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Mercury in the Amazon: A Comprehensive Review with Special Emphasis on Bioaccumulation and Bioindicators

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ABSTRACT

About 13 million people in 55 countries worldwide are directly engaged in artisanal mining activities. Gold represents the main mineral extracted by rudimentary techniques. Poor mining practices generate enormous social and environmental problems in most developing countries. In Latin America as many as 1 million artisanal gold miners are emitting about 200 tonnes of mercury annually to the environment. The majority of these illegal or informal operations are in the Amazon region. Misuse of mercury to amalgamate fine gold is an insidious occupational hazard for miners and for the environment; only recently have other sources of mercury been recognized. Forest fires, erosion of the river banks, flooding, vegetation and soil degassing are also responsible for releasing or mobilizing mercury into the Amazon environment. Assuming that the average atmospheric deposition rate from all sources of mercury emission the Amazon is between 10 and 16 $\mu g/m^2/a$, in an area of 5 million km^2, the Brazilian Amazon alone has been receiving 50 to 80 tonnes of Hg/a from different sources. As a consequence, a number of monitoring programs have reported high levels of methylmercury in fish in areas not even disturbed by mining activities.

A better knowledge of the reactions of metallic mercury with organic acids from sediments and darkwater systems is a key step to understand the factors which catalyze the methylation process. The mechanism in which organic complexes are directly bioaccumulated or transformed into methylmercury is still unknown. An understanding of mercury sources and methylation reactions are important to support the scientific background of the problem and establish solutions.

This paper raises some key points that must be addressed to understand mercury bioaccumulation in the Amazon. Specific issues evaluated include: different sources of mercury emission; Hg cycle components which are still poorly understood; the influence of organic matter on complexation of metallic mercury and its subsequent bioaccumulation. Earthworms are suggested as simple and effective bioindicators for fast, inexpensive assessments of the bioavailable species of mercury in contaminated soils. The methodology is discussed and results of preliminary tests with Hg-contaminated soils and synthetic Hg-tannate complexes are presented.

Keywords: mercury bioaccumulation, bioindicators, earthworm, artisanal mining, gold mining

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INTRODUCTION

Artisanal Gold Miners

Artisanal mining is the main environmental and social problem related to mining activities in developing countries. The economic structure of artisanal miners is not different from any other capitalist activity. The driving force for artisanal mining is maximum profit with minimum investment. However, the environmental damage caused by this attitude is now being recognized. Most people in developing countries become miners to escape complete social marginalization as a result of poor rural policies established by Governments (Veiga, 1997). Artisanal mining activities are usually an island of prosperity in a sea of poverty. The existence of informal mining is largely due to poverty, lack of alternative employment and a "get rich quick" mentality (Suttill, 1995).

In 1993, it was estimated that about 6 million of the world's 30 million mineworkers were engaged in artisanal mining in more than 40 countries extracting over 30 different types of mineral substances (Noetstaller, 1995). The International Labour Organization estimates that currently the number of artisanal miners is around 13 million (Table 1) in 55 countries and rising, which suggests that 80 to 100 million people worldwide depend on this activity for their livelihood (ILO, 1999). Due to its unique characteristic of being easily sold and not subjected to monetary instability of local Governments, gold is by far the main mineral being extracted.

Continent	**Number of Miners (million)**
Asia/Pacific	6.7 - 7.2
Africa	3.0 - 3.7
Latin America	1.4 - 1.6
Developed countries	0.4 - 0.7
Total	11.5 - 13.2

Table 1 - Employment in artisanal mining (source ILO, 1999)

A wide range of mining and mineral processing activities are classified as artisanal mining. This ranges from individual panning to large dredging operations. Quite often the terms artisanal and peasant miners are applied to make reference to low-tech manual panners. Even in large-scale operations, most of those miners do not follow conventional technical approach adopted by organized mining companies. The manner in which the work is carried out is the most significant identifying factor. Artisanal miners work based on instinct, need for feeding his family and paying bills. There is no previous “classical” geological exploration, no drilling, no proven reserves, no ore tonnage establishment and engineering studies (Table 2). The concept of survival is the constant, driving force for those miners.

Conventional Mining	**Artisanal Mining**
geology, drilling	feeling, testing
reserves	subsistence
engineering	curiosity, feeling
control	results
feasibility study	pay bills

sophisticated equipment	homemade devices

Table 2 - Main characteristics that differentiate Conventional Mining from Artisanal Mining

The term artisanal miners generally encompasses all small, medium, large, informal, legal and illegal miners who use rudimentary processes to extract gold from secondary and primary ore bodies. In many texts and legislations, the term "small-mining" is used to describe this type of activity but in fact, there are many mills and dredges in the Amazon with the capacity to process 3 to 4 million tonnes of ore. History has shown that without technical support and investment, primary ores are the worst nightmare for artisanal miners. So, artisanal activity is "naturally" controlled by the type of ore deposit.

The establishment of constant gold production by artisanal mining is a difficult task. Production numbers fluctuate considerably due to the illegal nature of the operations, existence of foreign currency black market and money laundering. In Latin America, virtually all countries have artisanal gold mining activities. It is estimated that as many as 1 million artisanal miners are currently mining for gold in Latin America and their production can be as high as 200 tonnes (6.4 Moz) of gold annually (Veiga, 1997). According to Gold Fields Mineral Service Ltd. (1999), the world gold production in 1998 totaled 2555 tonnes The contribution of artisanal miners is not reported but this might range from 500 to 800 tonnes/a.

Use of Mercury in Artisanal Gold Mining Operations

Although the use of mercury is illegal in most countries, mercury amalgamation is the preferred method employed by artisanal gold miners. When used correctly, mercury is an effective, simple and very inexpensive reagent to extract gold (1kg of Hg costs 1g of Au). Despite this, some miners are using cyanidation (*e.g.* Ecuador, Bolivia). A variety of mining and amalgamation methods are used in artisanal mining operations. The extent of mercury losses from a specific site is defined by Au-Hg separation procedures; mercury often is discharged with contaminated tailings and/or volatilized into the atmosphere.

