended to be used synonymously with bioaccumulation in aquatic organisms. This is because, bioconcentration, which is due to direct uptake from water by gill breathing animals, has been shown to be the major route of exposure for various classes of xenobiotics. Bioconcentration indeed used as a predictor for bioaccumulation/biomagnification in aquatic food chain[2]. This concentration effect is expressed as the ratio of the concentration of chemical in the organism to that in the water. Bioconcentration factors however can only be calculated in laboratory based on experiments where all factors can be controlled. In field based experiments, such as in the present study, it is not feasible to calculate bioconcentration factors due to the multitude of uncontrolled factors in the environment.

Bioaccumulation for many aquatic biota has been reported[3]. These include macrophyte, fish, invertebrates, benthic orgamisms, plankton and zooplankton[4,5]. At each trophic level of aquatic system, the bioaccumulation behaviour may be different. For macrophytes, bioaccumulation of metals appears to occur at rhizome and root. Biomagnification of metals also observed between small plankton and macrozooplankton. Fish and invertebrates did not seem to bioaccumulate metals but they showed bioaccumulation of polychlorinated organics with log-bioaccumulation factor of 4-11. Bioaccumulation is thus depended on the environment, species concern, trophic level and type of contaminants involved. One should not generalise results from various studies as bioaccumulation behaviour of aquatic system is unique.

Snails have been used for the purpose of bioindicator for contamination by industrial waste dump[6]. Bioaccumulation of metals and organic toxicants in snail

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species such as *Helix aspersa, Lymnaea stagnalis* and *Leptoxis praerosa* have also been reported[7-11]. This study was conducted to investigate the bioaccumulation behaviour of the metals, Cu, Fe, Mn and Zn by the freshwater snails, *Turretella* sp. found in the rivers of Borneo, East Malaysia. It was also to determine the bioaccumulation behaviour of this species of freshwater snail under natural environment.

MATERIALS AND METHODS

Snail and sediment sampling: The study areas were the Bole and Tembaling rivers in the Danum Valley Forest Conservation area at the south-eastern corner of Sabah and the Adis river, approximately 40 km from Kuching at the south west of Sarawak, both states are at Borneo East Malaysia. The Bole and Tembaling rivers are located in primary undisturbed forests but the Adis river flows through areas of agriculture. The freshwater snail, *Turritella* sp. was collected from nine locations, i.e. three locations for each river. At each site, a sediment sample was also collected together with the freshwater snail. Water quality of the Bole and Tembaling rivers was also measured during snail sampling.

Water analysis: The water pH, conductivity, dissolved oxygen and temperature was measured on site using a YSI meter. Total suspended solids and water hardness of the water samples were analysed in laboratory using standard procedures[12]. For total suspended solids, the analysis involved filtration of water samples, weighing and drying of the residues. For water hardness, it was calculated from the content of magnesium and calcium after these cations were determined using a Perkin-Elmer 2380 atomic absorption spectrophotometer.

Analysis of metals in snail samples: Before analysis of metals, the snail samples obtained from rivers were cleaned and then left in clean water 24 h to allow for excretion of waste. The samples were then dried in oven for a period of 24-48 h at 100°C until the weight became constant. This was then followed by acid digestion using a mixture of nitric-perchloric acids at a ratio of 5:1 in a microwave oven. For the study of bioaccumulation of metals in various parts of the snail, the samples were divided into the viscera, soft tissue and shell. Each component of the snail body was then dried and digested with nitric-perchloric acid mixtures. Metal analyses were carried out using a Perkin-Elmer 2380 atomic absorption spectrophotometer after acid digestion.

Analysis of metals in sediments: Sediment samples were first dried in an oven at 105°C for 2-3 days. It was then broken up and sieved through a sieve of mesh sized 0.63 μm. A certain amount of the sieved sediment was then digested with 5:2(v/v) of hydrochloric-nitric acids for 2 h at 90°C. This was followed by adding a mixture of nitric-perchloric acids in a ratio of 5:2 (v/v) to complete the digestion at room temperature. The resulting solution was then diluted before used for metal analysis with a Perkin-Elmer 2380 atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

The level of metals seems to vary depending on the sampling sites. Snails collected from the Bole River have significantly higher amount of trace essential metals when compared to those obtained from the Tembaling and Adis rivers ($\alpha = 0.05$). This demonstrates that there is influence of habitat on the level of some metals in the snails. Uptake of metal ions into aquatic organisms believed to involve the Free-ion Activity Model and water quality parameters such as pH, hardness and dissolved organic matter exert their influences on the model[13]. However, in terms of water quality, both the Bole and Tembaling rivers do not differ much although a slightly higher level of conductivity and water hardness is observed in the Tembaling River compared with the Bole River (Table 2). Therefore in the case of Bole and Tembaling rivers, water quality is unlikely to be the cause of different contents of metals in the snail samples. For Adis River, there was no water quality data available for comparison.

But when the level of metals of the sediment samples are examined (Table 1), it is clear that sediment samples from the Tembaling and Adis Rivers are very much lower in all metals analysed when compared with those from the Bole River. This trend is consistent with that observed for metals in the whole body of the snails. Indeed the levels of all trace essential metals in the snail are positively correlated with their levels in the sediment samples. The correlation is linear and strong as indicated by the correlation coefficients of 0.91-0.99 (Table 3).

The good correlation between all the metals in sediment and in the snail may be attributed to the feeding habit of the snails where the food source can be derived from the sediment.

