

**EARTHWORMS as a BIOINDICATOR of MERCURY POLLUTION  
in an ARTISANAL GOLD MINING COMMUNITY,  
CACHOEIRA DO PIRIÁ, BRAZIL**

by:

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## ABSTRACT

The health and welfare of millions of inhabitants of Latin America are directly and indirectly influenced by artisanal mining activities. The rudimentary methods that characterize artisanal gold mining, particularly in association with the misuse of mercury in gold amalgamation, often generate extensive environmental degradation, which persists long after mining activities cease. This is the case in the municipality of Cachoeira do Piriá, located in Pará, Brazil, where approximately 5,000 artisanal miners discharged more than four tonnes of mercury into soils, air and aquatic systems between 1980 and 2000. An area covering approximately 85 ha located adjacent to the town of Cachoeira is moderately to highly contaminated with Hg (>1000 ppb), although Hg contamination is dispersed across an area of approximately 2100 ha. Mercury discharged into the environment can undergo many changes, including the transformation to a readily bioavailable and highly toxic form of mercury, methylmercury. Due to its behaviour in the food chain, methylmercury poses a serious risk to humans reliant on fish as a protein source.

A laboratory methodology using the earthworm *Eisenia foetida* was developed in this research to assess mercury bioavailability in mine tailings and aqueous solutions, and examine the influence of certain factors on mercury bioavailability, in particular organic acids. The quick, inexpensive and simple earthworm protocol described herein was developed to prioritize contaminated sites based on the potential for mercury incorporation into biota with specific consideration of the technical and financial limitations inherent in artisanal mining communities.

Using samples collected in Cachoeira, this research indicates that the reaction of mercury with organic acids is an important pathway for the incorporation of mercury into the food chain. Earthworms (*E. foetida*) are capable of accumulating Hg from solutions and soils, and this potential increases markedly in the presence of organic acids. In addition, evidence suggests that earthworms may be converting mercury associated with organic acids to its highly toxic, readily bioavailable form, methylmercury, in the intestinal tract. The influence of organic acids on Hg bioaccumulation and intestinal methylation is extremely significant in terms of understanding biogeochemical cycling of Hg in darkwaters and assessing ecological and human health risks from Hg in these aquatic systems.

Due to the demonstrated bioavailability of Hg in soils, sediments and tailings, and elevated Hg levels in fish sampled in Cachoeira, the earthworm methodology was also applied to assess the suitability of local materials, specifically clay, lateritic and organic soils, for capping of Hg hot spots in order to inhibit its mobility. It was found that clay and lateritic soil have some capacity to inhibit Hg uptake, particularly in comparison to the organic-rich soil. However, these tests were conducted using distilled water, and increased bioaccumulation would be anticipated in the presence of organic acids. Non-technical measures (e.g. educational campaigns) to reduce Hg risks were also explored.

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# 1 INTRODUCTION

## 1.1 Statement of the Problem

One of the most significant sources of mercury pollution in many developing countries is from gold extraction by artisanal or small-scale miners. These informal miners resort to gold mining to escape poverty and social marginalization in regions virtually devoid of viable economic alternatives. As gold amalgamation using mercury is inexpensive, quick, simple and highly effective, it is the method of choice for most of the six million artisanal gold miners around the world. Throughout the 1990's in Latin America alone as many as one million artisanal gold miners emitted an estimated 200 tonnes of mercury annually to the environment.

Artisanal gold miners release mercury in two forms: metallic mercury and mercury vapour. Although inhalation of mercury vapour is a serious occupational hazard for artisanal miners, the conversion of re-deposited mercury vapour and metallic mercury into methylmercury, a readily bioavailable and highly toxic form of mercury, represents a critical health risk to residents consuming methylmercury contaminated food. Due to the behaviour of mercury in aquatic systems, mercury levels in carnivorous fish – a crucial protein source for many Amazonian communities – can be particularly high. Exposure to mercury through consumption or inhalation can result in a host of effects, including neurological damage and cancer. Given the severity of hazards associated with this highly toxic metal, potential mercury transformations *must* be well understood to effectively predict and prevent harmful human and environmental health effects. Mercury transformations in the environment are, accordingly, the subject of numerous research projects around the world. However, very few researchers are focusing on tools and solutions that are appropriate to mercury impacted marginalized communities in the developing world. A simple, low-cost method to prioritize contaminated sites and assess remedial technologies could contribute to this neglected, albeit crucial aspect of mercury pollution in artisanal mining communities

## 1.2 Outline of the Work

This work is primarily focussed on a laboratory methodology using the earthworm *Eisenia foetida*, devised to assess mercury bioavailability in mine tailings and aqueous solutions. Although a number of biological indicators are currently being employed to evaluate the toxicity and bioavailability of mercury, most methodologies are relatively costly and complicated. The quick, inexpensive and simple earthworm protocol described herein was developed to prioritize contaminated sites based on the potential for mercury incorporation into biota with specific consideration of the technical and financial limitations

inherent in artisanal mining communities. This methodology has also been adapted for use in the assessment of various remedial alternatives in impacted areas.

It was determined that field testing in a particular artisanal mining community was essential to validating the earthworm protocol. In the last 20 years, the municipality of Cachoeira do Piriá, located in the State of Pará, Brazil, has experienced a gold rush that has resulted in serious environmental and social consequences. During the 1980's, approximately 5,000 artisanal miners discharged more than four tonnes of mercury into soils, air and aquatic systems, thereby exacerbating the potential for mercury incorporation into the food chain and ultimately humans.

Cachoeira was selected as a field study site since the mercury pollution in various compartments (soil, water, biota) has been partially characterized by other researchers and mercury levels, particularly in certain species of fish, give cause for serious concern. Specifically, researchers from the Mercury Research Laboratory at the Brazilian National Department of Mineral Production (DNPM), the National Institute of Minamata Disease (NIMD) and the University of São Paulo have studied the distribution of mercury in soils, tailings and fish in Cachoeira. Researchers from the University of Surrey have also assessed mercury accumulation in humans, as well as pigs, sheep and cattle (Palheta and Taylor, 1995). In addition, Cachoeira is located in close proximity to a major centre, specifically Belém, the capital city of the State of Pará, where the National Department of Mineral Production (DNPM) mercury research laboratory is situated. Prior to this study, no efforts had been made to evaluate or develop measures for the mitigation of the risks to this community.

This work also provides general information about mercury in various compartments, i.e. terrestrial, aquatic, atmospheric and biological systems. This information is applied to existing conditions in Cachoeira in order to further contribute to an understanding of the behaviour of mercury in that municipality, as well as in similar artisanal mining communities. Technical and non-technical measures for mitigation of mercury pollution problems in Cachoeira are also presented.

### **1.3 Significance and Contributions of the Work**

The logic behind developing the earthworm methodology is primarily based on the absence of mercury assessment protocols that are appropriate to resource-limited artisanal mining communities. There are currently an estimated 160,000 artisanal mine sites around the world (Hilson, 2002). More than 2000 abandoned artisanal mine sites are located throughout the Brazilian Amazon alone. Individuals who reside

in these communities are left to contend with a legacy of deplorable socio-economic conditions and widespread environmental degradation, including extensive mercury pollution in gold mining areas.

Specifically, this research intends to make a significant contribution with the development of the low-cost, simple earthworm methodology. In applying the methodology, it also aims to provide information about the role of organic acids in mercury bioavailability, a topic that is poorly understood and plagued by confounding evidence. Furthering our understanding of the relationship between organic acids and mercury bioavailability has important implications, not only for artisanal mine sites, but for thousands of other mercury impacted sites around the World.

Using Cachoeira as a case study, this work identifies the benefits and limitations of various measures proposed for mitigation of mercury pollution. This is a substantial component of this work, first, as concrete solutions are typically overlooked by most mercury researchers and, second, this involves an integration of technical and socio-economic issues, a factor that is crucial to the successful implementation of any measures developed. Ideally, the findings derived from this thesis will advance the efforts towards developing viable solutions in mercury-impacted artisanal gold mining communities.

#### **1.4 Structure of the Thesis**

This thesis consists of nine chapters, including:

Chapter One – Introduction. The introduction briefly describes the justification for this work, identifies its purpose and objectives, and outlines the information contained herein.

Chapter Two – Mercury and Artisanal Gold Mining. This chapter reviews mercury use by artisanal miners with special emphasis on the Brazilian Amazon. Some of the other environmental and socio-economic consequences derived from artisanal mining are also highlighted.

Chapter Three – Mercury in the Environment. This section provides an overview of the major anthropogenic and natural sources of mercury in the environment, its partitioning and behaviour in various compartments, and pathways and impacts to human health. Biological indicators of mercury pollution are also discussed.

Chapter Four – Cachoeira do Piriá, Brazil. This chapter describes the physical and socio-economic environment in Cachoeira and outlines the history of artisanal mining. Prior assessments of mercury pollution are also discussed.

Chapter Five – Methodology. This section is comprised of an in-depth description of the earthworm protocol developed and the Cachoeira field sampling program methodology.

Chapter Six – Results. Results of the field sampling program in Cachoeira and laboratory experiments using the earthworms are detailed in this section.

Chapter Seven – Discussion. This chapter discusses the results and their implications for mercury risks for residents of Cachoeira. Measures to mitigate mercury pollution in Cachoeira, as well as other artisanal gold mining communities, are also identified. Concepts explored include remedial options, educational campaigns and consumption advisories, as well as the use of native resources for site remediation.

Chapter Eight – Conclusions. This conclusion discusses key findings derived from this thesis, including important results from the earthworm experimental program, uncertainties associated with its applicability, and recommendations for the use of this methodology, as well as other measures, in the management of mercury-polluted sites.

Chapter Nine – Recommendations. This chapter consists of recommendations specifically related to the earthworm protocol and its application, as well as some suggestions for mitigating mercury pollution in artisanal mining communities.

## **2 MERCURY AND ARTISANAL GOLD MINING**

### **2.1 Artisanal Gold Mining Defined**

Although artisanal mining has been widely recognized as the most environmentally and socially destructive mining activity in the world, it continues to be an essential source of livelihood in many developing countries, particularly in regions where economic alternatives are critically limited. The International Labour Organization (1999) estimates that the number of artisanal miners is currently around 13 million in 55 countries. From this, it has been extrapolated that 80 to 100 million people worldwide are directly and indirectly dependent on this activity for their livelihood. As gold is easily sold and not influenced by the instability of local governments, it is by far the most important mineral extracted by artisanal miners. Artisanal miners have been extracting gold in virtually all Latin American countries, but most mining activities take place in the Brazilian Amazon region. In 1989, more than one million gold miners were believed to be working at 2000 sites in the Brazilian Amazon producing an average of 100 tonnes of gold (Au) per annum (Feijão and Pinto, 1992). In 1995, the Brazilian contingent dropped to 200,000 - 400,000 extracting 30 to 50 tonnes (Veiga, 1997). Today, less than 200,000 miners are struggling to produce 20 tonnes/year, which according to IBGM (2001) represents 30% of Brazil's gold mining production. Other sources believe that the Brazilian "garimpos" (artisanal mining sites) have recently been producing only 10 tonnes of gold annually (Nery and Silva, 2000). With the exhaustion of the easily extractable ore, low gold prices and high fuel costs, it is safe to assume that most of the mining sites are currently being abandoned and that no effort is being made to rehabilitate pits or tailings impoundments (Veiga and Hinton, 2002).

Frequently, the terms artisanal, small-scale and peasant miners are applied to low-tech manual panners, although this term actually applies to individual panning to large dredging operations. In many regions in the Brazilian Amazon, for instance, a single mining operation moves as much as four million m<sup>3</sup> of material annually (Hinton et al, 2002). However, even in large-scale operations with highly mechanised extraction techniques, artisanal miners do not conduct the same kind of mine planning as organised mining companies, but employ methods that make the most money in the shortest period of time. The term artisanal mining seems to best encompass all definitions and designations, as it includes all small, medium, large, informal, legal and illegal miners who use rudimentary processes to extract economic minerals from secondary and primary ore bodies. The Portuguese labeled artisanal miners "grimpeiros" and Brazilians have adapted the name to "garimpeiros". For three centuries, the term garimpeiro was improperly limited to individual panners, but is currently applied to all artisanal miners. Other terms commonly applies to these activities include "garimpo" and "garimpagem", which respectively refer to

the worksite and the activities conducted by garimpeiros in general. In Brazil, these terms have negative connotations and are often associated with illegal or questionable activities.

Historic gold mining activities of colonial North America are very similar to artisanal practices employed for subsistence purposes today - both are characterized by very rudimentary and labour intensive equipment and methods. Mechanization significantly modifies artisanal mining operations as resource extraction can take place in a much shorter time frame. As miners seek opportunities elsewhere, this practice results in rapid abandonment of communities, in addition to the degradation of huge expanses of land associated with the excavation of even greater volumes of earth. Miners are eager to re-invest profits in expensive excavators, bulldozers and trucks, or look to private investors to scale-up production. Access to modern equipment, however, does not necessarily equate to efficient resource extraction.

The situation in Poconé, located in western Brazil, exemplifies what can occur when mechanization is not accompanied by appropriate use of technology. At the beginning of the 1990s, more than 4,500 miners in 100 sites were mining low-grade quartz veins in highly weathered rock using trucks and shovels. In the absence of geological information and traditional mining concepts such as planning, all of the capital invested in equipment was quickly lost. Even miners who had previously produced 10 tonnes of gold were facing bankruptcy. Because funding in Poconé and other locations is obtained outside of traditional financing systems, miners pay a high price for these failed ventures, in some cases, with their lives (Veiga and Hinton, 2002).

## **2.2 Current Practices in Artisanal Gold Mining**

Artisanal mining usually involves the extraction of secondary gold from alluvial, colluvial or illuvial material<sup>1</sup>, (i.e. free gold that is easily concentrated by gravity processes). For soft materials, such as weathered ores, miners typically employ hydraulic monitors, which involve the high-pressure application of water to ‘fluidize’ loosely consolidated materials. For alluvial ores, dredging of the river bottom sediments is the preferred method. Hydraulic monitoring typically results in extensive environmental degradation due to the sheer volume of material fluidized, the amount of water used and the lack of containment structures. Typical impacts include the modification of hydrologic regimes through creation of vast tailings “beaches” and diversion of rivers. In the north of Mato Grosso State, south of the Amazon

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<sup>1</sup> Alluvial gold has been transported by water; colluvial gold has been transported by erosion on land; and illuvial gold results from dissolution and subsequent precipitation within soil horizons.

basin, the impact caused by diversion of one river extended for more than 30 km (Hinton and Veiga, 2002).

Extraction of gold associated with primary ores (e.g. sulphide associated gold, often found at depth) can be far more complicated. Blasting with dynamite may be required and, if the operation is not limited to a small-scale excavation at the surface, underground tunnelling presents additional challenges (e.g. ventilation, hauling, tunnel stability, etc). Some operations in Latin America, Asia and Africa extract gold from quartz veins, which may contain extremely high grades (>10-20 g Au/tonne) (Hinton et al, 2002). Most gold is not occluded in sulfides and can be extracted by amalgamation or cyanidation without prior sulfide oxidation. Without access to capital, appropriate technology and skills, encountering gold associated with sulfides typically signifies the beginning of the end of artisanal mining involvement in these operations.

Comminution (i.e. crushing and grinding) is sometimes carried out to process primary ores (e.g. sulfide associated), although most gold is not liberated through these methods. Gold-laden tailings are subsequently re-processed, often using the same circuit. Although material derived from comminution processes is suitable for other mercury-free concentration methods, mercury is sometimes added during grinding, gravity concentration or after to amalgamate gold, as discussed below.

### ***2.2.1 Amalgamation Practices***

Amalgamation using mercury (Hg) is the preferred method employed by artisanal gold miners in the Amazon. When used correctly, Hg is an effective, simple and very inexpensive reagent to extract gold (1kg of Hg costs ~1g of Au) (Veiga, 1997). All attempts to eliminate Hg amalgamation by introducing alternative techniques, such as gravity separation, have been unsuccessful. In these cases, Hg use was reduced, but never eliminated. The widespread misuse of Hg associated with these activities has consequently generated thousands of polluted sites with impacts extending far beyond localized ecological degradation (Veiga and Meech, 1997).

Gold extraction using Hg consists of four main steps: amalgamation, separation of the mineral portion, amalgam decomposition and gold melting. Amalgamation is a process wherein Hg alloys with gold in a pulp of gold-bearing ore and water. A common practice during the gold rush in the Brazilian Amazon involved amalgamation of the whole, weathered ore, either by spreading Hg on the ground, on riffled concentration boxes or carpets. As Hg amalgamates with gold, the heavier amalgam 'sinks' and is

retained behind riffles or in carpet fibres (Figs. 2.1 and 2.2). As copper also forms amalgams with Hg, copper plates are alternately used to retain the Au-Hg amalgam. When conducting hydraulic monitoring, which involves application of high pressure water to ‘fluidize’ loosely consolidated weathered rock or soil, some miners spread large amounts of Hg on the ground in the belief that the ‘quicksilver’ will move throughout the slurry and capture all available gold. When this method is employed, more than 3 parts of Hg is lost to 1 part of gold produced. This practice is rarely or no longer used in the Brazilian Amazon, primarily due to the influence of public opinion and artisanal miners' associations.

Addition of Hg during comminution is less common, as the grinding process tends to pulverize, or ‘flour’, the Hg reducing its ability to coalesce and amalgamate. Greater quantities of Hg are therefore required to increase gold recoveries. The ratio of mercury used to gold produced, can be as high as 40:1, as can be the case for the quimbaliti crusher used in Peru (Hrushka and Medina, 2001) and muller mills used in China (Gunson, 2001).

Currently, most miners usually only amalgamate gravity concentrates (i.e. ore which has been crushed and separated by weight), a practice that contributes to significant reductions in Hg consumption and emissions. In some Brazilian “garimpos” (Poconé, for example), approximately 14 grams of Hg are required to amalgamate 1 kg of concentrate (ratio Hg:concentrate = 1:70) (Veiga, 1997). The undesirable mineral portion is separated from the Au-Hg amalgam by panning either in ‘waterboxes’, in pools excavated in the ground or at creek margins. The heavy mineral-rich amalgamation tailings frequently contain 200 to 500 ppm of residual Hg, which create ‘hot spots’, i.e. sites with extremely elevated Hg concentrations, when discharged into adjacent water bodies (Farid et al, 1991).

In some locations, artisanal miners use simple suction dredges to extract gold from river bottoms. This practice can result in a number of ecological and human health risks. Fatalities are common for miners outfitted with crude diving gear to ‘vacuum’ bottom sediments through a pipe. In order to recover gold from the gold-rich gravel, diluted pulps (~5% solids) are sent to on-board sluices where they are concentrated (Veiga and Hinton, 2002). Tailings from sluices are directly discharged back to the river generating a large silt plume, which can make the river uninhabitable for fish. Gravity concentrates from sluices are sometimes amalgamated with Hg on-board using high-speed blenders, which discharge Hg-rich amalgamation tailings directly into rivers (Pfeiffer et al., 1991). Alternately, amalgamation of concentrates typically takes place in pools excavated in the ground or at creek margins.



Figure 2.1 Riffler Sluice Box



Figure 2.2 Carpeted Trench

Excess Hg is removed from the amalgam by hand-squeezing the liquid Hg through a piece of fabric. The excess Hg is re-bottled and used again. Once the amalgam is obtained, which typically contains ~60% gold, it is retorted or simply burnt in pans. More than 95% of the Hg can be recovered through retorting, and this method therefore contributes to significant reductions in air pollution and occupational exposure. Despite the potential health and economic benefits of Hg recovery, retorting is more time consuming and complicated than the predominant technique: direct open-air burning of the amalgam in a pan or shovel using a blowtorch (Fig. 2.3).

The amalgam decomposition process, either with or without retorts, produces a sponge-like gold doré containing about 20g of Hg per kg of gold (CETEM, 1989). This is sold to gold shops in nearby villages or melted on-site to get rid of the remaining Hg and any other impurities. Mercury levels inside melting sheds are extremely elevated. In the event that fume hoods are used, they are typically rudimentary, consisting of only a fan that blows Hg vapours into the ambient atmosphere.



*Photo courtesy of M. Veiga*

Figure 2.3 Amalgam Decomposition with a Blowtorch and Shovel

### 2.3 Sources of Mercury Emissions in the Brazilian Amazon

In Brazil, as in many Latin American countries, the use of Hg in mining is illegal. Mercury imports are only permitted for certain registered industrial uses (electronic industries, chlorine plants, paints, dental, etc.), although the declared uses from these industries are declining. It was estimated that in 1989, out of a total of 337 tonnes of Hg imported into Brazil, more than 170 tonnes was illegally diverted to mining activities, and subsequently lost to the environment (Ferreira and Appel, 1991). In 1998 and 1999, 90 and 50 tonnes of Hg products respectively, entered Brazil from Russia, Spain, Algeria and Finland (DNPM, 2000). The intensity of Hg use is further exacerbated by its low price, even when sold in Brazilian garimpos at five times the international price (approximately US\$ 4.00/kg) (Veiga & Fernandes, 1990).

A ratio of  $Hg_{lost}/Au_{produced}$  is frequently used to estimate the amount of Hg discharged into a given environment. Veiga (1997) determined that this ratio typically ranges from 1 to 3 depending on whether the whole ore or concentrate is amalgamated, and whether a retort is used (see Table 2.1). Lacerda and Salomons (1999) suggested a range of 1.1 to 5, with an average of 1.3. In reality, as gold grades decrease, many miners actually tend to use more Hg in the hopes of increasing recovery. Consequently, a  $Hg_{lost}/Au_{produced}$  ratio of 1:1 is likely conservative. Ratios of up to 10:1 have been reported in some Chinese operations (Gunson and Veiga, 2001). So, although an estimated 20 tonnes of gold was produced in the Brazilian Amazon in 1999 (IBGM, 2001), it is reasonable to suggest that *at least* 20 tonnes of Hg was discharged into the environment in the region during that year alone. Although artisanal gold production has decreased significantly since the 1980s (compare with 64 tonnes in the same region in 1983), these outputs are nevertheless quite substantial. Estimates derived by Lacerda and Marins (1997) suggest that emissions from mining in the Amazon are around 78 tonnes per year. An estimated 3000-4000 tonnes of Hg have been emitted into the environment from artisanal mining activities in the Brazilian Amazon alone in the last two decades (Veiga et al, 1999).

Table 2.1 Estimated Ratio of Mercury Lost to Gold Produced  
(after Veiga, 1997)

Amalgamation method	Hg <sub>lost</sub> : Au <sub>produced</sub>
Whole ore	3
Concentrates, no retort	1
Concentrates with retort	0.001

Several estimates of Hg emissions at various phases in processing have been proposed based on assessments conducted at a number of garimpos in the Amazon. In one operation, around 70% of total Hg lost to the environment was released during open-air amalgam decomposition, 20% was discharged with

amalgamation tailings, and 10% released to the urban atmosphere when gold dealers melted the bullion without appropriate fume hoods (Farid et al, 1991). When retorts are used during amalgam decomposition, as little as 0.05% of Hg is lost to the environment (Farid *et al*, 1991), but in their absence, as much as 50% of the Hg introduced through amalgamation can be lost (Veiga, 1997). Although there are some uncertainties associated with these estimates, it is apparent that the amalgamation methods and Au-Hg separation procedures used in garimpos has a major impact on Hg releases at given operations. Mercury discharges using various processing methods are illustrated in Figure 2.4.

In a study by Marins et al. (1991), most of the Hg emitted from the 32 gold smelting shops was deposited near the emission source (i.e. within 1 km). Due to the low temperature at which Hg vapour is emitted from gold mines, some researchers suggest that emissions are predominantly controlled by lower, local wind currents, resulting in deposition within 2-3 km of gold shops (Borochoff, 2001). This is consistent with results from Alta Floresta, a town in the south of the Amazon Basin, where analyses of air and soil samples taken up to 500 m from gold shops had significant Hg concentrations that increased with proximity to the gold shop (CETEM, 1991). Due to this local deposition, exposure to Hg vapour is extremely high for people living in close proximity to gold smelters (Veiga and Hinton, 2002).

Metallic Hg discharged during processing can subsequently undergo transformations into volatile forms. Atmospheric transport of Hg to relatively distant regions or countries is probable (Villas Boas, 1999), particularly in association with forest fires. Forest fires are responsible for mobilizing extensive amounts of natural or anthropogenic Hg contained in biomass as well as the Hg emitted by miners and deposited nearby (Meech et al., 1995). Due to the high rate of deforestation by fire in the Amazon [29,059 km<sup>2</sup> in 1995 (Artaxo et al, 2000)], Hg emissions from wood combustion have been estimated at between 0.78 and 1.76 kg/km<sup>2</sup> of forest per year (Veiga et al., 1994; Lacerda 1995). Through analysis of aerosol particles, Artaxo et al. (2000) estimated that about 30% of the atmospheric Hg emitted in the Amazon region is associated with biomass burning (of natural and anthropogenic origin), and 63% from gold mining. Only 7% is from other sources, such as fossil fuel emissions.

The amount of Hg annually emitted by deforestation of the Amazon alone has been estimated between 8 and 80 tonnes of Hg (Veiga *et al*, 1994, Lacerda, 1995). These estimates primarily depend on the biomass distribution, the area burned and Hg levels in plants and organic matter (from 0.02 and 0.3 mg/kg). In one Amazonian forest fire, Hg concentrations as high as 1,000 mg/kg were measured in fine smoke particles (<2.5 µm) (Kaufman et al, 1992). Due to the high temperatures generated in forest fires, in conjunction with vertical air mixing characteristic of the Amazon region, Hg released from forest fires likely reaches a

higher elevation than that emitted from amalgam decomposition or evasion from natural sources (Artaxo et al, 2000).

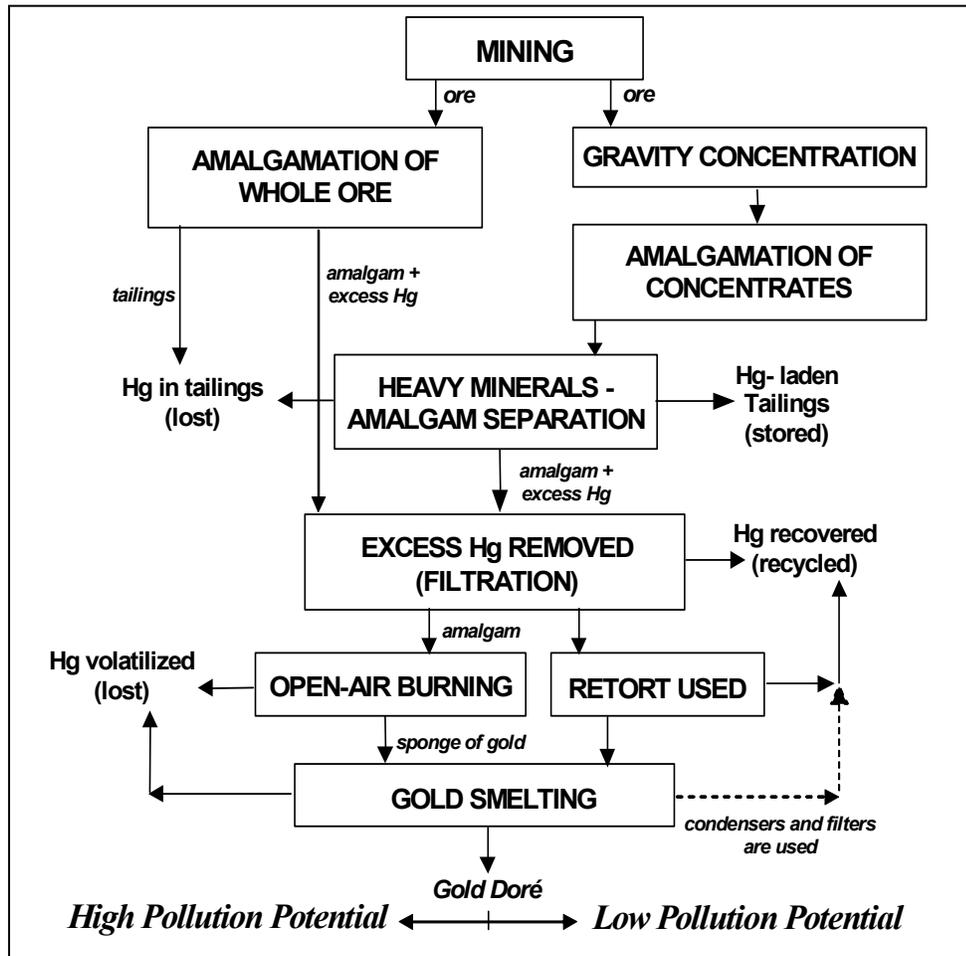


Figure 2.4 Main Steps Involved in Gold Recovery by Amalgamation. The right side of the diagram includes methods that can reduce Hg emissions (after Veiga and Hinton, 2002)

## 2.4 Other Environmental Consequences

A number of other ecosystem impacts can result from artisanal mining activities. Modification of hydrologic systems, for example, through relocation of natural drainages or construction of water reservoirs for use in hydraulic monitoring, can have a devastating effect on local ecosystems. Deforestation is also a major consequence (Akagi and Naganuma, 2000). For instance, it is estimated that up to 2300 km<sup>2</sup> of forest in Suriname alone will be destroyed by artisanal miners by 2010 (Peterson and Heemskerk, 2001). Although regeneration of these forests is anticipated as mining activities subside, it has been shown that regrowth tends to be slow as soil quality has been degraded significantly (Peterson and Heemskerk, 2001). In addition, as the mineral resource is depleted, community inhabitants become

increasingly dependent on agricultural activities for subsistence and additional deforestation may occur – ranching continues to be the primary cause of deforestation in the Amazon (MacMillan, 1995). As discussed, slash-burning practices common to the Amazon may exacerbate Hg releases into the atmosphere.

Deforestation coupled with the discharge of fine tailings into watercourses creates an additional problem – siltation. Siltation of rivers reduces light penetration and dissolved oxygen levels thereby making rivers uninhabitable for fish. As well, silt build-up may effectively modify the dimensions of drainages such that flooding occurs. With decreased organic matter with deforestation, the water retention capacity of soils also diminishes, thereby resulting in an increased potential for flooding. Fishery depletion and frequent flooding can obviously have detrimental effects on riverine communities and others dependent upon fish as a food source. As flooding tends to increase the net area of standing water, it also contributes to increases in malaria and other mosquito-transmitted diseases.

## **2.5 Socio-economic Implications**

Most artisanal mining communities around the world initially developed around the activities of a few miners who discovered a viable reserve of easily extractable surficial gold. News travels quickly and populations can rapidly grow to tens of thousands of miners working the same deposit independently. At Serra Pelada, Brazil, a gold rush occurred in the 1980s, bringing around 80,000 miners to work on a single massive deposit (Veiga and Hinton, 2002). In conjunction with the rapid growth and influx of money, women also migrate to the area to find employment either as cooks, prostitutes and occasionally gold panners.

In addition to the well-documented environmental consequences of these activities, these communities are also plagued by deplorable social conditions, both during operations and well after mining activities have ceased. The artisanal mining cycle is well known: discovery, migration, and relative economic prosperity are typically followed by resource depletion, out-migration and economic destitution. Drugs, prostitution, disease, gambling, alcohol abuse, and degradation of moral standards are frequent consequences of the chaotic occupation at mining sites. After depletion of easily exploitable gold reserves, sites are abandoned, and those who remain contend with a legacy of environmental devastation and extreme poverty.

### **3 MERCURY IN THE ENVIRONMENT**

In response to increased environmental and human health concerns of the public and scientific community, much work has been done to improve our understanding of the transformation of Hg to its most insidious forms. Although elemental Hg vapour ( $\text{Hg}^0$ ) can pose a serious health risk if inhaled, organic forms of Hg, specifically methylmercury (MeHg) is of greatest concern in terms of incorporation from food sources.

Natural processes and industrial activities introduce Hg into the environment in many different forms. As the toxicological impacts of Hg are largely dependent on speciation, understanding its transformations and the impacts of various chemical forms is vital to the prevention of harmful human and environmental health effects. Ultimately, the Hg species present in a given environment depends on the initial released form, the thermodynamic stability of this compound and the transformation rate of the released form to a more stable one (Baeyens et al, 1979). These issues must all be well understood to effectively mitigate contamination in Hg impacted areas.

#### **3.1 Sources of Mercury in the Environment**

Mercury is present in the atmosphere as a result of: volcanic activity and evasion from aquatic systems (Kim and Fitzgerald, 1986; Fitzgerald, 1979; Matheson, 1979); transpiration from plants (Lindberg et al, 1999), as well as anthropogenic sources (fossil fuel combustion, silver and gold mines, etc.). Current annual atmospheric discharges from anthropogenic activities are approximately 3600 to 4500 tonnes/a globally, which constitutes 50 to 75% of total atmospheric discharges (Fitzgerald, 1995; Mason et al, 1994). Since the beginning of the industrial revolution, the total amount of Hg in the atmosphere is believed to have increased 2 to 5 times (Boening, 2000).

