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Ecotoxicity of Mercury Contaminated Sediment Collected from Mabubi River (Geita district, Tanzania) to the Early Life Stages of African Catfish (*Clarias gariepinus*)

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ABSTRACT: The quality of Hg contaminated sediments in artisanal gold mining areas of Tanzania have to date only been assessed through bulk chemical analysis. However, measurement of contaminant levels alone has a limited ability to predict adverse effects on living resources. In this study we investigated the possible effects of Hg contaminated sediments from the river Mabubi which drains the artisanal gold mine on the hatching success, larval survival and growth of the African catfish (*Clarias gariepinus*). The tested species is a resident fish whose demersal behaviour keeps it in frequent contact with the sediment. Sediments collected downstream of the mine decreased survival and impaired growth of *Clarias gariepinus* but did not affect its hatching success. These effects were observed up to six kilometres downstream of the mining activity. The calculated 5 d-LC_{50} value (larval survival) was 1.75 µg/g Hg dw (95% CL of 0.72 – 2.53), the 5d-NOEC for hatching was >2.3 µg/g Hg dw and that for larval survival and growth was 0.23 µg/g Hg dw. In conclusion, chemistry and ecotoxicity results from this study suggest that sediments collected downstream of the mine adversely affect catfish and probably other fauna and as such present a considerable local environmental risk.

Key words: Artisanal gold mine, *Clarias gariepinus*, Mercury, Sediment

INTRODUCTION

In the last decade there have been considerable amount of research about Lake Victoria contamination with mercury (Hg) (Ikingura and Akagi, 1996, Kahatano and Mnali, 1997, Ikingura *et al*,. 1997, Kondoro and Makundi, 1998, Ikingura and Akagi, 1999, , van Straaten, 2000, Campbell *et al.*, 2003, Taylor *et al*., 2005, Chibunda *et al*,. 2008, Chibunda, 2008a, 2008b). Most of these studies were triggered by concerns about the gold (Au) ore processing practices in artisanal gold mines that use mercury amalgamation for extraction of gold. Most of the above studies identified that mercury accumulates more in sediments than in the water column. Sediments contamination in this area has only been assessed by using chemical analysis (Ikingura and Akagi, 1996, 1999; Kondoro and Makundi, 1998; LVEMP, 2002). Although, the information obtained through chemical analysis is valuable it has a limited ability to predict adverse effects on living resources as it does not integrate contaminant fluctuations over time (Canfield *et al*., 1994). Thus, in order to provide information on the ecological impact of sediment contamination on aquatic biota, toxicity bioassays are recommended (Canfield *et al*., 1994).

The objective of the present study was therefore, to establish the toxicity of sediments collected from mercury contaminated river to the early life stages of the African catfish (*Clarias gariepinus*). The tested endpoints included

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hatching success, larvae survival and growth of *C. gariepinus*. The choice of *C. gariepinus* was based on the fact that it occurs in most rivers in the Lake Victoria basin and is an economically important fish for the local communities (David, 1992). In addition, *C.gariepinus* has a demersal life style that makes it vulnerable to contaminated sediments (Bezuidenhout *et al.,* 1990; Adeyeye *et al.*, 1996). Like many other organisms early developmental stages of *C. gariepinus* are probably the most sensitive stages of its life cycle. As other aquatic fauna, *C. gariepinus* can be affected by mercury through the disruption of several processes such as the inhibition of metabolic pathways leading to failure of the energetic balance, cellular division, differentiation and cellular migration (Itow *et al.*, 1998). Bano and Hasan, (1989) showed that Mercury induced time-dependent alterations in lipid profiles and lipid peroxidation in different body organs of cat-fish. It has also been reported that mercury causes necrosis and degeneration of renal tubular cells and increase thickening of renal glomerular basement membrane in catfish (Kendall, 1975; Kirubagaran and Joy, 1988). Similarly, mercury has been shown to induce morphological changes in the respiratory surface of the catfish of the *Saccobranchus fossilis* species (Khangarot, 2003).

