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Environmental and human health risks of mercury released from artisanal gold mining in Tanzania

Thesis submitted in fulfillment of the requirements for the degree of Doctor (Ph.D) in Applied Biological Sciences: Environmental Technology

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Dutch translation of the title:

Risico's voor milieu en volksgezondheid van de kwikverontreiniging door artisanale goudontginning in Tanzania

Illustrations on the cover:

Front: An artisanal gold miner amalgamating mercury with gold concentrate using unprotected hands; an artisanal gold miner roasting mercury/gold amalgam on open air; local cattle grazing and drinking water from mercury contaminated river (Mabubi) near Mugusu mine; catfish (*Clarias gariepinus*) caught from Nungwe bay in Lake Victoria

Back: Open pit mining at Mugusu mine; crushing of the mined ore by using ball mills at Mugusu mine

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Environmental degradation and poverty are twins sisters, their parents are illiteracy and ignorance

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Ghent, January, 2008

Raphael

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List of abbreviations

AAS	Atomic Absorption Spectrophotometer
AIDS	Acquired Immune Deficiency Syndrome
AMREF	African Medical Research Foundation
ANOVA	Analysis of Variance
ATSDR	Agency for Toxic Substances and Diseases Registry
AVS	Acid Volatile Sulfide
EU	European Union
EPA	Environmental Protection Agency
FAO	Food Agriculture Organization
GGM	Geita Gold Mine
GDP	Gross Domestic Products
HIV	Human Immunodeficiency Virus
Hg	Mercury
ILO	International Labour Organisation
JECFA	Joint WHO/FAO Expert Committee on Food Additives
LOD	Limit of Detections
LOEC	Lowest Observed Effect Concentration
LVEMP	Lake Victoria Environmental Management Project
LVGF	Lake Victoria Gold Fields
MeHg	Methylmercury
MEM	Ministry of Energy and Minerals
UNIDO	United Nations Industry Development Organisation
US EPA	United State Environmental Protection Agency
NEMC	National Environmental Management Council
NOEC	No Observed Effect Concentration
NGO	Non Governmental Organisation
OC	Organic Carbon
OECD	Organisation for Economic Cooperation and Development
PEC	Probable Effect Concentration
PTWI	Provisional Tolerable Weekly Intake
SEAMIC	Southern and Eastern Africa Mineral Centre
SEM	Simultaneously Extracted Metals
SSSP	Statistics for Social Science Programme

TAFIRI	Tanzania Fisheries Research Institute
TB	Tuberculosis
TBS	Tanzania Bureau of Standards
TDS	Total Diet Study
TOC	Total Organic Carbon
UNEP	United Nations Environmental Programme
UNIDO	United Nations Industries Development Organisation
WHO	World Health Organisation

Chapter one

General introduction, literature review and conceptual framework of the study

1.0. General introduction, literature review and conceptual framework of the study

1.1. General introduction

1.1.1. Mercury

As an element, mercury (Hg) has always been present in the Earth's environment. However, human activities have dramatically changed where mercury occurs as well as the forms it takes in the environment. Over centuries, human activities have removed mercury from deep reservoirs in the earth and mobilized it in the biosphere, creating vast disruptions in the natural cycle of the element. Some examples of major sources of anthropogenic releases of mercury are:

- Coal-fired power and heat production
- Energy production from other fossil carbon fuels
- Cement production (mercury in lime)
- Mining and other metallurgic activities involving the extraction and processing of virgin and recycling mineral materials
- Mercury mining
- Artisanal gold mining
- Chlor-alkali production
- Dental amalgam fillings
- Manufacturing mercury containing products like thermometers
- Releases from waste treatment and cremation

Mercury is a potent neurotoxin. Exposure to mercury impairs the brain, kidneys and liver and causes, reproductive disorders, disturbances in sensations, impairment of speech and vision, hearing and walking difficulties, mental disturbances and death. Recently, chronic poisoning with mercury or its derivatives has been associated with cardiovascular related illness (Alan, 2005). Mercury contamination has become an important topic of scientific study shortly after the publication of Silent Spring (Carson, 1962). While there is no direct reference made to mercury in the text, Carson's book certainly spawned research in a wide range of environmental disciplines. Two incidents put mercury in the global spotlight for the first time. The first was an epidemic in south–western Kyushu, Japan, where during the late 1950s and early 1960s, a number of inhabitants of the Minamata bay region began experiencing acute neurological disorders. It was later discovered that these people had consumed excessive quantities of mercury contaminated fish and shellfish. The poisoning was caused by methylmercury, which had formed in the sludge discharges of a plant that had used mercuric oxide in sulphuric acid used during acetaldehyde production. Symptoms of this

disorder included neurological damage and disturbances of sensation and movement (Minamata disease) (McAlpine and Araki, 1958; MEJ, 1970). By the end of 1960, 111 cases of mercury poisoning had been reported. As of August 1965, 41 people had died from this Hg exposure (Klein and Goldberg, 1970). The second large incident involving Hg occurred in Iraq, where in 1971, thousands of people were poisoned following accidental consumption of seeds treated with mercury based pesticides (Bakir *et al.*, 1973). Numerous people experienced vision impairments and women gave birth to children with disorders such as mental retardation, vision and hearing impairments and coordination problems (Amin-Zaki *et al.*, 1979). By the late 1960s and early 1970s, these two poisoning incidents, attracted the attention of the scientific community which began to analyze the metal's environmental and human health impacts in great detail. Recent cohort studies in the Seychelles have shown that children who are born to mothers with mercury levels higher than 10 $\mu g\ g^{-1}$ in their hair, can exhibit neurobehavioral deficits later in life (Davidson *et al.*, 2006).

