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Heavy Metal Contamination in the River Toad, *Bufo juxtasper* (Inger), Near a Copper Mine in East Malaysia

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Mining of metals creates a potential source of heavy metal contamination in the environment. An open pit copper mine situated in the Northwest of Sabah, East Malaysia has been known to pollute its surroundings especially with discharges involving heavy metals (Murtedza et al., 1989). The main source of this pollution originates from the effluent resulting from the floatation process used in preparing copper concentrates. The effluent contains high levels of certain heavy metals and although treated, can still pose environmental problems especially those related to contamination of local rivers. Earlier studies on heavy metal content of these rivers indicated that river water and sediments contained high levels of certain metals. (Murtedza et al., 1984). Rice grown in an area irrigated with polluted river water also suffered contamination (Murtedza and Lee, 1986). The most recent survey of aquatic plants and animals found in the river system around the copper mine has also indicated a certain degree of such pollution (Markus et al, 1988). Although extensive investigations of heavy metal pollution has been carried out, none of the studies performed so far has included amphibians as indicator of heavy metal contamination in the area. As amphibians live both on land and in water, a study on the heavy metal content of these animals will thus enable a more extensive evaluation of the degree of contamination by heavy metals. Such a study will also allow the level of contaminants in amphibians to be viewed in relation to other indicators of contamination which were studied earlier. Anuran amphibians are well suited as indicators of certain types of environmental pollution, as their life cycles generally include both an aquatic and a terrestrial phase. Their diets are also known (herbivory as tadpoles, insectivory as adults) so that sources of uptake of pollutants may be discernible. *Bufo juxtasper* was chosen since it inhabits the rocky streams and rivers which exist in both a polluted and non-polluted condition in Sabah. Its' tadpoles are herbivorous feeding mainly on plant detritus, while adults feed principally on ants (which are polyphagous). Furthermore the large adult size of *Bufo*

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juxtasper , in which the size of the liver has an allometric relationship with body size, may allow for differentiation between larval and adult uptake through regression analysis. Thus concentration of pollutants acquired only during the larval phase should show a declining or negative slope as a function of body size in adults.

MATERIALS AND METHODS

Toads were collected from four different sites. Of these four sites, only one location was directly affected by effluent discharged from the copper mine while the other three were included for the purpose of comparison. The sampling site which was directly affected by discharge from the copper mine was the Mamut River (*ca* 6^0 5' N, 116^0 40' E). The other three sampling sites included the Langanan River situated within approximate 1 km of the Mamut River but not affected by the copper mine; the Tabin Wildlife Reserve (*ca* . 5^0 10'N, 118^0 30'E) known to contain mud volcanoes rich in minerals (Mazlin et. al, 1990); and the Sunsuron Ridge of the Crocker Range National Park (*ca* . 5^0 45' N, 116^0 5'E) composed principally of sandstone with no known source of heavy metal pollution. Sampling sites for Mamut River and Langanan River are shown in Figure 1.

A total number of 26 toads were caught and preserved in absolute alcohol until analysis. Before analysis, the entire liver from each toad was removed and then dried at 100°C until constant weight was achieved. These livers were then weighed (mean dry weight was about 0.6 g) and then digested with a 1:1 mixture of nitric and perchloric acids in a Kjeldahl Digester until complete dissolution of the liver tissue occurred. After digestion, the resulting digestate was diluted and the metal content was analysed using a Perkin-Elmer 2380 atomic absorption spectrophotometer. The metals analysed were Ag, Cd, Cr, Co, Cu, Ni, Mn, Pb and Zn. Blank solutions and the alcohol used in preserving the toad samples were also tested for metal content to ensure no contamination from laboratory procedures. For data analysis, non-parametric statistics (Mann-Whitney U test) was used to test the difference between means. Regression analyses were also employed. All statistics were computed on an Apple Mcintosh SE using a Statview program.