A common practice in many countries is to amalgamate the whole ore, either spreading mercury on the riffled concentration boxes or by using the old plate amalgamation method. In a few places where hydraulic monitors are used, some miners spread large amounts of mercury on the ground with the belief that the "quicksilver" will move on the dirt to catch all gold available. Amalgamation actually occurs after, when the riffled sluices retain mercury droplets and gold specks pumped with the ore. This gives the impression that gold is amalgamated on the ground. When this crude method is applied, the losses can be higher than 3 parts of mercury lost to 1 part of gold produced and the chance to trap gold is remote. Nowadays, several miners are amalgamating only gravity concentrates. This is an important evolution in artisanal mining methods, enabling significant decreases in Hg consumption and emissions.

When gravity concentrates are amalgamated, the mineral portion is separated from the amalgam by panning either in water boxes or in pools excavated in the ground or at creek margins. The heavy mineral-rich amalgamation tailings frequently contain 200 to 500 ppm of residual mercury, which creates “hot spots” when dumped into adjacent water bodies. In dredging operations, amalgamation is done on board using a blender and amalgamation tailings are steadily dumped into the rivers.

The most common method applied to remove excess mercury from amalgams is by manually squeezing it through a piece of fabric. The remaining amalgam usually consists of about 60% gold. When the amalgam is

centrifuged the mercury content in the amalgam drops to 20-30%. Only a few miners in Venezuela are using this centrifuging method.

Once the amalgam is obtained, it is retorted or simply burnt in pans. Retorts can be used to capture volatilized mercury and condense it, allowing the mercury to be recycled. This leads to Hg recovery above 95% and significant reductions in air pollution and occupational exposure. There are many types of retorts. Some are made with stainless steel while others use inexpensive cast iron. Mercury losses during retorting depend on the type of connections or clamps used. Unfortunately, the usual practice to separate Hg from gold is to burn the amalgam in a pan or shovel with a blowtorch. When this happens, Hg is accumulated in the miner's lungs, as evidenced by high Hg content in urine.

In some countries, miners recover mercury from amalgams through dissolution in nitric acid. Mercury can then be precipitated from solution using an aluminum or zinc wire. The major problem with this technique is the fact that, after precipitation, the solution still has some mercury and must be treated before disposal. Unfortunately, this never happens. In addition mercuric nitrate fumes are highly toxic. Human beings have a tolerance of only 0.05 mg per cubic meter of air for the prevailing compound in the process, mercury pernitrate - $Hg(NO_2)_2.H_2O$. A very serious risk is also present when mercury pernitrate contacts alcohol as fulminate ($Hg(CNO)_2$) can be produced. This compound explodes readily when dry and is used in blasting caps and detonators. Currently, gold miners in some parts of the world, such as Colombia are not precipitating mercury from nitrate solution. They simply discharge all mercuric solution into the water streams. Mercury in this form is readily available to be biotically or abiotically methylated.

When the amalgam is retorted, a gold doré is obtained. This is sold in villages to gold shops that melt the gold to get rid of some impurities before paying the miners. In fact, the doré still contains about 20 g of mercury per kg of gold, which is later released when gold is melted. This operation is usually carried out by the gold buyers under the miner's supervision. Mercury levels in the interior of these shops are extremely elevated. Fume hoods used for this are usually very rudimentary, consisting only of a fan which blows the mercury vapours out into the urban atmosphere. The exposure to mercury vapour creates an extremely serious hazard to innocent people living in cities near those gold dealers. In the video documentary "The Price of Gold", the BBC (1993) profiled a case of severe mercurialism in the Amazon caused by vapours emitted from a gold shop. A 60 year-old citizen who lived in an apartment on top of a gold shop received toxic vapours for 10 years. As a result, his neurological functions were dramatically reduced and he suffered from extreme muscle tremors.

When gravity concentrates are amalgamated properly (*i.e.* when retorts are used and gold is melted in adequate furnaces), mercury emissions are almost insignificant (Table 3). An example of effective and creative amalgam processing is currently being applied in Venezuela, where Amalgamation Centers have been constructed to increase gold recovery and reduce mercury emissions. Miners bring their gravity concentrates to these private or state-owned centers to be properly amalgamated, retorted and melted by specialized operators (Veiga and Beinhoff, 1997).

Amalgamation Method	**Hg_{lost} : $Au_{produced}$**
Whole ore	3
Concentrates, no retort	1
Concentrates, with retort	0.001

Table 3 - Mercury losses depend on the amalgamation method

In summary, mercury emitted by miners includes both the fraction lost to the atmosphere when the amalgam is inappropriately burned *and* the portion discharged with amalgamation tailings into aquatic environments. As much as 80% of the mercury initially introduced during amalgamation is lost to the atmosphere when no retort is used (CETEM, 1989). As well, the amount of mercury dumped with tailings is more significant when the whole ore is amalgamated.

Mercury Emissions in the Amazon

Gold mining activities are not the only source of mercury emissions. Other sources of mercury are usually underestimated in tropical environments. As mercury is extremely volatile compared to other metals, the atmosphere is a key compartment to be investigated. Some other natural and man-made sources of Hg emission and/or mobilization in the Amazon are listed below:

- geologic weathering and erosion
- evaporation from waters and soils
- run-off waters
- ancient gold and silver mining
- plant transpiration and decomposition
- waste incineration
- forest fires
- diffuse emissions

Soils are important sinks for atmospheric mercury deposition from all sources. Roulet and Lucotte (1999) studied the importance of soil erosion as a form to carry Hg from lithogenic and anthropogenic sources associated to particulate matter into the aquatic systems in Amazon. Organic matter can play a significant role in solubilizing mercury in these environments (Melamed, 1997)

Forest fires are believed to mobilize Hg contained in biomass and redistribute it into the atmosphere, either as vapour or attached to particulate. Today, with the high rate of deforestation by fire in South America, Hg emissions derived from wood combustion must be significant. The amount of Hg annually emitted by deforestation in the Amazon has been estimated between 8 and 80 tonnes of Hg (Veiga *et al*., 1994; Lacerda 1995). To a large extent, the estimate depends on the biomass distribution, the area burned and Hg levels in plants and organic matter (ranges from 0.02 and 0.3 mg/kg). Regardless of differences in emission estimates, the significance of the forest fire as a vector for Hg emissions in Amazon is indisputable. Concentrations as high as 1,000 mg/kg Hg were measured in smoke particles smaller than 2.5 µm in a forest fire in Amazon (Kaufman *et al*., 1992).