##### **3.1.1 Anthropogenic Sources**

The unique chemical and physical characteristics of Hg have made it a favourable component in many industrial and agricultural applications. For instance, it is the only metal that can exist as a liquid and gas at ambient temperatures and it has the capacity to amalgamate with several other metals (e.g. gold, silver, tin, copper, etc). Releases from fossil fuel combustion (i.e from coal-fired boilers and incinerators) are believed to emit more Hg to the atmosphere than all other point sources combined (Hanisch, 1998). World emissions from coal combustion are estimated at 700-3800 tonnes/a (Nriagu and Pacyna, 1988), with emissions in the US alone estimated at 300 tonnes per year (Chu and Porcella, 1995). An inventory

conducted by Prasad et al (2000) suggest that 272 tonnes/a are emitted from anthropogenic sources in North America alone. Although most US emissions are derived from combustion of municipal waste and coal (56% of 176 tonnes/a), Canadian and Mexican emissions are primarily attributed to smelting of non-ferrous ores (24 and 31 tonnes/a, respectively). Coal combustion in China is also a significant source of atmospheric Hg, with emissions in 1995 estimated at more than 300 tonnes (Gunson, 2001).

Some chloralkali plants still use metallic Hg as a cathode to amalgamate sodium in the electrolytic production of NaCl, resulting in Hg losses to air, water and soils (Malm et al, 1997). Other significant anthropogenic sources in modern times include the pharmaceutical industries, the electrical industry, mainly in the production of primary batteries, but also associated with arc discharge lamps, rectifiers, oscillators, etc. Metal ore roasting, refining, and processing are also important sources of emissions (Ebinghaus *et al*, 1999). Lead, copper, and zinc ore smelting alone emits about 100 tonnes of Hg to the atmosphere per year (Boening, 2000). Due to the toxicity of Hg and its compounds, it has also been extensively used in bactericides and fungicides for marine paints and agricultural applications.

One of the most consequential anthropogenic Hg sources in developing countries (and historically in developed countries) is from gold extraction by amalgamation with Hg. This practice is commonly accompanied by the discharge of large quantities of Hg into the environment, which consequently exacerbates the potential for deleterious human and environmental health effects (Lacerda, 1995, Meech et al, 1998). Mercury emissions from artisanal gold mining were discussed in Chapter 2.

During the last five hundred years, approximately one million tonnes of Hg has been extracted from cinnabar and other ores around the world (Hylander and Meili, 2002). In 1998, more than 2300 tonnes of Hg was produced (excluding China) at various mines around the world (UNDP, 2000). Of the 800,000 tonnes of Hg produced in the last 500 years, most was provided by three mines: Almaden, Spain (280,000 tonnes), Idrija, Slovenia (150,000 tonnes) and Mt. Amiata, Italy (100,000 tonnes) (Ferrara, 1999). It is suggested that Hg mines emit 10-30 tonnes of Hg to the atmosphere per year (Hylander and Meili, 2002).

Mercury is also produced in conjunction with gold, silver and copper. Yanacocha Mine in Peru, for instance, has produced an estimated 142 tonnes of Hg in conjunction with gold since 1994 (Veiga and Hinton, 2000). Prior to a 151 kg Hg spill on June 2<sup>nd</sup>, 2000, this Hg was sold to Peruvian companies (e.g. brokers) with an unknown end use. It has been speculated that most of this Hg was likely resold to artisanal miners. With the global attention brought to the spill, Yanacocha operators Minera Yanacocha S.R.L. briefly ceased sale to these brokers, but this has since resumed (Gibson, 2002).

Primarily due to increasing emissions from industrialized countries, atmospheric Hg is believed to have tripled in the last 100 years with a current rate of increase of about 0.6% per year (Fitzgerald, 1995). Between 1977 and 1990, Hg concentrations in the atmosphere have increased by 1.5% and 1.2% in the Northern and Southern Hemispheres, respectively (Pirrone et al, 1995). Estimates for total anthropogenic emissions have been somewhat variable: Hanisch (1998) estimated that 2000-6000 tonnes are emitted annually from human activities; Fitzgerald (1996) later suggested emission rates are 3600 to 4500 tonnes/a; and Nriagu and Pacyna (1988) proposed a range of 5800 to 29,100 tonnes/a. The latter estimate is much higher as emissions to soil (1600-15000 tonnes/a) and water (600-8000 tonnes/a) were included in addition to atmospheric inputs (3600-4500 tonnes/a). Discharge estimates for soil and water bodies include solid and liquid waste from industrial sources and sewage sludge dumping, as well as atmospheric deposition derived from anthropogenic sources.

### **3.1.2 Natural Sources**

Mercury is naturally present in many igneous, sedimentary and metamorphic rocks although concentrations of Hg are generally very low (<1 ppm). Mercury may, however, occur in much higher concentrations in certain mineralized areas, generally in the form of sulfide minerals, such as cinnabar (HgS), and to a lesser extent in metallic form (Fitzgerald, 1979). Mercury has been widely documented as a trace metal in many base metal ore bodies around the world, and is frequently found associated with native gold and to a lesser extent copper and silver (Kerfoot *et al*, 2000).

Some studies in different parts of the globe estimate that the natural Hg flux from earth's surface to the atmosphere ranges from 0.0014 to 10  $\mu\text{g}/\text{m}^2/\text{day}$  (Andren and Nriagu, 1979). Volatilization rates have been estimated at 0.001 – 0.005  $\mu\text{g}/\text{m}^2/\text{h}$  in mercuriferous belts and less than 0.001  $\mu\text{g}/\text{m}^2/\text{h}$  in “unimpacted” areas (Ebinghaus *et al*, 1999). Fluxes as high as 1  $\mu\text{g}/\text{m}^2/\text{hr}$  have been measured in some geothermal zones (Gustin and Lindberg, 2000). Degassing from Hg-bearing minerals is difficult to quantify, but estimates of 700 tonnes/a from soils have been suggested (Ebinghaus *et al*, 1999). Global emissions from volcanoes have been estimated at between 20 tonnes/a (Matheson, 1979) and 362 tonnes/a (Nriagu, 2001).

Vegetation is another potential source of Hg emissions. Mercury evaporation from forests have been estimated at 850 tonnes/a from vegetation and 80-160 tonnes/a from forest soils (Lindberg *et al*, 1996). Emissions from non-forested soils are estimated at 500-1100 tonnes/a.

As oceans are important sources and sinks of Hg, they are an integral component of the global Hg cycle (Mason et al, 1995a). Some research indicates that the flux of Hg<sup>0</sup> from the oceans to the atmosphere may be biologically mediated, as evidenced by evasion rates in zones of upwelling (i.e. nutrient-rich zones of high primary production) (Kim and Fitzgerald, 1986). Cyanobacteria are believed to facilitate most Hg reduction in seawater (Mason et al, 1995b). Open ocean and coastal waters emit approximately 9000 tonnes/a of gaseous Hg (Matheson, 1979).

### ***3.1.3 Atmospheric Deposition***

Atmospheric Hg derived from all anthropogenic and natural sources will eventually be re-deposited on the Earth's surface. Porcella (1995) estimated Hg deposition rates in the Northern Hemisphere to be 11 to 14 µg/m<sup>2</sup>/a and in the Southern Hemisphere, where industrial activities are less intense, to be 5 to 7 µg/m<sup>2</sup>/a. Deposition rates of Hg in dry, non-Hg mineralized areas, the deposition is estimated between 5 and 7 µg/m<sup>2</sup>/a. In wet conditions, such as those in rainforests, Hg deposition rates can double. Deposition rates in the Amazon are estimated to be between 10 and 16 µg/m<sup>2</sup>/a, with higher rates in closer to artisanal gold mining activities (Lacerda et al, 1999; Fosberg et al, 1999; Lacerda and Marins, 1997). Using this average deposition rate, and considering that the Amazon covers an area of 5 million km<sup>2</sup>, the Brazilian Amazon alone has been receiving 50 to 80 tonnes of Hg/a from atmospheric deposition (Veiga et al., 1999).

## **3.2 Mercury Bioavailability and Bioaccumulation**

### ***3.2.1 Mercury Speciation***

As Hg bioavailability is primarily dependent on its speciation, understanding the form of Hg in various compartments is vital to assessing its potential for incorporation into biota. Mercury is commonly found in the environment as mercuric salts, elemental Hg (vapour or metallic form), soluble complexes and sulfide minerals. Mercury transformations are generally limited by the presence of mercuric compounds (Hg(II)) (Fig. 3.1). Under oxidizing conditions, Hg(II) will be released from Hg minerals (mainly sulfides) or through oxidation of metallic Hg. It can also be released via demethylation, desorption from colloids or dissociation from inorganic or organic complexes. Once Hg (II) enters solution, it is promptly transformed and/or removed due to methylation, complexation, reduction, bio-assimilation and particulate scavenging (Sadiq, 1992; Mason *et al*, 1995a). Most reduced mercury (Hg<sup>0</sup>), which can occur as Hg vapour or elemental (or metallic) Hg, is lost to the atmosphere via evasion (Mason *et al*, 1995a), while

particulate bound Hg may be subject to settling or be transported, depending upon the local hydrologic conditions. The speciation and partitioning of Hg in various compartments (air, water, soil) is discussed in greater detail below.

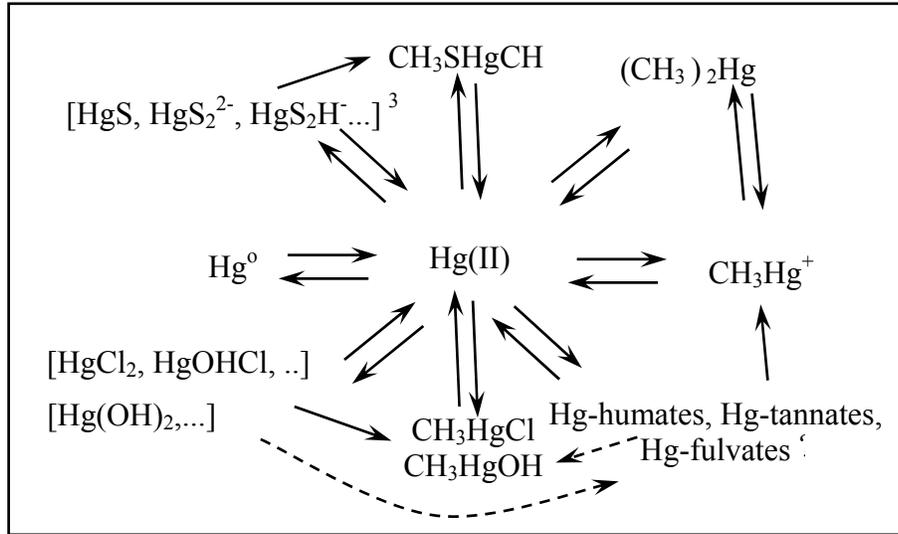


Figure 3.1 Mercury Transformations in the Environment

### 3.2.1.1 Speciation in Aquatic Systems

Mercury cycling in aquatic systems is dependent on exchanges between various compartments summarized as follows (CEQD, 2000):

- Wet or dry atmospheric deposition;
- Removal to sediments;
- Evasion to the atmosphere as  $Hg^0$ ;
- Methylation or demethylation processes; and
- Exchanges with associated surface waters (e.g. tributaries) or groundwater.

The form in which Hg is released into aquatic systems and conditions in that system (pH, Eh, etc) control its reactivity and transformation rate. As metallic Hg and Hg in mineralized forms (e.g. HgS) are relatively insoluble and biologically unavailable to organisms, it generally must oxidize (i.e. to Hg(II)) before significant biochemical impacts are observed. The solubility of metallic Hg is  $56 \text{ ng g}^{-1}$  and HgS is  $0.002 \text{ ng g}^{-1}$  (Schuster, 1991). In fast moving waters, metallic Hg oxidation rates are greater due to higher dissolved oxygen levels.

In the absence of humic substances, dominant forms of Hg(II) in low-sulfide freshwaters are  $\text{Hg}(\text{OH})_2$ ,  $\text{HgOHCl}$ , and  $\text{HgCl}_2$ ; methylmercury (MeHg) is mainly found as  $\text{CH}_3\text{HgOH}$  (Ulrich et al, 2001). As chloride concentrations increase, Hg(II) is found as  $\text{HgCl}^+$ ,  $\text{HgCl}_2$ ,  $\text{HgCl}_3^-$ ,  $\text{HgCl}_4^{2-}$  and MeHg is mainly as  $\text{CH}_3\text{HgCl}$  in seawater (Stumm and Morgan, 1981). At high sulfide concentrations, for instance in sulfide-rich sediment pore waters, Hg forms various poly- and bi-sulfides depending on pH, Eh conditions and sulfide ( $\text{S}^0$ ,  $\text{S}^{2-}$ ) concentrations, while MeHg is mainly found as  $\text{CH}_3\text{HgS}$  (Stumm and Morgan, 1981). Both Hg(II) or MeHg in various forms have a strong tendency to be adsorbed to particle surfaces, thus suspended matter plays an important role in Hg transport and sediments can serve as important Hg sinks (and sources).

### 3.2.1.2 Speciation in Terrestrial Systems

Mercury levels in soils are influenced by parent material (rocks), atmospheric exchanges (wet or dry deposition and volatilization), uptake by and degradation of organic matter (vegetation and animals), anthropogenic discharges (solid or liquid wastes) and hydrologic and mass movement processes that can transport Hg into aquatic systems. Mercury is found in soils, as with other metals, in various forms, as follows (*after* Schuster, 1991):

- Dissolved forms (soluble inorganic or organic complexes, MeHg);
- Non-specifically adsorbed to soil constituents (e.g. through electrostatic forces);
- Specifically adsorbed (i.e. due to covalent bonding);
- Chelated (i.e. involving multiple bonds with functional groups); and
- Precipitated (e.g.  $\text{HgS}$ ,  $\text{HgCO}_3$ , etc.).

Under mildly reducing conditions, Hg can precipitate in soils as  $\text{HgS}$ , which has extremely low solubility ( $0.002 \text{ ng g}^{-1}$ ) (Schuster, 1991). Highly soluble chloride and hydroxyl- forms of Hg(II) are expected to predominate under the oxidizing conditions found in surficial soils. Due to the affinity of Hg for sulphur (Schuster, 1991), the presence and abundance of humic substances and mineral colloids containing S-functional groups could significantly influence its retention (or mobility) in soils.

In the case of metallic Hg, the form used for amalgamation by artisanal miners, Hg discharged with tailings can oxidize to Hg(II) and subsequently be transformed to other species, or it can volatilize into the atmosphere. Most metallic Hg (>99%), however, remains as elemental Hg associated with tailings (Lacerda and Salomons, 1999). As elemental Hg is unreactive compared to other Hg species, (it is relatively stable at  $\text{pH}>5$  and at moderately oxidizing conditions), Hg in tailings is a long-term source of

contamination to impacted areas. Organic acids may solubilize Hg in terrestrial soils (Veiga et al, 1999) or erosional processes can mobilize tailings into aquatic systems over time where Hg is more likely to transform to soluble and bioavailable forms. Organic matter concentrations in soil solutions can range between 20 and 1400 mg L<sup>-1</sup> (Schnitzer and Kerndorff, 1981). Due to the disorganized manner in which artisanal mining activities are carried out, distribution patterns in terrestrial systems are difficult to determine (Lacerda and Salomons, 1999).

### 3.2.1.3 Speciation in the Atmosphere

Mercury is mainly emitted to the atmosphere as gaseous elemental Hg<sup>0</sup>, gaseous or particle associated Hg(II), and MeHg (Pai et al, 2000). Several factors have been suggested that contribute to the evasion of Hg including temperature, air movement and soil moisture content (Gustin et al, 1997). Rates of Hg vapour emissions from contaminated soils to the atmosphere have been shown to be directly proportional to Hg concentrations in the first 15 cm of soil (Lacerda and Salomons, 1999). Some research indicates that the flux of Hg to the atmosphere may be biologically mediated (Kim and Fitzgerald, 1986).

Mercury can undergo various chemical and physical transformations once in the atmosphere. These include oxidation by ozone, peroxide, sulfate or other compounds, photoreduction, adsorption and desorption from particulate, and removal processes (i.e. wet or dry deposition). Elemental Hg (Hg<sup>0</sup>), the dominant form in the atmosphere, has an atmospheric lifetime of 0.5-2 years and is mainly deposited through dry processes (Lin and Pehkonen, 1999; Artaxo et al, 2000). Methylmercury is believed to constitute < 3% of Hg in the atmosphere (Lin and Pehkonen, 1999). Approximately 3% of total atmospheric Hg consists of Hg(II) compounds (e.g. HgCl<sub>2</sub>, Hg(OH)<sub>2</sub>), which are also known as reactive gaseous Hg (RGM). Due to the high solubility and amenability for transformations, the importance of RGM in biogeochemical cycling is becoming increasingly apparent. A study by Lindberg and Stratten (1998) indicated that RGM, which is mainly a product of combustion processes, varies in concentration with temperature, solar radiation, O<sub>3</sub>, SO<sub>2</sub>, and total Hg vapour concentrations. Vegetated areas may be important sinks for RGM and, due to their high solubility, precipitation is an important form of RGM removal from the atmosphere. The formation of Hg-organic complexes once RGM is deposited into organic-rich aquatic systems has been suggested as an important mechanism in Hg cycling in darkwater systems, although little information is available on this matter (Veiga et al, 1999).

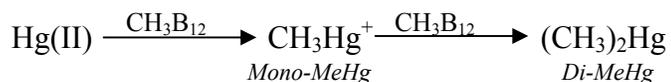
### 3.2.2 Methylation of Mercury

Methylmercury (MeHg) is an organometallic compound that is highly lipophilic and readily bioavailable. Due to the tendency of MeHg to be incorporated by biota, MeHg tends to increase in concentration up trophic levels of the food chain, i.e. it is biomagnified. For example, Hg concentrations can be more than a million times greater in predatory fish compared to the water they inhabit (US EPA, 1999). Consequently, inhabitants of communities dependent upon Hg laden fish as a primary food source are likely to have elevated levels of Hg in hair, blood and urine (Wheatley et al., 1995, Malm et al., 1997). The tendency of MeHg to bioaccumulate and biomagnify is typically more characteristic of hydrophobic organic contaminants than other trace metals (Mason et al., 1996).

A number of parameters and mechanisms have been identified as important to Hg methylation. These include the supply of Hg(II), composition of micro-organisms, pH, redox conditions, temperature, organic matter, and iron and sulfate concentrations. The prevailing theory is that methylation is facilitated by bacteria under anaerobic conditions in sediments (Ullrich et al, 2001, Jensen and Jernelov, 1969). Other proposed methylation sites include particulate surfaces to which Hg is sorbed (Windon *et al*, 1979; Sadiq, 1992), within the intestinal tracts of organisms, including fish and invertebrates (Veiga *et al*, 1999; Boening, 2000), on the external slime layer of fish (Rudd, 1995), and within roots of certain aquatic plants (Guimarães, 2001).

#### 3.2.2.1 Biotic Methylation

Mercury methylation by microorganisms is thought to be a Hg detoxification mechanism wherein a carbanion ( $\text{CH}_3^-$ ) is transferred to hydrated, complexed or ionic Hg(II) (Melamed, 1997). Methylcobalamin, which is a vitamin B<sub>12</sub> derivative, is believed to be the primary methyl group donor and the transfer can be facilitated within cell by enzymes, or externally (Ullrich et al, 2001). The formation of monomethylmercury (Mono-MeHg, or MMHg) and dimethylmercury (Di-MeHg, or DMHg) is described as follows (CEQD, 2000):



As DMHg is less stable than MMHg, particularly in the presence of light or acidity, it is typically not detectable in most surface waters (Mason et al, 1995a). Contrary to the reaction series described above, it has been speculated that DMHg actually forms directly from Hg(II) and rapidly decomposes to MMHg (Mason et al, 1995a). As DMHg predominates MeHg speciation only under very specific conditions, for

instance in deep ocean waters or at high pH/low O<sub>2</sub> conditions (Baldi et al, 1995), most studies of MeHg (formation, presence, abundance, etc.) generally refer to MMHg alone.

Enzymatic methylation is facilitated by metabolizing organisms, whereas non-enzymatic methylation involves the use of methylated products of metabolism (Ullrich et al, 2001). The latter process, extracellular methylation, is facilitated by enzymes involved in organic matter decomposition.

Although many Hg compounds have been shown to be toxic to microorganisms, many bacteria are resistant to Hg. A number of bacteria are believed to be capable of methylation, but sulfate-reducing bacteria (SRBs) are the primary methylators, particularly under anaerobic conditions (e.g. in sediments) (Ullrich et al, 2001, Hammerschmidt et al, 2001, Benoit *et al*, 1999, Kelly et al, 1995). Sulfidogens have the capacity to both methylate and demethylate, but methanogens are only capable of demethylation (Pak and Bartha, 1999).

The degradation of MeHg, or demethylation, involves the breaking the Hg-carbon bonds by microorganisms. In soils, enzymes that demethylate Hg produce Hg(II) and methane (CH<sub>4</sub>), as evidenced by lower CH<sub>4</sub> production in soils with a low total Hg to MeHg ratio (Bailey et al, 1999). Mercuric reductase may subsequently reduced Hg(II) to volatile species. Many types of organisms, aerobic or anaerobic, are capable of demethylation, but aerobic microorganisms seem to be the predominant demethylators (Ullrich et al, 2001). Because methylation and demethylation are both occurring, any MeHg measured reflects a net MeHg produced.

#### 3.2.2.2 Abiotic Methylation

Abiotic, or chemical, methylation is also possible in the presence of suitable methyl-group donors (Sadiq, 1992; Ullrich et al, 2001). Suggested donors include CHCl<sub>3</sub> and methanol (Falter and Wilken, 1999), as well as ethanol, acetic acid, and propionic acid (Akagi et al, 1977). Transmethylation reactions, wherein other methylated metals transfer methyl groups to Hg(II), have also been reported (Ullrich et al, 2001). Although their relative significance in natural systems has not been established, it is likely that both biotic and abiotic methylation could be occurring independently or in combination.

#### 3.2.3 **Mercury Bioaccumulation**

Methylmercury is very soluble and rapidly absorbed by biota. This is, in part, why concentrations of total Hg in water are usually very low, or often, undetected by analytical methods. As MeHg is more slowly

metabolized and eliminated than inorganic compounds, the overall result of continued uptake is a net bioconcentration in the organism over time. Once Hg enters biota (e.g. plants, animals), concentrations generally increase up levels of the food chain (i.e. it is biomagnified). Consequently, carnivorous fish are most likely to have high Hg concentrations in impacted areas. This biomagnification demonstrates that bioaccumulation through ingestion of MeHg is more important than accumulation via respiration, i.e. through the gills. Most Hg found in fish is in the methylated form (from 70 to more than 95% of the Hg in fish is in the form of MeHg), which is easily transferred to man since methylmercury is readily adsorbed by the intestines (Jugo, 1979).

Generally, inorganic Hg is believed to be poorly absorbed by the intestinal tract and so is less toxic (Loredo et al, 1999). However, uptake of inorganic Hg species may be important, particularly at lower trophic levels, as research has indicated that neutrally charged inorganic complexes are readily bioavailable to methylating bacteria (Mason *et al*, 1995c, Mason, 1996, Benoit *et al* 1999, Watras et al, 1998). This is mainly attributed to the lipophilic nature of neutral Hg compounds, both inorganic and organic, and their resulting ability to diffuse more effectively across cell membranes. The  $\text{MeHgCl}^\ominus$  species is thought to be most important as the diffusion rate of MeHgOH is 0.04 times the diffusion rate of its chloride counterpart (Mason et al, 1996). Once inside the organism, both Hg(II) and MeHg have strong affinities for anionic sulfur ligands in proteins and tissues (Gottfrey and Tjalve, 1991).

Organisms in terrestrial and aquatic systems are extremely important to Hg speciation as they facilitate the release of Hg fixed to organics into the water column or transfer Hg up trophic levels of the food chain. The assimilation of dissolved Hg compounds principally occurs at the primary production level (i.e. phytoplankton), although higher trophic levels may also be able to process Hg directly from waters. As secondary producers (i.e. zooplankton) consume primary producers, both MeHg and inorganic Hg are biomagnified; however it is well demonstrated that the transfer of the inorganic form is far less efficient (Lawson et al, 1998; Mason et al, 1995c). Mason et al (1995) suggest that inorganic Hg is not biomagnified like MeHg due to partitioning within phytoplankton cells. Membrane bound inorganic Hg is not transferred to zooplankton to the same extent as cytoplasm associated MeHg. This inefficiency continues up the food chain as exemplified by the increasing ratio of MeHg to inorganic Hg in higher organisms (Lasorsa et al, 1995).

Due to the bioavailability of MeHg and its tendency to biomagnify, the parameters influencing Hg bioavailability and bioaccumulation tend to concur with those controlling Hg methylation. For instance, as MeHg production has been shown to increase with decreasing pH, bioaccumulation generally also increases. Not all bioaccumulation studies, however, directly measure MeHg (i.e. many studies measure

total Hg concentrations in organism tissues). Thus, assessments of either Hg bioaccumulation or Hg methylation can provide insight into the mechanisms influencing Hg uptake. The primary factors controlling Hg methylation and bioaccumulation are discussed in greater detail below.

#### 3.2.3.1 Adsorption

Hydrous oxides of iron and aluminum, some clay minerals and organic matter have a strong capacity to adsorb mercuric compounds and so may inhibit the bioavailability of Hg to organisms. The capacity of clays to adsorb heavy metals is proportional to its ability to expand, as this relates to surface area (Fergusson, 1990). Accordingly, the adsorption capacity of clay minerals follows the series montmorillonite > vermiculite > illite > chlorite > kaolinite. As chemisorption, (i.e. specific adsorption) and co-precipitation of heavy metals is more important for hydrous oxides than physical (i.e. electrostatic) sorption, heavy metals retention by hydrous oxides is generally stronger than by clays (Fergusson, 1990).

Roulet et al (2001) found a strong relationship between the geochemistry of the hydrous oxides and Hg concentrations in several locations throughout the Amazon. Specifically, Hg had an increased affinity for the minerals when Al substitution for Fe in the oxide crystal structure was more significant. Interestingly, these correlations decreased in prominence with increasing organic matter, indicating the importance of organics in Hg desorption from mineral constituents.

In addition to desorption by organic acids, release of Hg and MeHg from hydrous ferric oxides (HFOs) may coincide with the release of iron at the redox boundary in sediments and aquatic systems (Miller and Mason, 2001), thereby rendering it available for bioassimilation or further transformations.

Desorption can be facilitated by various changes in physio-chemical and biological conditions (e.g. ionic strength, pH, Eh, etc). The importance of Hg desorption on bioaccumulation was demonstrated in an experiment involving two species of filter feeding mussels (*Mytilus edulis* and *Modiolus demissus*) (Breteler and Saksa, 1985). In this study, the desorption of inorganic Hg from sediment particles, which was directly linked to Hg uptake by mussels, was strongly correlated with organic matter ( $r = 0.86$ ).

#### 3.2.3.2 pH and Redox Conditions

These parameters are of fundamental importance to Hg bioavailability as its speciation is primarily a function of pH and Eh. These factors also influence processes including desorption-adsorption, the

composition and abundance of microbial populations, volatility, and bioaccumulation mechanisms in biota.

As acid rain precursors ( $\text{SO}_2$ ,  $\text{NO}_x$ ) and Hg are both by-products of coal combustion, the behaviour of Hg in acidic environments has become the subject of several studies. In a study of five Wisconsin lakes, Bloom et al (1991) correlated high Hg levels (as MeHg) in fish tissues with low pH. As bioconcentration was proportional to pH throughout a wide range (4.7 to 7.2), it was suggested that pH influences in-lake MeHg generation, and not necessarily bioaccumulation mechanisms. Consistent with these results, in a study of 15 Wisconsin Lakes, Watras et al (1998) found that MeHg in phytoplankton and zooplankton also increased with decreasing pH.

Redox conditions also have a demonstrated effect on the generation of MeHg. Watras et al (1995) modelled MeHg production in a dimictic lake (i.e. a lake that turns over twice annually) and proposed that MeHg was mainly produced within a layer of plankton located at the interface between oxic surface waters and the anoxic hypolimnion (cold, low oxygen bottom waters). Sulfate reduction and abundant microbial activity were also identified at this boundary. These results are consistent with those from a study of four forest lakes in Finland wherein maximum MeHg production rates at the sulfide maxima in the hypolimnion were also documented (Matilainen, 1995). Methylmercury generation at the oxic/anoxic boundary is not surprising, given the demonstrated importance of SRBs and supply of Hg(II) in Hg methylation.

Rates of MeHg production were also evaluated in anoxic versus aerobic conditions in a laboratory scale study by Porvari and Verta (1995). Methylmercury production was found to be significantly higher in anoxic columns containing humus and peat mixtures, compared to their aerated counterparts; however, demethylation rates were also higher in the anoxic systems. Incidentally, the relation between MeHg and total organic carbon in this study was very weak.

#### 3.2.3.3 Ion Concentrations

As Hg(II) is one of the reactants involved in methylation, its concentration influences MeHg production rates (Kelly et al, 1995). As methylation is a function of a number of factors, the relationship between Hg(II) supply and MeHg production can be variable. For instance, linear relationships between Hg(II) addition to a solution and MeHg formation have been demonstrated in laboratory studies (Rudd, 1995,

Lee et al, 1985). Alternately, the relationship between MeHg and Hg in a study of 15 Wisconsin lakes was described as a third-order polynomial (Watras et al, 1998).

Increasing concentrations of certain metal ions, particularly iron, have been shown to increase Hg methylation rates, as demonstrated in a field study of Hg in sediments of five lakes (pH 5 to 7) in Southern Finland (Matilainen et al, 1991). Although most methylation occurred under anaerobic conditions, MeHg was produced under aerobic conditions with increasing iron and manganese concentrations. Lee et al (1985) also documented methylation rate increases proportional to iron concentrations in the presence of fulvic acid.

#### 3.2.3.4 Sulfide and Chloride

The presence of sulfate and chloride have been demonstrated to enhance methylation, but not affect demethylation rates (Hintelmann *et al.*, 1999), although studies indicate that both processes are entirely inhibited in high sulfide conditions in sediment pore waters (Benoit *et al*, 1999). Although Hg-sulfides are believed to be relatively insoluble and unavailable to organisms, some evidence suggests that in sulfide-rich solutions, such as those found in tailings or sediment pore waters, there is a slow production of several soluble Hg-sulfide species (Paquette and Helz, 1995). The neutrally charged, soluble Hg-sulfide species ( $\text{HgS}^0$ ), which is expected to predominate under a wide range of conditions, is also believed to be bioavailable to methylating bacteria (Benoit *et al*, 1999, Hammerschmidt et al, 2001). Recent studies have also suggested that Hg methylation can be proportional to dissolved sulfide concentrations until a threshold, above which methylation is inhibited (Ullrich et al, 2001).

The influence of chloride concentrations and Hg methylation is not clear. Inhibition of methylation in marine sediments has been correlated to sulfide production from sulfate in seawater (and not chloride), although, as discussed above, high sulfide conditions do not necessarily limit methylation. Like Hg-sulfides, neutrally charged species of Hg-chlorides ( $\text{HgCl}_2$ ) may also be bioavailable to methylating bacteria (at least more so than their ionic counterparts), although little research has been conducted in this field (Ullrich et al, 2001). Watras et al (1998) found that MeHg and Hg concentrations in plankton were independent of chloride, even though aqueous chloride concentrations varied by a factor of 30. Contrary to these results, Rowland et al (1977) showed that Hg(II) ingested as a chloride can be methylated in less than 20 hours by intestinal bacteria of rats. They estimated that the total methylmercury synthesized from ingested inorganic Hg in man would be approximately 0.4 mg/day.

#### 3.2.3.5 Selenium

Certain studies indicate that selenium (Se) may have a protective influence on Hg bioaccumulation and toxicity in organisms, i.e. its presence may have an antagonistic effect. As Se can behave similarly to certain sulfur species, formation of a Hg-Se complex in organisms presumably inhibits the cellular damage and enzymatic function interference found when Hg complexes with sulfahydril groups. As most Se studies have been carried out at abnormally high Se concentrations, biological processes in the species being studied may not be reflective of natural responses. To assess the antagonistic effect of Se on Hg under natural conditions, Belzile et al (2000) sampled fish (perch and walleye) from ten lakes in Northern Ontario. High Se levels strongly correlated with low Hg concentrations in both species of fish (r-values from -0.79 to -0.97) and Hg bioaccumulation was below average levels in high Se lakes. Evaluations of these mechanisms at higher trophic levels, specifically in three species of seals (Baikal seal, Caspian seal, and Northern Fur Seal), also indicated evidence that Se is important to Hg detoxification (Kunito et al, 2001). This was primarily evidenced by accumulation of HgSe (mercuric selenide) in the livers of these animals. Due to the strong binding between Hg and Se, Se may also inhibit Hg methylation in soils, sediments and the water column (CEQD, 2000).

#### 3.2.3.6 Temperature

As cold temperatures decrease the volatility of Hg, Hg concentrations in aquatic systems will be higher in both aquatic and terrestrial systems (including vegetation) in colder climates, other factors being equal. Generally, however, as microbial activity increases with temperature, (optimum conditions are usually between 20 and 40°C (Brady and Weil, 1996)), methylation rates can also decrease with temperature. This relationship was observed in a study of Hg methylation in lichen (Morrison and Therien, 1991) and has been well demonstrated in many studies of lakes, wherein MeHg production rates increase significantly in warmer, summer months (Ullrich et al, 2001).