In the aquatic environment, inorganic mercury is methylated by micro organisms into its various organic forms. Organic mercury can occur as an organomercuric salt e.g. methylmercuric chloride, or as an organomercuric compound e.g. dimethylmercury. While the majority (about 95 to 99%) of mercury in aquatic ecosystems is in the inorganic form (Krabbenhoft, 1996), organic mercury complexes remain important for the mobility and bioavailability of mercury (Mahalingam, 2003). Mercury methylation is a predominantly biologically mediated process between dissolved inorganic mercury and, primarily, sulphate reducing bacteria (Driscoll *et al.*, 1994). The factors that influence the amount of methylmercury present in an aquatic system include the amount of dissolved inorganic mercury and the physico-chemical characteristics of the aquatic system such as pH, organic matter, dissolved sulphate and sediment sulphide (Pak and Bartha, 1998). Methylmercury is known to bioaccumulate in the aquatic food chain, and is the most toxic form of mercury to man (Krabbenhoft, 1996).

High concentrations of Hg in the aquatic system can lead to a range of ecological effects. For macro - invertebrate communities, Hg has been documented to decrease the abundance of metal sensitive taxa (e.g. some mayfly species), with a corresponding increase in the relative abundance of metals–tolerant taxa (e.g. some chironomids) and a decrease in the total biomass (Beltman *et al.*, 1999). In fish, Hg bioaccummulates in muscles, beyond certain concentrations induces adverse effects. Toxic effects are unlikely to occur in fish in the environment, except in the case of point source discharges. However, histological changes and effects on behaviour, reproduction, and development can occur

at water concentrations as low as 0.12 to 28 µg/L (Wiener and Spry, 1996). Sublethal concentrations of Hg affect sensitive species of fish through inhibition of reproduction (Dave and Xiu, 1991), reduction in growth rate, increased rate of histopathology (Kirubagaran and Joy, 1988; Berntssen *et al.*, 2003) and impairment in the ability to capture prey. Grippo and Heath (2003), demonstrated experimentally that fathead minnows (*Pimephales promelas*) foraging ability and capture speed were drastically reduced at 6.79 and 13.57 µg/L. Behavioural changes are attributed to alterations in neurotransmitter levels following Hg poisoning (Zhou, *et al.*, 1999).

Since the beginning of the industrial era, the concentrations of mercury in the global environment have increased three fold (Mason and Sheu, 2002). However, in the industrialized countries emissions have recently been shown to gradually decrease due to stringent regulations. Conversely, emissions in the developing world have been increasing, mainly due to intensive use of mercury in artisanal gold mining and the absence of restrictive legislation (UNEP, 2002). According to United Nations Industries Development Organisation (UNIDO) report of 2004, there are almost 15 million people working directly in the artisanal gold mining industry across the developing world.

The primary gold recovery method used by artisanal gold miners involves crushing of ore followed by amalgamation with mercury. Mercury tends to escape and contaminate the environment at different points of the gold extraction process. Although mercury has been used as a gold amalgamation agent for over 5000 years (Malm, 1998) it was not until the 1980s, following the gold rush in Brazil, that scientists began paying attention to its effects in artisanal mining communities. In these artisanal mines mercury associated problems are due to poor operational practices and careless handling of mercury. It has been estimated that around 20 to 40% of Hg is lost during the initial concentration and amalgamation stage, where inorganic mercury effluents are released directly onto soils and into streams and rivers (Ogola *et al.*, 2002). The remaining 60 to 80% of mercury is released directly into the atmosphere when gold amalgam is burned. This is usually performed in open air by individuals wearing no or minimum protective headgear (Ogola *et al.*, 2002; van Straaten, 2000a). During gold extraction, it has been shown that miners lose approximately between 1 and 2 grams of Hg per gram of gold produced (van Straaten, 2000a). Studies conducted in the major amalgamation areas around the world, identified the widespread use of mercury as a major risk to the environment and local human population (Paulo *et al.*, 2000). To address this concern, ministers and other government representatives from countries around the world addressed this question in 2005 at the 22nd meeting of the Governing Council of the United Nations Environmental

Programme (UNEP) in Nairobi –Kenya. At this meeting, it was anonymously agreed that artisanal gold mining was an environmental concern in the developing world more particularly for aquatic ecosystems and fishery products and that immediate action was needed. One of the affected countries is Tanzania, where it was estimated that about 450,000 people are engaged in artisanal gold mining (UNIDO, 2004).