In a comprehensive review, Porcella (1995) describes the mercury emission and deposition rates in the North and South Hemispheres. He believes that much of the background emissions are in the form of elemental Hg (Hg°) that evades from water surfaces, soils and vegetation. Conversely, forest fires and other high temperature emissions are likely to emit at least partially oxidized Hg in particulate and gas-phase forms of Hg. Upon evaluation of data compiled from different sources, the author estimates that the global emission of mercury from all sources is between 5000 and 6000 tonnes/a. Porcella also estimated that the mercury deposition rate in the northern hemisphere ranges form 11 to 14 µg/m²/a and in the southern hemisphere, where

industrial activities are less intense, from 5 to 7 µg/m²/a. In wet conditions, such as in forested areas, mercury deposition rates can double. In the central part of Brazil, von Tumpling *et al*. (1996) estimated a deposition rate of 67 to 151 µg/m²/a associated with mining activities and grassland fires. Lacerda and Marins (1997) considered the mercury deposition rate in the Amazon around 16 µg/m²/a, particularly near mining activities. In recent work using lake sediment cores, Lacerda *et al*. (1999) estimate the current Hg deposition in the Amazon basin ranges from 10 to 12 µg/m²/a. Fosberg *et al*. (1999) analyzed Hg in rain water and estimated the annual deposition of Hg in the Negro River basin, a region with very little mining influence, as 14.7 µg/m²/a. Assuming the average deposition rate from all sources of mercury emission the Amazon is between 10 and 16 µg/m²/a, in an area of 5 million km², the Brazilian Amazon alone has been receiving 50 to 80 tonnes of Hg/a from different sources.

It is not clear if all these calculations have taken into consideration the high levels of mercury emitted by artisanal miners in the Brazilian Amazon, but experts believe that about 3000 to 4000 tonnes of mercury were emitted by miners to the environment over the last 2 decades. In the same period it is estimated that 5,000 tonnes of Hg were emitted in all of Latin America by artisanal miners (Veiga, 1997). Most calculations of mercury emission from gold miners are extremely rudimentary but are usually based on the regional ratio of $Hg_{emitted} : Au_{produced} = 1$. Based on a similar ratio, Lacerda and Marins (1997) estimated that the annual mercury emission from mining activities in Brazil should be around 78 tonnes/a. Due to the scarcity of easily exploitable ores, the low price of the metal and high operating costs, artisanal gold activities have declined since the beginning of the 90's. Consequently, it is very difficult to establish the current gold production from artisanal miners and thus the amount of Hg being emitted.

Whether or not all mercury emitted to the atmosphere by miners travels long distances or is deposited near the emission source is a controversial point. According to Marins *et al*. (1991), the majority of Hg emitted from 32 gold smelting shops is deposited near the emission source (*i.e*. within 1 km). In Alta Floresta, a town in the South of the Amazon Basin, neither air analyses nor soil samples up to 500 m from gold shops show significant Hg concentrations in samples analyzed (CETEM, 1991). A simulation model of mercury emissions from gold shops in the same town concluded that Hg concentrations in air decrease quickly with distance (< 2km) from the source (Artarxo *et al*., 1999). Even when deposition is near the source, mercury from miners and gold shops can be re-emitted when a fire is ignited (Fig.1). The use of fire to clear forests or to control pest on pastures is actually a common practice in the Amazon. No detailed speciation has been conducted to characterize the forms of Hg being emitted from miners and gold shops.

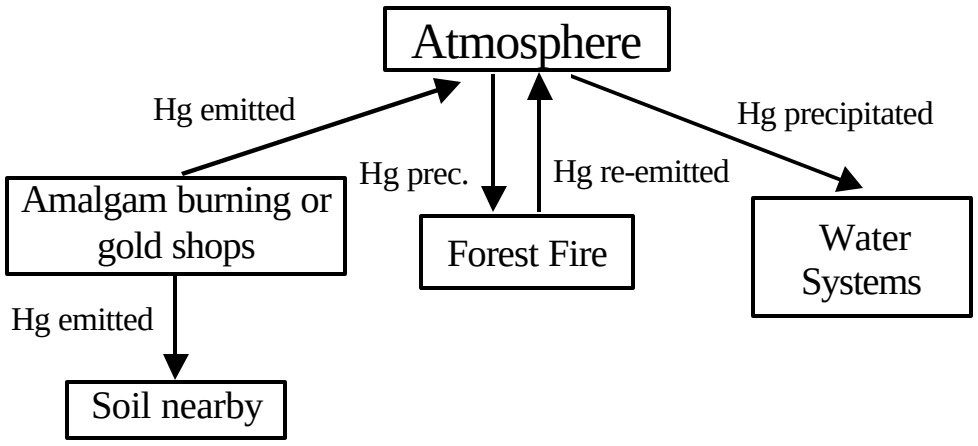


Fig. 1 - Forest fires redistributes Hg emitted by amalgam-burning

The recent discovery of water-soluble species of mercury in the atmosphere, named reactive gaseous mercury (RGM), has heightened concerns of toxicologists. Source measurements have indicated that RGM is formed in combustion processes (Lindberg, 1999). The nature of RGM is believed to consist of one or more simple Hg (II) compounds, such as $HgCl_2$. In Tennessee, the RGM form of mercury represents 3 to 5 % of the total gaseous mercury in the atmosphere (Lindberg and Stratton, 1998). In Florida, this species of mercury represents the dominant form of total mercury in the atmosphere associated with dry deposition (S. Lindberg - personal communication). Lindberg and Stratton (1998) indicated seasonal trends might exist in RGM concentrations; this variability was primarily associated with temperature, solar radiation, O_3, SO_2, and TGM. This research also suggested that vegetated areas may act as important sinks of RGM and, due to the high water solubility of the compounds, rainfall events are significant to RGM's removal from the atmosphere.

It seems reasonable to believe that most mercury emitted by miners is in Hg° form and the majority of this is deposited near the source. It is difficult to predict if a small portion of gaseous Hg° travels long distances, as the rainfall in the mining areas of the Amazon region is seasonal. From November to March the monthly precipitation is usually 100 to 300 mm and from June to August <10 to 30 mm. Reactive gaseous mercury might have a significant role in mercury deposition from other emission sources such as forest fires or other diffuse forms. However, little is known about how Hg° emitted by miners can be transformed into RGM and what is its relation to fish contamination in areas with no influence of gold mining.