### **3.2.4 *Humic Substances and Bioaccumulation***

Humic substances are believed to play a special role in Hg mobility and bioavailability (Meech et al, 1998). Mercury forms strong complexes with soluble organic acids and can be transported great distances in this form; organic acids may act as methyl group donors and thereby may promote either abiotic or biotic methylation and evidence exists to suggest that direct intestinal methylation of Hg-organic complexes may be possible. As many rivers and streams in the Amazon are darkwater (i.e. organic-rich) systems, organic acids interactions with Hg have particular relevance to many artisanal gold mines in that region. The role of organic acids in Hg transformations is discussed in detail below.

### 3.2.4.1 Humic Substances

Organic matter is a general term that refers to living biomass, recognizable decomposing tissues, and a somewhat amorphous mixture of highly degraded plant and animal tissues, known as humus (Fig. 3.2). Humus can further be subdivided into complex, decomposition-resistant humic substances, and simpler, less-resistant non-humic substances. Non-humic substances consist of carbohydrates, proteins, peptides, amino acids, fats, waxes and low-molecular weight acids (Sparks. 1995). The fractions of organic matter most relevant to trace metal behaviour in aquatic systems are humic substances. Humic substances are relatively high molecular weight, amorphous, yellow to black coloured substances that are relatively resistant to microbial breakdown.

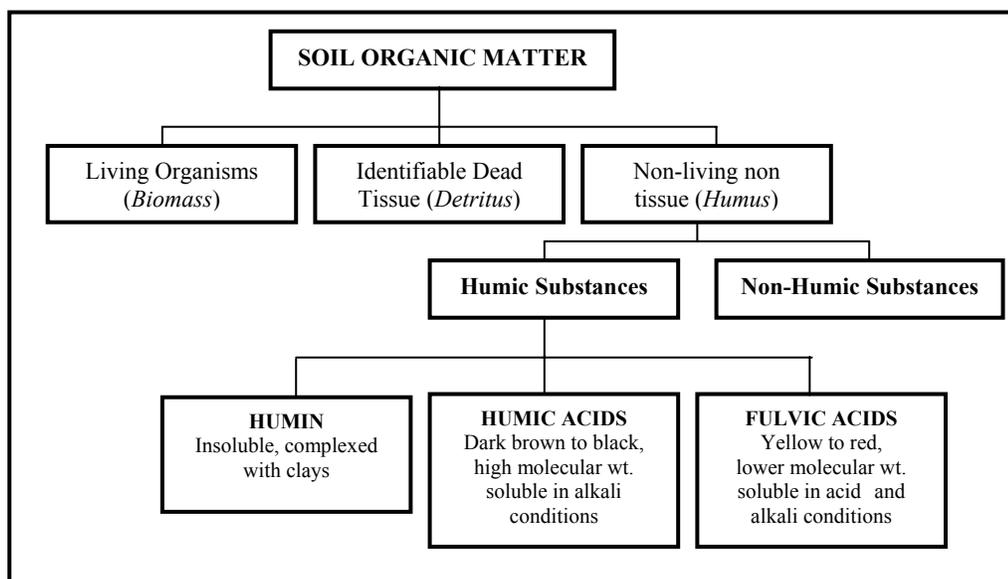


Figure 3.2 Classification of Components of Organic Matter  
(after Brady and Weil, 1996)

Humic substances can be further subdivided into humin, humic acid, and fulvic acid; these terms do not pertain to a single compound but a wide range of compounds with similar origins. Differentiation between these fractions is done primarily on the basis of solubility, but to a lesser extent, molecular weight, colour, and degradation resistance can also be used to distinguish them (Brady and Weil, 1996). Humin is insoluble under acid and alkali conditions; as well, humin is the darkest colour and highest molecular weight of the three compounds. Due to its insolubility and molecular weight, humin is expected to influence trace metals in aquatic systems mainly at the sediment-water interface. Humic acid is soluble in alkali conditions and is intermediate in colour, degradation resistance and molecular weight. Fulvic acid is

soluble in both acids and bases, is the lightest colour of the three types and has the lowest molecular weight and degradation resistance. Metal-organic complexes within the water column tend to be associated with humic and fulvic acid, which are sometimes known as organic acids. Molecular weights, which vary with pH, concentration and ionic strength, range between 500-5000 for fulvic acids and 3000-1,000,000 for humic acids (Sparks, 1995).

Despite their molecular size, the hydrophilicity of humic and fulvic acid functional groups enables these substances to remain in solution (Stumm and Morgan, 1981). This is particularly important when considering trace metal mobility. As humic substances can form stable complexes with metals (particularly if they are chelated), they can serve as effective carriers, transporting potentially toxic substances great distances. Subsequent dissociation of metals from organics can be facilitated by decreases in pH and extreme changes in concentrations of other chemicals (Kalbitz and Wennrich, 1998).

#### 3.2.4.2 The Adsorptive Capacity of Humic Substances

The metal binding capacity of humus is attributed to cation exchange processes (~1/3) and metal complexation (~2/3) (Schuster, 1991). As the speciation of Hg is dominated by uncharged complexes (i.e.  $\text{Hg}(\text{OH})_2^\circ$ ,  $\text{HgCl}_2^\circ$ ) specific adsorption to colloid surfaces is expected to be more important. However, as the adsorption capacity of soils is also a function of the cation exchange capacity (in accordance with the factors determining adsorption capacity: surface area > organic content > cation exchange capacity > grain size (Schuster, 1991)), discussion of both cation exchange and specific adsorption processes is warranted.

Like other colloids (e.g. clay minerals, hydrous Fe, Al-oxide minerals), humus colloids are highly charged and therefore tend to attract and adsorb cations or charged complexes from surrounding solutions. As  $\text{H}^+$  dissociates from functional groups with increasing pH, the negative charge on humus particles also increases. Conversely,  $\text{H}^+$  associations with organic matter increases with acidity and metals that are electrostatically bonded at the colloid surface desorb (Kalbitz and Wennrich, 1998). The ability of a colloid to adsorb positively charged ions or complexes, known as the cation exchange capacity (CEC), is a function of its surface charge. As the Point of Zero Charge (PZC) of most humic substances is between pH 2-3 (Pawlek, 2002), surface charges are typically negative in most natural waters.

Permanent charges on the surfaces of soil constituents are attributed to isomorphous substitution of ions with differing charges in the structure (for instance,  $\text{Mg}^{2+}$  for  $\text{Al}^{3+}$  in clayminerals), whereas variable

charges are pH dependent and result from broken bonds at the edges of the mineralogical phase. The permanent and variable charge characteristics of primary soil constituents vary considerably. Clay minerals typically have high permanent charge due to isomorphous substitution whereas HFOs and organic substances have high pH dependent charges.

Compared to other soil constituents, organics have an extremely high capacity to adsorb positively charged ions and complexes. The CEC<sup>2</sup> of organics, clay minerals, and HFOs have been estimated at 200 cmol/kg, 8-150 cmol/kg, and 4 cmol/kg, respectively (Brady and Weil, 1996). In some soils, it has been estimated that up to 80% of its CEC can be attributed to organic matter (Sparks, 1995).

As discussed, Hg typically occurs as *uncharged* chloride and hydroxides. Thus, specific adsorption is a particularly important complexation mechanism for Hg, as well as most heavy metals. Specific adsorption is defined as “adsorption caused by specific interactions between the adsorbate and the adsorbing surface, rather than by simple electrostatic forces” (Anon, 2002). “Free” cations can also specifically adsorb, although not as strongly as metal complexes (hydroxides, carbonates, etc) (Schuster, 1991). As ions are mainly covalently bound via their oxide and hydroxide groups, specific adsorption is extremely strong, often more so than chelated ions.

In the presence of other metals, complexation is controlled by competitive reactions for binding sites. The affinity of a ligand for specific metals generally follows the series (Alberts and Filip, 1998):



The relative abundance of these elements, either as ‘free’ cations or complexes, predominance of specific functional groups and heterogeneity of the humic substance influence complexation onto organic matter (Alberts and Filip, 1998; Benedetti *et al*, 1995; Ephraim and Allard, 1994). As Hg<sup>2+</sup> is a *soft* (Group III) cation (i.e. as determined by the ratio of the charges divided by the radius), it has an affinity for S-functional groups (e.g. S<sup>2-</sup>, SH<sup>-</sup>) following the series S>I>Br>Cl=N>O>F (Sparks, 1995). Methylmercury also tends to complex with *soft* ligands (Ullrich et al, 2001). It should be noted that the relative abundance of ligands considerably effects the predominance of certain complexes. The high metal complexing capacity of humic substances has mainly been attributed to carboxylic groups (4 to 8 meq g<sup>-1</sup>) although the presence of sulfidic groups may be more important for Hg (Allard and Arsenie, 1991). Ionic forms of

organic and inorganic Hg ( $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$ ) have both been shown to form very stable, lipophilic complexes with anionic sulfur ligands (Gottofrey and Tjalve, 1991).

### 3.2.4.3 Humic Substances and Mercury Speciation

The presence and abundance of organic substances can significantly affect the chemical, physical and biological behaviour of Hg in natural systems. Schnitzer and Kerndorff (1981) have shown that over a large pH range (4 to 9) when more than 20 mg/L of fulvic acid is added to solution, Hg becomes very soluble. The authors pointed out that Hg interacts with fulvic acid in partly hydrolyzed forms. Xu and Allard (1991) found the majority of Hg(II) in the presence of only 1 mg/L of fulvic acid is complexed, independent of the system pH (2.5 to 9). As organic acid concentrations in most aquatic systems range between 1 and 10 mg/L (Allard and Arsenie, 1991), Hg-organic complexes are the predominant Hg species in many systems. Melamed et al. (1991) experimentally demonstrated that humic acid solutions increase the solubility of metallic Hg, although the presence of calcium ions inhibits solubilization. Meech et al. (1998) found that Hg in the presence of organic acids forms soluble complexes at Eh levels below those observed in the Eh-pH diagram for inorganic soluble species. This research also demonstrated that a number of organic acids, including tannic and fulvic acid, are capable of solubilizing metallic Hg, despite its low solubility in water ( $56 \text{ ng g}^{-1}$ ). Wang et al (1997) reported a Hg complexing capacity of humic acid as high as  $170\text{-}420 \text{ mg g}^{-1}$ .

Certain factors have been demonstrated to influence Hg speciation in the presence of organic acids. These include the presence of specific metals ions and UV radiation, as discussed below.

#### Influence of Specific Metal Ions

Lee et al (1985) suggested that Hg methylation in the presence of humic substances is stimulated by the presence of certain metals ions. As prior research (Rogers, 1977; Nagase, 1982) had reported the effectiveness of fulvic acid in this process, various concentrations of fulvic acid were used ( $5 \text{ to } 20 \text{ mg L}^{-1}$ ). Two to four day incubations in darkness at varying Hg(II) concentrations and a range of pH (3 to 6.5) revealed that Fe had the most significant catalytic effect on methylation, following the series  $\text{Fe}^{3+} > \text{Cu}^{2+} \approx \text{Mn}^{2+} > \text{Al}^{3+}$ . The methylation rate was greatest at a pH of 4-4.5. Although the role of humic substances in Hg methylation is not understood, the research described by Lee et al suggests that the presence of other metals may exacerbate methylation, possibly by weakening the organic acid-Hg

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<sup>2</sup> A measure of the CEC, the cmol/kg is the centimoles of positive charge held by 1 kilogram of soil. For instance, 1 kg of organic matter can hold 200 cmol of monovalent  $\text{K}^+$  or 100 cmol of divalent  $\text{Hg}^{2+}$ . With an atomic weight of 200, 1 mol of Hg is 200g or 1cmol is 2g. Thus, 1 kg of organic matter can retain 200g of Hg (II).

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complex. As high Fe concentrations, as well as acidic conditions, are common in darkwater (i.e. organic-rich) systems; the methylation mechanism involving organic acids may be of extreme importance in Hg-impacted artisanal mining communities in many regions of the Amazon.

#### Influence of UV Radiation

Humic substances have also been shown to have a reductive capacity in aquatic systems and may account for as much as 70% of the volatile Hg released from some Hg contaminated streams (Allard and Arsenie, 1991). A study by Matthiessen (1998), which stated that organic acids may facilitate Hg(II) reduction to volatile Hg<sup>0</sup> while complexed to *or* dissociated from humic substances, determined that the amount of elemental Hg formed increased with pH. The role of humic and fulvic acids in reduction of Hg(II) seems to be further exacerbated in the presence of UV radiation. Allard and Arsenie (1991) observed a reducing capacity of 0.1 meq h<sup>-1</sup> by fulvic acid; these researchers also observed that Hg reduction by humic substances is decreased by factors, such as the presence of competing ions (particularly Cl<sup>-</sup>), air or methylation of carboxylic groups, and increased by light. Over a period of six hours of exposure to UV light, humic substances degrade by ~50% and remaining compounds decreased in molecular weight from 1800 to 300 (Allard et al, 1994). The 'new', less stable, low molecular weight organic acids mainly consisted of formic, acetic and oxalic acid. In the process of degradation, adsorbed metals are released and subject to transformations to less or more bioavailable species.

Light penetration may also influence levels of MeHg in biota from darkwaters. As the rate of MeHg photodegradation has been demonstrated to be 350 times faster than degradation by microorganisms (CEQD, 2000), MeHg in areas with greater light penetration, i.e. clearwaters, may be more susceptible to degradation and transformation to biologically unavailable species. This is inconsistent with results from a study by Costa et al (2001), which demonstrated that Hg photoreduction in freshwater increases with DOC concentrations.

#### 3.2.4.4 Organic Acid-Metal Complexes versus Organometals

Organometallic compounds involve metal binding to carbon atoms that do not dissociate reversibly with decreasing pH or changes in concentration (Manahan, 1994). Unlike organometallic compounds, organic acid-metal complexes can release metals with changes in environmental conditions (e.g. Eh, pH) or degradation of humic substances (for instance, as facilitated by UV radiation) (Allard et al, 1994). Particularly strong examples of complexes are chelates, which form when two or more functional groups bond with a metal (resulting in a ring-like structure).

Metals in organometallic compounds are generally bound to carbon atoms in the form of alkyl groups (e.g. ethyl in tetraethyllead), carbonyls, or in conjunction with electron donor groups, such as ethylene or benzene (Manahan, 1994). Due to their high toxicity and tendency to accumulate in organisms, metals that form alkyl compounds, particularly methyl derivatives, are of particular environmental concern (Stumm and Morgan, 1981, Fergusson, 1990). Metals that can be biomethylated include: Sn, As, Se, Pt, Au, Hg, and Pb (Stumm and Morgan, 1981). Mono- and di- methylmercury are examples of organometals.

#### 3.2.4.5 Humic Substances and Bioaccumulation

Primarily due to adsorption, many metals are believed to be less bioavailable when associated with organic substances, as well as other matrices (e.g. clay minerals). For instance, copper toxicity is believed to significantly decrease when complexed with humic or fulvic acids (Intl. Copper Assoc, 1999; Parametrix, 1999). Evidence also exists to the contrary. As organic acids promote desorption from soil or sediment particles, their presence may enhance the bioavailability of some metals, which has been shown for cadmium in the presence of fulvic acids at high pH (Ledin *et al*, 1996, Breteler and Saksa, 1985). Humic and fulvic acids have also been shown to enhance zinc and copper toxicity as they promote algal growth, which accumulate the metals and transfer them to fish and other aquatic organisms (Ohkuba and Okada, 1998).

The contribution of organic acids to Hg uptake in biota has been equally confounding. Some studies do suggest that inorganic or organic Hg associated with organic acids is less bioavailable than in non-adsorbed forms (Watras *et al*, 1998; Lawrence *et al*, 1999; Mason *et al*, 2000; Sjoblom *et al*, 2000). Conversely, positive correlations between MeHg and dissolved organic carbon (DOC) have been identified in many aquatic systems (Lee *et al*, 1985, Watrus *et al*, 1998). Although DOC competes with biota for inorganic and organic Hg, on a “unit carbon basis”, biota has a much stronger affinity for MeHg (Watras *et al*, 1998).

Waters in the Amazon Basin are characterized as ‘blackwaters’ or ‘darkwaters’, which are organic-rich, or ‘whitewaters’ or ‘clearwaters’, which are organic-poor and frequently have high levels of clayminerals in suspension (Kuchler *et al*, 1994). About 60% of the DOC in the Amazon River is comprised of humic substances, the main source being waters from the Rio Negro. The Rio Negro has extremely high concentrations of humic substances (6-7 mg/L), with a fulvic to humic acid ratio of 1.53 (Kuchler *et al*,

1994). As higher levels of MeHg have been documented in fish sampled from Amazonian darkwaters compared to whitewaters (Veiga et al, 1999), humic substances may play a particularly important role in the bioaccumulation of Hg. Direct intestinal methylation of ingested Hg complexed to organic acids may be possible in fish although very few studies have addressed this capacity. Guimarães et al (1998) documented methylation rates ( $10^{-2} \mu\text{g g}^{-1} \text{h}^{-1}$ ) in organic rich Amazonian sediments adjacent to darkwaters higher than those in whitewaters. High methylation rates have commonly been associated with low pH characteristic of organic sediments and darkwaters (Lacerda et al, 1995) as reflected by high Hg levels in fish from acidic darkwaters (Nakazano et al, 1999).

The capacity of floating or rooted macrophytes to methylate Hg in their living or decomposing roots was studied by Guimarães et al (2000). Net MeHg production was found to be an order of magnitude higher in the plants than in the underlying sediments (compare 1.8-35% of total Hg methylated in plants to 0.6% of total Hg methylated in sediments). In the Amazon, these macrophytes, or “floating meadows” are important habitats for various fauna, including fish. Consequently, this source of MeHg can be of great importance to biogeochemical Hg cycling in the Amazon Basin.

In studies of fish in Ontario lakes, Hg concentrations have been positively correlated with DOC in walleye and northern pike (Wren *et al*, 1991) and lake trout (McMurty *et al*, 1989). An extensive review of field data collected from 15 northern Wisconsin lakes suggested that Hg correlated to DOC was an indication of the supply and bioavailability of MeHg to lower trophic levels (Watras *et al*, 1998). This means that the behaviour of MeHg incorporated by primary producers paralleled the carbon cycle. Research has also indicated that MeHg associated with fulvic acid is more readily taken up by benthic organisms, specifically the mussel *Mytilus edulis*, than in the absence of the acid (Gagnon and Fisher, 1997).

Lawrence *et al*. (1999) found that the solubilization of particle-associated MeHg by the digestive fluids of benthic organisms decreased with increasing organic matter under marine conditions. This is consistent with a study of Hg uptake by *Chaoborus* (phantom midge) larvae, where incorporation of both dissolved Hg and MeHg decreased with increasing concentrations of humic substances (Sjoblom *et al*, 2000). A study of Hg in Adirondack lakes found that inorganic and organic Hg levels in the water column increased with DOC, but DOC also negatively correlated with Hg in fish tissues, suggesting complexation decreased bioavailability (Driscoll *et al*, 1995). A detailed geochemical study of total Hg and MeHg in the water column and sediments in the Patuxent River estuary, Maryland indicated that DOC concentrations correlate with total Hg, but not MeHg in this system (Benoit et al, 1998).

Experiments by Wang et al (1997) demonstrated that plant uptake of Hg in soils is significantly inhibited in the presence of humic acids. Using two types of soils and two varieties of vegetables (*Lactuca sativa* var. *angustana* Irish and *Brassica Chinesis*), researchers reported that Hg uptake decreased with increasing humic acid levels and bioaccumulation could not be measured at humic acid concentrations above 0.2 g kg<sup>-1</sup> in one of the soils. The mechanisms contributing to these observed effects were not discussed in this study, but may relate to the limited capacity of various plants to solubilize organic-associated Hg.

### **3.3 Mercury in Humans**

Mercury exposure is universally recognized as a potential health hazard for humans. There are two primary pathways for exposure to Hg by humans: inhalation and ingestion. Artisanal miners involved in amalgam decomposition through open-air burning and working or living in close proximity to gold shops are at a significant risk of inhaling toxic quantities of Hg. Other industries with risks of Hg vapour exposure include organized mining (particularly refining), chlorine and caustic production, and electrical industries (Stopford, 1979). As discussed, consumption of MeHg containing foods, particularly carnivorous fish, is a crucial route for exposure that warrants serious review.

#### **3.3.1 Inhalation**

Mercury vapour is almost completely absorbed in the lungs (Dietrich, 2000). Once Hg<sup>0</sup> is absorbed, it oxidizes and forms Hg (II) complexes that are soluble in many body fluids (Jones, 1971). Oxidation can be facilitated by red blood cells or directly within tissues (Malm et al, 1997). The time interval between passage of elemental Hg through the alveolar membrane in the lungs and complete oxidation is long enough to produce accumulation in the central nervous system (Mitra, 1986). Mercury can easily diffuse across the blood-brain barrier, which is the system that controls the exchange of metabolic material between brain and blood (Chang, 1979). Damage induced by Hg incorporation includes impairment of the blood-brain barriers capacity to regulate neural metabolism; malfunctions in enzymatic systems; and direct damage to cellular proteins (Chang, 1979). Kidneys are also highly susceptible to Hg accumulation. In the blood stream, Hg(II) bound to plasma proteins is filtered via the kidney and enters the proximal tubules. Mercury interactions with -SH (thiol) group proteins in the tubule membrane walls, this can alter the permeability of membrane walls and enzyme function in the kidneys (Chang, 1979). Functional impairment of the proximal tubule can result in excretion of 'useful' compounds that normally would be returned to the bloodstream.

The kidneys are mainly affected in exposures of moderate duration to considerable levels, while the brain is the dominant receptor in long-term exposure to moderate levels. Elimination occurs through excretion of urine and faeces, with a half-life in the urinary tract of 40 to 60 days (Malm et al, 1997) although total Hg elimination can take several years.

#### 3.3.1.1 Symptoms of Toxicity from Inhalation of Mercury Vapour

The symptoms usually associated with chronic Hg vapour exposure are erethism (exaggerated emotional response characterized by shyness, irritability, memory loss and insomnia), fatigue, anorexia, weakness, gingivitis and muscular tremors (Dietrich, 2000). Mild cases of Hg poisoning have many psychopathological symptoms that can be mistaken for alcoholism, or fever, malaria or other tropical diseases.

Acute exposure to Hg vapour can result in nausea, abdominal cramps, diarrhea, muscle aches and fever within a few hours of exposure. Inhalation of extremely elevated Hg vapour concentrations can also result in concentric constrictions of the visual field, swelling of the gums and, within a few days of acute exposure, a metallic taste in the mouth. This is followed by ulcers, tooth loss and formation of a blue line at gum margins (Stopford, 1979). As well, acute toxicity may produce persistent shortness of breath and interstitial fibrosis of the lungs.

#### 3.3.1.2 Assessing Exposure from Inhalation

Maximum levels in urine have been established by the WHO as 7 µg/L for unexposed people and 50 µg/L for people occupationally exposed to Hg vapour (WHO, 1991). The half-life of Hg in the brain is longer than in the kidney, thus urine levels would not be expected to correlate with neurological findings once exposure has stopped. Malm et al (1997) estimated the half-life of Hg in blood after long-term exposure ranges from 3.8 days in the 'rapid' phase (i.e. during intake and redistribution) and 45 days, in the 'slow' phase (i.e. during metabolism and excretion).

Experiments with animals indicate continuous exposure to Hg above 0.3 µg/m<sup>3</sup> of air may present a health hazard. Kobal and Dizdarevi (1997) found that subacute intoxication in mine workers occurred after 5 to 14 days of exposure to concentrations of 0.5 – 1.0 mg/m<sup>3</sup>. Acute Hg poisoning, which can be fatal or can cause permanent damage to the nervous system, has resulted from inhalation of 1,200 to 8,500 µg/m<sup>3</sup> of Hg (Jones, 1971).

At the Idrija Mercury Mine in Slovenia, mine workers were typically exposed to concentrations above maximum acceptable levels ( $0.1 \text{ mg/m}^3$ ) and periodically to concentrations of  $3 \text{ mg/m}^3$  (Kobal and Dizdarevi, 1997). Prior to the initiation of a health and safety programme, Hg intoxication in workers was high, as manifested by sub-acute and chronic symptoms including tremors, ptyalism (excessive saliva production), and digestive problems.

### **3.3.2 Ingestion**

Unlike metallic Hg, intestinal adsorption of MeHg is extremely high (>90%) (Jugo, 1979). The percentage of MeHg absorbed seems to be independent of the type of food consumed (Mahaffey, 2000). Neutrally charged species of MeHg easily penetrates cell membranes. Once MeHg diffuses across cell membranes, it complexes with sulfhydryl groups, thereby creating a concentration gradient into the cell (Jugo, 1979). The new complex formed mimics a common amino acid that is transported via the bloodstream and distributed to tissues throughout the body. Methylmercury in this form readily crosses placental and blood-brain barriers. Consequently, MeHg in cord blood is typically 20 to 30% higher than in maternal blood (Mahaffey, 2000).

Methylmercury accumulates in the central nervous system where it is slowly demethylated to inorganic Hg that persists in brain tissues (Mahaffey 2000). By ‘scavenging’ sulfhydryls associated with proteins, MeHg essentially disassembles or ‘depolymerizes’ certain membranes, inhibiting cell division and transport processes, thereby reducing the number of brain cells and neurons (Dietrich, 2000). This central nervous system damage results in a number of symptoms, described with other impacts below.

#### **3.3.2.1 Symptoms of Toxicity from Methylmercury Ingestion**

Concerns over ingestion of foods containing elevated levels of MeHg were first raised following international coverage in the 1950’s and 1960’s of the widespread contamination of fish and resulting effects on the residents of Minamata, Japan. Officially, 12,615 inhabitants of Minamata were finally recognized as being victims of MeHg poisoning, although many died before “Minamata Disease” was recognized in 1956 (MDMM, 1997). For more than 30 years, Chisso Corporation used inorganic Hg as a catalyst for acetaldehyde production. Methylmercury was a direct by- product of this process and was discharged into Minamata Bay, contaminating fish and shellfish on which the local community relied. Even after Chisso became aware they were responsible for Minamata Disease, they continued to discharge MeHg-containing wastewater into the Bay.

Although kidney problems are evident in acute exposures, long-term (i.e. chronic) exposure to moderate levels of MeHg results in severe neurological effects (Girard et al. 1995; Satoh, 1994, Suzuki, 1979). Symptoms of prolonged exposure to moderate levels of MeHg can include seizures, concentric constriction of visual fields, numbness of the extremities, impaired speech and hearing, irregular gait, muscle tremors, exaggerated emotional response and other mental symptoms, and potentially death (Murata et al, 2000, Deitrich, 2000). In cases of acute intoxication, muscular atrophy and mental disturbance are prominent. Incidences of Parkinson's disease have also been reported (Arimura et al, 2000). It has also been suggested that MeHg is also an endocrine disrupting compound, i.e. a chemical that can affect the glandular system, thereby impairing a wide range of functions including growth and reproduction processes (Keen, 1999).

Many factors can influence individual responses to MeHg including: genetics, age, sex, health status, and nutrition (Usuki, 2000). Effects of MeHg exposure on the central nervous systems of children can differ considerably from those to adults. These differences have been attributed to factors including reduced excretion, higher barrier permeabilities, less effective protective mechanisms, and different distribution in tissues of children (Jugo, 1979). Methylmercury readily crosses placental barriers and is considered to be a developmental toxicant (Grandjean, 1999); some studies indicate that concentrations of MeHg in foetal tissues are up to 1.3 times greater than in the mother.

Depending on the frequency and degree of exposure, effects can range from sterility, spontaneous abortion, to mild to severe neurological symptoms, including psycho-motor retardation and other developmental delays (Grandjean, 1999, Barbosa et al, 1995). Children exposed in utero are also at greater risk of developing cerebral palsy (Sakamoto et al, 2000; Barbosa et al, 1995) as well as limb malformations (Dietrich, 2000). The US EPA (1999) has classified MeHg as a possible human carcinogen and it is one of only two chemicals proven to be teratogenic (Dietrich, 2000).

#### 3.3.2.2 Assessing Exposure from Ingestion

In some food sources, MeHg can occur in association with other substances that biomagnify in the food chain (e.g. PCBs, dioxins) (Deitrich, 2000). These substances can also be neurodevelopmental toxicants and so may complicate diagnosis of Hg toxicity in some populations. As well, the symptoms of low-dose MeHg toxicity are similar to several other childhood learning disabilities, thus testing protocols must be sensitive to multiple variables (Mahaffey, 2000). In artisanal mining communities, Hg vapour can absorb

to hair and may interfere with hair samples (Akagi and Naganuma, 2000). Due to these and other interfering factors, attributing observed effects to MeHg toxicity can be difficult.

Levels of Hg in hair are commonly analyzed to assess MeHg accumulation in humans. Methylmercury is usually above 70% of the total Hg analyzed in hair (Vanconcellos et al, 1999). The normal Hg level in hair is less than 6 ppm and signs of MeHg intoxication can be observed at 50 ppm. 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 guideline for pregnant women (Skerfving, 1973). Many studies have shown levels of Hg above 50 ppm in riverine and aboriginal populations with fish dependent diets (Barbosa et al. 1997; Malm et al., 1997; Kehrig et al. 1997).

Mercury concentrations in blood are also used to evaluate contamination. Correlations between Hg in blood and hair have been derived from Swedish individuals, who are thought to have reached an equilibrium between dietary intake and body burden of Hg. Although results vary depending on the part of hair samples, hair values are approximately 300 times higher than blood (Nelson et al, 1971). The World Health Organization levels above which adverse effects are expected to occur are 200 µg/L and 50 ppm in blood and hair samples, respectively (WHO, 1990).

Although they are more complicated to sample, cord blood samples have been used to provide indications of adverse risks. The US EPA (2000) revised its reference dose (RfD) for MeHg to 0.1 µg/kgbw/day based on correlations with cord blood and an assessment program consisting of neurological development tests. Scores in the lowest 5% were linked to cord blood concentrations of 58 to 85 µg/L. Applying a safety factor of 10, a cord blood Hg concentration greater than 5-6 µg/L is considered to have an adverse risk.

Safe daily ingestion levels have been recommended by governmental and non-governmental agencies (e.g. WHO) as guidelines for consumption. The Total Daily Intake (TDI) proposed by Environment Canada is 0.2 µg/kg (ppb) per kg of body weight for women and children and 0.47 µg/kg (ppb) per kg of body weight for men (Mittelstadt, 2000). CONAMA (the Brazilian Environmental agency), Environment Canada and WHO all recommend Hg concentrations of 500 µg/kg as an upper limit in fish tissues for consumption. As 70 – 90% of mercury in fish is typically MeHg, several studies indicate that harmful effects (e.g. neurological effects, delayed onset of walking in children, psychomotor retardation) can occur at much lower allowable daily doses (0.06-0.1 µg/kg MeHg body wt) (Hoover et al, 1997).

Studies conducted in the New Zealand, Faroe Islands, Madeira Island and villages throughout the Amazon have documented symptoms of low-level chronic Hg toxicity (characteristic of Minamata disease) from consumption of Hg contaminated fish (Kjellstrom, T., 2000, Muratta et al, 2000, Mergler et al, 1999, Grandjean et al., 2000). In the case of the Faroe and Madeira Islands, biogeochemical oceanic Hg cycling has contributed to elevated Hg levels in pilot whales and black scabbard, respectively (Grandjean et al, 2000, Murata et al, 2000). In these regions, Hg in maternal hair was well correlated with auditory and visual impairment in children. In a study conducted in a high-fish consuming group in northern New Zealand, children were studied for a 6 year period using a number of psychological and behavioural tests in conjunction with results of MeHg analysis (Kjellstrom, 2000). This study correlated decreased IQs with increasing MeHg concentrations in hair of mothers while pregnant. As developmental neurotoxicity in children has been identified as the most sensitive health endpoint documented to date, neuropsychological and behavioural tests are increasingly being used to measure the effects of MeHg (Wizga, 2001). Directly correlating results with MeHg levels (i.e. in blood and hair), however, continues to be a challenge.

Extensive studies of mining, riparian and indigenous communities in gold-producing regions of the Brazilian Amazon also indicate that Hg-contaminated aquatic food chains are resulting in Hg accumulation in fish-dependent communities (Barbosa et al, 1997, Mergler et al, 2001). In this study, 54% of women of child-bearing age had MeHg concentrations in hair above 10 µg/g. Several other Amazonian studies have documented abnormalities in motor and visual function due to chronic MeHg exposure (Mahaffey, 2000). Occurrences of environmental and human health impacts from MeHg accumulation are currently being studied throughout Africa (Ikingura et al. 1997), in remote regions in China (Lin et al., 1997), in aboriginal communities in northern Canada (Wheatley et al., 1995, 1997), as well as in Africa, Philippines, Indonesia and Vietnam (Akagi and Naganuma, 2000). It is apparent that victims of chronic toxicity to low-levels of MeHg may not manifest the drastic array of symptoms typical of high-level chronic MeHg exposure as observed in Minamata, but chronic low-level MeHg exposure is a real phenomenon that still warrants considerable attention (Dietrich, 2000).

### **3.4 Mercury Bioindicators**

Bioindicators are organisms (e.g. plants, fish) used to identify the factors controlling pollutant toxicity and bioavailability and can ultimately be used to evaluate hazards where pollution is present. The bioavailability of metals, i.e. its capacity to be incorporated by organisms, 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 (Wen-Xiong *et al.*, 1998; Benoit *et al.*, 1999). Organism physiology, internal solubilization capabilities (Gagnon and Fisher, 1997), food quality (i.e. nutrients) and feeding behaviour also affect the assimilation efficiency of a metal (Wen-Xiong *et al.*, 1998; Lawrence *et al.*, 1999). Thus, an appropriate bioindicator organism must be reasonably well understood in terms of biological qualities and responses *and* be broadly applicable to various external (e.g. geochemical) conditions. A number of biological indicators are being used to assess Hg bioavailability and toxicity, although most established protocols are generally applied to assess a range of metals.