RESULTS AND DISCUSSION

The average content of nine metals examined in the toad livers are shown in Table 1. The liver copper content of *Bufo juxtasper* from all sampling sites appears to be extremely variable and abnormally high (range : 4-1020 ppm). This is in contrast to liver copper content of other species of animals which are generally low and fall within a small range. For example, the liver Cu content of mammals is generally in the range < 30 ppm (Beck, 1955; Smith and Rongstad, 1983), Aves : 10-100 ppm (Beck, 1955; Vermer and Peakall,

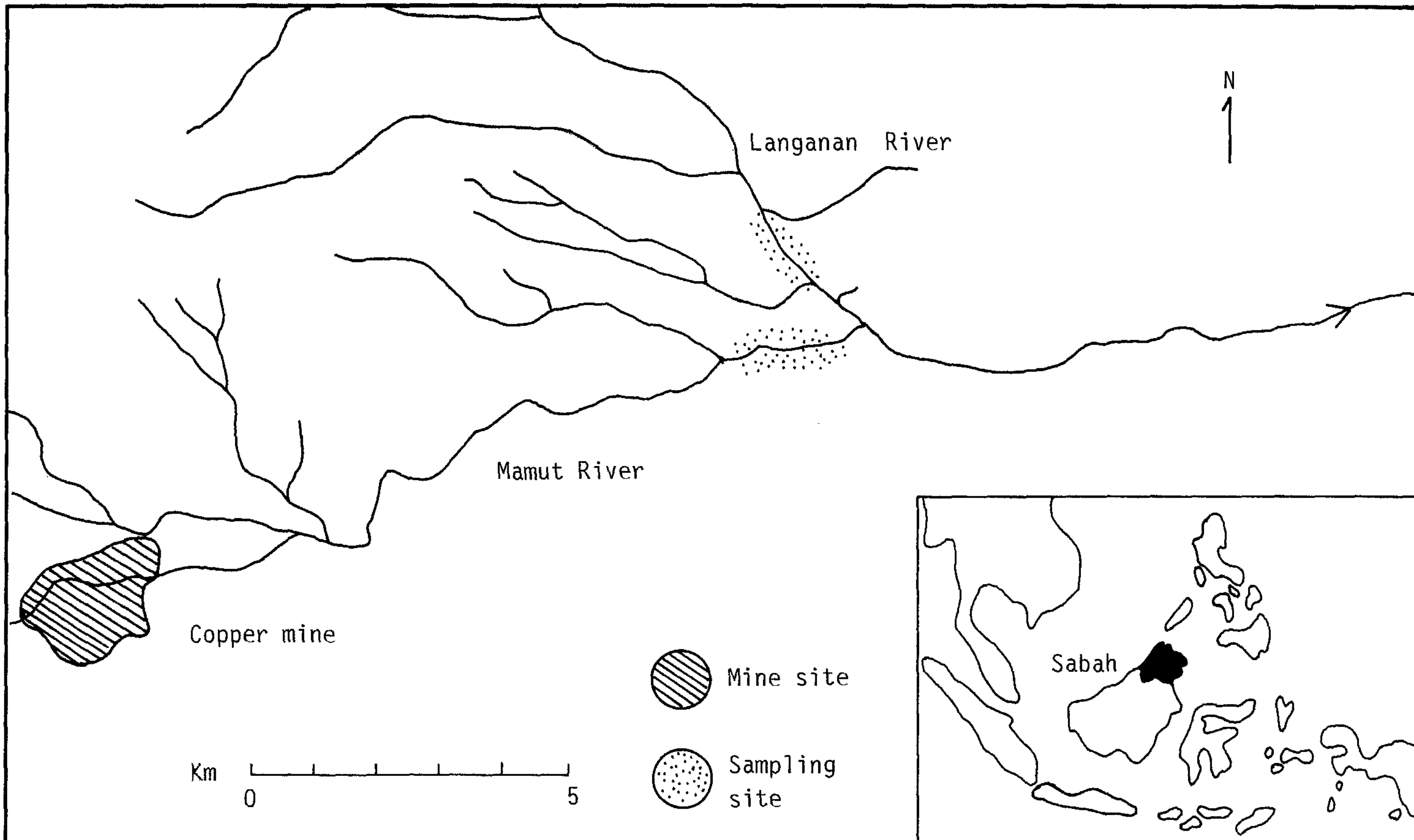

Figure 1. Map showing the Mamut River and Langanan River where toad samples were collected