Mercury Bioaccumulation in Darkwater Systems

The first evidence of mercury bioaccumulation in Amazonian fish was reported in 1984 by the Jacques Cousteau Society as a result of an expedition of the scientist to Serra Pelada in 1982 (Hacon, 1990). In 1991-92, an international team comprised of Brazilian and British scientists analyzed blood and urine from residents of Jacareacanga, an area not directly influenced by mining activities (Fig. 2). Fish is the main diet of this community, which is located 250 km upstream of the Tapajós River where "garimpo" activities in the Itaituba region are abundant. Considering normal Hg blood levels range from 6 to 12 $\mu g.L^{-1}$ (Krenkel, 1971), the gravity of the situation is evident.

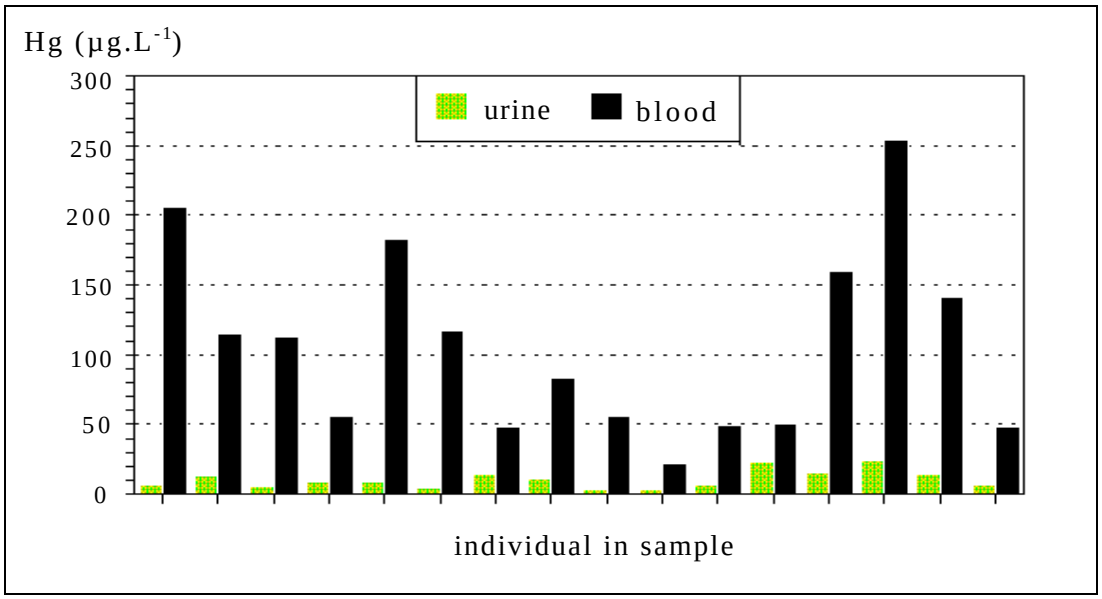


Fig. 2 - Hg in blood and urine of fish-eating people from Jacareacanga. (from GEDEBAM, 1992)

A number of monitoring expeditions took place in the following years. Many studies have established the extent of Hg contamination in fish in the Amazon region. Levels higher than 0.5 ppm Hg are mainly encountered in carnivorous species. Albert Rogerio B. Silva (personal communication), ex-director of the Secretariat of Industry, Commerce and Mining from State of Pará, gathered results of 8333 samples of sediments, water, and biological subjects from at least 30 research institutes from 10 different countries. Many of these studies determined that methylmercury is the main form of mercury found in the aquatic biota, constituting more than 70% of total Hg in muscles.

It has been recognized that methylmercury is mainly produced in sediments and subsequently released into the water column where it is rapidly accumulated by biota (Jensen and Jenerlov, 1969; D'Itri, 1972). The methylmercury production rate seems to primarily depend on (a) mercury complexing characteristics, (b) microbial metabolic activity of the sediment and (c) total inorganic mercury concentration in the sediment (Bisogni and Lawrence, 1975). The availability of Hg (II) in an environment is generally regarded as a limiting factor to the formation of methylmercury by biotic processes. A radiometric method, originally developed by Canadian scientists in the 80's, was adapted to tropical conditions by Guimarães *et al*. (1995) to determine the rate in which $^{203}HgCl_2$, as a source of Hg (II), is methylated in a sediment or in other substrates, such as aquatic plant roots (Guimarães *et al*., 1998). In the Amazon, higher methylation rates (10^{-2} $\%.g^{-1}.h^{-1}$) were found in organic rich sediments in dark water forest streams than in rivers with cloudy or clear waters. High methylation rates have commonly been associated with low pH characteristic of organic sediments and dark waters (Lacerda *et al*., 1995).

Some authors believe that the pH of sediments has less influence on methylmercury production, than the distribution of methylmercury between sediments and the water column (Miller and Akagi, 1979). A decrease in pH of one or two units has been found to double the amounts of Me-Hg released from sediment into the overlying water, but not actually stimulate methylation. As well, field observations have shown more Hg accumulation in fish living in acidic waters in Canada, Sweden and Finland (Verta, 1986; Lindqvist *et al*., 1991; Andersson *et al*., 1995; Hintelmann *et al*, 1995). In many areas in the Amazon basin, far away from

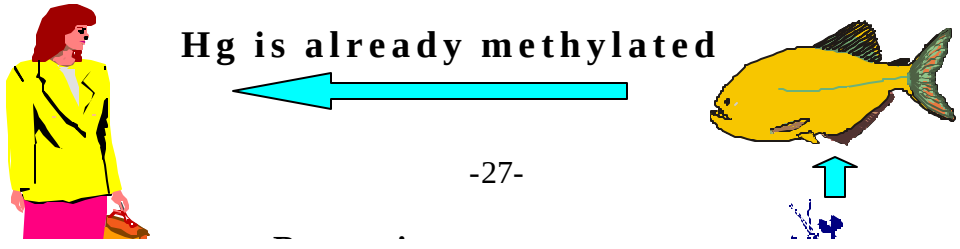
mining activities, fish samples have shown high levels of mercury (CETEM, 1991; Fosberg *et al*., 1999; Fadini and Jardim, 1999; Brabo *et al*., 1999; Kehrig and Malm, 1999; Meili *et al*., 1999). High levels of Hg has been encountered in fish from acidic dark waters (Nakazono *et al*., 1999) confirming previous predictions (Veiga, 1994; Tromans *et al*., 1996; Meech *et al*., 1998).