### **3.4.1 Fish**

Due to their importance in human consumption, relative ease with which they can be studied and the behaviour of Hg in aquatic systems, carnivorous fish are the most commonly employed indicator of Hg bioaccumulation. Many studies 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 and variable food sources of species.

Black piranha (*Serrasalmus rhombeus*) seem to be an ideal bioindicator in the Amazon 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 from the Tapajós River, specifically tucunaré (*Cichla ocellaris*), traíra (*Hoplias malabaricus*) and piranha (*Serrasalmus nattereri*), 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 traíra as a bioindicator while studying 15 dams in the interior of Venezuela. Incidentally, these researchers found high concentrations of Hg in 7 lakes, 5 of which were not influenced by mining. No single protocol or methodology has been adopted in the Amazon.

Baker *et al.* (2001) have developed a non-lethal method of fish tissue sample extraction for Hg analysis. Using a Tru-cut tissue biopsy needle (14 gauge x 7.6 cm cannula with a 20 mm notch), ~25 mg of tissue is extracted from fish anaesthetized with Alka-Seltzer. As Hg concentration increases with size and age, a wide size range of fish is needed to characterize Hg pollution in a given system. Using linear regression and analysis of covariance, comparisons can be made with a standardized size (550mm for trout and 250 mm for whitefish, in the lake evaluated).

Although laboratory protocols may not characterize the potential for Hg bioaccumulation as effectively as field sampling, laboratory methods can be used to identify variables influencing toxicity and adverse effects from Hg exposure. In Canada, established protocols exist to evaluate acute and sub-acute toxicity using brook trout, (*Salvenius fontinalis*), fathead and sheepshead minnow (AETE, 1997). The most traditional protocol involves a 96-hour static or flow through tests using Rainbow Trout to assess toxic affects of mine effluents. Another methodology developed by Environment Canada (1992) suggests the use of early life stage salmonid fish to assess sub-acute toxicity (Protocol1/RM/28).

### **3.4.2 Earthworms**

Earthworms may be a viable alternative to traditionally applied organisms (e.g. fish, people) as they are simple, well-studied creatures that have a demonstrated ability to accumulate heavy metals from polluted soils and other media (Ireland, 1983; Neuhauser *et al.*, 1985; Goats and Edwards, 1988; Rhett *et al.*, 1988; Edwards and Bohlen, 1996). 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 in these organisms. Very few studies have documented Hg concentrations in earthworm tissues (Martin and Coughtrey, 1982; Marquenie and Simmers, 1988; Rhett *et al.*, 1988; Braunschweiler, 1995) and even fewer (Beyer *et al.*, 1985; Yongcan *et al.*, 1998; Lawrence *et al.*, 1999) have addressed the biological and physiological elements that influence Hg bioavailability in these organisms.

The protocol derived as part of this thesis primarily evolved from a methodology developed by the American Society for Testing and Materials Standard Guide for Conducting Laboratory Soil Toxicity Tests for the Lumbricid Earthworm *Eisenia foetida* (ASTM-1676-95). Upon review of existing methodologies for earthworms and similar organisms and a range of contaminants (organics and inorganics), a methodology was derived that is applicable to evaluate metals associated with solids and solutions that can be applied under various conditions. Existing methodologies reviewed are summarized in Table 3.2.

Table 3.2 Major Earthworm Protocols and Studies

Protocol	Description	Comments
ASTM 1676-95: Standard Guide for Conducting Laboratory Soil Toxicity Tests for the Lumbricid Earthworm <i>Eisenia foetida</i>	Using the Earthworm <i>Eisenia foetida</i> a 28 day test was devised; involved the creation of a sphagnum based synthetic soil to be mixed with test media.	- acute toxicity test - interference of synthetic soil with metals possible; also variability of synthetic soils from test-to-test likely.
US EPA 600R94024: Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates  ASTM 1383-93a: Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates	Using the aquatic invertebrate <i>Lumbriculus variegates</i> a 28 day test for sediments; involved a fully saturated system and continuous flow-through of overlying water	- limited to sediments - requires collection and treatment of water
Lockheed Martin Environmental Restoration Program: Development and Validation of Bioaccumulation Models for Earthworms	Compared bioaccumulation of earthworms relative to study using 33 studies from 12 countries and developed bioaccumulation models for selected organics and inorganics	- does not provide specific methodology information on studies reviewed - numerical correlation between worms and substrate derived from various protocols
The Prediction of Field Toxicity of Chemicals to Earthworms by Laboratory Methods (Goats and Edwards, 1988)	Review of available methods for toxicity prediction using earthworms and subsequent development of new methods; evaluated various synthetic and natural substrates, modes of exposure and compared field methods for toxicity to the same series of contaminants	- evaluated contaminants in solutions only - acute toxicity test - standardized substrate (using filter paper or silica) deemed most appropriate for solution evaluation
Hazardous Materials Assessment Team (HMAT) 14 day Soil Test Using Earthworms	Involves addition of contaminant of concern with standardized mixture of sand, clay and peat and exposure of worms for 14 days.	- evaluated contaminants in solutions only - bioaccumulation test - contaminant interactions with prepared soil probable

### 3.4.3 Other Bioindicators

Comparatively few aquatic bioindicators have been developed to assess of sub-lethal Hg toxicity. Established protocols for the evaluation of acute toxicity of anthropogenic organic compounds using fresh water aquatic biota include methods for amphipods (*Hyalella azteca*) (EPA, 1994-2) and inland silversides (*Menidia beryllina*) (EPA, 1994), which use survival growth and reproductive capacity as endpoints African clawed frogs/FETAX (*Xenopus laevis*) (ASTM, 1997b) are also used and apply evaluation of developmental early stages as an endpoint.

Established sub-lethal protocols have been developed for aquatic organisms including brook trout (*Salvelinus fontinalis*), fathead and sheepshead minnow full life cycle, as well as *Daphnia magna*, *Ceriodaphnia dubia*, zebrafish (*Brachydanio rerio*), and mysid shrimp (AETE, 1997). Tests on algae are often reported as acute tests, although as algae reproduce so quickly within a typical study period (4-5 days), these tests can also be classified as chronic studies (Adams, 1993).

Seabirds have been shown to be effective as biomonitors of Hg pollution in marine environments as their diet, mobility and occurrence are well known and they can provide evidence of bioaccumulation by sampling eggs and feathers (Thompson *et al*, 1998; Monteiro, 1996). As certain species feed at distinct locations, specifically epipelagic (i.e. shallow) versus mesopelagic (below the eutrophic zone), they can be used to characterize Hg in certain marine food chains and, in conjunction with water dating techniques, can reflect Hg inputs during specific time periods.

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 Hg over the days, probably because the Fe-rich sediments had adsorbed any reactive Hg.

In similar experiments by Otchere *et al* (2000), which used the bivalves *Anadara (Semilia) senilis*, *Crassostrea tulipa* and *Perna perna*, factors such as “growth dilution” (i.e. when the intake level does not increase in proportion with the growth rate) and seasonal variability of conditions (e.g metabolism, salinity) were also considered. Although this research demonstrated that these bivalves do accumulate Hg, it reiterated the importance of incorporating site-specific variability (physical and environmental factors) into the assessment of results.

Plants have also been extensively used as bioindicators of metals uptake. Environment Canada suggests the use of macrophytes (*Lemna minor*) (Report EPS 1/RM/37- 1999) as bioindicators of aquatic toxicity from effluents. This test method measures growth inhibition by evaluating the effluents using frond dry weight (i.e. individual leaf-like structure and the smallest unit capable of reproducing) over a 7-day period. This test is limited in that results are difficult to correlate with impacts to other ecosystem components. Laboratory and field tests using lettuce (*Lactuca Sativa*) (EPA 1996; Folsom *et al*, 1991;

WES, 1989) have also been used, but this is not naturally occurring in most locations. Marsh Grass (*Echinochloa Crusgalli*), (Walsh et al, 1991), and Rye Grass (*Lolium Perenne*) (ASTM E1598-94; ASTM, 1997) have been also used as toxicity monitors using survival and growth as endpoints, but salinity of the tested soils can significantly influence the results and similarities to certain weeds can complicate field applications.

Intuitively, the best bioindicator for MeHg bioaccumulation is human beings; however, there are ethical issues associated with collecting biological samples from individuals in many cases, as the donors are not always properly informed of the results. Although hair analysis is affected by external factors, such as use of dyes and Hg<sup>0</sup> vapour exposure, the simplicity of sampling and analysis make it an amenable indicator for toxicological assessment of MeHg exposure.

## 4 CACHOEIRA DO PIRIÁ, BRAZIL

In the last 20 years, the municipality of Cachoeira do Piriá (hereinafter referred to as Cachoeira), located in the State of Pará, Brazil, has experienced a surficial gold rush that has resulted in serious environmental and social consequences. During the 1980's, more than 10,000 people, including 5,000 artisanal miners, were attracted to abundant and easily accessible colluvial and alluvial gold (Veiga et al, 2000). Currently, the remaining 8,427 inhabitants of the town of Cachoeira mainly subsist on agriculture (crops and livestock) and sparse gold mining activities. Mercury and Hg-rich tailings from past and current mining operations have been directly discharged into adjacent creeks, thereby exacerbating the potential for Hg incorporation into the food chain and ultimately humans. Many of the 17,000 residents of the municipality of Cachoeira reside adjacent to watercourses and rely on local fish stocks and are therefore susceptible to the impacts of artisanal mining.

### 4.1 Site Description

The town of Cachoeira do Piriá is located approximately 250 km east of Belém, the capital of Pará State, Brazil (Fig. 4.1). Cachoeira can be accessed from Belém via a paved highway (Route BR-316), which runs directly through the town. The municipality of Cachoeira is located on the border of the state of Pará with Maranhão in a region called Pará-Maranhão. The municipality covers an area of approximately 1.8 million km<sup>2</sup>.

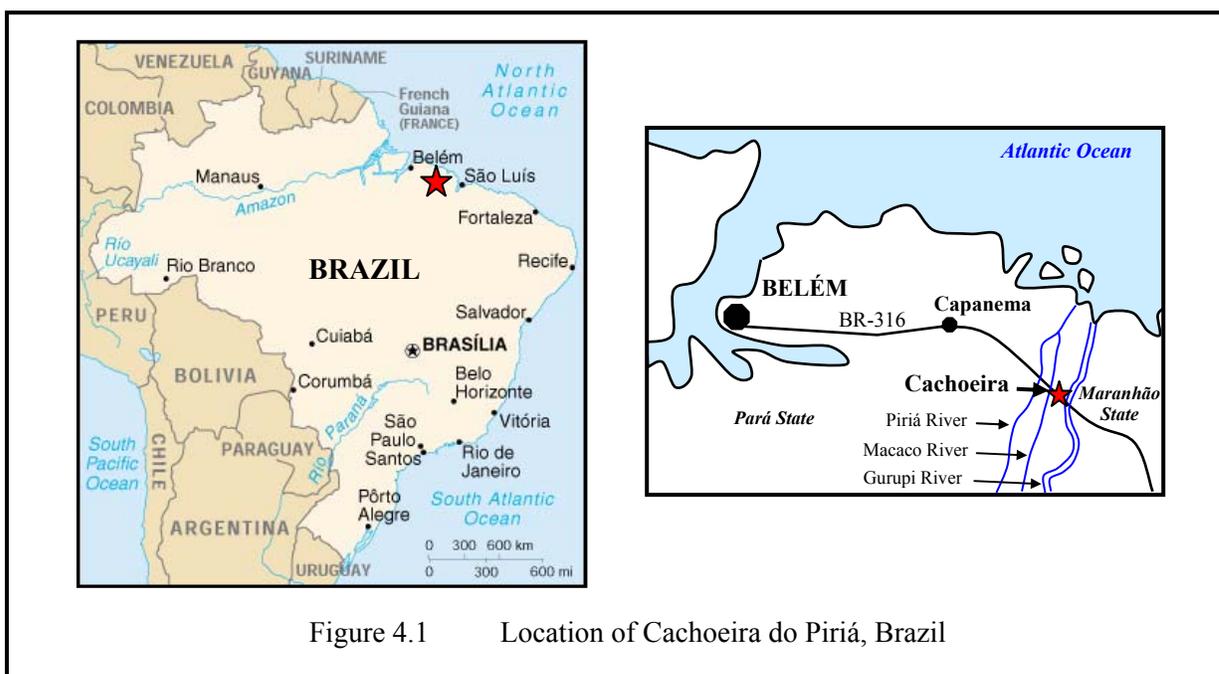


Figure 4.1 Location of Cachoeira do Piriá, Brazil

The municipality of Cachoeira is located in the northeastern Amazon Shield of Brazil (Cinits, 1998). The town of Cachoeira is situated on the top of a topographic rise and is surrounded by comparatively flat terrain. Elevations ranged from 30 to 70 metres in the flat areas up to about 200 metres above sea level. The town site was probably strategically chosen by original settlers as exposure to gentle winds at this elevation provides relief from the frequently hot and humid climate (typical temperature range 29 – 35 °C; mean ave. temp ~30°C).

#### **4.1.1 Physical Environment**

##### **4.1.1.1 Geology**

The geology of Cachoeira is dominated by a mafic-to-felsic, metavolcano-sedimentary sequence of an Archean to lower-Protozoic age (Hannon, 1999). The Maracacume Complex that dominates the area surrounding the study site consists of lenses of ultramafic metavolcanics, mafic and intermediate intrusives/volcanics, and metatuffs of variable composition, all of which intruded by porphyritic dacite. Most lithologies have undergone extensive deformation, i.e. folding, shearing, faulting, greenschist facies metamorphism and hydrothermal alteration (CML, 2000).

##### Gold Occurrences

Gold in the Gurupi Gold Province, a 30,000 square kilometre area extending across portions of Pará and Maranhão states, is mainly found in Precambrian lithologies that were exposed during the Cretaceous and Tertiary time periods (Hannon, 1999). The Gurupi Gold Belt is cut by the 90 km long, 5 km wide Tentugal shear zone, with which gold mineralizations are frequently associated (Cinits and Ewert, 1998). Saprolite hosted deposits worked by garimpeiros in the region were derived from quartz veins (ave. 50 cm diam) in schistose rocks associated with the Gurupi and Santa Luzia Formations. The Gurupi Formation mainly consists of low-grade metamorphic rocks (arkoses, phyllites and metagreywackes) and the Santa Luzia Formation is a sequence of biotite-muscovite-garnet-staurolite-graphite schists (Cinits and Ewert, 1998).

Due to the extensive tropical weathering characteristic of the Amazon, gold can be chemically and physically re-mobilized in lateritic profiles. Laterites are soils derived from the intense weathering of basalt (mafic) parent rock (Makasa, 1998). Common to high rainfall, tropical climates, laterites have been depleted of nearly all elements except iron and aluminum oxides. Due to solubilization and re-precipitation mechanisms, gold enrichment can be observed throughout lateritic profiles. As gold tends to be dispersed, it is effectively exploited using mass mining methods, hence the hydraulic monitors

frequently employed by artisanal miners. In some locations in the Gurupi Gold Province, alluvial or placer deposits subsequently experienced cementation through laterization (Hannon, 1999).

### Surficial Geology

The surficial geology found in Cachoeira is typical of tropical climates. A 2.0 metre thick, dark to reddish brown A-horizon<sup>3</sup> grades downward into a 2.0 to 3.0 metre thick dark red clay rich B horizon<sup>4</sup>. This overlies a variably weathered, clay rich saprolite C-horizon<sup>5</sup>, which continues to depths of approximately 40 metres. Iron and aluminum oxides characteristic of the lateritic soil horizons are abundant.

#### 4.1.1.2 Surface Water Hydrology

Two major rivers bound the area surrounding the village of Cachoeira – Rio Gurupi to the east and Rio Piriá to the west. Rio Gurupi marks the border between the States of Pará and Maranhão. The tributary Rio Macaco runs nearly parallel with Rio Piriá until their convergence (Fig. 4.1). Topography and field observations suggest most drainage within the mining area flows towards the north. Both rivers are ‘darkwaters’, i.e. abundant in organic acids. Two smaller creeks (Curretela and Barriquinha creeks) run through or adjacent to garimpo impacted areas, then into a wetland-type zone before discharging into Rio Macaco (Fig. 4.2). The banks and beds of both creeks predominantly consist of, or are armoured with, tailings. A man-made reservoir (“Lake Cachoeira”), constructed mainly to retain water for gold processing, is located in the southern region of the study area. The local topography, proximity of the reservoir to the town and water quality observations indicates that the creek that discharges into the reservoir receives considerable quantities of raw sewage from adjacent, upslope houses.

Rainfall in the region is highest between December and May (average 29 cm per month) and lowest between June and November (average 11 cm per month) (Atlas, 2000). Total precipitation averages 2.1 m/a.

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<sup>3</sup> A-horizons are the uppermost mineral horizons. They typically are darker in colour than underlying horizons due to the presence of partially decomposed organic matter and are often coarser in texture due to transport of fines downward.

<sup>4</sup> B-horizons are subsurface horizons where accumulation of materials by illuviation has occurred. In humid regions, iron and aluminum oxides and silicate clays dominate this horizon.

<sup>5</sup> C-horizon is the unconsolidated material underlying the A and B horizons that is generally unaffected by processes in the overlying soils. As weathering continues, the C-horizon may become a part of the overlying horizons.

#### 4.1.1.3 Vegetation

Due to the intense rainfall characteristic of the Amazon Basin, tropical rain forest, or selva, is typical of the region. However, opening of the paved highway (BR-316) has resulted in settlement, and with it widespread deforestation by large-scale slash burning. Although dense jungle persists along river margins and in areas with limited road access, vegetation in the municipality is now dominated by grasses and plantation crops.

#### **4.1.2 *Socio-Economic Environment***

Although the region was sparsely populated for centuries, the presence of the paved highway, combined with the 1980's gold rush, catalyzed an influx of people to Cachoeira. During this decade, an estimated 10,000 people immigrated to Cachoeira in search of gold and related opportunities. In recent years, a new agrarian policy began distributing plots to landless citizens, thereby attracting many more people to the region. The National Institute of Agrarian Reform (INCRA) provides labour and material (e.g. bricks, lumber, and cement) for housing construction to those who paid R\$2500 (~ \$1700 US) for a plot. Plots are located where no water, roads or power are available. The City Hall has issued permits to build houses using INCRA resources in illegal areas (*i.e.* plots owned by others or on mining concessions).

In 1996, the municipality of Cachoeira do Piriá was formally created and, according to the municipal government, currently provides up to 80% of local employment through jobs on municipally owned plantations and ranches. This conflicts with information derived from other local sources, which suggests that the municipality employs closer to 25%. Although municipal workers earn considerably less than minimum wage, formalization of the local government has meant several benefits to the community. The construction of a new school, a health centre, access to first aid, a garbage collection service and the purchase of two ambulances are excellent examples. Malaria is common in the municipality (more than 1000 cases in the year 1999 had been registered in the local health post), but 100% of municipality residents have been vaccinated against yellow fever (MGC, 2000).

In 1998-2000 a major power grid was constructed and electricity is now locally available to the town of Cachoeira, although more extensive infrastructure (e.g. phone service) is limited to the adjacent towns of Capanema and Castanhal, located about 95 and 180 kilometers west of Cachoeira, respectively. Prior to the installation of the power grid, many homes were serviced by generators. Despite the presence of the power grid, some homes continue to be without electricity.

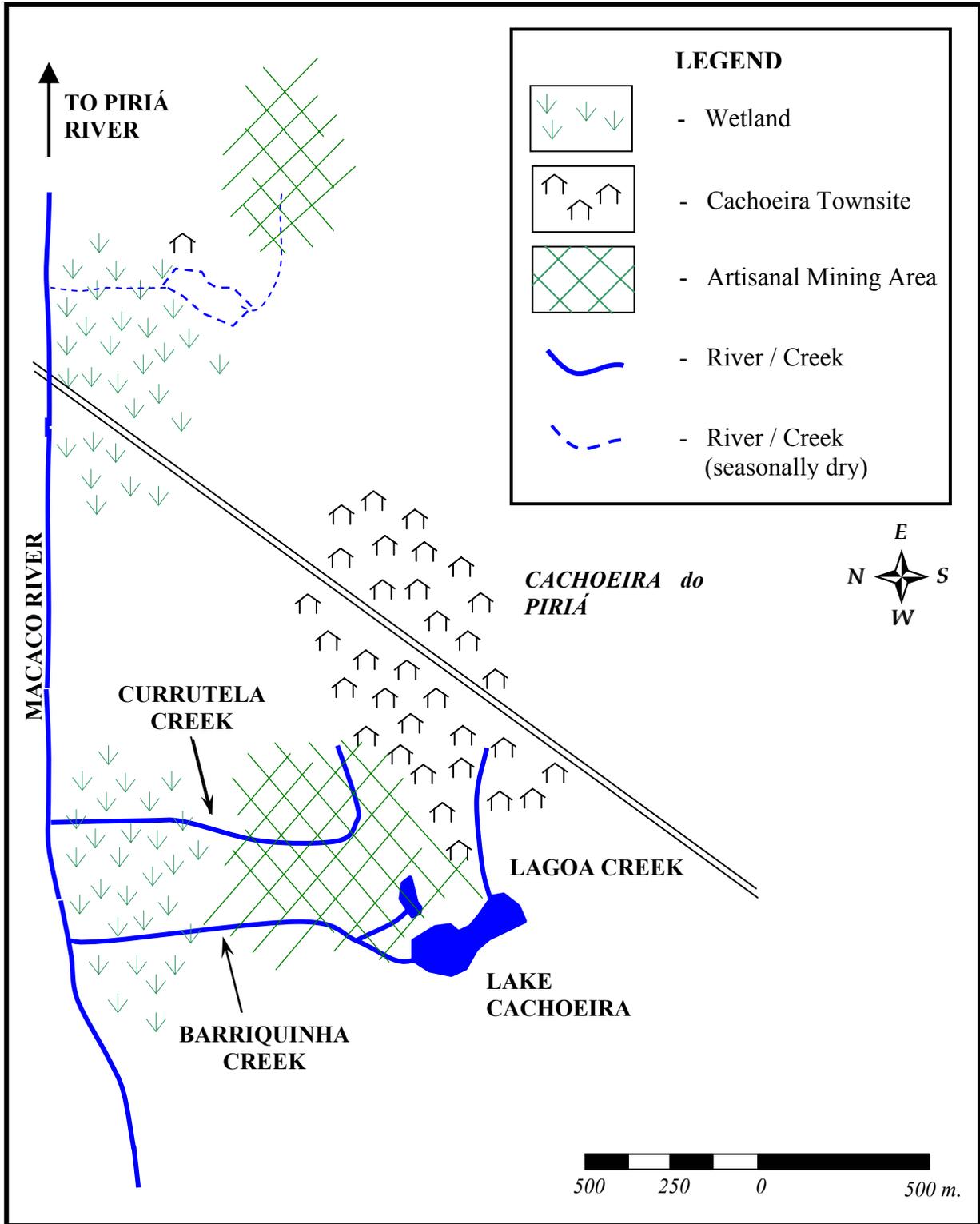


Figure 4.2 Study Area - Cachoeira do Piriá, Brazil

Installation of a series of groundwater wells brought potable water to the community in the late 1990's. Drinking water is supplied to the town primarily using this system although homes do not have direct service and must obtain water from a central water tower. There are currently 13 water posts serving the municipality. Prior to this, drinking water was collected from rainwater in large towers. Town inhabitants continue to use water from local creeks and a man-made impoundment as wash-water; some of these waters are expected to receive sewage discharges from adjacent homes.

There are 42 schools in the municipality of Cachoeira serving a total of 4,184 students (MGC, 2000). Although an upgrading program for teachers has commenced, very few of the 156 teachers in the system are qualified to teach secondary school. Thus, most education is limited to primary school. Two public day-care centres in the community serve 200 children.

Despite the advances provided by the new municipal government, proper sanitation is non-existent and standards of living are generally poor. The legacy of past activities is also evident in the composition of families. The violence and prostitution associated with the "golden years" of mining and the out-migration that followed has left many households – and the care of children, the sick and elderly - the sole responsibility of women. The economic strain on many families likely plays a part in the child prostitution that persists in the community. Although municipal authorities contend that child prostitution does not exist in Cachoeira, local sources suggest as many as 80% of the prostitutes in the community are girls aged 12-18 (Cerveira, 2000).

#### 4.1.2.1 Agriculture

Agriculture, mainly rice crops and cattle farming, is the primary means of existence for village inhabitants today (Veiga et al, 2000). Four thousand head of cattle have been provided by the municipal government for milk production and an additional 4000 for leather (MGC, 2000). Areas used for cattle pasture must be burned every 7 to 10 years to control pests and encroachment of jungle vegetation. Slash-and-burn methods are also employed for crop farms, although nutrient depletion and invasion of weeds typically results in abandonment within two to three years (Camill, 1999). In addition to rice, municipally owned plantations produce crops of bananas, beans, corn, açai fruit, and cassava (MGC, 2000). In addition to the larger scale economic agricultural activities, most houses have small adjoining gardens, mainly for personal consumption.

#### 4.1.2.2 Fisheries

The Macaco, Piriá, and Gurupi rivers and Lake Cachoeira all contain fish subject to consumption by local residents. Studies conducted to date have not addressed consumption rates by area residents or the existence of an economic fishery. However, given the economic conditions in the villages and the availability of fish in nearby rivers, frequent consumption is anticipated. Mercury levels in local fish are presented and discussed in Section 5.2.

#### 4.1.2.3 Artisanal Mining

Like many artisanal mining communities around the world, Cachoeira initially developed around the activities of a few miners who discovered a viable reserve of easily extractable surficial gold. Their efforts did not go unnoticed and as many as 5,000 miners were attracted to the region during the 1980's. The population quickly grew to 10,000. In conjunction with this rapid growth and influx of money, women also migrated to the area to find employment either as cooks, prostitutes and occasionally gold panners.



Figure 4.3 Miners using Hydraulic Monitors in Cachoeira

During the 1980's, an estimated 4 tonnes of gold was extracted from surficial ores (Veiga et al, 2000). During this time, the town of Cachoeira also became the main centre for gold exchange in the Gurupi region (Palheta, 1993). With the depletion of easily extractable ore and decline of gold prices in the late 1980's, many miners subsequently left the region. Currently, less than 20 artisanal miners are active on the study site, extracting only a few grams of gold, mainly through reprocessing of old tailings. With the

decline of artisanal mining activities in the last decade, violence and prostitution have also subsided in the community, but any indications of wealth have also vanished.

#### *History of Mining and Exploration Activities*

Small scale, alluvial mining has been taking place in the Gurupi region over the course of several centuries. Gold is believed to have been produced in the area since the 17<sup>th</sup> century, although most official documentation of these activities only dates to the 18<sup>th</sup> century (Cleary, 1992). The history of exploration and mining in the region is summarized in Box 4.1.

## **4.2 Previous Assessments of Mercury Pollution**

Mercury pollution in various compartments (soil, water, fish) has been partially characterized by researchers from the Mercury Research Laboratory at the Brazilian National Department of Mineral Production (DNPM), the National Institute of Minamata Disease (NIMD) and the University of São Paulo. Dulcedeia Palheta from the University of Surrey, UK, has also assessed mercury accumulation in humans, as well as pigs, sheep and cattle (Palheta, 1993; Palheta and Taylor, 1995). These research programs are described briefly below, but in-depth discussion of Hg distribution in Cachoeira is deferred until Section 6.0.

### ***4.2.1 Soil and Sediment***

The DNPM has conducted extensive sampling of soil, tailings and sediment in Cachoeira (Cid de Souza and Anjos, 2002). In soils taken from residential areas, Hg levels ranged from 0.03 to 11.02 ppm, with highest concentrations in surficial soils near gold shops. Mercury concentrations in sediments, which were highest near tailings discharge points, ranged from 0.01 to 11.7. Some correlation ( $r=0.65$ ) was observed between Hg concentrations and the percentage of organic matter (2 to 9%) in sediments.

### ***4.2.2 Water***

Palheta and Taylor (1995) sampled river water from sites along the Gurupi, Piriá and Macaco Rivers, as well as drinking water from the village of Cachoeira. Levels in Gurupi River and Lake Cachoeira reached 800 ng/L but most samples (six out of ten) were below the detection limit of 200 ng/L. Drinking water samples were also below this detection limit (<200 ng/L). Mercury levels well below the aquatic life standard for water (0.1 µg/L ; CEPA, 2001) are not surprising given the tendency of Hg to partition to particulate matter.

#### Box 4.1 - The History of Exploration and Mining in Cachoeira and the Surrounding Region

- 1857* – The Montes Aureos Brazilian Gold Mining company unsuccessfully attempts to explore the region of Montes Aureos;
- 1884* – The Baron of Capanema enlists mining engineer Arrojada Lisboa to conduct a geological survey of gold occurrences, with the intention of mining. Logistical difficulties and the hostility of native people ultimately impede this venture.
- 1935* – The Ministry of Agriculture (SFPM) studies the region and subsequently commissions a detailed survey of the Gurupi River area, including the locations of garimpos and other significant mineral occurrences. During this time period, garimpeiros were intensively extracting gold from both placer deposits and quartz veins in saprolite (decomposed phyllites and schists).
- 1941 – 1946* – Gold is mined from altered saprolitic veins between the Guarimanzal and Chega-Tudo Creeks in Gurupi by the Ferreira brothers. The Ferreira brothers and Anaconda Copper conduct intense exploration in the area.
- 1946 – 1947* – Mineração Brazil Canada S.A. (“Brascan”) conduct trenching, pitting, and diamond drilling over a 16km strike length across the Site in Cachoeira.
- 1973* – Grupo Badin (subsidiary Mineração Arapiranga Ltda.) conduct exploration in the region and construct an experimental pilot plant to extract gold from secondary deposits. This venture was deemed economically unfeasible. Companhia de Desenvolvimento Agro-Pecuario Industrial do Estado do Pará (“CIDAPAR”) acquires rights to the property at Cachoeira and conducts diamond drilling, mainly in the same area where Brascan had operated.
- 1975 – 1990* – A gold rush begins following discovery of new mineralizations throughout the region. Noranda signs an agreement with CIDAPAR to explore the Cachoeira property and subsequently conducts a multi-phase exploration program. Noranda ceases operations at Cachoeira in 1978. Once easily accessible surficial gold is recovered, garimpo activity in Cachoeira and the surrounding area subsides, although a few garimpeiros continue to operate.
- 1980’s* – With high gold prices, garimpo activity is rejuvenated and continues into the early 1990’s. During this period, up to 4 tonnes of gold is believed to have been extracted by more than 5000 garimpeiros working in Cachoeira. These activities result in pits over several hundred metres long and up to 45–50 metres deep and several shafts and tunnels, which follow high-grade quartz veins.
- 1985 - 1992* – The Brazilian company Companhia Paraense de Minerios (“CPM”) acquires rights and begins extensive, multi-phased exploration through their subsidiary Mineração Xopoto Ltda. Rights are sold by CMP in 1992 due to in-house legal and political reasons.
- 1993* – Mineração Capanema Ltda. (50% owned by Consulta Engenharia and Mineração, 50% by Mineração Santa Lucia) acquire exploration and mining rights. A review of data compiled by CMP is conducted and resources are subsequently re-estimated. Construction of an on-site recovery plant begins and a program for mining and processing saprolitic ore and treatment of tailings and waste rock is proposed, although none of these ventures come to fruition. Consulta Engenharia appeared to operate the project.
- 1996* – The property rights are offered by Mineração Capanema Ltda. to Barrick, who subsequently conduct a brief assessment of the site.
- 1997* – The exploration and mining rights to the Cachoeira property are acquired by a subsidiary of TVX Gold, Companhia Nacional de Mineração (“CNM”). It is not known whether CNM conducted exploration in Cachoeira.
- 1998* – Brazilian International Goldfields (“BGZ”), of Vancouver, BC, through Brazilian Goldfields Part. S.C. Ltda. signs an option agreement (effective September 24, 1998) with CNM to acquire the Cachoeira property. Extensive exploration is subsequently conducted by BGZ.
- 1999* – Goldfields Ltd. From South Africa enters a joint venture with BGZ. Exploration activities in Cachoeira continue under the direction of Goldfields.
- 2001* – Cachoeira property returned to CNM after BGZ defaulted acquisition payment to CNM.

### 4.2.3 Biota

Fish, cattle, sheep and pigs were sampled by various researchers to ascertain the abundance of Hg in the food chain of Cachoeira.

University of Surrey researchers (Palheta, 1993) purchased fish from local markets or caught fish themselves in local waters, whereas DNPM researchers contracted a local fisherman to catch fish. Neither of these studies identified the originating water body or specific location where fish were caught. Fish species common to both studies included traíra (*Hoplias marabanicus*), acará (*Ciclossoma sp.*) and mandii (*Pimelodus Blochii*). Surrey researchers also sampled species of piaba (*Pempheris schomburgki*), tucunare (*Cichla sp.*), cachorrinho de padre (*Cernicichla sp.*) and pacu (*myleus sp.*). In both studies, omnivorous or carnivorous fish species (i.e. traíra, mandii, tucunare) had significantly higher Hg levels than herbivorous species. Levels in traíra exceeded World Health Organization (WHO) guidelines of 500ppb in almost 70% of the fish sampled. Results from all fish studies are presented in Section 6.2.