MATERIAL & METHODS

Sediment samples were collected from river Mabubi, which drains Mugusu artisanal gold mine. The river runs for 10 km before entering Lake Victoria through Nungwe bay. This river is very shallow, with average depths varying between 0.5 to 3 meters depending on the season and level of precipitation (Chibunda *et al*, 2008c). Water temperature during the sampling period ranged between 24°C to 26°C and had dissolved oxygen ranging from 4.2 to 6.1 mg/L. Sediment samples were collected at an average depth of 1.8 meters from five sites (points), which were identified as A, B, C, D and E (Fig. 1). Point A was located two kilometers before the gold processing area and it was considered as a reference point. Point B, C and D were located at a distance of three, six and nine kilometers downstream the gold processing area, respectively. Point E was located at the river mouth inside Lake Victoria. About three kilograms of superficial sediment (~5 cm) was collected with a core sampler into 10 N nitric acid washed glass jars and kept in cool boxes with ice and transferred to the laboratory. At the laboratory large debris were handpicked and sediment homogenized by stirring and wet – sieved at 300 µm to remove indigenous fauna. Thereafter, sediment was stored at 4 °C in darkness until analysis and testing, which commenced within two weeks. The sediments were analyzed for heavy metal content (described below), as well as for organic carbon content, fine particle fraction (63µm), Acid Volatile Sulfides (AVS) and Simultaneously Extracted Metals (SEM).

The sediment particle sizes were measured by wet sieving according to the method described by Gee and Bauder (1986). Percentage organic carbon (%OC) was measured by using the volatile solids technique which involved drying sediments and burning off organic matter in a furnace for 16 hours at 550°C (ASTM, 1992). The % OC was calculated based on the change in sediment weight before and after ignition (ASTM, 1992). AVS was determined at the Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University in Belgium by using the modified diffusion method according to Leonard *et al.*, (1996). The container with sediment collected from point B broke during transportation from Tanzania to Belgium and it was therefore not analysed for AVS and SEM.

The tested sediments were sampled for chemical analysis on day 0 of the experiment. Sediments were analysed for Cu, Pb, Cr, Zn, Cd, and Hg. The analysis of Cu, Pb, Zn, Cr and Cd was performed by using Atomic Absorption Spectrometer (GBC 906) (USA) in the flame mode. Briefly, water was decanted from the sediment samples and the sediments were air-dried in an air-conditioned room set at 25°C and 65% relative humidity. Sediments were further dried in an oven at 40°C for 48 hours and then milled using an agate planetary micro-mill (Fritsh) from Laval lab - Canada. The resulting fine dry powder was used for digestion, which was done by using an aqua regia digestion system (3:1 parts of HCl to HNO_3). Dry powdered sediment (0.5 g) was mixed with 1.5 ml concentrated HCl and 0.5 ml concentrated HNO_3 in a graduated test tube and digested on a hotplate at 95°C. The digest was