1.1.2. Artisanal gold mining in Tanzania

Tanzania has great potential in the gold mining industry. Artisanal miners have worked on gold extraction since the independence of this country in 1961. Also, prior to independence, mining activities contributed considerably to the country's gross domestic product (GDP). However, at that time gold was produced solely by large-scale gold mines. The post-independence political structure emphasized state-owned production, including in the mining sector. However, the state enterprises which were entrusted to develop the gold sector, had inadequate technological, human and capital resources. As the large mines collapsed, individuals resorted to informal artisanal gold mining activities. These informal activities were not officially recognized and most gold was illegally smuggled out of the country. Only in the late 1980s and early 1990s did the government recognize the need to control the activities of artisanal miners with the view of eliminating smuggling (Mwaipopo *et al.*, 2004). To date, most artisanal gold miners basically conduct their operations on an informal basis without adhering to laws, regulations and technologies. They shift from one site to another, working on both registered and unregistered land.

1.1.3. Use of mercury in artisanal gold mines in Tanzania

In most artisanal gold mines in Tanzania, mining involves digging pits using simple tools such as hand hammers, picks, hoes and shovels. After mining the ore, gold is recovered through a repeated series of crushing, grinding and gravity concentration followed by an amalgamation process in which liquid mercury is added and mixed with ground gold ore concentrates in order to produce a gold/mercury amalgam. Subsequently the amalgam is fired in open air to produce mercury vapour and elemental gold. During these various stages of the process, mercury is introduced into the environment through disposal of processing water and tailings, while open-air firing of the amalgam releases mercury vapour to the atmosphere. In Tanzania, it is estimated that, every year gold miners release about 10 to 30 tonne of Hg in the environment (Shoko and Veiga, 2003; Veiga, 2003; Taylor *et al.*, 2005). However, apart from generalized information, there is no quantitative data on the

actual amount of mercury being used and subsequently released into the environment from specific mining centres.

1.1.4. Mercury contamination in Lake Victoria

In Tanzania, artisanal gold mines are mainly found in the Lake Victoria goldfields which are situated in the Geita, Kahama, Musoma, Mugumu, Tarime, and Biharamulo districts (Mwaipopo *et al.*, 2004). Many of these mines are located close to Lake Victoria, which is the world's second largest fresh-water lake and the largest in Africa with a surface area of 68,800 km^2. Lake Victoria is important for food (fish) production, transport, communication, water supply for domestic use, agricultural and industrial use, waste disposal, recreation, sports and biodiversity conservation. Studies conducted along the major amalgamation areas around Lake Victoria, reported widespread use of Hg and contamination of the environment (Kondoro and Mikidadi, 1998; van Straaten, 2000a: Kahatano and Mnali, 1997, LVEMP, 2002, Ikingura *et al.*, 1997; Taylor *et al.*, 2005). It has been estimated that approximately 12 tonnes of mercury are released into Lake Victoria annually. Chemical measurements have indicated that Hg levels in filtered water collected from mine draining rivers and lake waters range between 0.02 µg/L to 5 µg/L, and sediment concentrations range between 1.5 µg g^{-1} dw to 136 µg g^{-1} dw (Kondoro and Mikidadi, 1998; van Straaten, 2000a; Kahatano and Mnali, 1997; LVEMP, 2002; Ikingura *et al.*, (1997; Taylor *et al.*, 2005). These Hg levels in water and sediment exceed the Tanzania standards for drinking water (1 µg/L) (TBS, 2003) and the recommended minimum toxic threshold in the sediment quality criteria for Canada (i.e. of 0.2 µg g^{-1} dw) (Haines *et al.*, 1994). However, there are no reports on whether the measured concentrations of Hg in Lake Victoria can adversely affect the local biota.

1.1.5. Mercury accumulation in fish

Studies conducted in artisanal gold mining impacted areas around Lake Victoria basin indicate a progressive increase with time in levels of mercury in some species of fish. Ikingura and Akagi (1996) reported Hg levels ranging from 0.069 to 0.117 µg g^{-1} wet weight (ww) in nile perch collected in Geita district waters. Machiwa *et al.*, (2003) found higher levels of Hg in nile perch from the same area (0.095 to 0.396 µg g^{-1} ww). Furthermore, results from a recent study conducted at the Rwamagasa artisanal gold mine, indicated that Hg concentrations in *Haplochromis* spp were up to 2.06 µg g^{-1} ww (Taylor *et al.*, 2005). Tanzania, like most other African countries does not have its own guidelines and safety standards or limits for the mercury content in fish intended for human consumption. Instead, it uses standards formulated by the World Health Organisation (WHO) which

proposes not to consume fish containing Hg levels of 0.5 μg g^{-1} ww or more. It is recommended that the population restrains itself from consuming on a regular basis, species exceeding these values. Nonetheless, because actual fish consumption patterns of poor local communities which subsist on fishing and fish consumption in Tanzania are inadequately known, it is unknown if these internationally set limits are protective to the health of these populations. To date, it is not known if their current Hg intake through fish consumption can result in adverse effects.