It is well known that dissolved organic matter (say fulvic acid) forms more stable and predominant complexes than any of the inorganic species (Duinker, 1980; Xu and Allard, 1991). The presence of fulvic acids (FA) is an important parameter that enhances solubility of organic matter and associated mercury. Schnitzer and Kerndorff (1981) have shown that over a large range of pH (4 to 9) when more than 20 ppm of FA is added to solution, Hg becomes very soluble. The authors pointed out that Hg interacts with fulvic acid in partly hydrolyzed forms. Melamed *et al*. (1999) experimentally demonstrated that humic acid solutions increase the solubility of metallic mercury but the presence of calcium ions inhibits Hg solubilization.

When organic acids contact metallic mercury, in interstitial waters for example, soluble complexes are formed at lower Eh levels than those observed in the Eh-pH diagram for inorganic soluble mercury species (Tromans *et al*., 1996). As oxygen is likely the main electron donor in the complex formation reaction, Hg oxidation is controlled by oxygen diffusion in water. So, when Hg-contaminated sediments ("hot spots") exist in shallow creeks with considerable dissolved oxygen available or atmospheric Hg is deposited on top of organic soils the possibility of formation Hg-rich soluble complexes is high. The run-off waters can easily transport these contaminants to water streams. For deep sediments, available oxygen is likely to be extremely low and non-replenished (Meech *et al*., 1998). The formation of Hg-organic complexes when reactive gaseous mercury (RGM) is deposited in darkwater systems may be a significant mechanism worthy of detailed investigation. However, no information is currently available on this matter. It seems reasonable to assume that the RGM-organic matter reactions occur preferentially in the water column and less intensely in the sediments, although the residence time of these complexes in the water is not known. Currently, most studies addressing interactions of Hg with organic matter focus on understanding the chemistry and bioaccumulation of these Hg-organic complexes.

How these Hg-organic complexes transform into methylmercury is unclear. Since fulvic acids are known to be methyl-group donors, methylation of these complexes seems to be feasible through either biotic or abiotic processes (Mannio *et al*., 1986; Verta *et al*., 1986). The soluble Hg complexes can also be adsorbed by colloidal organic matter, which serve as substrate for methylating bacteria.

An intriguing aspect that deserves special attention is the potential for direct bioaccumulation of these Hg-organic complexes. Since most Hg found in fish flesh is already methylated, if these complexes are directly bioaccumulated into invertebrates (Fig. 3) or in fish, then methylation may be occurring in the intestines of the organisms. Rowland *et al* (1977) showed that Hg (II) ingested as a chloride can be methylated in less than 20 hours by intestinal bacteria. They estimated that the total methylmercury synthesized from ingested inorganic mercury in man is approximately 0.4 mg/day. No information is available on intestinal methylation of Hg-organic complexes.



Mercury Bioindicators

All studies in the Amazon have shown that carnivorous (piscivorous) fish accumulate more Hg than other species; however, it is difficult to compare Hg levels in fish from different sites due to different migration habits of species. Another problem is that many carnivorous fish have omnivorous habits and consume other sources of food, such as seeds. Black piranhas (*Serrasalmus rhombeus*) seem to be an ideal bioindicator as 80% of their diet is fish-based, they do not make long migrations, and they mainly live in quiet waters (Goulding, 1980). Unfortunately, black piranha is not found in all areas of the Amazon.

Roulet *et al*. (1999) have found that some carnivorous fish (tucunaré-*Cichla ocelaris*, traíra-*Hoplias malabaricus* and piranha caju-*Serrasalmus nattereri*) from the Tapajós River show very good correlations between Hg content, weight and standard length. Consequently, these researchers believe it is possible to use some of these species as bioindicators of Hg contamination from different Amazonian sites. Rondon and Perez (1999) adopted the 250g *Hoplias malabaricus* as a bioindicator while studying 15 dams in the interior of Venezuela. Incidentally, these researchers found high concentrations of mercury in 7 lakes, 5 of which had no evidence of mining influence.

CETEM (1991) used aquatic snails as bioindicators in Poconé, Brazil. These freshwater mollusks (*Mariza sp*.), with a diameter from 5 to 15 cm, are herbivorous with low mobility and an enormous water filtering capacity. The test procedure consisted of analyzing a group of 15 of snails (30 grams as total wet weight) after they lived in cages for 15, 30, 45 and 60 days in contact with highly Hg-polluted ferruginous sediment. The organisms showed low incorporation of mercury over the days, probably because the Fe-rich sediments had adsorbed any reactive mercury.

Intuitively, the best bioindicator for methylmercury (Me-Hg) bioaccumulation is human beings; however, there are ethical issues associated with collecting biological samples from individuals as, in many cases, the donors do not have any knowledge of the results. Although hair analysis is affected by external factors, such as use of dyes and Hg° vapour exposure, the simplicity of sampling and analysis make it an amenable indicator for toxicological assessment of Me-Hg exposure. Assessments of Hg concentrations in blood and fish suggest that a direct relationship exists between the two. Clarkson (1973) compiled results from other authors which showed that, for a 70 kg individual, Hg in blood (ppb) = 0.95 x Hg (mg) daily intake from fish. Swedish individuals, who are considered to have reached the equilibrium between dietary intake and body burden of mercury, exhibit a direct relationship between Hg in blood and hair. Hair values are about 300 times higher than blood, although this depends on which part of the hair is sampled (Nelson *et al*., 1971). In this case, a correlation between Hg in the hair in ppm (H), mass of fish consumed daily in grams (W_f) and Hg concentration in fish in ppm (F) is approximately obtained: $H = 0.285 \times W_f \times F$.