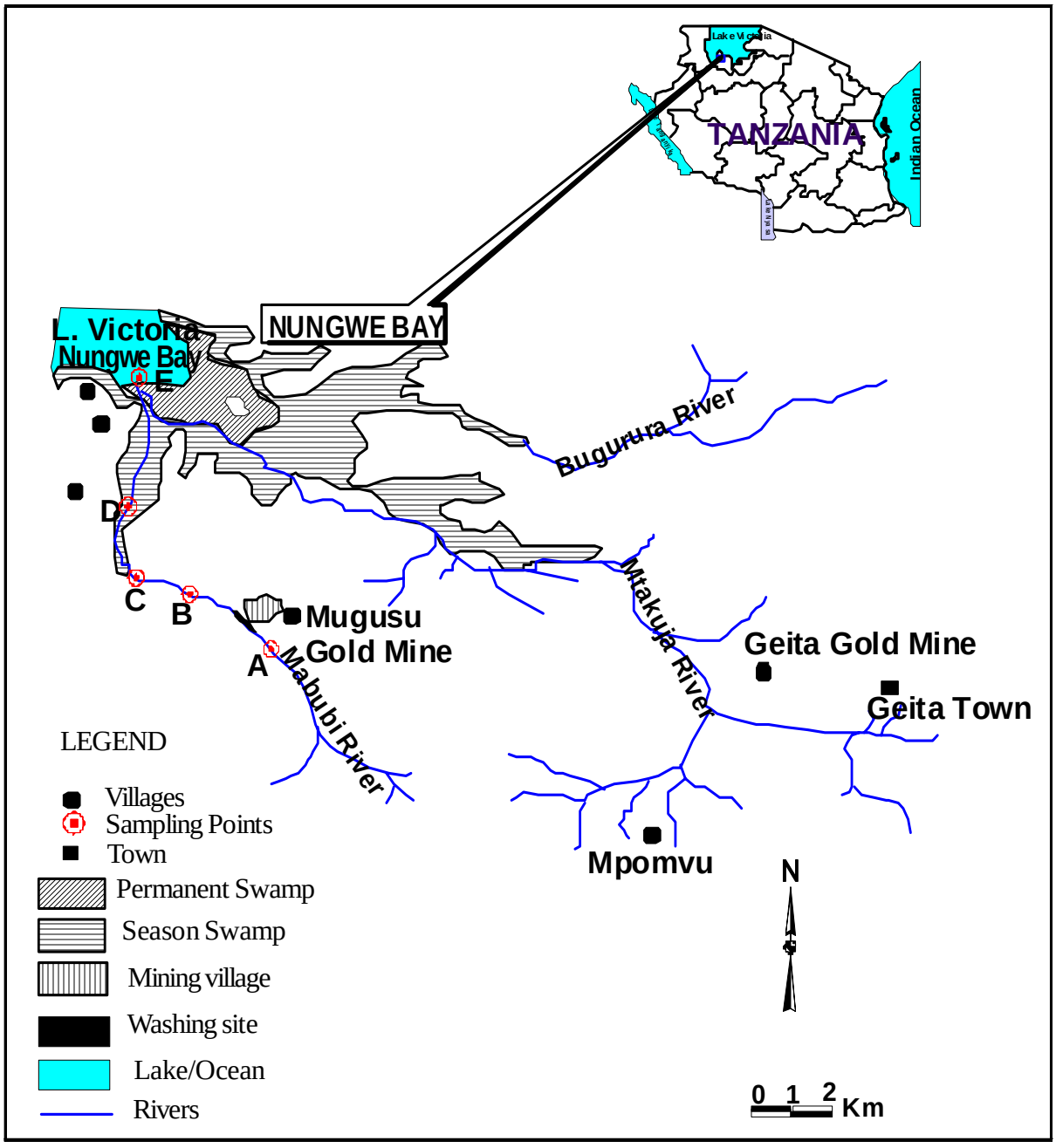

Fig. 1. Map of Geita district to show sampling points

cooled to room temperature and diluted with deionized water to 10 ml. After settling overnight, samples were ready for analysis.

Total mercury analysis was done by using Atomic Absorption Spectrophotometer (ASS) with cold vapour generation technique (ICP Ultima 2, Horiba Jobin Yvon, France). Briefly, sediment samples were air dried in an air conditioned room set at 25°C and 65% relative humidity. Sediments were further dried in an oven at 45°C for 48 hours and then milled using an agate planetary micro-mill (Fritsh) from Laval lab -Canada. The resulting fine dried powder was used for digestion. 0.5 g of sediment was mixed with 4.5 ml concentrated HCl and 1.5 ml concentrated HNO_3 in a graduated test tube and digested on a water-bath at 70°C. Subsequently 5 ml of samples was mixed with 10 ml 6M HCl in a 50 ml test tube. After adding 1ml of KI-ascorbic acid solution, the mixture was vortexed before measuring. Acid washed

glassware, analytical grade reagents and double distilled and deionised water were used in the analysis. In order to check purity of the chemicals used, one blank was run every 10 samples. There was no evidence of contamination in these blanks. Analytical quality control was ensured through the analysis of replicates of Hg standard solutions and the control reference material [BCR-580 for mercury with Hg 132 ± 3 µg/g from European Commission DG Joint Research Centre (IRMM)]. The recovery percentage was 84% and the results were therefore not corrected for recovery. All samples were analysed in duplicates. The detection limit was 0.02 µg/g and 0.02 µg/L. More details on this method are given by Chibunda, (2008b).