1.1.6. Mercury contamination of domestic animal products and food crops

Consumption of mercury contaminated fish is usually considered the main route of human exposure to mercury. In developing countries, however, there may be other important sources. In Tanzania for example, domestic animals like cattle, domestic fowl and ducks are usually kept in a free range system where the animals are allowed to graze/feed/scavenge freely near or in mercury contaminated environments. During the dry season the mercury contaminated rivers around the mines are the only available watering points for animals (own observation). Crops cultivated in the vicinity of gold ore sluice/washing areas, such as yams, sugarcane and rice have been found to accumulate high levels of mercury (LVEMP, 2002). It is therefore possible that natural pastures for domestic animals around such areas are similarly contaminated. Domestic chicken and ducks have been reported to peck and feed in amalgamation ponds of gold mines (Lyatuu, 2002). In the same study, this author reported mercury concentrations up to 0.373 μg g^{-1} and 0.032 μg g^{-1} ww in liver and lung samples of ducks, respectively. To date, there is no information on the contribution of animal products produced from animals reared in and around gold mining areas to Hg exposure to consumers in Tanzania. Similarly, studies that have been conducted to estimate dietary exposure of local population to mercury in artisanal gold mining areas have been largely confined to exposure from Hg contaminated fish (Ikingura and Akagi, 1996; Machiwa, 2003; Taylor *et al.*, 2005). These studies thus ignored possible exposure from other food items such as tubers, rice and sugarcane, crops known to be cultivated in Hg contaminated soils.

1.1.7. Regulation of mining sector and environmental pollution in Tanzania

1.1.7.1. Regulation of mining sector

The institutions responsible for establishment and enactment of laws and regulations in the mining sub-sector in Tanzania are the Ministry of Energy and Minerals (MEM) and the regional and district administration authorities. The mandate of these regulating authorities is derived from the Tanzania Mining Act of 1998. The ministry, through the Commissioner of Minerals is responsible for regulating the activities of mining on behalf of the government. This commissioner in consultation with the Minister of minerals is responsible for granting prospecting rights and mining claims, offering technical assistance, monitoring of production and safety in the mines. Zone mining officers are charged with the responsibility of assisting the commissioner in performing his/her duties in their respective areas of jurisdiction. Regional and District administrations have the task of maintaining law and order in mine camps; providing social services like schools, health centres; settle land disputes between local communities, individuals, claim holders or mining companies. However, the main handicaps of these governmental institutions are the lack of funds, uncommitted leadership, weak or bad planning and management, and poor technical facilities to reach artisanal mines (Mwaipopo *et al.*, 2004).

1.1.7.2. Regulation and monitoring of environmental pollution in Tanzania

Management of environmental pollution in Tanzania is mainly regulatory in nature, and is governed by one umbrella legislative act, the National Environmental Management Act of 2004. This act is enforced through the National Environmental Management Council (NEMC) which is charged with responsibility of carrying out environmental audits, enforce and ensure compliance of the national environmental quality standards, and disseminate manuals, codes or guidelines relating to environmental management. Thus, in principle conventional pollution arising from mining is also monitored and regulated by NEMC. Local government authorities have the responsibility for regulating environmental pollution in their respective areas. Under the district councils there are environmental committees at the districts, ward and village levels, all of which have regulatory responsibilities concerning environmental pollution. In addition, there are non-governmental organisations (NGOs) which make a significant contribution to the regulation, advocacy and management of environmental pollution in Tanzania. These organisations are, however, constrained by lack of resources and/or qualified manpower.

For the efficient regulation of environmental pollution, monitoring of environment quality is a pre-requisite. The monitoring process encompasses repetitive observation, of one or more chemicals or biological elements, according to a pre-arranged schedule over time and space, and using comparable and standardized methods. There are various environmental monitoring methods that may be used to assess risks of contaminants and classify the environmental quality of the ecosystem. These methods include chemical measurements, bioaccumulation, and biological effects assessments, health and ecosystem integrity evaluations (Van der Oost *et al.*, 2003). In artisanal gold mining areas in Tanzania, the only type of biomonitoring studies which have been performed to date are the measurement of Hg concentrations in fish (Ikingura and Akagi, 1996; LVEMP, 2002; Machiwa *et al.*, 2005) and the analysis of Hg in human hair (Ikingura and Akagi, 1996; Harada *et al.*, 2001). These studies can, however, not be used to assess the potential risks to the aquatic ecosystems.