So, a 70-kg person consuming 200 g daily of "non-contaminated" fish containing 0.3 µg/g Hg, as commonly observed in riverine populations of the Amazon (Barbosa *et al*., 1995; Castilhos and Bidone, 1999), would be expected to consume 0.86 µg of Hg per kilogram of body weight and may show around 11 ppm of Hg in hair samples. This is clearly an approximation since many site-specific variables must be taken into account. The time following fish consumption also plays an important role in Hg blood levels. The most recent guideline from Health Canada recommends as the allowable daily ingestion level something below 0.2 µg of Hg per kilogram of body weight for women of child-bearing age and children and 0.47 µg for men

The normal Hg level in hair is less than 6 ppm and signs of Me-Hg intoxication can be observed at 50 ppm. Methylmercury readily crosses placental barriers and is considered to be a developmental toxicant (Grandjean, 1999), thus hazardous effects to the fetus are possible when only 20 ppm is analyzed in the hair of pregnant women (Krenkel, 1971; Malm, 1991). Levels of 10 ppm must be considered as the upper limit for pregnant women (Skerfving, 1973). Many studies have shown levels of mercury above 50 ppm in riverine populations and Aboriginal people in the Amazon with fish dependent diets (Barbosa *et al*. 1997; Malm *et al*., 1997; Kehrig *et al*. 1997). Methylmercury is usually above 70% of the total mercury analyzed in hair (Vanconcellos *et al*. 1999).

Bioindicators play an important role in identifying the factors controlling Hg toxicity and bioavailability and can ultimately be used to evaluate hazards where Hg pollution is present. Recent studies have demonstrated that the bioavailability of metals in terrestrial and aquatic systems is dependent upon a number of geochemical and biological factors. The presence of organic matter (Gagnon and Fisher, 1997, Standley, 1997), colloidal particles and certain minerals, such as sulphides (Melamed, 1997) or Fe, Mn oxides (Gagnon and Fisher, 1997), influence speciation and/or sorption mechanisms and therefore bioavailability of various metal compounds (Benoit *et al.*, 1999; Wen-Xiong *et al*., 1998). Organism physiology, internal solubilization capabilities (Gagnon and Fisher, 1997), food quality (*i.e.* nutrients) and feeding behavior also affect the assimilation efficiency of a metal (Lawrence *et al.*, 1999, Wen-Xiong *et al*., 1998). Thus, an appropriate bioindicator organism must be reasonably well understood in terms of biological qualities and responses to be broadly applicable to various external (*e.g.* geochemical) conditions. Earthworms may be a viable alternative to traditionally applied organisms (*e.g.* fish, people) as they are simple, well-studied creatures that can provide indications of bioavailability in a short time frame at relatively low costs.

Earthworms as Bioindicators

Substantial evidence indicates that earthworms accumulate heavy metals from polluted soils and other media (Edwards and Bohlen, 1996; Goats and Edwards, 1988; Rhett *et al.*, 1988; Neuhauser *et al.*, 1985; Ireland, 1983). Earthworms are particularly suitable for the assessment of contaminant bioavailability for a number of reasons. They ingest large quantities of soil and are in full contact with the substrate they consume. They constitute up to 92% of the invertebrate biomass of soils and participate in many food chains, acting as a food source for a wide variety of organisms including birds, fish, insects, various mammals, and reptiles (Ireland, 1983; ASTM E1676-95). In addition, they are easily bred, have been extensively studied, and are approved for use in toxicity testing by the US EPA, the European Economic Community and the Organization for Economic Cooperation and Development (ASTM E1676-95). Despite these factors, little information exists concerning the uptake of Hg and MeHg in these organisms. Very few studies (Braunschweiler, 1995; Rhett *et al.*, 1988; Marquenie and Simmers, 1988; Martin and Coughtrey, 1982) have documented Hg concentrations in earthworm tissues and even fewer (Lawrence *et al*, 1999; Yongcan *et al*, 1998; Beyer *et al*., 1985) have addressed the biological and physiological elements that influence Hg bioavailability in these organisms.

In this paper, we present a methodology using the earthworm *Eisenia foetida*, commonly known as the barnyard or tiger worm, to evaluate the bioavailability of Hg in both tailings and aqueous solutions. This methodology can also be applied to soils and sediments, as well as a wide range of inorganic and organic contaminants. Results indicate that *E. foetida* are capable of accumulating Hg and a positive correlation exists between Hg concentrations in worm tissues, the substrate they consume and the length of exposure (a dose-response relationship). Depuration (*i.e.* post-exposure starvation) time was also compared to ensure analytical results were indicative of Hg in tissues and not material retained in the gut. In addition, this research program investigated the effect of natural organic acids as mediators of Hg bioavailability. Two series of tests were conducted wherein metallic Hg was dissolved in tannic acid and "fed" to the worms in a substrate of paper and silica sand. Total Hg and MeHg were analyzed to assess whether methylation of Hg was occurring in the substrate, directly within the worms (*e.g.* in the intestines), or in the tannic acid-Hg solution. The results of analysis revealed that the ratio of MeHg:Total Hg was up to 160 times higher in worm tissues than both the tannic acid-Hg solution and the substrate at the conclusion of the test periods. This result is particularly important in darkwater systems, such as the Amazon, where naturally occurring organic acids may be facilitating methylation internally within organisms. An additional series of jar tests was completed wherein worms were put in direct contact with heavy metal laden tailings. Mercury and other metals (*e.g.* Cd, Pb, Zn) in this media were in a sulphide form and were consequently not bioavailable.

METHODS AND MATERIALS

Organism Culturing and Selection. *E.Foetida* were initially acquired from a local composting cooperative and cultured in a dark plastic, ventilated bin on a diet of either alfalfa pellets or a mixture of vegetable food waste. Worms were hand-selected for testing on the basis of sexually maturity, as evidenced by the presence of a clitellum, size (0.25 to 0.3 g wet weight), and liveliness. Prior to use, the chosen worms were stored for 24 hours on damp filter paper to void their gut contents.

Tannic Acid-Hg Jar Tests. Two separate series (TA1, TA2) of these tests were conducted. Metallic mercury (TA1: 3.03 g and TA2: 6.18 g) was added to 0.005M tannic acid (0.3 and 1.0 L volumes) and

vigorously stirred for one (TA1) and three (TA2) days to promote dissolution. Total Hg concentrations in the tannic acid solutions were 696 ppb (TA1) and 1150 ppb (TA2). Prior to full scale testing, 3 to 5 worms were exposed to the pure tannic acid solution (pH 4.1) in Petri dishes to provide some indication of acute responses. Most specimens died within two hours. Subsequent adjustment of the pH (to 5.85 and 6.02, respectively) enabled long term habitability for the worms.