Fertilized eggs and larvae of C. *gariepinus* were obtained from the Kingolwila National Fish Breeding Centre in Morogoro, Tanzania. Hand stripped eggs were artificially fertilized with sperm taken from males collected from the wild according to de Graaf and Janssen (1996). Fertilized eggs (2 to 4 cell stage) and 24 hour old larvae were used in this study.

Each test sample was replicated five times. After thorough mixing, aliquots of 30g of sediments were dispensed into 200 ml glass vessels. To keep the eggs visible, a 300 µm Teflon mesh was inserted at the sediment water interface. Then, 120 ml of aerated deionized water (58.8 mg $CaCl_2$, 25 mg $MgSO_4$, 13 mg $NaHCO_3$, and 1.15 mg KCl per L) was carefully added in such a way that disruption of the sediment layer was minimal. Fifty percent of the water was renewed every 24h. The test vessels were kept in a temperature bath (28 ± 1°C) for 12 h before the introduction of fertilized eggs. Ten eggs were introduced in each vessel using a plastic pipette within two hours of fertilization. Recording of hatched and dead eggs/ embryos was done every 12 h. Hatching was defined by the number of larva present. Eggs that did not hatch after 48 h were counted and considered dead. Temperature, pH, dissolved oxygen, hardness and ammonia were measured every 24 hours (i.e. before water renewal). Water samples were taken with a pipette from approximately 1 cm above the sediment surface without causing any disturbance (to avoid contamination of the overlying water with sediment particles).

The experimental design was similar to that of the sediment-embryo test described above, except that the Teflon mesh was not inserted in the testing vessels. Ten larvae (24 h old) were carefully introduced into each vessel by using a glass pipette. The viability of each larva was checked by observing the swimming motion in the water column as they were added. Like in the sediment-embryo test, temperature, pH, dissolved oxygen, hardness and ammonia were measured before each water renewal. Larvae were not fed during the exposure period. To avoid water quality deterioration, dead larvae were removed before each water renewal. A larva was considered dead when it did not respond to probing by using a glass rod. At the end of day five, surviving larvae were removed from the test vessels, counted and preserved in 10% carbonate-buffered formalin. Total length (mm) measurements were done by using a microscale calliper.

The percentage survival and the total length data were arcsine square root transformed, then tested for normality and homogeneity using the Shapiro-Wilkinson test. Differences between the treatments were determined with ANOVA (Fisher LSD test). The LC_{50} values were calculated by using the probit analysis method according to Finney (1971).

RESULTS & DISCUSSION

The physical characteristics of the natural sediments are presented in Table 1. The sediment collected upstream of the gold mine (point A) was relatively coarser than the sediment samples taken downstream of the mine. The clay percentage was 62.5% at point B and 54.3% at point D. Organic carbon (OC) percentage varied from 5% before the gold mine to 1.2% at point D. Point E which was located at the river mouth (in the lake) had the highest percentage of OC (38.2%). Concentrations of metals and AVS measured in the samples collected from the Mabubi River are shown in Table 2. Sediment sampled from point B had the highest concentrations of Hg, Cr and Zn with values of 2.3, 55.2 and 51.04 µg g^{-1} dw, respectively. The concentrations of Hg were 76 times higher than those found at point A.Physico-chemical conditions in the exposure assays are summarized in Table 3. All measured parameters were within the recommended values for chemical

testing with fish early life stages (OECD guideline 210).