1.2. The aim of the thesis

The main aim of this doctoral study was to assess the environmental and human health risk of Hg released from artisanal gold mining activities. For this purpose the Mugusu artisanal gold mine which is located in Geita district, was selected as a typical representative of most artisanal gold mines in Tanzania. The specific objectives of the study are described in detail in section 1.4.1, and include:

- To establish the current status of mercury use and environmental contamination at Mugusu artisanal gold mine and explore the level of awareness among gold miners on the effects of mercury on the environment and human health;
- To asses the ecotoxicity of Hg contaminated sediment from artisanal gold mining impacted watersheds in Tanzania using sediment assays with a benthic invertebrate;
- To study the ecotoxicity of Hg contaminated sediment from Mugusu artisanal gold mine impacted watershed (river Mabubi) on resident fauna using the early life stage assay with an endemic fish species;
- To study the influence of fish consumption patterns on Hg exposure and associated health risk to local human communities ;
- To establish the contribution of animal food products produced by animals reared in and around artisanal gold mining areas, to the Hg exposure of local populations;

- To estimate mercury exposure to people residing in artisanal gold mining settlements through food intake by using a Total Diet Study (TDS) approach;
- To monitor current Hg exposure to gold miners at the Mugusu mine through head hair analysis.

1.3. Literature review

1.3.1. History of use of mercury in gold mining and environmental contamination

Gold is probably the first metal to be known to the early hominids. They found it as nuggets and spangles in the soils and stream sands, and were undoubtedly attracted by its intrinsic beauty, great malleability, and virtual indestructibility (Aysen, 2003). The principal source of gold in primitive times were streams, although there is considerable evidence in certain gold belts (e.g. Egypt and India (Kolar) that eluvial deposits, auriferous gossans, and the near surface parts of friable (oxidized) veins were mined. The eluvial and alluvial placers were worked in the crudest manner by panning or the simplest form of sluicing. The first record of gold mining dates back to 4000 BC. During antiquity, most gold on the world market came from Egypt, the Balkans, Anatolia, and India (Rafal Swiecki; 2002). Gold was recovered principally by crushing and washing. Crushed ores were ground into powder form and was subsequently shaken by water. After shaking, quartz powder would form a foam layer and the settled sludge would contain gold (Rafal Swiecki; 2002).

The Romans were the first to develop the method of amalgamation for gold recovery as an alternative to the ancient water shaking method. With this method, sands or ground ores containing gold were mixed with metallic mercury to produce gold amalgam and the mixture was then put into leather bags. Amalgam would leak out of the bag and the mixture would be kept inside. This method was used extensively for many centuries by Spanish colonizers in South America. It is estimated that between 1550 and 1880, nearly 200,000 tons of mercury was released into the South American environment by this technology (Malm, 1998). Because of lack of better technologies and capital, wherever there is ore with microscopic gold particles gold has been purified as in the old days by mercury amalgamation. This practice is still wide spread in the artisanal gold mines in most developing countries.

UNIDO (2004) estimated that the number of artisanal gold miners has increased to approximately 15 million people worldwide. Since 1998, annual gold production from artisanal gold mines has constituted 20 to 30% of the global gold production (UNIDO, 2004). This report identified artisanal gold mining in more than 50 countries (Table 1-1).

Table 1-1: Countries with small scale gold mining by 2003

Country	Reference	Country	Reference
Benin	Yager *et al.*, 2002	**Malaysia**	Priester and Hentschel, 1992
Bolivia	Maurice-B *et al.*, 2000	**Mali**	MMSD, 2002
Brazil	Veiga, 1997	**Mauritania**	Mbendi, 2004
Burkina Faso	ILO, 1999	**Mexico**	Veiga, 1997
Burundi	Priester and Hentschel, 1992	**Myanmar**	UNESCAP, 2003
Cambodia	Sotham (2001)	**Mongolia**	Tumenbayar *et al.*, 2001
Central R. Congo	Yager *et al.*, 2002	**Mozambique**	Zacarias and Manuel, 2003
Chad	Mobbs, 1996	**Nicaragua**	Veiga, 1997
Chile	Silva Bruna, 2001	**Niger**	Alfa, 2000
China	Gunson and Veiga, 2004	**Nigeria**	Priester and Hentschel, 1992
Colombia	Veiga, 1997	**Papua New Guinea**	Crispin (2003)
Costa Rica	Veiga, 1997	**Peru**	Hruschka and Medina, 2001
DRC	ILO, 1999	**Philippines**	Drasch *et al.*, 2001
Dominican Republic	Veiga, 1997	**Russia**	Appel *et al.*, 2003
Equador	Veiga 1997	**Rwanda**	Priester and Hentschel, 1992
Ethiopia	Labonne, 2002	**Senegal**	Bermudez –Lugo, 2002
French Guyana	Frèry *et al.*, 2001	**South Africa**	Mahlatsi and Guest, 2003
Gabon	Priester and Hentschel, 1992	**Sudan**	Ibrahim, 2003
Gambia	Dolley, 1996	**Suriname**	Veiga (1997)
Ghana	Babut *et al.*, 2003	**Tanzania**	van straaten, 2000a
Guinea	Labonne, 2002	**Togo**	Yager *et al.*, 2002
Guinea-Bissau	Dolley, 1996	**Uganda**	World Bank, 2003
Guyana	Couture and Lambert, 2003	**Venezuela**	UNIDO, 2004
Honduras	Veiga, 2003	**Vietnam**	Trung, 2001
India	Siddaiah, 2001	**Zambia**	Dreschler, 2001
Indonesia	Veiga, 2003	**Zimbabwe**	Shoko and Viega, 2003
Ivory Coast	Yager *et al.*, 2002		
Kenya	Yager, 2002		
Kyrgyzstan	Appel *et al.*, 2003		
Lao PDR	Boungnaphalon, 2003		
Lethoto	Coakley, 2002		
Liberia	Babut *et al.*, 2003		
Madagascar	Shoko and Viega, 2003		
Malawi	Drescheler, 2001		