Table 1 : Heavy metal content in the liver of the toad, *Bufo juxtasper* (ug/g dry tissue)

Location	Ag	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Mamut River [a](n=5-6)	[b]**1.20** [c]**(0.66-1.90)**	**3.08** **(1.42-5.23)**	**3.24** **(0.71-2.54)**	**0.76** **(0.00-2.40)**	**437.6** **(80.7-1020.)**	**7.0** **(2.0-12.9)**	**21.4** **(12.6-49.2)**	**12.8** **(6.91-15.8)**	**164.9** **(122.4-206.9)**
Laganan River (n=9-10)	**0.57** **(0.00-2.40)**	**1.48** **(0.42-3.42)**	**3.81** **(0.00-11.2)**	**1.15** **(0.00-6.00)**	**116.5** **(4.15-299.5)**	**11.0** **(4.50-21.4)**	**6.35** **(0.00-28.4)**	**9.79** **(1.72-18.7)**	**133.6** **(104.2-172.9)**
Tabin Wildlife Reserve (n=6)	**2.50** **(0.61-6.00)**	**0.45** **(0.00-1.60)**	**3.42** **(0.00-12.0)**	**3.20** **(0.00-7.20)**	**273.4** **(116.8-414.4)**	**7.07** **(4.90-9.70)**	**1.01** **(0.00-3.00)**	**11.8** **(4.05-19.1)**	**94.1** **(85.8-102.4)**
Crocker Range Park(n=4)	**0.69** **(0.21-1.63)**	**1.18** **(0.70-2.50)**	**1.10** **(0.00-2.19)**	**3.37** **(0.50-11.4)**	**45.6** **(8.72-127.4)**	**3.68** **(2.90-4.30)**	**1.95** **(1.10-3.00)**	**1.35** **(0.00-2.04)**	**166** **(39.1-244.2)**

a. Number of samples

b. Mean metal content

c. Figures in parentheses represent the range of metal content

1979);pices : 10-70 ppm and reptiles : < 20 ppm (Beck, 1955). Unfortunately, there is no data available on the liver Cu content of *Bufo juxtasper* to which the present results could be compared with. However, the results presented here may be compared with those for a different species of toad, *Bufo marinus* which was known to contain liver Cu of 10-2000 ppm (Beck, 1955; Goldfisher et al., 1970). The high level and wide variation of Cu in the liver of *Bufo marinus* are similar to those observed for *Bufo juxtasper* in this study. Such elevated concentration of hepatic copper did not seem to induce toxicity although as much as 2 ppm of the metal would be toxic to the tadpole of the toad *Bufo melanostictus* (Khangarot and Ray, 1987). The ability of toad to tolerate high levels of hepatic copper is attributed to the localization of the metal in liver lysosomes where it is made innocuous (Goldfisher et al., 1970).

In order to assess the relative contamination of *Bufo juxtasper* from the areas experiencing direct pollution from copper mine, mean liver metal content of toad from the Mamut River was compared with the mean values for toads collected from the three non-polluted sites, namely, the Langanan River, Tabin Wildlife Reserve and Crocker Range National Park. The results of the comparison (after Mann-Whitney test) are shown in Table 2. The results demonstrated that Cd and Ni content of toad livers from the Mamut River site were significantly higher compared to all three non-polluted sites with Ni and Cd consistently higher ($p < 0.01$ and < 0.05 respectively). Cu content was also significantly greater ($P < 0.05$) for the Mamut River samples, compared to those of Langanan River and Crocker Range National Park. Pb was significantly higher ($p < 0.01$) compared to the Crocker Range National Park samples while Zn was higher than that of samples from Tabin Wildlife Reserve ($P < 0.01$). No significant difference was observed between the contents of Ag, Co, Cr and Mn in the liver of *Bufo juxtasper* collected from Mamut River and the other three sites of comparison.