For the full-scale tests, 25g of shredded, kaolin-based paper and 175g (TA1) or 100g (TA2) of fine silica sand were added to nine 500mL acid washed, glass jars. The pH adjusted tannic acid-Hg solution (80 mL) was then added and jars were manually shaken to homogenize. Groups of 25 to 30 worms were weighed and added to each jar. The populations were left relatively undisturbed for the duration of the tests (14 or 28 days). Two additional jars were completed without silica, but the moisture content was inhabitable for the earthworms – all specimens in these jars died within 5 days. Silica sand not only retains some moisture, but is also used by worms for grinding during the digestion process.

At the conclusion of the exposure period, worms were removed from each jar, carefully washed and dried, counted and weighed. Observations such as motility, light sensitivity and physical qualities (*e.g.* discolouration) were documented to provide some indication of toxic responses. Cleaned worms were then placed in Petri dishes with damp filter paper for either 24 or 72-hour depuration periods, then re-washed and re-weighed. Worms from two jars (OCT99-7, OCT99-8) were kept in mixtures of 15g of clean paper towel and 50g of silica saturated with 50 mL of distilled water for a period of 5 days. Prior to analysis these worms were also depurated for 24 hours.

Nine jar tests were completed with the following experimental specifications:

- Two Jars: 14 day test, 24 hour depuration;
- Two Jars: 14 day test, 72 hour depuration;
- Three Jars: 28 day test, 24 hour depuration;
- Two Jars: 28 day test, 5 day clean paper feeding, 24 hour depuration.

Sample Preparation and Analysis. Following post-depuration washing and drying, worms were placed in 250ml, acid washed Erlenmeyer flasks and digested in 20 mL of 0.7M nitric acid. Distilled water was then added until a volume of 120 mL was reached. Samples were split into 60 mL volumes, poured into acid-washed polyethylene containers and promptly frozen. One of the two samples was kept as a duplicate and the other submitted for total Hg (wet weight) analysis by CVAA. Samples submitted for MeHg analysis were not digested but frozen immediately following post-depuration washing and drying. Methylmercury was analyzed by Cebam Analytical Inc, Seattle, Washington, US.

Total Hg and MeHg in the tannic acid-Hg solution was analyzed directly by the aforementioned methods. At the conclusion of the 28 day test period, substrate material (*i.e.* tannic acid saturated paper and silica sand) was leached with distilled water. Leaching involved the addition of 300 mL of distilled water to 178g of substrate. The combined material was shaken vigorously for several minutes. Leachate (198 mL) was subsequently extracted using a vacuum filter and submitted for MeHg and total Hg analysis.

Hg-rich Mine Tailings Jar Tests. Earthworms were put in direct contact with heavy metal laden mine tailings from British Columbia to assess uptake of various metals including Hg. Tailings were combined with different media in three 900 mL jars as follows:

- 175g tailings, 25g shredded brown paper, 1g bread yeast;
- 175g tailings, 25g shredded brown paper;
- 175g tailings, 25g peat, 10.3g $CaCO_3$.

Peat was used as a source of organic acid to investigate its influence on metal availability. Deionized water (125 mL) was added to each jar. Like the tannic acid-Hg jar tests, worms were pre-purged and cleaned, then placed in the substrate for periods of 16 and 29 days. Digestion and total Hg analysis of worms were conducted as described above. Full metals scan was also conducted using ICP-MS.

RESULTS AND DISCUSSION

The worms not exposed to any solution but cultured in the same medium as the test worms as well as the control worms which underwent the same test protocols, but fed a solution of tannic acid alone (<0.5 ppb Hg), had tissue concentrations below 40 ppb (Table 4). In the tannic acid-Hg jar tests, Hg concentrations in worm tissues ranged from 0.7 to 7.3 ppm. Bioconcentration factors, or the ratio of Hg concentrations in worm tissues to the substrate, averaged 3.7. The most bioconcentration occurred in populations which exhibited toxic responses to their environment (*e.g.* discolouration, impaired mobility). Conversely, lowest Hg concentrations were detected in the healthiest populations. It is probable that variability between populations can be primarily attributed to insufficient homogenization of the worms in the jars or the type of paper used. Successive tests intend to enhance mobilization of worms within jars and use a more absorbent paper capable of distributing solution more evenly. Table 4 summarizes results of the jar tests.

Sample No.	Test Description	Hg in Tissues (ppb)	Hg in Substrate (ppb)	Bioconcentration Factor
OCT99-1	14d, 24hr dep	2837	828	3.4
OCT99-2	14d, 24hr dep	2449	828	3.0
OCT99-5	14d, 72hr dep	3695	828	4.5
OCT99-6	14d, 72hr dep	1614	828	1.9
CAM99-1	28d, 24h dep	861	278	3.1
OCT99-3	28d, 24hr dep	2499	828	3.0
OCT99-4	28d, 24hr dep	749	828	0.9
OCT99-7	28d, 5d feed plus 24h dep	7305	828	8.8
OCT99-8	28d, 5d feed plus 24h dep	4055	828	4.9
14d Average	-	2649	828	3.2
28d Average	-	3094	718	4.1
Overall Average	-	2896	767	3.7
Control worms	28 d, 24 hr dep	35	<0.5	-

Table 4 - Results of Tannic Acid-Hg Jar Tests

Although 35 to 40% more Hg was, on average, accumulated during the 28-day tests, the populations were generally healthier and more responsive than the 14-day jar tests. The average weight per worm actually

increased for the 28-day populations. This overall improved condition could be due to the reduced stress associated with the extended acclimatization period. As healthier, less stressed organisms are supposed to be more indicative of natural uptake, the 28-day tests are recommended for implementation of this methodology.

Depuration times (24h, 72h, 5days plus 24h) were also compared during this study. The average worm weight loss was about 30% greater in the worms fed clean paper for 5 days following the test; despite this, Hg concentrations were up to 40% higher in these populations. It is possible that during the additional 5 days, Hg containing gut contents were more thoroughly assimilated than other tests. The overall weight decrease may be indicative of more complete purging. As shown in Figure 4, Hg concentrations in tissues generally increased with purging time. The physiological explanations for these responses are currently being studied in greater detail – results of subsequent test programs should provide more insight into this matter. A 5 day "clean" substrate feeding (plus 24 hour depuration) following a 28 day test provides the most reliable results.