Source: UNIDO, 2004

Artisanal and small-scale gold mining is an essential activity in many developing countries as it provides an important source of income, particularly in rural regions where economic alternatives are limited. The term 'artisanal mining' encompasses small, medium, informal, legal and illegal mining activities which use rudimentary processes to extract mineral substances worldwide (Priester *et al.*, 1993). The choice of extraction technique is what differentiates conventional and artisanal mining. An artisanal miner is driven by the need to survive; to feed his/her family and to pay bills. Such an operation is based on instinct; there is no previous "classical" geological exploration, proven reserves, ore tonnage estimates or engineering studies. Despite the small-scale and individual nature of the artisanal mining activities, the resulting environmental impacts can be considerable. When a large number of individuals excavate a single site, the resulting pit diameter can be as large as 2 km. This is for example the case in Serra Pelada, an infamous artisanal mining site in the Brazilian amazon where, during the 1980's, more than 80,000 miners gathered to extract manually about 90 tonnes of gold from the same open pit (Veiga, 1997).

1.3.2. Amalgamation and mercury releases

Although the use of Hg in mineral processing is illegal in most countries, amalgamation is the preferred method employed by artisanal gold miners. This is because Hg is easy to use, available and inexpensive. Its normal liquid state has a unique capacity to form an amalgam or bind with most metals except iron and platinum (UNEP, 2002). The wetting of gold by mercury is not alloying, but a phenomenon of moderately deep sorption, involving some interpenetration of the two elements (UNEP, 2002). As the surface tension of mercury is greater than that of water, but less than that of gold, Hg adsorbs onto the surface of gold particles. In addition, mercury acts as a dense medium; gold sinks into the mercury while the lighter gangue material floats on top. When the resulting amalgam is heated, the mercury vaporizes and gold is left on the heating plate. Mercury is usually discharged with tailings and/or volatilized into the atmosphere (Gunson and Veiga, 2004).

1.3.3. Current sources of mercury to the market

Despite a decline in global mercury consumption (global demand is less than half of that of 1980 levels), supply from competing sources are still available. Currently, Spain, China, Kyrgyzstan and Algeria dominate mercury mining with several mines being state-owned (UNEP, 2002). There are also reports of small-scale, artisanal mining of mercury in Russia (Siberia), Outer Mongolia, Peru and Mexico. Large quantities of mercury have come onto the market as a result of ongoing substitution and closing of mercury-based chlor-alkali production in Europe and other regions.

Market analysis indicates that 700 to 900 metric tons per year of recycled mercury (corresponding to about 30 percent of the recorded primary production) has been marketed globally since the mid 1990's, of which the majority originated from chlor-alkali production facilities. It is possible that within the current decade and beyond, vast supplies of mercury will become available from conversion or shutdown of chlor-alkali facilities using the mercury process, as many European countries envisage r a phase-out of this process before 2010 (UNEP, 2002). In most cases, Hg enters developing countries through legal channels for legitimate use (e.g. dental filings), but is then diverted to artisanal gold mining operations (Veiga *et al.*, 2004).

1.3.4. Mercury chemistry and transformation in the environment

1.3.4.1. Chemistry

Mercury occurs naturally in the environment and exists in a large number of forms commonly designated as "species". Like lead or cadmium, mercury is a constituent element of the earth, with an average abundance of approximately 0.05 $\mu g\ g^{-1}$ in the earth's crust, with significant local variations. Mercury ores that are mined generally contain about one percent mercury, although the strata mined in Spain typically contain up to 12 to 14 percent mercury (Lin and Pehkonen, 1999). While about 25 principal mercury minerals are known, the only deposits that have been harvested for the extraction of mercury are cinnabar (mercuric sulphide). Mercury is also present at very low levels throughout the biosphere. Its absorption by plants may account for the presence of mercury within fossil fuels like coal, oil and gas, since these fuels are formed from geologic transformation of organic residues (Lin and Pehkonen, 1999). In pure form, it is known alternatively as "elemental" or "metallic" mercury (also expressed as Hg (0) or (Hg0). Mercury is rarely found in nature as pure liquid metal, but rather within compounds and inorganic salts. Mercury can be bound to other compounds as monovalent or divalent mercury (also expressed as Hg (I) and Hg (II) or Hg^{2+}, respectively). Many inorganic and organic compounds of mercury can be formed from Hg (II). Elemental mercury is a shiny, silver-white metal that is a liquid at room temperature and is traditionally used in thermometers and some electrical switches. If not enclosed, at room temperature some of the metallic mercury will evaporate and form mercury vapours. Mercury vapours are colourless and odourless. The higher the temperature, the more vapours will be released from liquid metallic mercury. Some people who have inhaled mercury vapours report a metallic taste in their mouths (Siblerud, 1990).