Laskowski and Hopkin[10] reported that the garden snail *Helix aspersa* assimilated on average 60% of Zn and 90% of Cu from food fortified with these metals. The ratio of the concentration of metal in *Torritella* sp. to the concentration in the sediment is between 1-2 for Zn and Cu but much lower for Fe and Mn (Table 1). *Turritella* sp. appears to be able to accumulate both Zn and Cu when compared with Fe and Mn. In a study on bioaccumulation of Cu by the *Helix aspersa*[7], it has been found that the

Table 1: The average content (n = 6) of Cu, Fe, Mn and Zn determined in the freshwater snail, *Turritella* sp. and sediment samples from the Bole river (BR), Tembaling river (TR) and Adis river (AR)

Metal ($\mu g\ g^{-1}$)	*Turritella* sp.	Sediment	Ratio: snail /sediment
Cu			
BR	63.10±5.60	61.60±0.50	1.02
TR	58.30±4.50	47.10±2.00	1.23
AR	31.61±2.63	22.76±1.40	1.39
Fe			
BR	741.40±35.90	60315.00±1.00	0.01
TR	487.40±78.30	41698.00±1343.00	0.01
AR	368.70±19.60	27570.00±2585.00	0.01
Mn			
BR	926.70±134.50	1668.00±44.00	0.56
TR	440.80±34.70	909.70±89.80	0.48
AR	107.60±11.20	254.70±107.60	0.42
Zn			
BR	119.90±5.50	124.10±10.60	0.97
TR	124.70±20.50	68.80±5.90	1.81
AR	716.40±127.00	577.20±10.70	1.24

Table 2: The range of water quality of the Bole and Tembaling rivers at Danum Valley where both snail and sediment samples are collected

Water quality	Bole river	Tembaling river
Temperature (°C)	24.70-25.00	23.60-23.70
Conductivity (μS/cm)	148.20-163.04	196.80-203.05
pH	7.68-7.82	7.75-7.86
Dissolved oxygen (mg/L)	7.66-7.79	7.24-7.80
Total suspended solids (mg/L)	2.71-6.62	6.17-10.46
Water hardness (mg $CaCO_3$/L)	75.68-94.01	106.19-106.34
Chemical oxygen demand (mg/L)	13.20-15.60	8.80-13.20

Table 3: The relationship between the content of metals in the *Turritella* sp. and sediment samples collected near to the snail samples

Metal (sediment-snail)	Relationship (n = 9)	Correlation coefficient (R^2)
Cu	Y = 1.11X-12.56**	0.9163
Fe	Y = 83.43X-14.64	0.9344
Mn	Y = 1.70X+99.01	0.9878
Zn	Y = 0.77X+8.46	0.9527

** Y = concentration of metal in sediment samples
X = concentration of metal in snail samples

Table 4: The average content (n=9) of metals in various body parts of the freshwater snail, *Turritella* sp. collected from east Malaysia

	Metal ($\mu g\ g^{-1}$)			
Body parts	Cu	Fe	Mn	Zn
Viscera	99.6±0.3	685.8±54.8	5320.0±623.0	232.7±45.3
Tissue	33.7±1.6	186.1±7.9	129.8±10.4	91.5±7.39
Shell	4.9±0.3	47.7±4.8	37.3±1.3	149.3±12.7

snail could bioaccumulate up to 1.1-2.5 times of Cu through the ingestion of food alone. For the freshwater snail, *Leptoxis praerosa*, feeding experiments had shown that this species of snail could bioaccumulate significant level of Cu when feed with food containing 564 $\mu g\ g^{-1}$ Cu. However, no Zn bioaccumulation was observed even the food contained up to 20,000 $\mu g\ g^{-1}$ Zn. The observed Cu bioaccumulation behaviour of *Turritella* sp. was in agreement with other studies involving different species of snails.

In general the concentrations of metals in the whole body of the *Turritella* sp. followed the sequence of Fe, Mn>Zn>Cu. This is similar to those of sediments where concentrations of Fe> Mn>Zn>Cu. Although the ratio of Fe or Mn concentrations in *Turritella* sp. to that in sediment is low, the amount of Fe and Mn in the body of the snail is actually almost 10 times higher than that of Cu. Thus, it is likely that *Turritella* sp. also ingesting Fe and Mn from the sediment and the strong relationship between Fe or Mn in sediment with that in the snail's body supports this assumption (Table 3).

The distribution of all four metals in various body parts of *Turritella* sp. such as viscera, soft tissue and shell was depicted in Table 4. Obviously, the distribution of Fe, Mn and Cu is mainly in the viscera where significantly higher level (α = 0.01) of these metals are found when compared to the soft tissue and shell. The shell of the animal contained much less Cu, Fe and Mn when compared to the viscera and the soft tissue. Study using *Helix aspersa* demonstrated that Cu accumulated mainly in the soft tissue whilst Zn in the viscera[9]. The amount of Cu and Zn in shell did not exceed 5% of those found in soft tissue of the *Helix aspersa* but increased significantly with exposure[10]. For *Turritella* sp., the distribution of Cu and Zn in the shell, especially Zn has exceeded 5% of the soft tissue concentration. In fact, there is no difference in the distribution of Zn in all three body parts of the *Turritella* sp.

This study has shown that under natural environment, the freshwater snail, *Turritella* sp. was capable of bioaccumulates trace essential metals, especially Zn and Cu. The accumulation behaviour was strongly dependent on the amount of trace metals in the sediment and this indicated that ingestion may be the main route to the uptake of these essential metals. Most of the trace essential metals were accumulated in the viscera, followed by soft tissue and snail shell.

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