Blood and hair samples collected from pigs, cattle and sheep in Cachoeira indicated that Hg had accumulated in these domestic animals (Palheta, 1993). Highest concentrations (average 27 µg/L) were evident in the blood of pigs and levels in local animals were 30 to 45% higher than in control animals. Mercury concentrations in the blood and hair of sheep (average 1.82 µg/L and 0.24 µg/g, respectively) were lowest of all animals sampled and were slightly greater (10-20%) than in control animals. The percentage of total Hg that was in organic form (i.e. MeHg) ranged from 37 to 99.7% in pigs and 32 to 99% in cattle. Mercury levels in animal hair were not correlated with blood and, as Hg concentrations in tissues were not determined, the implications of these results with respect to human consumption were not ascertained.

#### 4.2.3.1 Humans

Blood, hair and urine samples were collected from garimpeiros, villagers and river dwellers (people living along the Gurupi River) by the University of Surrey (Palheta and Taylor, 1995). Results of Hg analysis are presented in Table 4.1.

Several of the garimpeiros and river dwellers sampled had Hg levels in hair that exceeded the 10 ppm warning limit and the 15 µg/L exposure index (WHO, 1990), which suggests these individuals may also be frequent consumers of Hg-laden fish<sup>6</sup>. However, both blood and hair samples were below WHO levels

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<sup>6</sup> As discussed in Section 3.3., Mercury in Humans, exposure to Hg through ingestion is best evaluated by Hg levels in blood and hair. Levels in urine (and to a lesser extent blood) are more indicative of exposure through inhalation.

(200 µg/L and 50 ppm, respectively) wherein neurological damage is expected to occur. WHO established maximum levels in urine (7 µg/L for unexposed people and 50 µg/L for people occupationally exposed to Hg vapour). Several garimpeiros had Hg levels in urine well above WHO guidelines. It should be noted that some researchers contend that notable toxic responses can occur at levels below guidelines set by the WHO (Lebel et al, 1997).

Table 4.1 Mercury Concentrations in Human Subjects  
(after Palheta and Taylor, 1995)

Sample	Group	Range of Results	Number of Samples
Blood (µg/L)	Garimpeiros	2.0 – <b>29.3</b> µg/L	10
	Villagers	2.7 – 9.6	3
	River dwellers	1.0 – <b>64.7</b>	10
	Reference values	1.7 – 9.9	-
	<i>WHO Guideline</i> <sup>1</sup>	<i>15 µg/L; 200 µg/L</i>	-
Urine (µg/L)	Garimpeiros	1.0 – <b>155</b> µg/L	20
	Villagers	1.0 – 2.5	5
	Reference values	0.1 – 6.9	-
	<i>WHO Guideline</i> <sup>1</sup>	<i>7.0 µg/L; 50 µg/L</i>	-
	Hair (ppm)	Garimpeiros	0.4 – <b>32</b> ppm
Villagers		0.8 – 4.6	5
River dwellers		0.2 – <b>15</b>	10
Reference values		<2	-
<i>WHO Guideline</i> <sup>1</sup>		<i>10 ppm</i>	-

1. Maximum Level for Exposure based on WHO Guidelines. 'Unexposed' limits presented first, followed by occupational exposure limits, if available.

## **5 MATERIALS AND METHODS**

This research program consists of an assessment of Hg in various compartments (soil, sediment, and biota) to further characterize its distribution in Cachoeira, and development and application of a methodology using earthworms to better understand risks to the community of Cachoeira and provide a basis to evaluate measures for mitigation.

### **5.1 Soil, Sediment and Tailings Sampling**

The primary objective of the soil and sediment-sampling program was to assess the abundance and distribution of Hg in aquatic and terrestrial systems, particularly in relation to garimpeiro activities at the site. Sampling took place at the end of the rainy season (May-June, 1999) and when water levels, and Hg dilution in rivers, were moderately high and the beginning of the dry season (July, 2000), when water levels had decreased.

The sampling program was initially based upon a grid system (100 m by 100 m) in terrestrial areas, and equidistant intervals (~100m) along rivers and creeks; however, accessibility ultimately controlled the location of sample collection. Seventy-three sediment samples were taken: along both creeks that run through the study site, along the Rio Macaco, as well as from the reservoir (Lake Cachoeira), a wetland zone adjacent to recently active mining areas, and another wetland near a historically active area. Sediment samples were also collected 2-3 km downstream from the site on the Macaco River, approximately 300-400 m before its confluence with the Piriá River. Six additional samples of amalgamation tailings (i.e. from the base of sluices) were also collected. Thirteen samples were also collected in residential areas to assess the influence of local atmospheric Hg transport from amalgam decomposition. The sampling program intended to obtain samples at equal distances (200 m) along drainages and in a grid pattern in terrestrial systems, but locations were ultimately controlled by accessibility. A Global Positioning System (GPS) was used to determine the coordinates (+/- 5m) of sampling locations.

Soils and sediments (0.5 to 2.0 kg) were collected as discrete samples, that is, within a 0.5 m radius. Tailings samples (2.0 to 3.0 kg) were composed of five “grab” samples, or compound samples, collected within a 2 m area. All samples were packed in plastic bags, sealed and maintained at a cool temperature until subsequent refrigeration at 0°C.

Analysis of total Hg in these samples was conducted by the Brazilian National Department of Mineral Production (DNPM) Mercury Laboratory (Belém, Brazil) and ACME Laboratories (Vancouver, Canada) by Cold Vapour Atomic Adsorption (CVAA). This analytical method is described in Appendix III.

At all sediment sampling points, *in situ* measurements of pH, EC, temperature and dissolved oxygen were taken in waters. The following models of meters were used: TOA HM-14P (pH), TOA CM-14P (EC), and TOA DO-14P (DO).

## **5.2 Fish Sampling**

Local fishermen were contracted to catch fish from specified locations: the Macaco River adjacent to the study site and 2-3 km downstream (adjacent to the riparian community of Bela Vista); Lake Cachoeira; and the wetland adjacent to historic mining activities. Smaller drainages (Curretela, Barriquinha and Lagoa Creeks) and the westernmost wetland do not support fish. Fish species collected were traira (*Hoplias marabaricus*), acará (*Ciclossoma sp.*), aracu (*Anastomidae sp.*), mandii (*Pimelodus blochii*), and jejú (*Hoplerythrimus unitaeniatus*).

Fish were weighed and measured prior to dissection. Samples were separated in triplicate for subsequent distribution to analytical laboratories (two samples to the Center of Mineral Technology Research (CETEM), one sample to the DNPM laboratory). Portions of edible tissues were extracted from fish and stored under freezing conditions (-20°C). At the laboratory, tissue samples were homogenized and analyzed for total Hg via cold vapour atomic adsorption (CVAA). Methylmercury was analyzed by GC separation and CVAFS detection (EPA Method 1630) by the DNPM laboratory (Appendix III).

## **5.3 Earthworm Methodology**

The protocol described herein primarily evolved from a methodology developed by the American Society for Testing and Materials Standard Guide for Conducting Laboratory Soil Toxicity Tests for the Lumbricid Earthworm *Eisenia foetida* (ASTM-1676-95), in conjunction with other methodologies and research programs (discussed in Section 3.4.2). Upon review of existing methodologies for earthworms and similar organisms and a range of contaminants (organic and inorganic), a methodology was derived that is applicable to evaluate metals bioavailability that can be applied under various conditions.

### **5.3.1 Mercury Bioavailability in Solutions**

These tests intended to assess the influence of organic acids on the bioavailability of metallic Hg, the form of Hg predominantly discharged by artisanal miners.

Organism Culturing and Selection. *Eisenia foetida* were initially acquired from a local composting cooperative and cultured in dark plastic, ventilated bins on a diet of horse manure mixed with straw. 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. These factors, in particular worm weight, were deemed to be important as they are indicative of worm health and their capacity to endure the stress of a new environment. Prior to use, the chosen worms were stored for 24 hours on damp filter paper to void their gut contents.

Solution Preparation. Eight series of solution tests were conducted wherein metallic Hg (2 to 6 g) was added to 1 litre of 1.61 g/L tannic acid, 0.05 g/L fulvic acid or 0.05, 0.125, 0.25 g/L humic acid. (As these acids tend to be heterogeneous, the molecular weight, and thus molarity, could not be determined for the humic and fulvic acid. The tannic acid was a chemically prepared substance with a known composition (0.005M)).

The Hg-laden solutions were vigorously stirred for 24 – 72 hours to promote dissolution. Solutions were collected, filtered and refrigerated in plastic 1L containers until their use.

Jar Preparation. Prior to full scale testing, 3 to 5 worms were exposed to the organic acids in Petri dishes to provide some indication of acute responses. Most specimens died within two hours. Subsequent adjustment of the pH solutions using NaOH enabled long term habitability for the worms (Ideal worm habitability at pH 5-8). Various paper types and consistencies were also initially evaluated: paper towel, shredded; white Kaolin-based paper, shredded; and cellulose, which was formed by creating a pulp from Kaolin-based paper, drying the pulp at 25°C, then breaking down the dried pulp in a blender. It was observed that better results were achieved using cellulose. Due to the variable absorption capacity of different paper types and consistencies, different moisture contents were also evaluated. Optimal moisture content for worm habitability typically ranges between 30 and 45%.

Contents of the 900mL jars consisted of a cellulose-based substrate, mixed with fine silica sand and the solution to be assessed. Silica not only retains some moisture, but is also used by worms for grinding during the digestion process. As the methodology evolved, there was some variation in the number of

worms, proportion and preparation of the cellulose substrate, exposure time and moisture content. Experiments were conducted in duplicate or triplicate. All experimental configurations of jar tests are detailed in Table 5.1. Prior to addition of the worms, jar contents were homogenized via hand-mixing and shaking of the jar, or mixing in a blender.

Control worms were also collected from the culturing bin prior to the test and digested and analysed (as described below). All jars were prepared in duplicate and non-Hg containing were assessed concurrent with Hg-laden solutions.

Post-exposure Handling. At the conclusion of the exposure period, worms were removed from each jar, carefully washed and dried, counted and weighed. Observations such as mobility, 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 (from Series Two) 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 (i.e. starved to void gut contents) for 24 hours. Depuration was necessary to ensure that subsequent whole worm analysis was indicative of Hg levels in tissues and not residual particles retained in the intestinal tract.

Sample Preparation and Analysis. Following post-depuration washing and drying, all worms were placed together in 250ml, acid washed Erlenmeyer flasks and digested in 20 mL of 0.7M nitric acid. After 24 hours, the solution was slowly heated under a fumehood and reduced to 10 mL. 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 refrigerated. One of the two samples was kept as a duplicate and the other submitted for total Hg (wet weight) analysis by CVAA. Worm samples submitted for MeHg analysis were not digested but frozen immediately following post-depuration washing and drying. Methylmercury was analyzed by GC separation and CVAFS detection (EPA Method 1630) by Cebam Analytical Inc, Seattle, WA (Appendix III).

The solutions (organic acids and distilled water) were submitted for total Hg (and MeHg, for some samples) analysis by the aforementioned methods. At the conclusion of the 28-day test period, substrate material (i.e. organic acid or distilled water saturated paper and silica sand) from Series Two and Three was leached with distilled water. Leaching involved the addition of 300 mL of distilled water to 200g substrate. The combined material was shaken vigorously for several minutes. Leachate was subsequently

extracted using a vacuum filter and submitted for MeHg and total Hg analysis. All paper and silica sand used, as well as the horse manure culturing substrate, was also submitted for total Hg analyses to assess their contribution to Hg uptake.

A minimum of one 'blind' duplicate (or 10%) was also included in every batch of samples submitted to analytical laboratories (i.e. samples were numbered alphanumerically and duplicates were out of sequence with each other). Laboratories also randomly selected samples from each batch for duplicate analysis.

### **Specific Descriptions of Solution Experiments:**

Six different series of solution jar tests ("Series A") were carried out in order to satisfy various objectives. In addition to the specific objectives described below, all tests intended to evaluate the efficacy of the methodology and assess the influence of organic acids on Hg bioavailability.

#### Series A-One and A-Two:

These series' intended to assess the influence of different lengths of exposure (14-day or 28-day) to Hg dissolved in tannic acid (1150 ppb), and different depuration times (24-h, 72-h, 5-day clean paper feeding followed by 24-h depuration) following exposure. As two of the earliest experiments conducted, these series' also attempted to evaluate the experimental design.

#### Series A-Three:

This series was conducted to assess the validity of results obtained in Series A-Two (28-d exposure, 24-h depuration) and provide additional information about uptake at a different Hg dosage (3650 vs. 1150 ppb Hg in tannic acid).

#### Series A-Four:

This series consisted of a 7-day habitability assessment for exposure to 3 different concentrations of humic acid. Unlike the synthesized tannic acid used in previous experiments, the dried humic acid used consists of molecules of varied molecular weights<sup>7</sup>. Consequently, the true concentration of humic acid in the solutions could not be determined. Specified masses were added to distilled water (0.05 g/L, 0.125 g/L and 0.25 g/L) based on amounts used in previous experiments that assessed the leaching capacity of humic acid (Melamed, 1997). A paper substrate with an enhanced capacity for moisture retention (shredded paper towel) was also used for these experiments. The pH was within an acceptable range (6.0 – 7.6) for worm habitability and was not modified.

#### Series A-Five:

This experiment intended to evaluate the bioavailability of Hg associated with humic acid (0.25 g/L) and tannic acid (0.005M). After following the metallic Hg dissolution procedures employed in previous organic acid experiments, solutions were analyzed (TA 8150 ppb; HA 3780 ppb) and applied to synthetic substrates, as per previous tests.

#### Series A-Six:

This series intended to compare the MeHg levels in worms exposed to Hg in humic (6850 ppb Hg), fulvic (15.4 ppb Hg) and tannic acid (7500 ppb Hg). Following exposure, worms were frozen immediately following post-depuration washing and drying. The substrate was leached with distilled water (300 mL added to 200g substrate). Leachate was subsequently extracted using a vacuum filter.

The experimental configuration of each series of jar tests is presented in Table 5.1.

### **5.3.2 Mercury Bioavailability in Soils and Sediments**

Soils evaluated in the jar tests were divided into four main types, as follows. These classifications were derived from field and laboratory identification methods, combined with mineralogical studies conducted in a resource evaluation of the property (Hannon, 1999) and information concerning the composition and weathering of oxisols (Brady and Weil, 1996).

Tailings - Tailings consist of fine to medium orangish-brown sand. The mineralogical composition of tailings is predominantly quartz and Fe, Mn-oxide minerals. Organic carbon in tailings is <0.001%.

Lateritic soil - Lateritic soil samples were composed of orange-brown clay to clayey fine sand. Mineralogy is dominated by hydrous Fe, Al-oxides (goethite, hematite, gibbsite) with silicate clays (kaolinite) and minor quartz.

Organic soil - Organic soils were predominantly light brown to yellowish orange silty clay to fine sandy silt. All soils were abundant in fibrous organics and blebs of dark brown staining (moderate to highly decomposed organics). Based on values for similar A-horizon

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<sup>7</sup> As discussed in Section 3.2.4, molecular weights of humic acids can range between 3,000 and 1,000,000.

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oxisols, organic carbon content is estimated at 3%. Mineralogy is mainly Fe-oxides, with some quartz and 1:1 silicate clay minerals.

Clay sediment - Sediments were described as light gray organic clays with occasional blebs of dark brown staining. Clay is predominantly composed of kaolinite.

Organism culturing and selection, post-exposure handling, and sample preparation and analysis for tests in soil and sediments were the same as described for the assessment of solutions. Jar preparation was also the same, although tailings, soils or sediments were used in lieu of silica sand. Experimental configurations are described in Table 5.2.

Tailings, Soil and Sediment Preparation. In Series B-Four through B-Seven, all solids were pre-dried to ensure the moisture content was the same in concurrent tests. In order to prevent volatilization of Hg from samples, this was carried out over a 24-hr period at a temperature of 20-25°C. Dried samples were ground in a fine grinder to a grain size passing No. 200 mesh (0.074 mm screen).

The ‘nugget effect’ can play a significant role in Hg sampling and analysis. When dispersed in the environment, metallic mercury forms droplets often resulting in a heterogeneous distribution. In order to reduce the influence of the nugget effect, samples collected were put through a sample splitter a minimum of three times in order to achieve more homogeneous mixing.

In all tests, the moisture content was determined prior to experimental use by weighing a representative sample, placing the sample in an oven at 30°C, then re-weighing the dried sample.

Soil paste pH was also determined for soil and sediment samples (ASTM, 2001). This involved addition of 10g of soil (passing 10 mesh screen) and 10 or 50 mL of distilled water (a 1:1 or 5:1 ratio of water:soil, respectively) in 100 mL erlenmeyer flasks and mixing on a shake table for 1 hour. After the mixture settled for an additional hour, the soil paste pH was measured (using a calibrated Accumet Model 50 pH meter).

Total Metals Analysis. Substrate and digested worms from Series B-Four of the experiments were also submitted for total metals analysis to assess the influence of other substrate constituents on Hg uptake. This analysis was performed by Acme Analytical Laboratories Ltd. (Vancouver, BC) by ICP-ES.

Shake Flasks. The solubility of Hg in the presence of humic acid (0.25 g/L), tannic acid (0.005M) and water was also evaluated for a Hg-laden tailings sample (Hg concentration 10.5 ppm). This involved placing 50 g of tailings in a 250 mL erlenmeyer flask with 125 mL of solution and shaking at medium intensity on a shake table for a 24 hour period. The slurry was subsequently filtered through Ahlstrom 900 series filter paper and the solution was submitted for analysis.

Adverse Effects Test. The influence of Hg accumulation in worm tissues on burrowing speed was also evaluated to determine whether this could be used to assess adverse effects. Ten worms from each of the jar tests in Series B-Six (Table 5.2) were randomly selected and placed at the surface of a synthetic soil consisting of cellulose (60 g), fine silica sand (100 g), and distilled water (100 mL). A bright (100 W) light was directed ~ 40 cm from the surface of the synthetic soil and the speed at which all worms burrowed into the material was recorded.

*Specific Descriptions of Soil and Sediment Experiments:*

Six different series of soil and sediment jar tests (“Series B”) were carried out in order to satisfy various objectives. In addition to the test objectives described below, these experiments also sought to evaluate the applicability of the methodology to soils and sediments.

Series B-One

This 7-day test intended to assess the habitability of mine tailings for earthworms at three different moisture contents (31, 37 and 45%).

Series B-Two:

This series examined the influence of an organic-rich soil (140 ppb Hg) and tannic acid (<0.5 ppb Hg) on the bioavailability of Hg in tailing with a Hg concentration of 1805 ppb.

Series B-Three:

This series was designed to evaluate the influence of different concentrations of humic acid (0.25 g/L, 0.125 g/L and 0.05 g/L) on Hg bioavailability in tailing with a Hg concentration of 1180 ppb Hg. As the molecular weight of the humic acid used was heterogeneous, the true concentration of humic acid in the solutions could not be determined.

Series B-Four:

These tests assessed the influence of different soils on Hg bioavailability in tailings. Organic-rich soil (20 ppb Hg), a lateritic soil (150 ppb Hg), and a clayey sediment (70 ppb Hg) collected from different locations in Cachoeira were combined with amalgamation tailing (10500 ppb Hg) in a series of jars. Distilled water was used to increase the moisture content of jars to within an acceptable range (50%).

Series B-Five:

This series examined the influence of humic and tannic acid (0.25 g/L HA and 1.61 g/L or 0.005M TA) on Hg bioaccumulation from tailing and lateritic soil.

Series B-Six:

This series compared the bioaccumulation of Hg in different soil samples collected in Cachoeira. The intent was to compare the potential for incorporation of Hg into the food chain at different locations. Organic-rich soils (20 and 205 ppb Hg), lateritic soils (90, 150, and 270 ppb Hg), clay-rich sediments (70 and 440 ppb Hg) and tailings (2925, 3730, and 10500 ppb Hg) were evaluated.

Series B-Seven:

Series B-Seven assessed the influence of tannic acid on Hg bioavailability in lateritic soil (135 ppb Hg), tailings (3730 ppb Hg) and a 50:50 mixture (1933 ppb Hg) of the two.

The experimental configuration of each series of jar tests is presented in Table 5.2.

Table 5.1 Mercury Bioavailability in Solutions – Jar Preparation

Series (# jars)	Number of Worms per jar	Paper / Cellulose (g)	Paper/Cellulose Preparation	Silica Sand (g)	Hg Solution Preparation	pH adjustment	Solution (mL)	Moisture content (%)	Comments
A-One (4)	25-30 (~ 10 g)	25 g	Shredded, kaolin-based white paper	175 g	3.03 g Hg <sub>metallic</sub> 1L 1.61 g/L TA	4.11 → 6.06	80 mL TA, W	29 %	Homogenized jar contents by hand-mixing. (14 day test; 24 hr or 72 hr dep)
A-Two (10)	25-30 (~ 10g)	25 g	Shredded, kaolin-based white paper	100 g	6.18 g Hg <sub>metallic</sub> 1L 1.61 g/L TA	4.04 → 6.02	80 mL TA, W	39 %	Homogenized jar contents by hand-mixing. (28 day test; 24 hr dep or 5-day clean paper feeding plus 24 hr dep)
A-Three (4)	25 (~ 10g)	25g	Shredded, kaolin-based white paper	100 g	5 g Hg <sub>metallic</sub> 1L 1.61 g/L TA	3.69 → 6.02	100 mL TA, W	44 %	Homogenized jar contents by hand-mixing. (28 day test; 24 hr dep)
A-Four (5)	10 (~4 g)	10g	Shredded paper towel	40 g	0 g Hg <sub>metallic</sub> 1L 0.05 g/L HA; 0.125 g/L HA; or 0.25 g/L HA	6.90 7.2 7.6	60 mL HA <sub>3</sub> , W	55 %	HA Habitability assessment; pH within acceptable range; Homogenized jar contents in blender. (7 day trial)
A-Five (10)	30 (10 – 12g)	25g	Shredded paper towel	100 g	2 g Hg <sub>metallic</sub> 1L 0.25 g/L HA; 1L 1.61 g/L TA;	6.42 4.41 → 6.00	120 mL HA, TA	49 %	Homogenized jar contents in blender. (28 day test)
A-Six (12)	20 (~ 7g)	20g	Cellulose	60g	2 g Hg <sub>metallic</sub> 1L 0.05 g/L HA; 1.61 g/L TA; or 0.05 g/L FA	6.7 3.87 → 6.03 5.9	80 mL HA, TA, FA	50%	Homogenized jar contents in blender. (28 day test; 24 hr dep)

Note: 1. TA = tannic acid; HA = humic acid; FA = fulvic acid; W = distilled water.  
 2. All organic acid solutions assessed in the presence and absence of Hg with the exception of Series Five, which evaluated earthworm habitability in HA.  
 3. All tests performed in duplicate

Table 5.2 Mercury Bioavailability in Soils and Sediments – Jar Preparation

Series (# jars)	Number of Worms per jar	Paper / Cellulose (g)	Cellulose Preparation	Tailings, Soil or Sediment (g)	Solution (mL)	Moisture content (%)	Configuration	Comments
B-One (6)	10 (~ 4 g)	10 g	Shredded, kaolin-based white paper	50 g 75 g 100 g	50 mL W	45 % 37 % 31 %	T <sub>Hg</sub> (50g) + W T <sub>Hg</sub> (75g) + W T <sub>Hg</sub> (100g) + W	Tailings habitability assessment Homogenized jar contents by hand-mixing. (7 day trial)
B-Two (6)	25 (~ 10 g)	25 g	Shredded, kaolin-based white paper	100 g	100 mL TA, W	44 %	T <sub>Hg</sub> + TA T <sub>Hg</sub> + W T <sub>Hg</sub> + S <sub>org</sub> + W T <sub>Hg</sub> + S <sub>org</sub> + TA	Homogenized jar contents by hand-mixing. (28 day test)
B-Three (12)	30 (~ 12 g)	10 g	Shredded paper towel	200 g	80 mL HA <sub>3</sub> , W	28%	T <sub>Hg</sub> + HA <sub>0.05 mL</sub> T <sub>Hg</sub> + HA <sub>0.15 mL</sub> T <sub>Hg</sub> + HA <sub>0.25 mL</sub> T <sub>Hg</sub> + W	Homogenized jar contents in blender. (28 day test)
B-Four (14)	20 (~ 7 g)	20 g	Cellulose	60 g	80 mL W	50%	T <sub>Hg</sub> + W T <sub>Hg</sub> + S <sub>org</sub> + W T <sub>Hg</sub> + S <sub>lat</sub> + W T <sub>Hg</sub> + Sed <sub>org</sub> + W S <sub>org</sub> + W S <sub>lat</sub> + W Sed <sub>org</sub> + W	Homogenized jar contents in blender. (28 day test)
B-Five (8)	20 (~ 7 g)	20 g	Cellulose	60 g	80 mL TA, HA	50%	T <sub>Hg</sub> + TA T <sub>Hg</sub> + HA S <sub>lat</sub> + TA S <sub>lat</sub> + HA	Homogenized jar contents in blender. (28 day test)
B-Six (14)	20 (~ 7 g)	20 g	Cellulose	60 g	80 mL W	50%	T <sub>Hg</sub> + W S <sub>lat</sub> + W S <sub>org</sub> + W Sed <sub>org</sub> + W	Homogenized jar contents in blender. (28 day test)
B-Seven (6)	20 (~ 7 g)	20 g	Cellulose	60 g	80 mL HA	50%	T <sub>Hg</sub> + HA S <sub>lat</sub> + HA T <sub>Hg</sub> + S <sub>lat</sub> + HA	Homogenized jar contents in blender. (28 day test)

Note: 1. Solutions: TA = tannic acid; HA = humic acid; FA = fulvic acid; W = water.  
 2. Soils: T<sub>Hg</sub> = Hg containing tailings; S<sub>lat</sub> = lateritic soil; ; S<sub>org</sub> = organic-rich soil; Sed<sub>org</sub> = organic-rich sediment.  
 3. All tests performed in duplicate.

### 5.3.3 Methylation Potential of Earthworms

In order to explore the potential for methylation within the intestinal tract, the presence of sulfate reducing bacteria (SRBs)<sup>8</sup> within the digestive tract of earthworms was evaluated using a qualitative biological activity reaction test (BART) for SRB detection. The experimental protocol is detailed in Appendix III and summarized as follows (*after* Baldwin, 2001):

- Wash a 1-litre flask with soap and rinse thoroughly (at least four times), the last rinse being in distilled water. [Soak overnight in 1% nitric acid if you are going to be doing metal analyses].
- Put a clean stir-bar in the flask and place it on the stirrer
- Add 100mL of SRB stock salt solution, 500mL of distilled water and 1g of yeast extract.
- Fill up to the 880 mL mark with distilled water.
- Autoclave at 120°C for 15 minutes and cool to room temperature.
- Adjust the pH to 7.5 by adding concentrated NaOH.
- Add 2.5 mL of ethanol and 0.200 mL of methanol using a micropipette.
- Bubble N<sub>2</sub> through the media in the flask for two minutes.
- Add 10 mL/L-solution of stock ascorbic acid solution and 10 mL/L-solution of stock thioglycolic acid solution.
- Add the media to clean culture bottles (capped test tubes) leaving a little headspace at the top of each bottle.
- Add 10% inoculum (i.e. 10mL inoculum into 90mL solution) to each bottle with a pipette. Add the inoculum into the bottom of the bottle so as to minimize exposure to oxygen. The ‘inoculum’ consists of worms that have been finely chopped using an ethanol-cleaned, dry scalpel in the anaerobic fumehood.
- Add additional media to eliminate headspace. Firmly cap the bottles.
- Incubate statically at 30°C.

The presence of SRBs in samples is evidenced by the generation of a black iron sulfide precipitate, such that the darker the sample the more SRBs are present. As conditions in the substrate are aerobic (moisture content 25-30%), SRBs are not anticipated within the jar substrate material.

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<sup>8</sup> As discussed in Section 3.2.2, SRBs are the bacteria believed to be primarily responsible for Hg methylation.

## 6 RESULTS

### 6.1 Soil, Sediments and Tailings

Results of the soil and sediment sampling program are summarized in Table 6.1; the distribution of Hg is shown in Figure 6.1. Mercury concentrations in sediments collected from wetlands and creeks on the study site ranged from 5 ppb to 10,440 ppb (average 695 ppb). Background Hg levels, as indicated by sediments collected upstream from the site, ranged from 3 ppb to 15 ppb (average 8.4 ppb). This indicates that Hg in the Cachoeira drainages could be attributed to past mining activities there. Two to three kilometres downstream from the site, immediately before the confluence with the Piriá River (adjacent to the riparian community of Bela Vista), sediment samples had Hg concentrations ranging from 70 to 440 ppm, suggesting garimpo activities at Cachoeira may have a downstream influence.

Terrestrial soils collected in various areas of the town ranged from 445 to 11,021 ppb Hg, with the highest values obtained near the amalgam decomposition areas (Fig. 6.1). Average natural Hg concentrations in soils are estimated at 100 ppb (Cid and Anjos, 2002). Tailings samples concentrations ranged from 120 to 10,500 ppb.

Table 6.1 Mercury in Soil and Sediments in Cachoeira do Piriá

Region	Range (ppb)	Mean [Median] (ppb)	No. of Samples
Macaco River (on-site and downstream)	5 – 2980	329 [132]	12
Macaco River (downstream 2-3 km) <sup>1</sup>	70 – 440	167 [83]	4
Macaco River (upstream – “background” levels)	4 – 15	8.4 [8.5]	5
Lake Cachoeira	100 – 10,440	1207 [1123]	8
Currutela Creek	140 – 455	290 [385]	5
Barriquinha Creek	32 – 1720	972 [398]	4
East Wetland (downstream of old mining area)	10 – 1885	551 [100]	9
West Wetland (downstream of recent mining area)	32 – 3730	1052 [1090]	8
Soils (residential area)	32 – 11021	1392 [758]	14
Tailings (recently active mining area)	120 – 10,500	3844 [1060]	4

1. Adjacent to the community of Bela Vista.
2. Concentrations in ppb ( $\mu\text{g}/\text{kg}$ ) in dry weight.

Detailed descriptions of the samples collected and relevant information concerning their locations are presented in Appendix II, Field Sampling Notes.

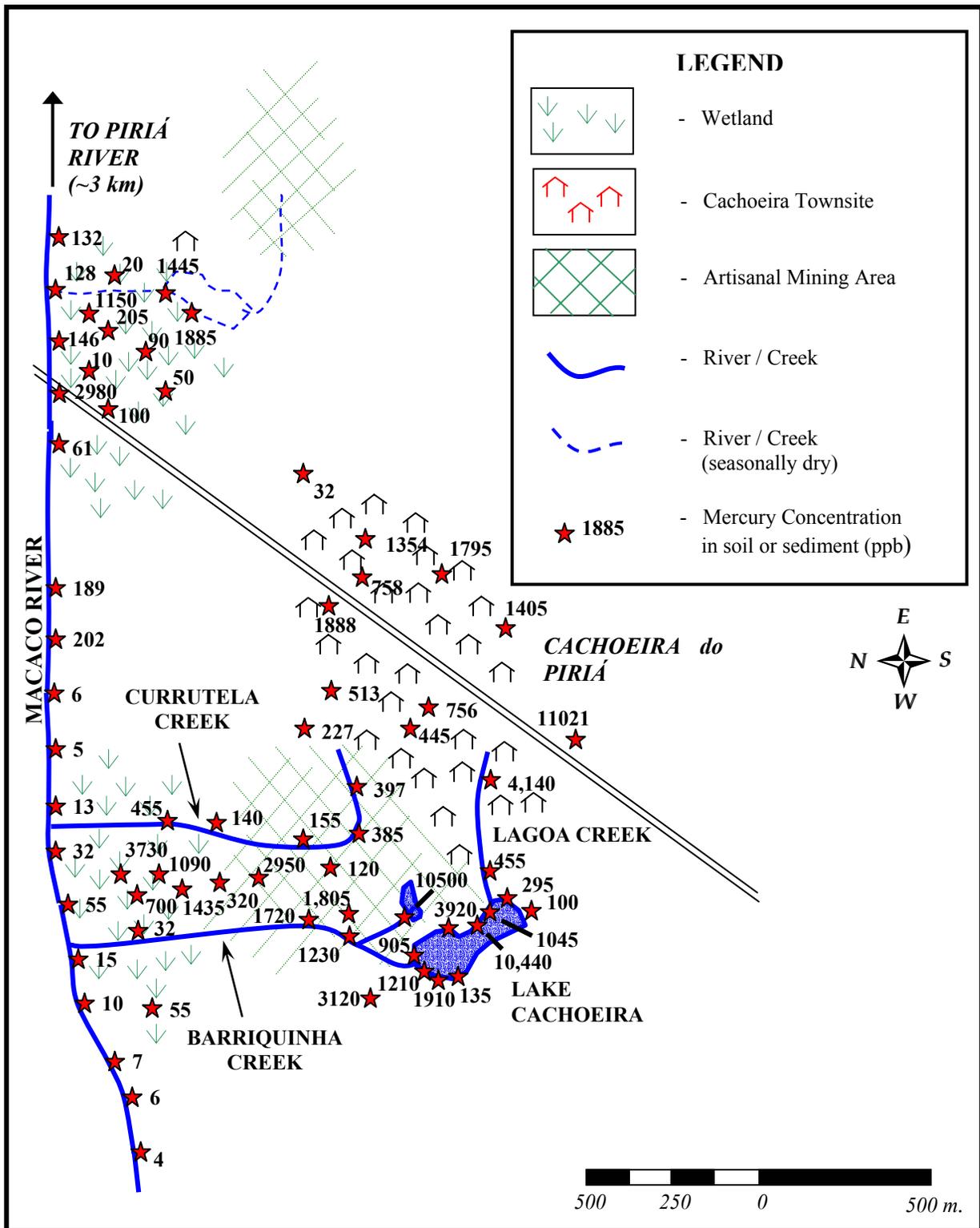


Figure 6.1 Mercury Concentrations in Soil, Sediment and Tailings Samples

## 6.2 Fish

Mercury concentrations within the seven species of locally consumed fish ranged from 45 ppb to 3433 ppb and averaged 334 ppb, but tissue concentrations differed considerably between species of fish (Table 6.2). The WHO guideline and CONAMA legislation for concentrations in fish is 500 ppb. All of the jejú exceeded this level and most traíra and mãe rosa fish samples were above this level. Carnivorous species of fish are subject to frequent selected for consumption over other species.