The relatively higher level of Cu, Cd and Ni in the liver of *Bufo juxtasper* may be directly related to the contamination of Mamut River. Examination of heavy metal content of the river water had indicated unusually high amount of Cu and slightly elevated amount of both Cd and Ni (*Lye, 1988*). Although the river water also contained abnormally high amount of Mn (*Lye, 1988*), the liver Mn content of toad from the area was not significantly elevated. This implies no accumulation of Mn in the *Bufo juxtasper* liver. There is no data available for the content of heavy metals in aquatic organisms found in the Mamut River, but studies on the heavy metal content of fish and snails in rivers affected by other parts of the mine showed contamination mainly by Cu, Cd, Pb and Zn (Jumat and Maimunah, 1989). For Pb and Zn, it is difficult to conclude whether contamination has occurred as the content of these metals in the toad samples from the Mamut River were only significantly higher than one of the three sampling sites compared (Table 2).

Table 2 : Comparison between the mean liver metal content of toad, *Bufo juxtasper* from Mamut River with samples collected from Langanan River, Tabin Wildlife Reserve and Crocker Range National Park.

Site	Metal								
Compared	Ag	Cd	Co	Cr	Cu	Mn	Ni	Pb	Zn
Langanan River	-	*	-	-	*	-	**	-	-
Tabin Wildlife Reserve	-	**	-	-	-	-	**	-	**
Crocker Range National Park	-	*	-	-	*	-	**	**	-

- Indicates no difference between mean metal content

* Significantly higher at $p < 0.05$

** Significantly higher at $p < 0.01$

Results from the simple regression analysis on the liver weight and metal content showed that only Pb and Zn were correlated to the liver weight. Pb exhibited negative correlation while Zn demonstrated positive correlation. For metal to metal correlation studies on all metals, only the Cu - Ag pair indicated positive correlation. The correlations observed are shown in Table 3 .

Table 3. Equations of the regression lines for the [Pb] - liver weight (W), [Zn] - liver weight (W) and [Ag]-[Cu] correlations in *Bufo juxtasper* liver.

Equation*	Correlation	Significance
[Pb]= -0.75W + 1403.77	-0.55	<0.005
[Zn]= 0.092W + 975.44	0.65	<0.005
[Cu]= 14.05[Ag] + 429.77	0.68	<0.005

*Concentration [ ] in ug/g (dry basis)

The existence of correlation between Pb and Zn contents with liver weight made it difficult to conclude about the level of contamination of these two metals in the toad livers as discussed earlier. A negative correlation between liver Pb and weight indicated accumulation of Pb in the larval phase of *Bufo juxtasper* but such accumulation declined relative to body weight when the toad entered the adult phase. The observed positive correlation of Zn and liver weight may be explained by the mechanism of Zn uptake by the hepatocyte. Homeostatic control of Zn in liver is known to occur via accumulation of Zn in a "zinc pool" which is regulated by Zn metallothionein or Zn metaloproteins (Nriagu, 1980). Larger liver size presumably enables the tissue to accumulate and regulate more Zn, thus, a higher content of Zn was observed in larger liver samples when compared to smaller ones. In the case of a positive correlation between liver Cu and Ag content (Table 3), it is difficult to explain why such correlation existed apart from the possibility that the two metals exhibit similar chemical behaviour within liver cells. In fact, silver is commonly used to stain liver copper in the study of Wilson's disease (Goldfisher, 1967).

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