Total Hg and MeHg were analyzed to assess whether methylation of Hg was occurring in the substrate, directly within the worms (*e.g.* in the intestines), or in the tannic acid-Hg solution. The results of analysis revealed that the ratio of MeHg:Total Hg was up to 2400 times higher in worm tissues (32.2 ppb) than both the tannic acid-Hg solution (0.059 ppb) and the substrate (0.013 ppb). This result is particularly important in darkwater systems, such as the Amazon, where naturally occurring organic acids may be facilitating methylation internally within organisms. Despite this, MeHg (32.2 ppb) constituted only around 1% of the total Hg in worm tissues (from a 28-day test), which is considerably lower than measured values in higher organisms. It is possible that, as earthworms are consumed (*i.e.* as the Hg moves up the food chain), it is subject to further methylation internally within other organisms.

The mine tailings jar tests suggest that these organisms can also be used to characterize the bioavailability of a range of heavy metals. Among other metals, Hg, cadmium (Cd), lead (Pb) and zinc (Zn) were accumulated by earthworms, but not bioconcentrated over the course of 16 and 29-day tests. For example, Hg in tailings was measured at concentrations up to 19.2 ppm, but only reached concentrations of 0.071 ppm in worm tissues. Bioavailability was likely inhibited as most metals, including Hg, were present in the form of sulphide minerals. The presence of peat in the jars did not significantly influence results. This methodology can potentially be applied to the assessment of bioaccumulation of metals from mining wastes (*e.g.* metals mobilization associated with ARD). This could be conducted in conjunction with kinetic tests for a more comprehensive evaluation.

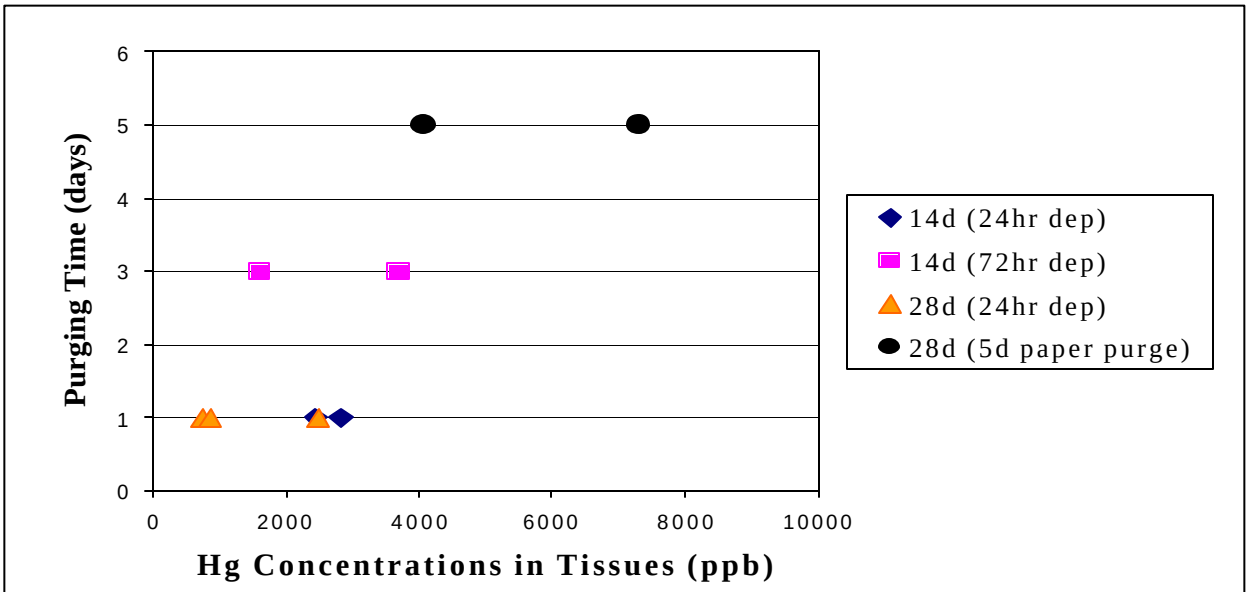


Fig. 4 – Total Mercury Concentrations in Worm Tissues versus Purging Time

CONCLUSIONS

Misuse of mercury to amalgamate fine gold is an insidious occupational hazard for miners and for the environment; other sources of mercury are currently being investigated. Forest fires, erosion of the river banks, seasonal flooding, vegetation and soil degassing, as well as other industrial activities are also responsible for releasing mercury into the Amazon environment. The recently discovered species of atmospheric Hg, reactive gaseous mercury, may play a significant role in transferring Hg to the aquatic environment. In fact, its has been recognized over many years that fish from darkwater systems accumulate more Hg than fish from other environments, in both the presence and absence of mining activities.

The reaction of metallic mercury with organic acids from sediments and darkwater systems is definitely an important pathway for mercury bioavailability. The mechanisms in which organic complexes are directly bioaccumulated or transformed into methylmercury require further study.

Bioindicators play an important role in identifying the factors controlling Hg toxicity and bioavailability and can ultimately be used to evaluate hazards where Hg pollution is present. The development of easily implemented, low-cost, less sophisticated methods can be beneficial for rapid diagnosis of potentially hazardous situations, particularly in regions such as the Amazon where resources are limited and pollution is widespread. Earthworms (*E. foetida*) are capable of accumulating Hg and other metals. A positive correlation exists between Hg concentrations in worm tissues, the substrate they consume and the length of exposure (a dose-response relationship).

The methodology presented can be easily employed within a short period of time at a relatively low cost. Thus, this procedure can be applied as a tool for the assessment of Hg and other metal bioavailability in polluted soil, sediments, tailings and liquid effluents. Future research intends to focus on three main areas. First, the influence of naturally occurring organic acids on the facilitation of methylation, particularly within organisms, will be investigated in greater detail. Next, the physiological mechanisms controlling Hg and other metal intake and the possible synergistic or antagonistic effects of other elements (*e.g.* selenium, calcium) will be addressed. Finally, correlations between *E. foetida* and the substrate they consume, as well as between worms and various well-studied fish species (*e.g.* tucunaré, traíra and piranha) will be quantified. As many correlations already exist between these fish and people, if a link can be established with fish and worms a "gap can be bridged" and the earthworms can be used to assess human health hazards in impacted areas.

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