The effect of the sediments collected from the river Mabubi on *C. gariepinus* are summarised in Table 4. Compared to the reference sediment, in general sediments collected downstream from the mine adversely affected the survival and growth of the *C.gariepinus* larvae, but not the hatching success of the embryos. Sediments collected at point B significantly reduced survival and inhibited growth of the catfish larvae and the samples collected from point C impaired growth ($p<0.05$). Sediments collected from points D and E did not affect the survival, growth or embryo hatching success. Based on these results, the calculated 5 day LC_{50} (larval survival) was 1.75 µg/g Hg dw (95% CL of 0.72 – 2.53) and the NOEC for hatching was >2.3 µg/g Hg dw and that for larval survival and growth was 0.23 µg/g Hg dw.

From these results it appears that high levels of metals are found near the gold washing site

Table 1.Physical characteristics of the sediments sampled from different points along the Mabubi River (Geita, Tanzania)

Sampling Points	% Sand	% Clay	% Silt	% OC
A	18.3	29.0	47.7	5.0
B	10.64	62.5	26.3	0.56
C	6.76	56.3	36.0	0.91
D	4.71	54.3	39.8	1.20
E	6.0	33.0	22.8	38.2

Table 2. Concentrations of metals, Acid-Volatile Sulfide (AVS), Simultaneously Extracted Metals (SEM) to AVS molar ratio in sediments collected along the Mabubi River (Geita district, Tanzania)

Sampling points	Metal concentrations (µg/g dry weight)						∑SEM (µmoL/g dw)	∑AVS (µmoL/g dw)	SEM/AVS ratio
	Cu	Pb	Cr	Zn	Cd	Hg			
A	1.09	21	31	26	0.62	0.03	0.87	1.06	0.82
B	2.41	34	55.2	51.04	0.86	2.3*	n.a	n.a	n.a
C	1.15	36	43	43.06	0.43	1.6*	1.85	0.03	59.27
D	3.35	17	19	25.2	0.86	0.23	0.98	0.65	1.49
E	3.17	13	6.7	26.56	0.85	0.08	0.67	2.02	0.33
PEC	149	128	111	459	4.98	1.06	n.a	n.a	n.a

PEC = Probable Effective Concentration , * exceeds PEC, n.a= not applicable

Table 3. Mean values (± SD) for different physico-chemical parameters monitored in the assay

Sampling Point	pH	Temperature °C	Ammonia (mg/L)	Hardness (mg/L as $CaCO_3$)	Oxygen (mg/L)
A	6.6 ± 0.13	27.5 ± 0.15	0.0	243 ± 1.2	5.2 ± 0.78
B	7.2 ± 0.45	28.4 ± 0.79	0.5	247 ± 2	4.5 ± 0.93
C	8.0 ±0.63	27.0 ± 1.1	0.3	238 ± 2.7	5.5 ± 0.84
D	7.8 ± 0.37	28.2 ± 0.45	0.0	248 ± 0.8	4.7 ± 0.67
E	6.6 ± 0.46	27.0 ± 0.64	0.0	241 ± 1.3	5.3 ± 0.92

Table 4. Survival, hatching and growth of C.gariepinus exposed to Hg contaminated sediment

Sampling points	A	B	C	D	E
Eggs hatching %	60	56.8	58.3	58.8	58.7
Larval survival %	76	43*	63*	77	83
Larval mean length (mm) ± SD	6.3 ± 0.67	4.8 ± 0.68*	6 ± 0.67	6.6 ± 0.72	7.3 ± 0.84

***Significantly different from the reference sediment (A)**

and decrease downstream. Tanzania lacks it own sediment quality guideline for metals. Measured metal concentrations in sediment were therefore compared with the consensus-based sediment quality guideline values referred to as the Probable Effect Concentration (PEC) proposed by MacDonald *et al.* (2000). The PEC guideline was selected for comparison because various evaluations have demonstrated that this guideline provides a unifying synthesis of the existing sediment quality guidelines and reflects causal rather than correlative effects (MacDonald *et al*., 2000). In the present study, the concentrations of Hg at point B and C exceeded the PEC. Similarly, the concentrations of Hg in sediment collected from point B and C exceeded the sediment guidelines proposed for Ecuador (0.45 µg/g Hg dw), Washington State-USA (0.41 µg/g Hg dw) and Canada (0.14 µg/g Hg dw) (Eisler, 2005).These guidelines provide concentrations of metals above which adverse effects are expected to occur. Therefore sediments collected from these two points are likely to cause adverse effects to local fauna.