Table 6.2 Mercury Concentrations in Fish Samples collected from Lake Cachoeira, the Macaco River and the East Wetland

Species	Range (ppb)	Mean] (ppb)	No. of Samples	% above 500 ppb	Trophic Level
Traíra ( <i>Hoplias malabaricus</i> )	186 – 3433	925	38	68%	Carnivorous
Mandii ( <i>Pimelodus blochii</i> )	45 – 286	108	21	0%	Omnivorous
Acará ( <i>Ciclossoma sp.</i> )	107 – 770	347	19	21%	Herbivorous
Jejú ( <i>Hoplerythrimus unitaeniatus</i> )	835 – 1712	1274	2	100%	Carnivorous
Mãe Rosa ( <i>Centropomus parallelus</i> )	400 – 904	667	8	63%	Carnivorous
Piaba ( <i>Pempheris schomburgki</i> )	103 – 339	215	7	0%	Herbivorous

1. Data derived from samples collected by UBC Researchers and the DNPM (1999-2000).
2. Mercury concentrations in ppb ( $\mu\text{g}/\text{kg}$ ) wet weight.

Based on results from all studies in Cachoeira, the percentage of total Hg present as MeHg in fish tissue ranged from 80 to 100% and averaged 87%.

## 6.3 Earthworm Protocol

Two main types of earthworm tests were conducted. First, the earthworm methodology was employed to evaluate the bioavailability of Hg in solutions, with particular emphasis on the influence of organic acids. Second, tests using soils, sediments and tailings from Cachoeira were used to compare Hg bioavailability in specific locations, soil types, and in conjunction with different organic acids.

### 6.3.1 Mercury Bioavailability in Solutions

Results of this research program indicate that earthworms (*E. foetida*) are capable of bioaccumulating Hg from Hg-laden substrate they consume. The primary objectives of the experiments to assess Hg bioavailability in solutions were to:

- (1) Evaluate the efficacy of the earthworm methodology.
- (2) Determine the influence of specific variables (e.g. types and concentrations of organic acids, moisture contents) on Hg bioavailability.
- (3) Verify whether Hg-organic complexes are readily bioavailable.

Results for each series are described below. Detailed results are presented in Appendix IV.

Series A-One and A-Two:

These series' intended to compare Hg uptake given different lengths of exposure (14-day or 28-day) and different depuration times (24-h, 72-h, 5-day clean paper feeding followed by 24-h depuration).

Concentrations in worm tissues in the tests ranged from 1614 ppb to 7305 ppb in jars containing the Hg-laden tannic acid solutions, and 35 to 39 ppb in "control jars", i.e. jars where "barren" tannic acid solution was applied (<0.5 ppb) (Table 6.3). Populations with highest Hg concentrations in tissues exhibited toxic responses post exposure. Documented responses include: yellowish discolouration, impaired mobility, and diminished light sensitivity (as evidenced by a reduced tendency to burrow in the presence of bright light), which were also symptoms observed in other studies (Lofs-Holmin and Bostrom, 1988).

Table 6.3 Results of Series A-One and A-Two Tannic Acid Solution Worm Exposure Jar Tests

<b>Test Description<sup>1,2</sup></b>	<b>Ave. Hg in Tissues (ppb)</b>	<b>Std Dev.</b>	<b>Hg in Substrate (ppb)</b>	<b>Bioaccumulation Factor<sup>3</sup></b>
14-d exp, 24-h dep	2693	244	828	3.3
14-d exp, 72-h dep	2655	1041	828	3.2
28-d exp, 24-h dep	2499	875	828	2.9
28-d exp, 5-d paper plus 24-h dep	5680	1625	828	6.9
control worms (28-d test, 24-h dep)	37	2	<0.5	-

1. All tests conducted in duplicate.

2. exp = exposure time; dep = depuration time

3. Bioconcentration Factor (BCF) = Hg concentration in tissue/Hg concentration in substrate

4. Mercury concentrations in ppb (µg/kg) wet weight.

Depuration is a significant variable of these tests, as contents remaining in the intestinal tract can skew results. Whole worm analysis should reflect Hg accumulated by the organisms and not particles retained in the intestinal tract. Results for the 14-day tests and the 28-day (24-h depuration) were quite comparable, which is consistent with steady state, but Hg concentrations were up to 127% higher in worm populations fed clean paper for 5 days following the test. As silica sand was not used in jars during the 5-day clean paper feeding, worms could not ingest sufficient quantities resulting in significant weight loss.

This was further corroborated by observations that paper in jars was mainly ‘intact’ (i.e. unprocessed) at the conclusion of the 5-day period. As Hg concentrations are calculated based on worm weight ( $\mu\text{g}$  Hg per kg worm), a low weight results in high Hg concentrations.

As worm survival throughout the duration of the test was high (>90%) and bioaccumulation was established, the experimental design was deemed acceptable. In addition, the bioaccumulation factor (BAF)<sup>9</sup>, which is the concentration of Hg in worm tissues divided by that in the substrate, was comparable for 14-day and 28-day exposures. According to ASTM E 1022-94, this relationship indicates that a 28-days exposure period is sufficient to represent steady state. Steady state occurs once equilibrium has been reached between the rate of intake and excretion of a substance. In order to ensure steady state was achieved, a 28-day exposure period was selected for subsequent experiments.

Series A-Three:

Using the 28-day exposure / 24-h depuration protocol established in the initial series of tests, Series A-Three was conducted to assess the validity of results obtained in Series A-Two and provide additional information about uptake at a different Hg dosage. The Hg concentration was almost 3 times higher in the Series A-Three tannic acid solution than in the A-Two series (3650 vs. 1150 ppb Hg). Mercury concentrations in tissues in the four replicates ranged from 2310 ppb to 4964 ppb and averaged 4359 ppb (std dev 1463 ppb).

Table 6.4 Results of Series A-Three Tannic Acid Worm Exposure Jar Tests

Test Description <sup>1</sup>	Hg in Tissues (ppb)	Std Dev.	Hg in Substrate (ppb)	Bioaccumulation Factor <sup>3</sup>
28-d exp, 24-h dep <sup>2</sup>	2499	875	828	2.9
“	3863	1463 (average 4359)	1424	2.7
“	4964		1424	3.5
“	2310		1424	1.6
“	6296		1424	4.4
control worms <sup>4</sup>	391	28	13	-

1. exp = exposure time; dep = depuration time

2. From Series A-Two – average of duplicates.

3. Bioaccumulation Factor (BAF) = Hg concentration in tissue/Hg concentration in substrate

4. Average of duplicates.

5. Mercury concentrations in ppb ( $\mu\text{g}/\text{kg}$ ) wet weight.

Series A-Four:

<sup>9</sup> BAF = Bioaccumulation Factor. The BAF represents the ratio of the Hg concentration in worm tissues to the substrate. A BAF above unity indicates that bioconcentration is occurring. The efficacy of the BAF in establishing

Bioaccumulation factors ranged from 1.6 to 4.4 (ave 3.1, std dev. 1.0) and were similar to the BAF derived from the 28-day 24-h depuration test in Series A-Two (2.9). With the exception of the 3<sup>rd</sup> replicate (2310 ppb), worm tissue concentrations were 55 to 152% greater in the high-dose jars (Series A-Three) compared to the lower dose Series Two jars, suggesting a dose-effect relationship<sup>10</sup>.

This series consisted of a 7-day habitability assessment for exposure to 3 different concentrations of humic acid. Mortality was below 10% during the 7-day test for all tests. Mobility and light sensitivity were not impaired through exposure to the humic acid and a constant humidity was maintained throughout the test. Little variation was observed between the 3 different humic acid concentrations. These observations indicate that humic acid may be suitable for subsequent experiments.

#### Series A-Five

This experiment intended to evaluate the bioavailability of Hg associated with humic acid (3780 ppb Hg in 0.25 g/L HA) compared to tannic acid (8150 ppb Hg in 0.005M TA). Worm health and jar conditions in the first week of exposure appeared to be excellent in jars with and without Hg. In subsequent days, worm health declined steadily in all jars and 100% of the worms perished.

A number of factors were considered in determining the cause(s) of death. Mercury toxicity was considered, but mortality also occurred in jars that were “Hg-free”, i.e. control jars. The moisture content in jars was measured, but was within acceptable ranges (40 – 51%; ave 47%, std. dev. 4.5%). An additional possibility included the presence of a foreign, toxic substance (e.g. mould, certain microbes). Due to insufficient resources, this possibility could not be evaluated. As the paper within the jars was relatively undigested, it is probable that this new paper source was not suitable, for example, due to insufficient or biologically unavailable organic carbon or presence of a compound toxic to worms. It was determined that subsequent experiments should use an alternate paper source. Subsequent experiments used the pre-processed cellulose paper source, as described in Section 5.3.1.

#### Series A-Six:

This series intended to compare the MeHg levels in worms exposed to Hg in humic (6850 ppb Hg), fulvic (15.4 ppb Hg) and tannic acid (7500 ppb Hg). As shown in Table 6.5, MeHg concentrations in worms from all jars were in the same general range, although, due to low total Hg concentrations in the fulvic acid jars, levels in worms were up to 90% less than in the TA and HA jars.

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steady state, assessing metal bioavailability and associated risks has come under some scrutiny and is discussed further in Section 7.1.

<sup>10</sup> A “Dose-Effect Relationship” means that a given dose results in an observed or measureable effect. In this case, the measurable effect is bioaccumulation of Hg, which increases in concentration as the dose increases.

Table 6.5 Results of Methylmercury Jar Tests

Description <sup>1</sup>	Worms		Substrate		BAF
	MeHg (ppb)	% of Total Hg	MeHg (ppb)	% of Total Hg	MeHg
HA + Hg	5.22	0.005	0.033	$2 \times 10^{-7}$	157.9
HA + Hg	6.19	0.005	0.018	$1 \times 10^{-7}$	336.6
TA + Hg	7.42	0.009	0.008	$5 \times 10^{-8}$	876.4
TA + Hg	4.22	0.005	0.009	$5 \times 10^{-8}$	446.6
FA + Hg	3.14	0.020	0.007	$2 \times 10^{-5}$	442.3
FA + Hg	3.11	0.010	0.006	$2 \times 10^{-5}$	536.2
Average	4.88	0.009	0.014	$7 \times 10^{-6}$	466

1. Hg concentration in solutions:  $Hg_{Humic} = 6850 \mu\text{g/L}$ ;  $Hg_{Tannic} = 7500 \mu\text{g/L}$ ;  $Hg_{Fulvic} = 15.4 \mu\text{g/L}$ .

2. Mercury concentrations in ppb ( $\mu\text{g/kg}$ ) wet weight.

The Bioaccumulation Factor (BAF) in all jars was two orders of magnitude above unity, suggesting that: (a) worms are producing and subsequently incorporating MeHg in the intestinal tract following ingestion, or (b) MeHg is produced in the substrate and quickly incorporated by the organisms, released into the atmosphere, or converted back to inorganic Hg. This is discussed further in Section 7.1.

### 6.3.2 Mercury Bioavailability in Soils and Sediments

Experiments that applied the earthworm protocol for soils and sediments ultimately intended to:

- (1) Compare the effectiveness of different naturally occurring materials in inhibiting Hg bioavailability, and
- (2) Further explore the influence of organic acids on Hg bioavailability.

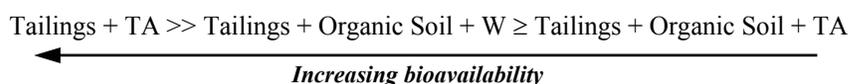
Tests involving gold amalgamation tailings and other soils (The “B” series’ of tests) indicate that Hg is bioavailable to earthworms in direct tailings and soil contact tests. Results for each series are described below. Detailed results are tabulated in Appendix IV.

#### Series B-One:

This 7-day test intended to assess the habitability of mine tailings for earthworms at three different moisture contents (31, 37 and 45%). Worm mortality was low (<10%) over the 7-day exposure period and minimal toxic responses (i.e. discolouration, reduced mobility, constrictions of segments) were documented in the jars. Little variation was observed between the six jars, suggesting the tailings at the moisture contents within the range assessed were habitable for worms.

Series B-Two:

This series examined the influence of an organic-rich soil (140 ppb Hg) and tannic acid (<0.5 ppb Hg) on the bioavailability of Hg in HFO-rich tailing (1805 ppb Hg). After 28 days of exposure, it was found that worms accumulated 254 - 538% more Hg from the tailings-tannic acid mixture (ave. 587 ppb Hg<sub>tissues</sub>; BAF 1.12) than when tailings were mixed with organic-rich soil with water (W) or tannic acid (TA) (ave. 166 and 92 ppb Hg<sub>tissues</sub>; BAF 0.56 and 0.31, respectively). This suggests that the organic soil may be binding some Hg, thereby inhibiting its uptake. As Hg uptake was comparable when both water and tannic acid were added to the tailings-organic soil mixtures, the binding capacity of the soil may exceed the capacity of the organic acid to solubilize Hg. The bioavailability series derived from this experiment is summarized as follows:



Series B-Three:

This series was designed to evaluate the influence of different concentrations of humic acid (0.25 g/L, 0.125 g/L and 0.05 g/L) on Hg bioavailability in tailings (1180 ppb Hg).

Table 6.6 Relative Influence of Humic Acid Concentrations on Hg Uptake from Tailing

	HA 0.25 g/L	HA 0.125 g/L	HA 0.05 g/L	Water
Influence on Hg Uptake from Tailing	++	+	+	-
Hg concentration in Tissues (ppb)	129	97	98	48

++ - relatively strong, positive influence on Hg bioaccumulation.

+ - positive influence on Hg bioaccumulation.

- - comparatively negative influence on Hg bioaccumulation.

Bioaccumulation of Hg was 33% higher in the most organic-rich humic acid solution (ave. 129 ppb Hg<sub>tissues</sub> in HA<sub>0.25 g/L</sub>) than the less potent solutions (ave. 97 ppb Hg<sub>tissues</sub> in HA<sub>0.125 g/L</sub>; 98 ppb Hg<sub>tissues</sub> in HA<sub>0.05 g/L</sub>). Bioaccumulation from tailings combined with water was the lowest (ave. 48 ppb Hg<sub>tissues</sub>). The relative influence of the organic acids on Hg bioavailability in tailings is described as follows:

Series B-Four:

These tests assessed the influence of different soils (organic-rich soil - 20 ppb Hg; lateritic soil - 150 ppb Hg; clayey sediment - 70 ppb Hg) on the bioavailability of Hg associated with high-Hg tailing (10500 ppb Hg). As shown in Table 6.7, the total mass of Hg was virtually the same in the substrate of each of the

three ‘mixed’ jars. Uptake was greatest in the jars where pure tailings were examined (ave. 1370 ppb  $Hg_{tissues}$ ) and the lowest when mixed with the clayey sediment and lateritic soil (ave. 840 and 918 ppb  $Hg_{tissues}$ , respectively). Bioaccumulation in mixtures with the organic soil was 36 to 49% higher (ave. 1247 ppb  $Hg_{tissues}$ ) than with other substrates, suggesting the presence of organics may be promoting uptake. This influence is described below.

Table 6.7 Relative Influence of Different Soils on Mercury Uptake from Tailing

	Tailings	Organic-rich Soil + Tailings	Lateritic Soil + Tailings	Clayey Sediment + Tailings
Influence on Hg Uptake from Tailings	++	++	-	-
Hg concentration in Tissues (ppb)	1370	1247	918	840
Hg concentration in Substrate (ppb)	4667	2339	2367	2349
Bioaccumulation Factor (BAF)	0.3	0.5	0.4	0.4

++ - relatively strong, positive influence on Hg bioaccumulation.

+ - positive influence on Hg bioaccumulation.

- - comparatively negative influence on Hg bioaccumulation.

Bioaccumulation in the organic-rich soil, lateritic soils and clayey sediment were also evaluated in the absence of the tailings. The least bioaccumulation was measured in the clayey sediment and the most bioaccumulation (aside from the tailings mixtures) was observed in the organic-rich soil, although the organic-soil contained considerably less Hg. Bioaccumulation was 3 to 21 times higher in the tailings mixture than the “clean” soils alone.

Table 6.8 Relative Mercury Uptake from Different Soils

	Tailings	Organic-rich Soil	Lateritic Soil	Clayey Sediment
Hg concentration in Tissues (ppb)	1370	429	122	37
Hg concentration in Substrate (ppb)	4667	9	67	37

Series B-Five:

This series examined the influence of humic and tannic acid on Hg bioaccumulation from tailing (10500 ppb) and lateritic soil (150 ppb). In the case of tailings, tannic acid was almost twice as effective as humic acid at increasing bioavailability (compare 5590 ppb  $Hg_{tissues}$  (TA solution); and 2930 ppb  $Hg_{tissues}$  (HA solution)). Uptake was considerably lower in the absence of organic acids, i.e. when distilled water was used (ave. 1370 ppb  $Hg_{tissues}$ ).

Bioaccumulation from the lateritic soil was also significant in the presence of organic acids (3180 ppb  $Hg_{tissues}$  (TA solution); and 3399 ppb  $Hg_{tissues}$  (HA solution)). These numbers are particularly significant as the Hg concentration in the lateritic soil is relatively low (150 ppb). Uptake from the laterite in the

absence of organic acids is an order of magnitude lower than in the organic acid tests (ave 121 ppb Hg<sub>tissues</sub>).

The influence of organic acids on Hg uptake from tailings and lateritic soil can be compared as follows:

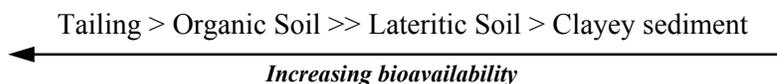
Table 6.9 Influence of Tannic and Humic Acid on Hg Uptake from Tailings and Laterite

<i>Influence on Hg Uptake from:</i>	Tannic Acid (1.61 g/L)	Humic Acid (0.25 g/L)	Water
<i>Tailing</i>	++	+	-
Hg concentration in Tissues (ppb)	5590	2930	1370
<i>Lateritic Soil</i>	++	++	--
Hg concentration in Tissues (ppb)	3180	3399	121

- ++ - relatively strong, positive influence on Hg bioaccumulation.
- + - relatively positive influence on Hg bioaccumulation.
- - comparatively negative influence on Hg bioaccumulation.
- - comparatively strong, negative influence on Hg bioaccumulation.

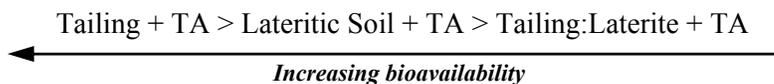
Series B-Six:

This series compares the bioaccumulation of Hg in different soil samples collected in Cachoeira. Organic-rich soils (20 and 205 ppb Hg), lateritic soils (90, 150, and 270 ppb Hg), clay-rich sediments (70 and 440 ppb Hg) and tailing (2925, 3730, and 10500 ppb Hg) were evaluated. Average bioaccumulation in the different substrates is summarized as follows and detailed in Appendix IV: Results of this series were consistent with those presented in Table 6.8.



Series B-Seven:

Series B-Seven assessed the influence of tannic acid on Hg bioavailability in lateritic soil (135 ppb Hg), tailing (3730 ppb Hg) and a 50:50 mixture (1933 ppb Hg) of the two. Series B-Four indicates that mixing tailing with a lateritic soil may reduce uptake in the presence of distilled water, but Series B-Five suggests that Hg bioavailability is high in laterite when organic acids are added. Thus, this test was needed to further ascertain the influence of organic acids on Hg bioavailability in these soil mixtures.



In the presence of tannic acid (0.005M), bioaccumulation was highest in the pure tailing (102 ppb) and lowest in the tailing:laterite mixture (58 ppb Hg). Interestingly, despite the lower Hg concentration in substrate, Hg in laterite was ~75% higher than in the mixture with tailing (compare 96 ppb and 58 ppb).

#### 6.3.2.1 Shake Flasks

As bioavailability is often a function of the solubility of species, a shake flask experiment was conducted to assess the potential for different solutions to solubilize Hg associated with tailing (10500 ppb Hg). Results indicate that tannic acid (1.61 g/L) has the greatest solubilization potential (210 µg/L) and distilled water has the lowest capacity to dissolve Hg associated with tailing (12 µg/L). Humic acid (0.25 g/L) also solubilized Hg (110 µg/L), but to a lesser degree than the tannic acid. The influence of various solutions on the solubilization of Hg associated with tailings is described as follows:

Tannic Acid > Humic Acid >> Water  
←  
*Increasing solubilization capacity of Hg associated with tailing*

#### 6.3.2.2 Influence of Other Elements on Hg Bioavailability

Multiple elements were analyzed for substrate and worm tissues in Series' B-Four, B-Five and B-Six in order to assess uptake of other metals and metalloids and ascertain the influence, if any exists, of these elements on Hg bioaccumulation. It was found that earthworms accumulate other soil constituents, with highest bioaccumulation factors observed for the essential elements, potassium and calcium. Zinc, which is a potentially toxic element, was also bioconcentrated. Most bioaccumulation factors, however, were well below unity. Results of bioaccumulation tests for selected metals are shown in Table 6.10.

Table 6.10 Bioaccumulation by Earthworms of Selected Elements in Soils and Tailings

Element	Average Concentration in Control Worms <sup>1</sup>	Average Concentration in Tissues <sup>1,2</sup> (range)	Average Concentration in Substrate <sup>1</sup> (range)	Average Bioaccumulation Factor <sup>3</sup> (range)
Aluminum (Al)	3.36	<b>23.1</b> (4.1 – 63)	4865.5 (488 – 8663)	0.01 (0.00 – 0.04)
Arsenic (As)	3.96	<b>35</b> (0.93 – 98)	580 (0.4 – 1338)	0.3 (0.00 – 0.04)
Calcium (Ca)	722	594 (252 – 881)	127.1 (37.5 – 712.5)	7.5 (1.0 – 23.0)
Cobalt (Co)	1.53	<b>3.6</b> (1.2 – 9.4)	25.7 (1.5 – 49.3)	0.30 (0.04 – 1.43)
Copper (Cu)	1.43	<b>3</b> (1.4 – 4.8)	42 (1.1 – 89)	0.2 (0.0 – 2.6)
Iron (Fe)	110.5	<b>158</b> (27 – 413)	36,509 (3638 – 62,475)	0.01 (0.00 – 0.02)
Potassium (K)	1194.4	891 (571 – 1458)	169 (37.5 – 375)	11.6 (2.6 – 39)
Nickel (Ni)	0.03	<b>0.7</b> (0.04 – 4.4)	100.1 (4.1 – 168)	0.01 (0.01 – 4)
Zinc (Zn)	0.01	<b>15.3</b> (7.4 – 22.7)	18.8 (0.8 – 39)	4.2 (0.22 – 26)

Note: 1 All concentrations in parts per million (ppm = µg/g).

2. Concentrations in tissues of worms exposed to Cachoeira soils and tailings. Concentrations in excess of levels in control worms are highlighted and italicized.

3. BAF = concentration in tissue / concentration in substrate

A correlation coefficient (r) was calculated to assess the linear relationship between Hg in worm tissues and other elements in both the substrate and in worm tissue. The formula for the correlation coefficient is given in Equation 6.1 (Bowen and Weisberg, 1980).

$$r = \frac{N \sum XY - (\sum X)(\sum Y)}{\sqrt{|N \sum X^2 - (\sum X)^2| |N \sum Y^2 - (\sum Y)^2|}} \quad \text{Equation 6.1}$$

In this equation 'r' is the correlation coefficient, 'N' is the number of samples assessed, 'X' is the concentration of the other element in worm tissues, and 'Y' is the Hg concentration in worm tissues. The square of the correlation coefficient (r<sup>2</sup>) is indicative of the variance from the linear prediction such that an r<sup>2</sup> of 1.0 is a perfect prediction, and an r<sup>2</sup> of zero indicates that the independent variable (X) cannot be associated with the 'dependent' variable (Y). It should be noted that this correlation coefficient is determined using a linear model, and strong nonlinear relationships may exist.

Table 6.11 Correlation Coefficients between Selected Elements and Hg in Worm Tissues

Element	Substrate		Worm Tissues	
	r	r <sup>2</sup>	r	r <sup>2</sup>
Al	0.20	0.04	-0.13	0.02
As	0.31	0.06	0.32	0.10
Ca	-0.10	0.01	0.21	0.05
Co	0.45	0.20	0.22	0.05
Cu	0.48	0.23	0.37	0.13
Fe	0.47	0.22	0.17	0.03
K	-0.29	0.08	-0.37	0.14
Ni	0.44	0.19	0.41	.017
Zn	-0.04	0.001	0.24	0.06

Note: Elements shown were selected on the basis of the strength of the correlation and possible importance with respect to uptake (e.g. Ca, Fe).

None of the elements analyzed correlated with Hg concentrations in tissues. Due to the multiple factors influencing the transformation of Hg to bioavailable species, it is likely that several variables are acting in conjunction with one another to promote or inhibit Hg uptake.

### 6.3.3 Methylation Potential of Earthworms

Methylmercury was analysed in worm tissues and substrate in two series of tests (A-One and A-Six) in order to assess whether methylation of Hg was occurring in the substrate, directly within the worms (e.g. in the intestines), or in the organic acid-Hg solution. Methylmercury concentrations in tissues ranged from 3.11 to 32.2 ppb with bioconcentration factors ranging from 157.9 to 2476.9 (Table 6.12). This is 1-2 orders of magnitude greater than the bioconcentration factors observed for total Hg in previous experiments suggesting that (a) MeHg is more readily incorporated as it is produced, and/or (b) worms are producing MeHg within the digestive tract.

In order to explore the potential for intestinal methylation, the presence of sulfate reducing bacteria (SRBs)<sup>11</sup> within the digestive tract of earthworms was evaluated using a qualitative biological activity reaction test (BART) for SRB detection. The presence of SRBs in samples is evidenced by the generation of a black iron sulfide precipitate, such that the darker the sample, the more SRBs were present (Fig. 6.3). As conditions in the substrate are aerobic (moisture content 25-30%), SRBs are not anticipated within this material.

<sup>11</sup> As discussed in Section 3.2.2, SRBs are the bacteria believed to be primarily responsible for Hg methylation.

Table 6.12 Methylmercury Accumulation by Earthworms in Jar Tests

Description	Worms		Substrate		BAF
	MeHg (ppb)	% of Total Hg	MeHg (ppb)	% of Total Hg	MeHg
HA + Hg	5.22	0.005	0.033	$2 \times 10^{-7}$	157.9
HA + Hg	6.19	0.005	0.018	$1 \times 10^{-7}$	336.6
TA + Hg	7.42	0.009	0.008	$5 \times 10^{-8}$	876.4
TA + Hg	4.22	0.005	0.009	$5 \times 10^{-8}$	446.6
TA + Hg	32.2	0.012	0.013	$4 \times 10^{-3}$	2476.9
FA + Hg	3.14	0.020	0.007	$2 \times 10^{-5}$	442.3
FA + Hg	3.11	0.010	0.006	$2 \times 10^{-5}$	536.2
Average	4.88	0.009	0.014	$7 \times 10^{-6}$	466

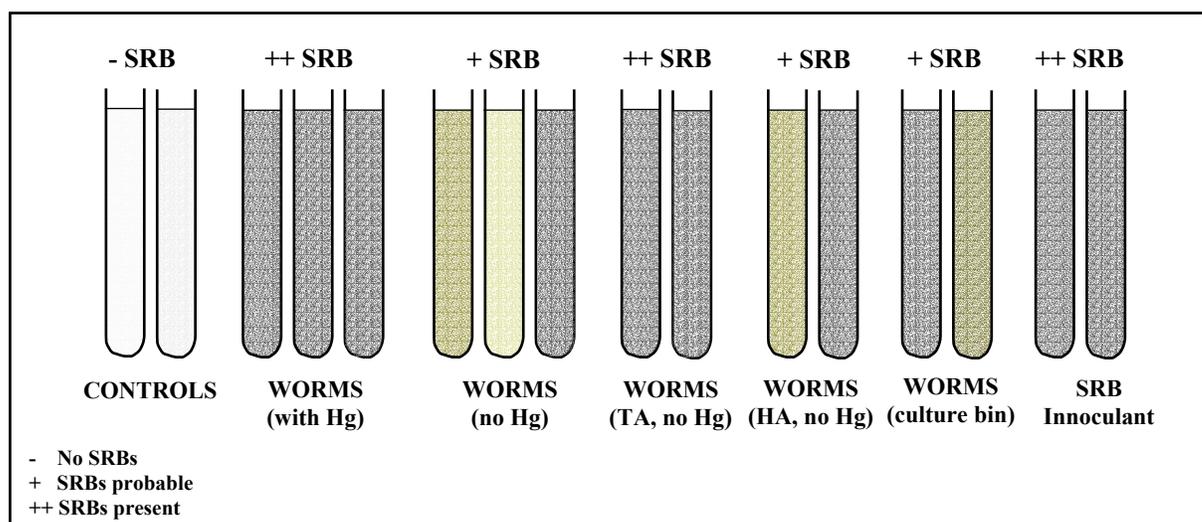


Figure 6.3 Results of SRB Test  
(Earthworms from Tannic and Humic Acid Jars)

Eight of twelve worm-containing test tubes tested positive for SRBs and the remaining 4 samples suggested the presence of SRBs. It is probable that longer incubation would promote SRB growth in the test tubes suggesting the presence of SRBs. Control test tubes exhibited a negative result for SRBs. It is apparent that worms do contain SRBs and therefore may facilitate methylation within the digestive tract. These results are particularly intriguing as evidence of intestinal methylation by earthworms, as well as other organisms, would compel researchers to re-assess their understanding of the transfer of Hg within food chains.

### 6.3.4 Quality Control

#### 6.3.4.1 Reproducibility of Results

A number of measures were implemented to assess the quality of data presented in this thesis. These include the following:

- All jar tests were conducted in duplicate or triplicate.
- During digestion of tissue samples, one duplicate was retained for future analysis, if necessary. In the event that analytical results were inconsistent, duplicates of tissue samples were submitted.
- A minimum of one “blind” sample (or 10% of the total number of samples in a batch) was included in every batch submitted for laboratory analysis. Sample numbering was such that the sample contents could not be inferred.
- The analytical laboratory conducted a minimum of one internal duplicate per batch of samples.
- Two series’ of samples was submitted to multiple laboratories for a comparison of results. One series of solutions was sent to both ASL Laboratories (Vancouver, BC) and Acme Analytical Laboratories, Ltd. (Vancouver, BC). Another series of soils was analyzed at the DNPM Mercury Laboratory (Belém, Brazil), the CETEM analytical laboratory (Rio de Janeiro, Brazil) and Acme Analytical Laboratories, Ltd.

As shown in Table 6.13, there is reasonable agreement between blind samples submitted to the laboratory and their duplicates. Internal laboratory duplicates have excellent reproducibility. There is significantly variability between laboratories, suggesting fundamental problems with the main laboratory used (Acme) or outside laboratories. Duplicates of experimental work range significantly and are also a serious concern with the earthworm protocol. This issue is addressed further in Section 7.1 of the Discussion.

Table 6.13 Reproducibility of Results

<b>Replicate Type</b>	<b>% Difference (average)</b>	<b>% Difference (range)</b>	<b>% Difference (standard deviation)</b>	<b>N</b>
“Blind” replicates	9.1	1.3 – 23.3	7.9	11
Internal laboratory	3.2	0.0 – 8.9	2.3	11
Re-submitted duplicates	15.0	3.0 – 21.7	22.2	3
Inter-laboratory	41.5	9.1 – 141.2	33.4	18
Experimental Duplicates	29.0	0.6 - 130	41.4	57

#### 6.3.4.2 Background Concentrations

Several times throughout the experimental program, background concentrations of Hg and other elements in cultured, unexposed worms were determined to assess natural levels in these organisms. Paper and silica sand used in the experiments were also analyzed for Hg, as well as other elements and these amounts were included in calculations of concentrations in substrate. The manure used for culturing was also sampled and submitted for analysis. These results are summarized in Table 6.14 and detailed in Appendix IV.