The metallic form is refined from mercuric sulphide ore by heating the ore to temperatures above 540°C. This vaporises the mercury which are then captured and cooled to form the liquid metal mercury. Inorganic mercuric compounds include mercuric sulphide (HgS), mercuric oxide (HgO) and mercuric chloride ($HgCl_2$). These mercury compounds are also called mercury salts. Most inorganic mercury compounds are white powders or crystals, except for mercuric sulphide, which is red and turns black after exposure to light. Some mercury salts (such as $HgCl_2$) are sufficiently volatile to exist as an atmospheric gas. However, the water solubility and chemical reactivity of these inorganic (ionic) mercury gases lead to much more rapid deposition from the atmosphere than for elemental mercury (UNEP, 2002).

When mercury combines with carbon, the compounds formed are called "organic" mercury compounds or organomercurials. There is potentially large number of organic mercury compounds (such as dimethylmercury, phenylmercury, ethylmercury and methylmercury). However, by far the most common organic mercury compound in the environment is methylmercury. Like the inorganic mercury compounds, both methylmercury and phenylmercury exist as "salts" (for example, methylmercuric chloride or phenylmercuric acetate). When pure, most forms of methylmercury and phenylmercury are white crystalline solids. Dimethylmercury, however, is a colourless liquid (UNEP, 2002).

Being an element, mercury cannot be broken down or degraded into harmless substances. Mercury may change between different states and species in its cycle, but its simplest form is elemental mercury, which in itself is harmful to humans and the environment. Once mercury has been liberated from either ores or from fossil fuel and mineral deposits hidden in the earth's crust and released into the biosphere, it can be highly mobile, cycling between the earth's surface and the atmosphere. The earth's surface soils, water bodies and bottom sediments are thought to be the primary biospheric sinks for mercury (UNEP, 2002).

1.3.4.2. Mercury transformation in the atmosphere

The atmospheric speciation plays an important role in the long-range transport of mercury, as well as in the deposition mechanisms. In the atmosphere, most mercury (95 to 99 percent) exists as gaseous Hg. The remainder is generally comprised of gaseous divalent mercury and mercury associated with particulates (Lin and Pehkonen, 1999). Gaseous methylmercury may also exist in air at measurable concentrations, especially near mercury emissions sources. Oxidized and particulate

mercury are more likely to be deposited than Hg (0) because they are more soluble in water and are scavenged by precipitation more easily. As a result, oxidized and particulate forms of mercury are thought to comprise the majority of deposited mercury, even though they make up only a few percent of the total amount of mercury in the atmosphere (Lindberg, 1998). Wet deposition is thought to be the primary mechanism for transporting mercury from the atmosphere to surface waters and land (Lindberg, 1998). In the Great Lakes area in the US, for example, wet deposition is believed to account for 60 to 70 percent of total mercury deposition (Eric *et al.*, 2005).

1.3.4.3. Mercury transformation in the aquatic environment

The important processes which affect Hg mobility in the aquatic environment are shown in Figure 1.1. Methylmercury is formed in the aquatic environment by microbial metabolism (biotic processes) and by chemical processes that do not involve living organisms (abiotic processes). The formation of methylmercury in aquatic systems is influenced by a wide variety of environmental factors. The efficiency of microbial mercury methylation generally depends on factors such as microbial activity (Gilmour *et al.*, 1992) and the concentration of bioavailable mercury (rather than the total mercury pool), which in turn are influenced by parameters such as temperature, pH (Miskimmin *et al.*, 1992), redox potential and the presence of inorganic and organic complexing agents (Ullrich *et al.*, 2001). Certain bacteria also demethylate mercury which decreases levels of methylmercury, thereby forming some natural constraints on the build-up of methylmercury in certain environments (Pak and Barther, 1998). Bacterial demethylation has been demonstrated both in sediments and in the water column of freshwater lakes. Degradation of methylmercury and phenylmercury by freshwater algae has also been described (Ullrich *et al.*, 2001).

Purely chemical methylation of mercury is also possible if suitable methyl donors are present (Weber, 1993). The relative importance of abiotic versus biotic methylation mechanisms in the natural aquatic environment is not fully understood, but it is generally believed that mercury methylation is predominantly a microbially mediated process (Ullrich *et al.*, 2001). Methylmercury is the predominant mercury species in fish. The US EPA states in a mercury overview paper that in most adult fish, 90 to 100 percent of mercury content is methylmercury (Hill *et al.*, 1996). As a consequence, the US EPA recommends that the cheaper total mercury chemical analysis should be used for the evaluation of the risks from consuming local fish. They further suggest that total Hg concentrations should be used as if mercury was present as 100 percent methylmercury in order to be most protective of human health.