The presence of higher Hg levels in sediments sampled downstream of the mine compared to those taken from upstream locations is the result of the use of mercury in the gold extraction process. The occurrence of other metals like Cu, Pb and Cr in the river sediment sampled downstream of the mine can be explained by their natural presence in the mined ore. It has been shown that, complexes of these metals like arseno-pyrite ($FeAsS_2$), CuS_2 and $FeO.Cr_2O_3$ are abundant in tropical gold bearing soils (Lacerda, 1997; Oyarzun *et al.,* 2004). These metals are released into the tailings during gold processing.

The biological effects observed at point B and C in Table 2, corresponded with Hg levels that exceeded the probable effective concentrations of the sediment quality guidelines proposed by MacDonald *et al.,* 2000 of 1.06 µg/g dw for Hg . Similarly, the concentrations of Hg in sediment collected from point B and C exceeded the sediment guidelines proposed for Ecuador, Washington State-USA and Canada. Because these guidelines propose limits of metal concentrations above which effects are likely to occur; it is very likely that the elevated concentrations of Hg at these points caused the observed adverse effects on survival and growth of *C.gariepinus* larvae.

However, like other metals the toxicity of mercury to aquatic organisms depends on species sensitivity, the concentration of mercury and its bioavailability. In natural settings, metal bioavailability depends on a variety of factors such as adsorption to particles, complexation by organic matter (e.g humic and fulvic acids), presence of other cations (Driscoll *et al.*, 1998), and pH (Klinck *et al.,* 2005). It has been established that mercury can react with sulfide (generated in sediments by sulphate-reducing bacteria) and render it biologically unavailable (Di Toro *et al*., 1990; Miller *et al.,* 2007). The observed toxicity of sediment in the present study may be the result of low concentrations of metal binding substances in the sediment samples which might have increased the bioavailability of metals. Indeed, metal toxicity in sediment occurs only when the concentrations of metals are in excess of the sulfides or organic carbon content and/or other metal binding substances (Macdonald *et al.,* 2000). In fact, it has been shown that there is no toxicity if the SEM to AVS ratio is one or less because of the available free metal will be bound to the AVS phase (Ankley, 1996; Burton *et al*., 2005). In the present study, the ratio of the sum of SEM to AVS concentrations was less than one only for sediments collected from points A and E (Table 2). These findings suggest that metals were readily bioavailable in the sediments sampled from points C and D. In addition, artificial sediment contained 2.5% of organic carbon which is higher than that measured in the field sediments collected from points B (0.56%), C (0.91%) and D (1.2%). This difference in organic carbon content may partly explain the observed difference in toxicity to *C. gariepinus* early life stages.

An alternative, but plausible explanation for the observed toxicity in these sediments might be the additional toxicity of the other metals that were measured in sediments i.e. cadmium, copper, chromium and lead. Indeed, mercury has been shown to have additive and possibly synergistic interactions with Cu, Cd and Pb (Fernàndez and Beiras, 2001). No chemical analysis of organic pollutants was performed as in this area there is

no known polluting organic chemicals industry, or farmers in the neighbourhood using chemical fertilizers, herbicides or pesticides (Kitula, 2006). Given that in the present study survival and growth of catfish larvae were affected at lower mercury concentrations than the Hg levels which have been recorded in other artisanal gold mining impacted watersheds in Tanzania; it is possible that the ecology and biodiversity of these water bodies are at risk. For example, mercury content which was measured in sediments collected from Bulyanhulu and Isingile rivers were 5.35 µg/g Hg dw and 2.84 µg/g Hg dw respectively (Kahatano and Mnali, 1997; Taylor *et al.*, 2005).

CONCLUSION

In conclusion, the chemistry and ecotoxicity results from this study suggest that use of mercury in artisanal gold mining activities contaminates the environment and adversely affects the local biota. It is therefore recommended that use of mercury for gold extraction should be discouraged.

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