Table 6.14 Concentration of Hg and Selected Elements in Background Material<sup>1,2</sup>

<b>Material</b>	<b>As</b>	<b>Ca</b>	<b>Cu</b>	<b>Co</b>	<b>Fe</b>	<b>Hg</b>	<b>Ni</b>	<b>Zn</b>
Background worms	0.8	3970	2.6	2.7	2000	0.04	1.2	2.4
Paper	2.1	19000	5.0	0.1	2000	0.4	0.5	19.1
Silica Sand	1.2	1000	6.2	0.7	56000	0.8	5.5	3.3
Manure	1.6	132000	4.2	1.2	30000	0.7	3.8	20.4

1. Average concentration of multiple samples shown. Detailed results presented in Appendix IV.

2. Concentrations in µg/g (ppm) dry weight.

Mercury levels in the background substrates and worms were negligible compared to those observed in Hg exposed populations. Mercury levels in background worms (i.e. worms sampled directly from culture bins) differed from worms in control jars, i.e. jars containing substrate material without Hg (Table 6.15). This indicates that the substrate is providing a source of Hg for the worms, in particular in jars containing soils. As discussed in Section 7.1, it may be necessary to incorporate control jar data into evaluations of exposure in Hg-laden jars, i.e. a relative BAF may be needed which accounts for the interference of the “low Hg” substrate. In conjunction with prior experiments, these results also reaffirm the importance of organic acids in the bioavailability of Hg associated with laterite.

Table 6.15 Summary of Hg Concentrations in Control Worms

<b>Description of Control Jars<sup>1</sup></b>	<b>Hg Conc. in Solution or Soil (ppb)</b>	<b>Hg Concentration in Control Worm Tissues (ppb)</b>	<b>Hg Concentrations in Background Worm Tissues<sup>2</sup> (ppb)</b>	<b>% Difference</b>
<i><u>Solution Experiments:</u></i>				
Humic Acid Solution	<0.5	61	40	55%
Tannic Acid Solution	<0.5	63	40	58%
Fulvic Acid Solution	<0.5	36	40	10%
Water	<0.5	45	40	13%
<i><u>Soil-Sediment Experiments:</u></i>				
Organic-rich Soil + W	20	409	40	923%
Lateritic Soil + W	135	121	40	203%
Clayey Sediment + W	70	37	40	8%
Lateritic Soil + TA	150	3180	40	7850%
Lateritic Soil + HA	150	1045	40	2513%
Lateritic Soil + W	150	38	40	8%

1. Soils below 200 ppb are considered “control” jars. All solutions in control jars had Hg concentrations <0.5 µg/L.
2. Background levels in unexposed worms, i.e. those sampled directly from culture bins.
3. Averages of multiple samples presented. Complete results tabulated in Appendix IV.

## 7 DISCUSSION

Based on the results presented in Chapter 6, this chapter intends to answer four main questions:

1. How effective is the earthworm methodology?
2. What information can be derived about the role of organic acids in Hg bioavailability?
3. What are the implications of the results for Cachoeira?
4. How effective are soils native to Cachoeira in reducing the bioavailability of Hg?

### 7.1 Earthworms as Bioindicators of Hg Pollution

There are three main elements for discussion with respect to the earthworm methodology. First, what is the basis for and implications of specific variables used in the methodology? Related issues range from the validity of applying different parameters to the direct influence of certain variables on Hg uptake. Second, what are the implications of the results obtained from this methodology, particularly with respect to the role of organic acids? And third, what are the main uncertainties associated with the methodology and how do these affect both the validity of the method and results?

#### 7.1.1 Influence of Experimental Parameters

Results generally indicate that Hg in soils and solutions is bioavailable to earthworms using the protocol described. However, a number of variables appear to influence the extent of uptake and/or the applicability of the method. These variables are described in greater detail below.

##### 7.1.1.1 Experimental Design Parameters

*Different exposure times* were evaluated in Series' A-One and A-Two in order to provide a basis for selecting a constant exposure time for subsequent experiments. One of the most significant criteria of bioaccumulation protocols is that the exposure period must be sufficient to establish steady state. It has been suggested that once the bioaccumulation factor (BAF), which is the concentration of Hg in worm tissues divided by that in the substrate, is constant, steady state has been achieved (ASTM E 1022-94). As the BAF calculated for the 14-day and 28-day exposures was comparable, it was determined that a 28-day period would be sufficient to satisfy the steady state criteria. Although a shorter exposure time may also have been adequate, this conservative exposure time was selected in order to maintain confidence that steady state was being achieved.

In addition, other established invertebrate protocols selected a 28-day exposure period. These include the ASTM Standard Guides for earthworms and freshwater invertebrates (ASTM 1676-95 and ASTM 1383-

93a, respectively), and the US EPA protocol for freshwater invertebrates (US EPA 600R94024). Most methods that employed shorter exposure periods (ranging from a few hours to 14-days) were developed specifically to assess acute toxicity. The primary exception is the lesser-known Hazardous Materials Assessment Team 14-day earthworm methodology, which assesses bioaccumulation.

**Depuration time**, or the length of time during which worms were starved in order to void contents of the intestinal tract, was another significant variable evaluated in early series' of tests (Series A-One and A-Two). As contents remaining in the intestinal tract can skew results, sufficient depuration ensures that whole worm analysis reflects Hg accumulated by the organisms. Mercury concentrations were up to 127% higher in worm populations fed clean paper for 5 days following the test than worms depurated for 24-hours (Table 7.1).

Table 7.1 Influence of Depuration Time on Hg Bioaccumulation in Worms

Exposure Period <sup>1</sup>	Depuration Period	Ave. Hg in Tissues (ppb) <sup>2</sup>	Bioaccumulation Factor
14-day	24 hr	2693	3.3
14-day	72 hr	2655	3.2
28-day	24 hr	2499	2.9
28-day	5-day paper + 24 hr dep	5680	6.9

1. All tests conducted in duplicate. Substrate Hg concentration in all jars was 828 ppb.
2. Average of tests conducted in duplicate.

As greater 'flushing' of the intestinal tract would promote lower Hg concentrations through the whole worm analysis, this result is contrary to anticipated results. It is possible that during the additional 5 days, contents remaining in the intestines were more thoroughly assimilated. Alternately, as silica sand was not used in jars during the 5-day clean paper feeding, worms could not ingest sufficient quantities resulting in significant weight loss. As Hg concentrations are calculated based on worm weight ( $\mu\text{g Hg per kg worm}$ ), high Hg concentrations in the 5-day paper fed worms can be attributed to low worm weight.

Increased metal excretion in response to stress, such as extensive starvation, has been documented by some researchers. This was the case for lead, following a 4-day starvation period (Richards and Ireland, 1978). The influence of starvation was evidenced by similar worm tissue lead levels in worms maintained in non-contaminated soils post-exposure to contaminated soils than worms analysed immediately post-exposure to contaminated soils. This behaviour was attributed to the energy demands related to sequestering heavy metals in the organisms, i.e. in the absence of a food source, energy stores were depleted and worms were unable to retain the metal. As Hg, like Pb, is believed to be predominantly

stored in the chloragogenous<sup>12</sup> tissue of earthworms, there is the potential for a similar release mechanism with excessive starvation. Thus, a shorter depuration time may limit Hg losses from worm tissue. Existing invertebrate protocols (Goats and Edwards, 1988; Rhett et al, 1988, ASTM, 2001) most commonly employ a 24-hour depuration period.

**Moisture content and pH** were two of the most significant environmental factors in the jar tests. Optimal pH and moisture contents for worm habitability typically range between 5-8 and 30 - 45%, respectively (ASTM, 1995). In accordance with these requirements, solutions used in jar tests were modified using NaOH to obtain a suitable pH (typically 6), and added in sufficient amounts to achieve acceptable moisture contents. These factors, however, can modify the speciation and bioavailability<sup>13</sup> of Hg, and therefore influence the validity of observations derived from bioaccumulation.

**Organic carbon** in the substrate is also an important design parameter. Worm growth, reproduction and survival can drastically change in relation to the levels of organic carbon. As organic carbon is depleted over time, levels must be sufficient to sustain worms for the duration of the test. The ratio of organic carbon in substrate to dry weight of organisms at the start of a 28-day test should be at least 50:1 (EPA, 1994). Based on an average cellulose concentration of paper of 60% (Anon, 2002), the organic carbon contribution of paper in the jar tests is approximated at 13-15g. Thus, 25 g of paper is sufficient to sustain worms (dry weight ~0.25 g per jar) throughout the exposure period. With the additional contribution provided by organic acids and soils, the 50:1 requirement is easily exceeded.

#### 7.1.1.2 Measured or Calculated Parameters

**Bioaccumulation Factors** (BAFs) are often used to express bioaccumulation, which occurs when a portion of a substance is retained by an organism. BAFs are unitless values calculated by dividing the steady state tissue concentration of a substance in tissues by the steady state concentration in food plus the surrounding living environment (e.g. water for aquatic organisms, air for humans). BAFs have been extensively used to evaluate parameters linked to sublethal effects of heavy metals in biota (Parametrix, 1995). For instance, Popovic and Perovic (1999) found that the tendency of chemicals to bioconcentrate is strongly related to its lipophilicity (i.e. hydrophobicity). In this study, BAF differences observed in various trophic levels were correlated to lipid contents, which varied inversely with chemical elimination

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<sup>12</sup> Chloragogenous tissue, which is composed of modified epithelial cells, comprises a thick layer around the intestine (Ireland, 1983). This tissue, which functions much like a liver, contains substances including carbohydrates, proteins, lipids and is known to have a high CEC. Although accumulation of Hg in these tissues has not been proven, research by Yongcan et al (1998) indicates that this tissue is particularly susceptible to damage from Hg, indicating cellular interaction.

deficiency of an organism. Even though several variations of the BAF have been derived (Blight and Dyer, cited by Geyer et al, 2000), most modifications have been applied to lipophilic organic compounds and their applicability to metals have not been extensively evaluated.

The BAF may not be a suitable indication of risk in highly contaminated areas (Sandoval et al, 2001). In these situations, bioaccumulation may be appreciable, but the resulting BAF may be low, suggesting comparatively low-risk conditions. For instance, in Series B-Five, worms exposed to laterite saturated with tannic acid accumulated approximately 1 ppm of Hg, but the BAF was 19. The high BAF implies a moderately high risk in comparison to worms with a BAF of 1.4, which was the case for worms exposed to tailings saturated with tannic acid. In the latter test, Hg concentrations in worms exceeded 5ppm - this obviously presents a much greater risk to wildlife consuming worms. In the earthworm jar tests, the BAFs ranged from 0.07 to 102 and averaged 7.8.

A ***Dose-Effect Relationship*** is one of the factors that demonstrate the validity of a chronic toxicity protocol. This is established when a given dose results in an observed or measurable effect. In this case, the measurable effect is bioaccumulation of Hg, which increases in concentration as the dose increases. Using data measured in the solution jar tests using tannic acid (Series' A-One and A-Two), a linear relationship ( $r = 0.6$ ) was observed between the dose and bioaccumulation, as follows:

$$y = 6.1x + 4351$$

where  $y$  = Hg concentrations in worm tissues (ppb) and  $x$  = Hg concentrations in the substrate (ppb). It should be noted that this relationship was derived from a statistically insignificant number of tests (10 results). Due to the confounding influence of other parameters (e.g. organic carbon, mineralogy), distinct relationships were not established for soil and sediment jar tests. As shown in Figure 7.1, Hg levels in substrate did not correlate with those in worm tissues.

***Worm mortality*** below 10% is one of the most important criteria of bioaccumulation protocols (ATSM, 1995; Fairbrother and Kapustka, 2000). With the exception of Series A-Five, worm survival throughout the duration of jar tests was high (>90%). This, in conjunction with other factors (e.g. evidence of bioaccumulation) attests to the soundness of the experimental design.

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<sup>13</sup> Metal uptake increases with moisture content consistent with the increase of soluble metal species have been observed by researchers (Ireland, 1983). Moisture content can also influence bioavailability as it relates to O<sub>2</sub>



Despite this relationship, cobalt in soil and worm tissues correlated poorly with Hg in worm tissues ( $r^2$  of 0.2 and 0.05, respectively). As discussed in Section 6.3.3.2, relationships between Hg and other elements in soils or tissues could not be established. It is possible that the influences of other elements, if present, were confounded by the synergistic and antagonistic effects of other variables.

The **soil type** is a parameter that is of fundamental importance in terms of Hg bioavailability in jar tests. Potential influences include the following:

1. The presence of various minerals (e.g. Fe-oxides) influence Hg associations and its potential for solubilization in the presence of different solutions (i.e. water, organic acids).
1. The grain size of soils influence the moisture content, habitability for worms (worms have an aversion to coarse soils), and the ability of worms to solubilize soil constituents (as it relates to surface area). In order to limit the interference caused by variable grain sizes, all soils were ground to <200 mesh (<0.074 mm) prior to use in jar tests.
2. The abundance of organic matter varies with soil types. This is significant both in terms of worm health, as well as its role in Hg bioavailability.

As described in Section 5.3.2, four types of soils were evaluated in the jar tests: tailings, consisting of fine to medium quartz sand and HFOs; laterite composed of HFOs with kaolinite and minor quartz; organic soils, whose composition was similar to the lateritic soils with moderately to highly decomposed organics; and clay sediment predominantly composed of kaolinite. Organic matter contents were lowest in tailings (0.01%) and highest in the organic soils (3-11%), with intermediate values in the lateritic soils and clayey sediments (1-4%).

As demonstrated in Series' B-Two, B-Four, B-Five and B-Six, the most bioaccumulation was observed in tailings, with relatively high uptake in organic soils and the least uptake in laterite and clayey sediments.

Tailings > Organic Soil >> Lateritic Soil > Clayey sediment  
←————— *Increasing bioavailability* —————→

Not surprisingly, in tests where different soils were mixed with Hg-laden tailings, the ability of the different soil types to inhibit uptake from tailings followed the same series, i.e. the clayey sediment and laterite were most effective in preventing bioaccumulation. Although uptake in mixtures of tailings and organic soil was slightly lower than that observed in tailings alone, it was 36-49% higher than with the other soils, suggesting organics may promote uptake.

In the presence of organic acids the bioavailability of Hg associated with lateritic soil, as well as tailings, increased significantly, suggesting some exchange processes may be occurring. Specifically, Hg bioaccumulation when organic acids were applied to the lateritic soil and tailings was 2-28 times higher than in experiments wherein only water was applied. This, in conjunction with the high relative uptake observed for the organic-rich soil, suggests the role of organic matter in Hg bioaccumulation is particularly important. In addition, the comparatively low Hg bioavailability in association with the clayey sediment suggests that Hg associations with HFOs may also be significant. HFOs are abundant in the lateritic and organic soils, and are found to a lesser extent in tailings. This phenomenon is discussed in greater detail in Section 7.1.2, below.

### ***7.1.2 Organic Acids and Mercury Bioavailability***

Organic acids (i.e. humic, fulvic and tannic acid) may contribute to the bioaccumulation of Hg by earthworms in two ways. Organic acids may:

1. Enhance the bioavailability of Hg by effectively desorbing Hg associate with soil constituents and forming Hg-complexes; and
2. Promote the methylation of Hg either within worm intestines or in the substrate.

In theory, organic acids may also promote worm health (which may in turn influence bioaccumulation) with the contribution of organic carbon to the system. However, this contribution is low relative to the requirements of the worms. Based on the concentrations of organic acids in the solutions added to each jar, less than 1% of the organic carbon needed for the 28-day test (approximately 12 g/jar) is supplied by the solution. Consequently, the influence of organic acids on worm health is likely quite insignificant.

The other potential influences of organic acids, i.e. on Hg solubilization and methylation, are discussed in greater detail below.

#### **7.1.2.1 Organic Acids and Mercury Solubility**

Although the capacity of organic acids to leach metals from lateritic and other soils has been recognized (Valix et al, 2001; Bowles, 1988; Baker, 1973), the link between Hg associated with organic acids and bioaccumulation is less understood. Results of the jar tests strongly indicate that Hg bioavailability in *both* tailings and HFO-rich soils does increase markedly in the presence of organic acids. These findings are summarized as follows:

- In solution tests wherein worms were exposed to mercury associated with tannic, humic and fulvic acid, bioaccumulation factors for MeHg ranged from 158 to 876, indicating that earthworms may be producing readily bioavailable MeHg in the intestinal tract.
- In tailings jar tests involving three concentrations of humic acid (0.25, 0.125, 0.05 g/L) and distilled water, up to 33% more bioaccumulation was observed in the highest humic acid concentration than the lower humic acid concentrations. Bioaccumulation was 200 to 270% higher in humic acid jars than in jars to which distilled water was applied.
- In jar tests containing tailings and lateritic soil, Hg bioaccumulation in jars with tannic and humic acid solutions was 213 to 2809% higher than in distilled water jars.
- In comparisons of the influence of different soils on uptake from tailings, bioaccumulation was 36 to 49% higher in the presence of organic-rich soils than with clayey sediment or lateritic soil. Although distilled water was added to jars, organics in the soil were moderately to highly decomposed suggesting the presence of residual organic acid.

It has been suggested that the bioavailability of metals to terrestrial invertebrates primarily relates to its capacity to dissociate from its mineral associations (Fairbrother and Kapustka, 2000). As evidenced by the tests involving soils from Cachoeira, organic acids may contribute to the solubilization of Hg from soils, particularly when it is associated with HFO minerals. Hg bioaccumulation was up to 28 times greater when humic and tannic acid was applied to lateritic soil than distilled water. In comparison, Hg bioaccumulation was up to 4 times higher in tailing jars when organic acids were applied than when distilled water was used. It is probable that the solubilization capacity of organic acids depend on the form of Hg present and its association with soil or tailings constituents.

In the case of the HFO-rich lateritic and organic soils, Hg is associated with HFOs through specific adsorption, also known as chemisorption or co-precipitation (Fergusson, 1990). This behaviour may, in part, be attributed to the significantly smaller negative surface charge of HFO minerals compared to organic matter. As discussed in Section 3.2.4.2, the negative surface charge of goethite and gibbsite is two orders of magnitude smaller than organic matter<sup>14</sup>. Mercury displacement from soil constituents may be followed by complexation of organic acids with Hg compounds and/or the soil surface. Coating of clay minerals and HFOs by humic substances is well documented, with estimates of 52-98% of all soil organic matter bound to the surface of clay-sized particles (Sparks, 1995). Under these circumstances, displaced Hg has a greater potential to be complexed by 'free' or clay-associated organic acids. Approximately 1-

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<sup>14</sup> Brady and Weil (1996) estimate the negative surface charge of gibbsite and goethite at 4 cmol/kg and organic matter at 200 cmol/kg.

3% of organic acid ligands consist of sulfidic groups (e.g. sulfhydryl), to which Hg has a strong affinity. Although other functional groups, such as carboxylate, are typically more abundant, if multiple functional groups coordinate with a Hg compound, than chelation of Hg will result.

Unlike HFO-rich soils, Hg in tailings is mainly present as metallic Hg (~99%)(Lacerda and Salomons, 1999). The solubility of metallic Hg is extremely low, but as tailings are eroded or mobilized, exposure to different environmental conditions, in particular the presence of organic acids, may contribute to Hg solubilization and/or other transformations. Metallic Hg is highly soluble when mixed with organic acids (Meech et al, 1995), as evidenced by Hg concentrations of 6850 and 7500 ppb in humic and tannic acid solutions, respectively, as used in Series-A of the earthworm experiments. The solubilizing capacity of organic acids was also observed in shake flask experiments, where 10 to 20 times as much Hg was leached from tailing using humic and tannic acid, respectively, in comparison to distilled water. This behaviour can be attributed to the relatively high stability of elemental Hg under mildly oxidizing conditions – in the presence of a complexing agent, such as organic acid, this stability is reduced and Hg(II) more readily complexes with suitable ligands.

As demonstrated in shake flasks and jar tests, the bioavailability of Hg also differs depending on the type of organic acid evaluated. Generally, more Hg bioaccumulation was observed when tannic acid was used, compared to humic and fulvic acid (Table 6.5). However, this observation has limited relevance as the molecular weights of the humic and fulvic acids were not known. If molecular weights were known, the same molarity could be achieved in solutions, which would be conducive to a more effective comparison of the solutions. Even if this were the case, due to the heterogeneity of these substances in nature<sup>15</sup>, the significance of such a comparison in field applications is limited. Generally, lower molecular weight components, such as tannic acid, are those most rapidly cycled and therefore make Hg cycling more dynamic.

It is apparent that organic acids play an important role in the bioavailability of Hg associated with lateritic and other soils. The link between Hg desorption from soil constituents and its subsequent bioaccumulation is, however, unclear. As MeHg is the most bioavailable form of Hg, the influence of organic acids on the transformation of Hg to its methylated form is an intriguing topic that warrants further discussion.

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<sup>15</sup> The molecular weights of tannic, humic and fulvic acids can vary significantly (500->20,000; 3,000-1,000,000; and 500-5,000, respectively). In addition, the abundance of different functional groups also varies. These factors significantly influence the complexation capacity of organic acids.

### 7.1.2.2 Organic Acids and Methylation Potential

One concept specifically investigated in this research program is whether organic acids contribute to the methylation of Hg. Key results on this topic derived from this research include:

- Methylmercury bioaccumulation factors (BAF = 158 to 2477) that were 1-2 orders of magnitude greater than those observed for total Hg in previous experiments. This suggests that MeHg is more readily incorporated as it is produced, and/or worms are producing MeHg within the intestinal tract.
- The presence of SRBs in the intestinal tract of earthworms was strongly indicated by biological activity reaction tests. SRBs are widely believed to be the primary methylators of Hg.

Although the mechanisms supporting MeHg production in the presence of organic acids are not known, three scenarios for Hg methylation in the jar tests are apparent:

1. Methylation is occurring in the substrate.
2. Methylation is occurring directly within the intestinal tract of earthworms.
3. Methylation is occurring both within the substrate and earthworms.

Methylation of Hg-organic complexes seems to be feasible through either biotic or abiotic processes. Abiotic methylation of Hg in the presence of humic substances has been suggested by several researchers (Rogers, 1977; Nagase, 1982; Ullrich et al, 2001). Interestingly, under these conditions, the generation of MeHg may be stimulated in the presence of certain cations, particularly  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  (Lee et al, 1984). The basis for this argument is that the Hg methylation increases as complexation between Hg and organic acid becomes weaker. Due to the abundance of Fe-oxides in the experiments, and Eh-pH conditions that support the presence of soluble  $\text{Fe}^{3+}/\text{Fe}^{2+}$ , this mechanism may be significant.

The prevailing theory, however, is that methylation is facilitated by bacteria under anaerobic conditions. As jars are not fully saturated during the tests (i.e. moisture contents are ~30%), oxygen levels are high and a different microbial community is supported than under anaerobic conditions. Given the abundance and diversity of bacteria and enzymes active in earthworm digestion (Laverack, 1963), in addition to the reducing environment in the intestinal tract, conditions within the earthworm may be suitable for Hg methylation. In addition to SRBs, enzymes involved in organic matter decomposition have also been shown to facilitate methylation. Although Hg methylation within the intestinal tract of worms has not been definitively established, as the conditions for Hg methylation are present, it is certainly an issue that warrants further exploration.

As the percentage of Hg that consisted of MeHg was low (<1%), the potential for direct bioaccumulation of ingested Hg-organic complexes should be considered. Although the Hg:organic complex is relatively

strong (particularly in comparison to other metals), the low pH conditions of the intestinal tract, combined with the presence of organic matter decomposers and other bacteria, may support the dissociation of Hg from the organic acid. In this case, Hg may be in a form that can diffuse across intestinal walls, or may bond with proteins (e.g. –SH thiol group proteins) or other substances, supporting its incorporation into the organism in an inorganic form.

### **7.1.3 *Uncertainties***

There are a number of uncertainties associated with the successful application of the earthworm methodology in evaluations of Hg-contaminated soils and solutions and its interpretation in Cachoeira. Considerable uncertainty can be derived from the assumptions made in this research program. Important assumptions include the following:

- As worms have been cultured under the same conditions throughout the research program, a given population of worms used in one experimental series does not differ significantly from worms used in another series (i.e. in terms of initial health, uptake, etc);
- Sample collection and laboratory methods sufficiently limit external contamination;
- Conditions within jars (e.g. Eh, pH) do not vary appreciably from series to series;
- Despite the limited number of tests conducted, trends observed in data are sufficient to make statements about Hg bioavailability; and
- Observations made in the laboratory experiments (i.e. with respect to Hg bioavailability) are transferable to the field.

Failure of any of these assumptions inevitably presents serious questions with respect to the validity of the results obtained.

Transferring laboratory results to the field is fraught with uncertainty. Although the earthworm experiments may indicate high or low bioavailability in samples from certain locations, issues such as the volatility of metallic Hg, seasonal variability of rainfall, and the somewhat heterogeneous distribution of Hg, all influence the value of any lab results obtained. Different conditions in the field, such as temperature, Eh, pH, etc., also limit the transferability of lab results. Any remedial measures derived from this lab work also face comparable uncertainties, in addition to issues such as the long-term efficacy of any measure employed and implementation of any measure without full understanding of its impacts in a given system (e.g. modifications to the hydrologic regime).

When dispersed in the environment, metallic Hg forms droplets often resulting in a heterogeneous distribution or “nugget effect”. This distributive behavior may explain the differences between inter-laboratory results, as described in Section 6.3.4. On average, samples analyzed in different laboratories varied by 42%. Samples collected in Brazil were hand-mixed prior to splitting, but this may have been insufficient to obtain representative samples. Upon arrival to Canada, dried samples were put through a sample splitter a minimum of three times in order to achieve more homogeneous mixing. Thus, there may have been less influence of the nugget effect in the jar tests, but some of the variability between duplicates (average 29%) could be attributed to this.

Although efforts were made to limit the subjective influence of the researcher, qualitative interpretations and observations can also influence results. For instance, decreased mobility and severe discoloration are prevalent observations in worms from jars with extremely high Hg concentrations. Field classification of soil samples can also be somewhat subjective (e.g. silty medium sand can be mistaken for fine-to-medium sand).

The largest uncertainty in this research relates to the lack of statistical evidence. Despite the limited number of jar tests conducted, this research does identify some important trends and relationships, in particular with respect to the bioavailability of Hg associated with HFOs and tailings and the potential for intestinal methylation of Hg by earthworms.

## **7.2 Implications for Cachoeira do Piriá**

The implications of Hg pollution in Cachoeira primarily relate to the current extent of Hg contamination in the food chain and the potential for Hg in other compartments to become bioavailable.

### **7.2.1 Mercury Distribution**

#### **7.2.1.1 Soil, Sediment and Tailings**

Based on the results of the soil, sediment and tailings sampling program, an area of approximately 460,000 m<sup>2</sup> (46 ha) is moderately to highly contaminated with Hg (i.e. concentrations in soil and sediments are above 1000ppb). The 310,000 tonnes of tailings distributed across the study area is predominantly located in the region is directly adjacent to the Cachoeira townsite and includes the banks and bottom of fish-bearing, artificial Lake Cachoeira (Fig. 7.2).

The elevated concentrations found outside of this zone, particularly those in the drainages and the downgradient wetland zone, are mainly the result of erosion of tailings. As shown in Figure 7.1, runoff from the main mining area can enter Barriquinha or Currutela Creek (water depths of 0.1-0.3m), although much of the drainage passes through the wetland zone (water depths 0.5 – 1.0m) prior to discharge into the Macaco River. Mercury levels in the Macaco River are elevated above upstream concentrations (i.e. background) but are significantly lower than “on-site” levels (i.e. in Currutela and Barriquinha Creek and the wetlands). As discussed further below, Hg levels in fish caught in the Macaco River are elevated, suggesting that “on-site” watercourses may be important sites for transformation of Hg to more bioavailable forms.

As the aquatic systems surrounding Cachoeira are darkwaters<sup>16</sup>, once Hg enters these drainages, its mobility and bioavailability will inevitably be modified. As shown in Figures 7.3 and 7.4, revegetation of tailings is well underway.

Mercury distributed throughout the site may also be a consequence of atmospheric Hg deposition. As shown in Figure 7.2, residential soils had Hg concentrations up to 11021 ppb. As Cachoeira was a major centre for gold exchange in the region, elevated levels in soils could be attributed to the historical and current presence of gold shops. Due to the low temperature at which Hg vapour is emitted from gold shops, most deposition is believed to be in close proximity to the emission points (Borochoff, 2001).

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<sup>16</sup> As discussed in Section 3.2.4.5, darkwater systems are typified by low productivity (*i.e.* in terms of living biomass), low dissolved solids, and high concentrations of organic matter in sediments and the water column.

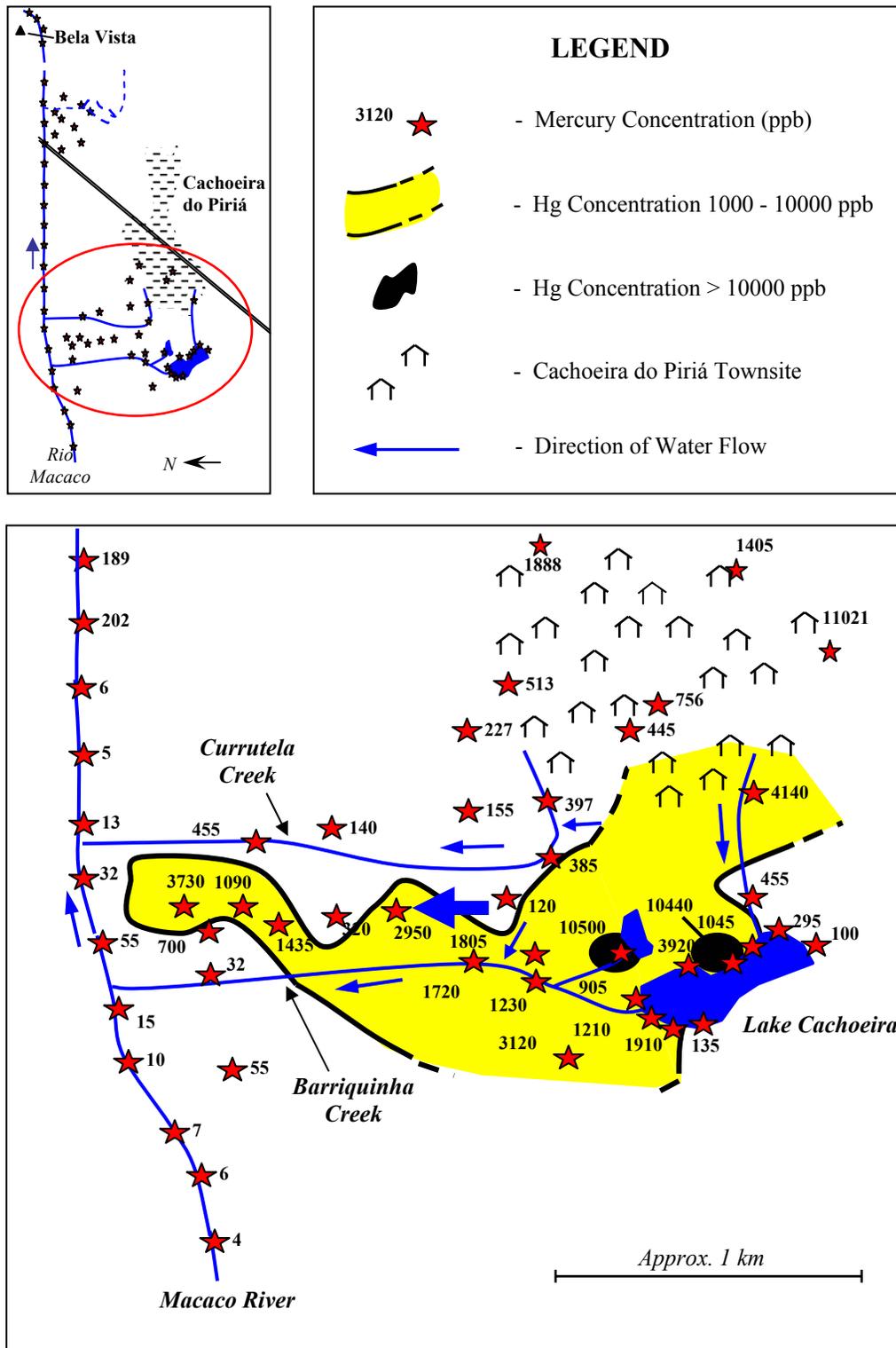


Figure 7.2 Contour Map of Mercury Concentrations in Soil, Sediment and Tailings in Cachoeira



Figure 7.3 Tailings Discharge Area



Figure 7.4 Drainage from Tailings Area entering Barriquinha Creek

### 7.2.1.2 Biota

The bioavailability of Hg in soils – as well as its distribution across the study site - is heterogeneous in Cachoeira, i.e. Hg bioaccumulation in earthworms does not exhibit any strong spatial patterns (Fig. 7.5). The exception is significantly decreased levels of earthworm bioaccumulation 2-3 km downstream from the study site (i.e. adjacent to the community of Bela Vista). This may be attributed to typically lower Hg concentrations in these soils in conjunction with the fact that the Bela Vista sediments mainly consist of clay (kaolinite).

As indicated in the results of the earthworm experiments, Hg bioaccumulation correlates poorly with other elements, but generally increase in the presence of organics. Cid de Souza (2002) established a correlation ( $r = 0.64$ ) between total Hg in soils and organic matter ( $Hg_{ppb} = -196.6 + 130.7(\%OM)$ ) in Cachoeira, although this investigation was limited to clay-rich sediments from the Macaco River. Numerical correlations between Hg in worm tissues and total Hg or organic matter in soils, tailings and sediments were not evident in earthworm jar tests results, but the highest BAFs were, however, from organic-rich samples collected from densely vegetated areas (see soils with 270 and 1180 ppb, Fig 7.4). The results shown in Figure 7.4 are derived from tests involving the application of distilled water. As demonstrated in the earthworm experiments, organic acids tend to exacerbate this uptake significantly.

This has long-term implications for residents of Cachoeira. Despite declining artisanal mining activities in the study area, the potential for Hg incorporation into the food chain may actually be increasing as revegetation and erosion of tailings progresses. The wetland, through which much of the drainage from the mining area passes, is likely an important source and sink of Hg and may exacerbate the potential for Hg bioaccumulation. Wetlands are widely recognized as significant sources of MeHg to other water bodies (Rudd, 1995; Ullrich et al, 2001), in part due to high microbial activity (Kelly et al, 1995), and the presence of organic acids, as indicated by the results of the earthworm experiments and other studies (Guimarães et al, 1998; Lacerda et al, 1995).

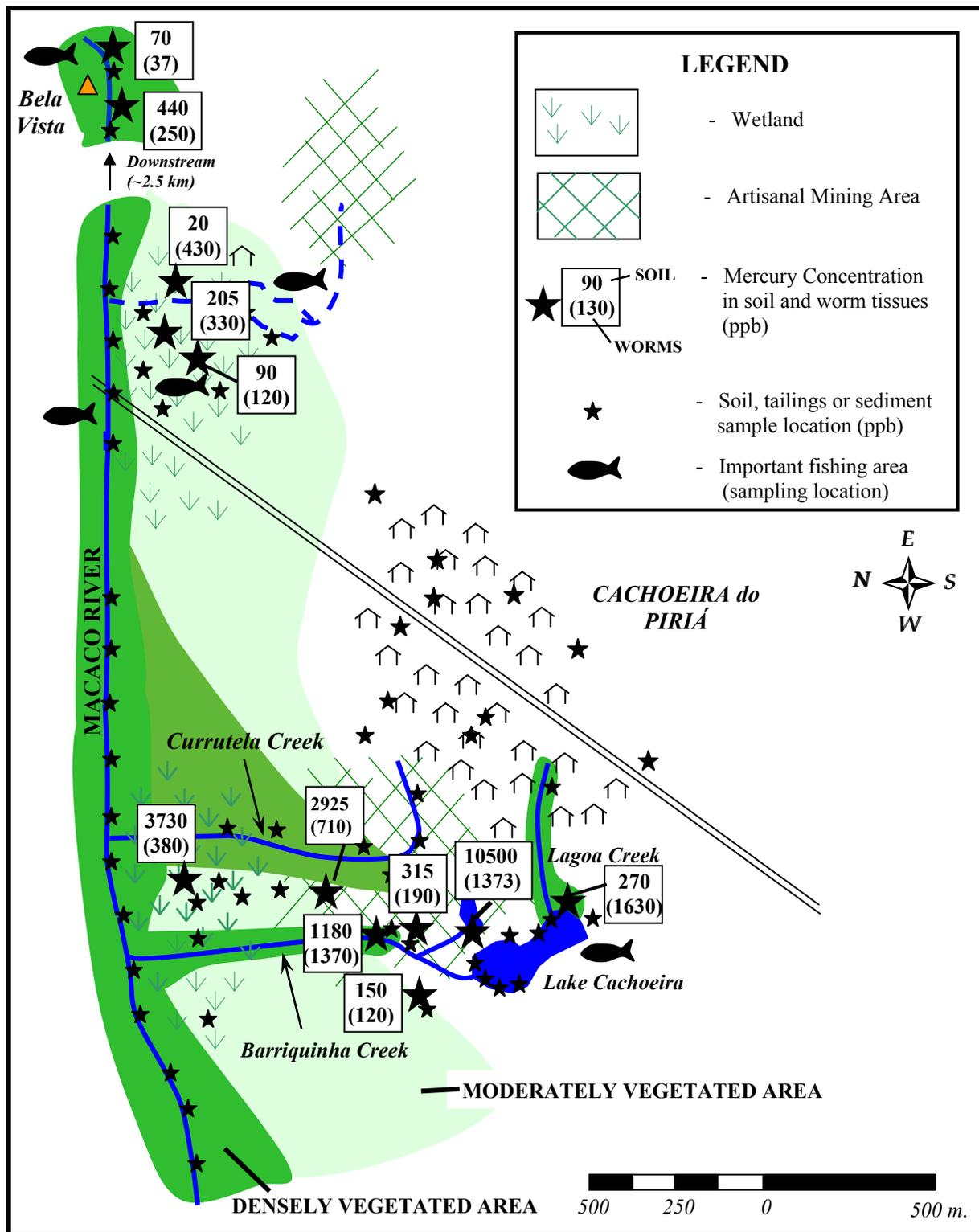


Figure 7.5 Bioavailability of Hg in Soils, Tailings and Sediments in Cachoeira

## Fish

Given the high Hg levels in soils and sediments, in conjunction with the abundance of organic acids, elevated Hg levels in carnivorous fish in the Cachoeira region are not surprising. Frequently consumed carnivorous species, traíra and jejú, which were caught in Lake Cachoeira, on the Macaco River downstream from the study site, and in the wetland area in the “old” mining area (Fig. 7.5), had Hg tissue concentrations which exceeded WHO guidelines (500 ppb) in 68 and 100%, respectively, of samples (ave. 987 ppb). Lower levels detected in the omnivorous fish mandi (ave. 108) could be attributed to physiological or dietary differences between species. Mercury concentrations in fish from a given locale generally vary with species, migratory habits, age, size, dietary habits, and properties of the aquatic system, among other factors (Silva Brabo et al, 2000). Herbivorous species, such as acará (ave. 347), exceeded this guideline in only 21% of samples.

Fish collected several kilometres downstream from the site at the confluence with the Piriá River (adjacent to the community of Bela Vista) also had elevated Hg levels, suggesting that the effects to biota from mining activities in Cachoeira extended downstream a considerable distance. In this location, traíra and mandi had tissue Hg concentrations of 560ppb and 50ppb, respectively (Palheta, 1995).

As sampling took place at the end of the rainy season (May-June, 1999) and the beginning of the dry season (July, 2000), Hg was only slightly diluted in rivers in comparison to the rainy season. As observed by Palheta (1993), rainy-season dilution tends to result in lower Hg levels in fish than in the dry season. Thus, Hg levels in fish may be higher than results presented indicate, particularly in ponds and lakes, such as Lake Cachoeira.



Figure 7.6 Fishing in Lake Cachoeira

### 7.2.2 Community Risks

Exposure to Hg through diet is influenced by several factors including ingestion frequency, species consumed and seasonal variability of these preferences. Informal interviews conducted with residents of Cachoeira and Bela Vista indicated that fish consumption habits vary considerably between individuals and at different times. Frequent consumption of abundant (and generally more flavourful) carnivorous species, such as traíra, was confirmed, although residents essentially consumed what was caught. Frequency of ingestion ranged from 0 to 7 times per week in the town of Cachoeira and up to 21 times per week (2-3 times/day) in the riparian community of Bela Vista, located 2-3 km downstream on the Macaco River. Research in other Amazonian communities have correlated increased fish consumption with poverty levels, thus gold miners tend to consume the least fish in artisanal mining communities (Malm et al, 1995). Daily ingestion rates were not determined in Cachoeira, but upper estimates 110 g/day<sup>17</sup> were established in a similar Amazonian community (Hacon et al, 1997). Estimates of 200 g/day have been proposed for riparian communities (Barbosa et al, 2001) and were deemed reasonable for the downstream community of Bela Vista, an impoverished, non-mining, river-dependent community.

Although factors such as gender, age, and metabolism can influence the retention and subsequent effects of Hg, simplistic assessments of risk can be conducted using consumption rates, body weight and Hg levels in fish (Table 7.3). The safe daily ingestion level recommended by Environment Canada is 0.2 µg/kg (ppb) per kg of body weight for women and children and 0.47 µg/kg (ppb) per kg of body weight for men. Thus, a 50 kg (~110 lb) woman consuming 200g of fish daily (about the size of a fillet) is ingesting about 17 times the recommended limit if carnivorous species are predominantly consumed. As children generally weigh considerably less than 50kg, with regular fish consumption they can exceed the recommended safe level to an even greater degree. As shown, a child living in Bela Vista may be consuming more than 50 times the safe daily Hg ingestion levels.

As shown in the simplified assessment presented in Table 7.3, all age and gender categories may be at risk from ingestion of Hg-contaminated fish. In addition, several studies indicated that harmful effects (e.g. neurological effects, delayed onset of walking in children, psychomotor retardation) can occur at much lower allowable daily doses (0.06-0.1 µg/kg MeHg body wt) than those previously thought (Hoover et al, 1997). As approximately 87% of the total Hg in fish from Cachoeira consists of MeHg, risk may be even more serious than anticipated.

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<sup>17</sup> Estimates of daily rates of ingestion were derived from weekly consumption levels.

Table 7.3 Exposure to Mercury from Fish Ingestion

Exposure Group	Body Weight (kg) <sup>1</sup>	Daily Ingestion Rate (kg/d) <sup>2</sup>	Hg Conc. in Fish (µg/kg)	Exposure Rate (µg/d) <sup>3</sup>	Recommended Max. Safe Exposure Rate (µg/d) <sup>4</sup>	Times above Safe Limit
<i>Cachoeira do Piriá</i>						
Adult – Male	60	0.050	845	0.70	0.47	<b>1.5</b>
Adult – Female	50	0.050	845	0.85	0.2	<b>4.3</b>
Child (1-4 yrs)	15	0.050	845	2.82	0.2	<b>14.1</b>
<i>Bela Vista</i>						
Adult – Male	60	0.200	845	2.82	0.47	<b>6.0</b>
Adult – Female	50	0.200	845	3.38	0.2	<b>16.9</b>
Child (1-4 yrs)	15	0.200	845	11.27	0.2	<b>56.3</b>

1. Body Weights from a survey of the Amazonian community of Alta Floresta (Hacon et al, 1997).

2. Ingestion Rates from Barbosa et al (2001) and Hacon et al (1997).

3. Fish concentrations assume 85% consumption of carnivorous species (Traíra) and 15% herbivorous species (Pacu). Average concentrations in Traíra and Pacu are 987 ppb and 40 ppb, respectively.

4. Based on Safe Exposure Levels recommended by Environment Canada (Mittelstadt, 2000).

Despite the apparent risks, this basic exposure assessment has a number of limitations:

- Data concerning weight, consumption rates and habits, etc of the population in Cachoeira were not determined through in-depth studies. As discussed, information was derived from informal interviews with residents of Cachoeira and Bela Vista, and data derived from other studies.
- Other exposure pathways have not been considered as they are expected to have lesser impacts on the community. Mercury levels in water are significantly below levels warranting concern and, due to the decline in mining activities, Hg releases into the atmosphere are believed to be low. However, those involved in amalgam decomposition (and others in close proximity to this activity) face an additional risk from exposure.
- People who have built houses on old tailings may be exposed to Hg through inhalation. Rates of Hg volatilization have not been estimated in Cachoeira, although degassing rates from garimpeiro-generated tailings at other locations range between 0.12 – 1.28 µg m<sup>-2</sup> (Lacerda, 1995). WHO has established a risk occupational exposure risk<sup>18</sup> at 50 µg m<sup>-2</sup>. Based on this information, a risk of exposure to Hg vapour from old tailings may exist although the effects are strongly dependent upon the individual and length of exposure.
- Ingestion of Hg-contaminated soil may present an additional risk for children. The US EPA (1999) estimates that 1-3 year old children ingest approximately 135 mg of soil daily.

<sup>18</sup> WHO occupation exposure guidelines and values for degassing from tailings are integrated over an 8 hour period.

- Slash burning, which releases additional Hg into the atmosphere, occurs frequently in the area. This exposure pathway has also been excluded from the assessment due to insufficient information.

Despite the limitations of the simplified assessment, it is apparent that some members of the population, in particular women and children consuming appreciable quantities of fish, are at some risk. Mitigative measures, including remedial options and consumption advisories, could reduce risks from Hg exposure significantly.

### 7.2.3 Measures for Mitigation

Simple, low-cost methods to reduce the risks from Hg pollution are critically needed in many artisanal gold mining communities throughout the world. Although preventative measures, such as the introduction of clean technologies and more effective government policies, could make great strides in reducing the social and environmental impacts of these activities, the legacy of artisanal mining in the communities that remain post-mining must also be addressed. The most enduring solutions in these communities are those that consider the socio-economic characteristics of artisanal gold mining communities, as well as the technical complexity of Hg in the environment.

In Cachoeira, Hg is both dispersed in the environment and concentrated in “hot spots.” Suggested responses to dispersed and concentrated Hg pollution scenarios are presented in Figures 7.7 and 7.8. Based on these recommendations, combined with financial and technical conditions in the community, suitable remedial and management options are described below:

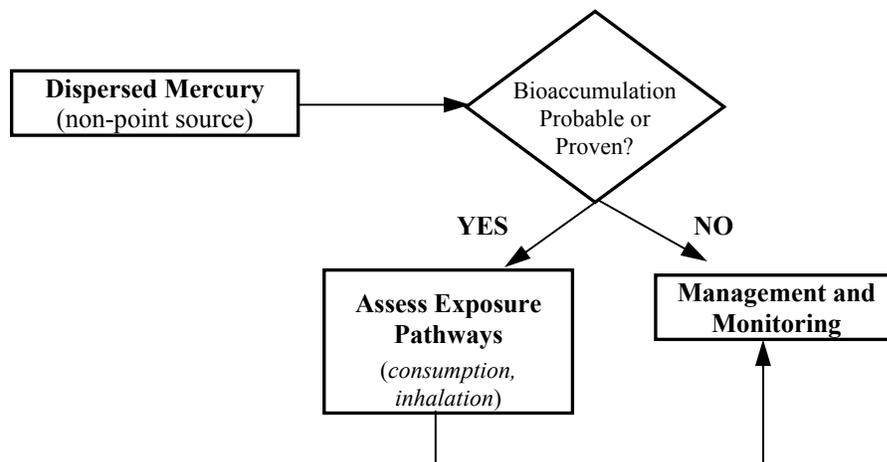


Figure 7.7 Appropriate Responses to Dispersed Mercury Contamination  
(After Hinton and Veiga, 2001)

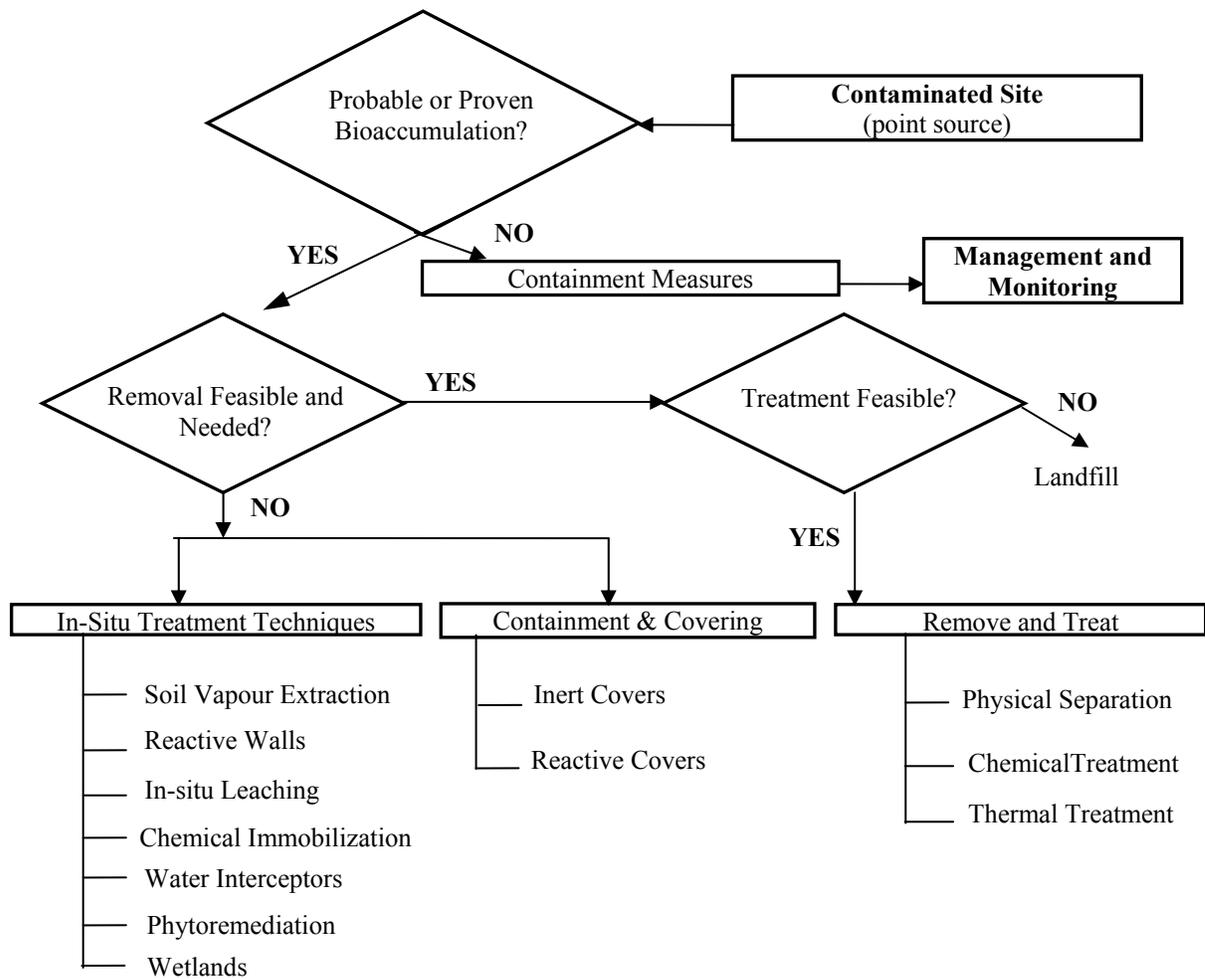


Figure 7.8 Appropriate Responses to Concentrated Mercury Contamination  
(After Hinton and Veiga, 2001)

### 7.2.3.1 Remedial Options

In recent years, several technologies have been developed to remediate Hg in soils, sediments and groundwater. Each method developed is applicable under specific site and contaminant conditions; primary limitations include geologic heterogeneity, cleanup costs and time. Based on the technical characteristics and financial circumstances in Cachoeira, the following technologies were considered:

*Wetland Remediation* –The use of wetlands for Hg immobilization is a somewhat controversial issue as wetland-type environments are intrinsically amenable to the conversion of metallic Hg to more bioavailable forms. Despite this potential, the US Department of Energy (Anon, 2000) has had notable success in significantly limiting the amount of MeHg generated (<1 ng/L) in an artificial wetland, mainly by controlling the amount and form of sulfur present. Mercury bound to decomposed organic matter descends to the bottom of anaerobic cells where it reacts with gypsum to form relatively insoluble sulfide. Ultimately, the wetland will treat up to 1 million gallons of water daily. As much of the drainage from the

tailings discharge area passes through a wetland, the role of this system in Hg transformations in Cachoeira should be studied further.

*Permeable or Impermeable Walls* - These approaches are geotechnically-engineered to prevent the off-site migration of contaminants. Design parameters include emplacement depth, wall thickness and permeability. Barriers may be designed to surround the contaminated zone entirely or remove the potential for groundwater flow through the source. As Hg mobilization in Cachoeira is believed to be mainly associated with erosion of tailings, walls would be constructed to inhibit tailings mobilization. Due to the intense precipitation in the region (>2 m/a) and the continued influx of organic acids into the tailings area, these options are not expected to be particularly effective. In addition, walls would require long-term maintenance, and could have negative implications for the hydrological regime (e.g. by inducing flooding).

*Phytoremediation/phytoextraction* - Phytoremediation is a promising, albeit unproven technology, wherein plants assimilate and concentrate metals from soils. Metal recovery typically occurs through subsequent harvesting and combustion of plants (Anderson et al, 1999). Plants that accumulate substantial quantities of metals from soils, known as hyperaccumulators, may be species specially developed for extraction of certain metals, or native species possessing a resistance to toxic effects of specific metals. This technique holds much promise for the cost effective remediation of shallow soils over a fairly widespread area, but issues such as limited access to vegetation by wildlife and time required for clean-up must be addressed. A preliminary study using tailings from Cachoeira has shown promising results with respect to uptake of gold (Anderson, 2002). More detailed study of gold and Hg uptake in plants will commence in Cachoeira in 2002.

*Capping of "hot spots"* - Mobilization of Hg in artisanal mining sites is predominantly associated with the erosion of tailings. Capping of tailings would not only prevent the mobilization of tailings into local drainages, but would inhibit oxidation of Hg associated with tailings and limit interactions with organic acids. Low permeability materials, such as compacted clays, natural soils mixed with stabilizers or bentonite, or geosynthetic membranes, are frequently used to inhibit infiltration. Design considerations include grading of surfaces to direct drainage and the long-term integrity of the seals. Elements such as weather effects (e.g. desiccation of clays), damage from burrowing animals or vegetation or degradation of synthetics by sunlight can all be significant.

Due to the financial limitations of the community, the potential for use of native materials to cap hotspots was evaluated in the earthworm methodologies. These tests intended to assess the influence of the

materials on uptake in areas where cap erosion has occurred, resulting in some mixing of the capping soil with the tailings. Findings are summarized as follows:

- *Organic-rich Soils* are fine grained (i.e. low permeability), have a high capacity to retain metals and are abundant at the study site. However, results of earthworm experiments indicate that Hg bioavailability is high relative to other soils evaluated. Thus, these soils are not recommended for use as caps.
- *Clayey Sediments* are fine grained (i.e. low permeability) and have a high capacity to retain metals, as well as organic acids. As these sediments are primarily composed of kaolinite, problems with swelling and shrinking are not anticipated. Clayey sediments are abundant along the Macaco River; however, given the extensive area requiring capping (~0.5 km<sup>2</sup>), the volume of clay required would inevitably damage watercourses. Earthworm experiments indicate that these soils are the most effective at inhibiting uptake from tailings.
- *Lateritic Soils* have been used extensively in a number of geotechnical applications (e.g. road building), are fine-grained (low permeability), have a high metal retention capacity and are abundant in the study site area. These soils are comparable to clayey sediments in terms of their ability to inhibit Hg bioaccumulation in the presence of water; but are less effective in the presence of organic acids.

Although the clayey sediments show promise as capping materials, their use in Cachoeira is limited by accessibility and the likelihood of damaging watercourses in their excavation. Although the laterite is less effective in the presence of organic acids, it is attractive in terms of its availability at the site. Further study of this remedial option, in particular additional bioaccumulation and geotechnical studies, is recommended.

#### 7.2.3.2 Community Based Measures for Risk Reduction

Non-technical measures for reduction of risks from Hg pollution include educational campaigns and consumption advisories. Examples of public information media developed in response to high-risk situations (e.g. Hg spills and contamination of biota) are presented in Appendix V. As discussed below, distribution of literature (e.g. pamphlets), such as those presented in Appendix V, is only one means of communicating the risks of Hg pollution.

##### Educational Campaigns

It is fundamental that any educational initiative is accompanied by audience-appropriate information. Some efforts have included the distribution of information packages, such as pamphlets, comics and videos, while others have relied on verbal communication through community meetings or door-to-door

campaigns. In 1985, the Secretary of Mining of Goiás State, Brazil, started a campaign promoting retorts that included a brochure illustrating the effects of mercurialism (Veiga, 2001). Impotence was stressed as one of the initial symptoms, which is somewhat inaccurate, but extremely effective in capturing the attention of miners. Key considerations of educational campaigns include the following:

- Educational level of the audience, in particular literacy;
- Characteristics of audience members, including role in the community (miner, health care worker, homemaker, etc) and factors such as age, gender, etc/;
- Presence of organizations in the community;
- Indigenous knowledge; and
- Appropriate communication methods (e.g. visual, oral, group, one-on-one).

As mining activities have essentially ceased in Cachoeira, efforts there should focus on responding to risks from existing pollution. In communities with active mining, campaigns should also address clean technologies and providing capacity to organize and work within regulatory frameworks. These issues are addressed in greater detail in Hinton et al (2002).

#### Consumption Advisories

As many communities have a cultural and economic reliance on fish, the communication of risks from Hg pollution and recommendations for dietary modifications must not be recklessly implemented. When the risks of consumption of Hg-contaminated fish were conveyed to an aboriginal community in Northern Canada, the resulting decrease in fishing activities – an important economic and cultural activity – resulted in increases in alcoholism and suicide rates and, with the change in diet, substantial increases in diabetes (Wheatley and Paradis, 1996). As most residents of Cachoeira have relatively diversified diets, this issue is most significant for fishermen living in the town and riverine populations (e.g. residents of Bela Vista). Resources developed to communicate consumption advisories must make the same considerations as other educational tools, but the impacts of such measures on the community must also be assessed.

As it is well demonstrated that programmes developed by the local people participating in them (i.e. bottom-up measures) tend to be most effective and enduring, educational campaigns would likely be most successful if members of the artisanal mining community were directly involved with their development and implementation. Doing so will increase the potential that the materials and methods will be audience-appropriate, will strengthen the relationship between researchers and community members, and will improve the likelihood that creative solutions can be achieved.

## 8 CONCLUSION

This research was undertaken to develop a laboratory methodology using the earthworm *Eisenia foetida* to assess mercury bioavailability in mine tailings and aqueous solutions, with specific applications in artisanal gold mining communities. The community of Cachoeira do Piriá, where an estimated 4 tonnes of Hg has been discharged into the environment in the past two decades, was selected as a site to validate the efficacy of the methodology. Characterization of Hg pollution in Cachoeira and identification of measures for risk mitigation supplement this work.

The following conclusions were derived from the development and application of the earthworm methodology:

- Earthworms (*E. foetida*) are capable of accumulating Hg and other metals from solutions and soils. Factors contributing to uptake could not be determined, although it is strongly indicated that organic carbon is significant.
- A positive correlation generally exists between Hg concentrations in worm tissues and the substrate they consume (a dose-response relationship), although this relationship is not well-defined and does not correlate with other variables (e.g. uptake of other elements present in the substrate).
- A 28-day exposure is deemed acceptable in order for earthworms to achieve steady state in terms of Hg uptake.
- Worm survival was over 90% and health was generally good throughout the duration of the exposure period, satisfying these established criteria for bioaccumulation protocols.
- Reproducibility between experimental duplicates was poor (42% difference). This is a frequently encountered setback in experiments using biological populations, but it indicates the need for additional replicates to provide statistically significant data. The ‘nugget’ effect observed for metallic Hg may have played a role in this disparity.
- Reproducibility of other quality control measures was acceptable. Differences between “blind” replicates submitted for laboratory analysis and internal laboratory duplicates were 7.9% and 2.3%, respectively.
- Bioaccumulation Factors for MeHg ( $BAF_{ave} = 466$ ) were two orders of magnitude greater than factors for total Hg. This suggests that Hg is produced in the substrate and quickly incorporated

into the organisms and/or worms are producing and subsequently incorporating MeHg in the intestinal tract.

- The latter hypothesis, intestinal methylation of MeHg, is strongly supported by biological activity reaction tests that indicate the presence of SRBs in the intestinal tract of earthworms is. SRBs are widely considered to be the primary methylator of Hg.

The reaction of metallic Hg with organic acids from sediments and darkwater systems is definitely an important pathway for Hg bioavailability. This is indicated by the following results:

- Mercury uptake from tailings increases with increasing concentrations of humic acids. Bioaccumulation from tailings was 200-270% higher in the presence of humic acids than water.
- In tests wherein various solutions were applied to tailings and lateritic soil, earthworms accumulated 2-4 and 26-28 times more Hg, respectively, in jars containing organic acids (humic and tannic acid) than in jars containing distilled water.
- In shake flask experiments, 10 to 20 times more Hg was dissolved from tailings mixed with humic and fulvic acids compared to distilled water. The solubility of metals has been directly linked to their bioavailability.
- In comparisons of the influence of different soils, Hg bioaccumulation was 36-49% higher from organic-rich soil than with clayey sediment or lateritic soil. BAFs for organic-rich soil, lateritic soil, and clayey sediment were 22, 1.2 and 0.5, respectively.

These results strongly indicate that the formation of Hg-organic complexes (i.e. Hg-humates and Hg-tannates) contribute to Hg bioaccumulation. This is extremely significant in terms of the biogeochemical cycling of Hg in darkwaters and related ecological and human health risks associated with these organic acid-rich aquatic systems.

Conclusions specific to Hg pollution in Cachoeira and measures for its mitigation are described as follows:

- An area covering approximately 85 ha located adjacent to the town of Cachoeira is moderately to highly contaminated with Hg. However, Hg contamination is dispersed across an area of approximately 2100 ha and extends 2-3km downstream from the mining area.
- Mercury levels in soils, sediments and tailings range from 5 ppb to 10500ppb (ave. 695ppb) and are heterogeneously distributed.

- Mercury in sediments collected upstream from the site on the Macaco River, which passes through the impacted area, have concentrations ranging from 3 ppb to 15 ppb. This indicates that Hg on and downstream from the study site is attributed to mining activities there.
- Hydrologic, geologic and geochemical data indicates that the tailings discharged in the mining area are further dispersed into creeks and a downgradient wetland zone. These watercourses, in particular the organic-rich wetland, seem to be important in terms of subsequent Hg mobilization into the fish-bearing Macaco River and the transformation of Hg to MeHg.
- Certain species of carnivorous fish (traíra, jejú) from Cachoeira exceed WHO Hg consumption guidelines in 68-100% of the samples. These fish are species that are frequently consumed by local residents.
- A simplified assessment of Hg exposure from ingestion of fish indicated that men, women and children in Cachoeira may be consuming 1.5, 4.3 and 14.1 times Safe Hg Ingestion Levels. Men, women and children in the downstream, riparian community of Bela Vista may be exceeding these levels by 6, 16.9 and 56.3 times, respectively.
- Capping of Hg “hot spots” was identified as a viable technique to reduce the mobilization of tailings, and thus Hg, into watercourses. Evaluations using the earthworm protocol indicated that clayey sediments are suitable in terms of their ability to reduce Hg bioaccumulation. Lateritic soils were also generally effective except in the presence of organic acids.
- Non-technical measures to reduce Hg risks from fish consumption include educational campaigns and the consumption advisories. A more detailed social assessment of the community is needed before undertaking such programs.

Easily implemented, low-cost methods can be beneficial for rapid diagnosis of potentially hazardous situations, particularly in regions such as developing countries where resources are limited and pollution is widespread. The earthworm methodology presented herein can be easily employed within a short period of time at a relatively low cost. With some additional refinement, this procedure may become a very effective tool for the assessment of Hg in polluted solutions, soils and tailings.

## 9 RECOMMENDATIONS

It is apparent that many knowledge gaps exist in understanding of the behaviour of Hg in the environment, in the use of bioindicators to assess pollution and evaluate remedial options, and in the development of mitigative measures appropriate to artisanal mining communities. Recommendations specifically derived from the main topics of this research are presented below.

Based on the results of earthworm experiments, subsequent research on this topic should address the following issues:

- Replication of the experiments on a scale sufficient to attain statistical certainty about the results;
- Scrupulous field sampling and sample-splitting methods, which limit the ‘nugget effect’ of Hg;
- Development of measures to link laboratory results more directly with field parameters. This could involve development of a field method to assess bioaccumulation (e.g. using cages invertebrates placed in impacted soils);
- Linking Hg bioaccumulation with adverse effects, such as impairment of reproduction rates or growth, would support the practical application and interpretation of results derived from bioaccumulation protocols;
- Determination of specific characteristics, such as molecular weights and functional groups, of humic, fulvic and tannic acid, in order to ascertain their relative roles in influencing Hg uptake (i.e. in additional earthworm experiments);
- Rigorous assessment of the methylation potential of earthworms. This may involve detailed studies of the physiological aspects of Hg uptake, combined with Hg solubilization and methylation experiments using intestinal fluids. This may also include further characterization of conditions in the intestinal tract (e.g. microbial community composition).

Application of the earthworm method in Cachoeira, combined with an assessment of pollution effects and mitigative measures, yielded the following recommendations:

- In order to sufficiently ascertain impacts to area residents, a more detailed risk assessment is recommended. Detailed study of consumption habits and the risks from other sources (e.g. residing on tailings) should be conducted.
- The relationship between organic acids and Hg uptake from tailings and lateritic soils is a topic of significance in many artisanal mining communities throughout the world. The mechanisms

influencing this behaviour should be further evaluated through both geochemical and biological studies.

- Remedial measures appropriate to artisanal mining communities, including the use of native materials in capping hot spots, require further study. This may involve more detailed geochemical, biological and geotechnical assessments of capping materials, or research into alternatives technique. As phytoremediation involves recovery of gold in conjunction with Hg, this may be a sustainable and highly effective method in the future.
- Educational programs, such as consumption warnings for certain fish species and health issue advisories, have been implemented in other impacted villages. A comparable program could be employed in Cachoeira.

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