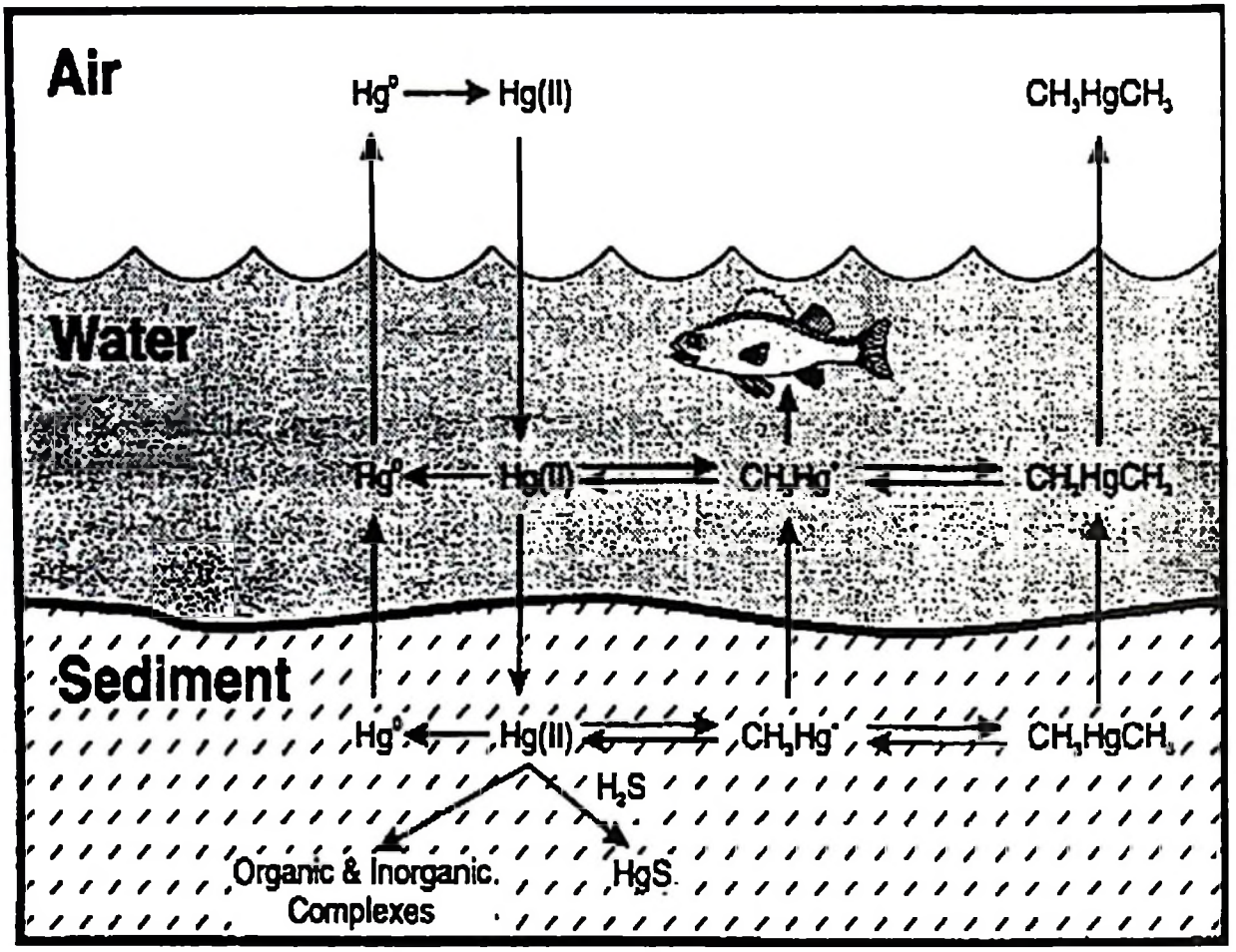

Figure 1.1. Important processes which affect the mobility of mercury in aquatic systems (source: US EPA, 1997)

1.3.4.4. Mercury species and transformation in soil

Mercury that is found in soil originates from either airborne, geogenic or anthropogenic (Schlüter, 2000). Air borne mercury includes Hg (II) and Hg (0) which is deposited on the soil surface by dry and wet deposition. Geogenic soil mercury includes Hg (II), Hg (I) and Hg (0) which is the product of recent release from ore deposits and bedrock by oxidation and weathering, or from geothermal sources (Schlüter, 2000). Soil conditions are usually favourable for Hg to form inorganic or organic complexes. This complexing behaviour controls to a large extent the mobility of mercury in soil. Much of the mercury in soil is bound to bulk organic matter and is susceptible to wash out in runoff only when attached to suspended soil or humus. For these reasons mercury has a long retention time

in soil and as a result, the mercury accumulated in soil may continue to be released to surface waters and other media for long periods of time, possibly hundreds years (Schuster, 2004).

1.3.5. Mercury toxicity

1.3.5.1. Effects to human

The toxicity of mercury to humans and other mammals depends on its chemical form. Thus symptoms and signs are rather different in individuals exposed to elemental mercury, inorganic mercury compounds, or organic mercury. The sources of exposure are also markedly different for the different forms of mercury. For alkylmercury compounds, among which methylmercury is by far the most important, the major source of exposure is the diet, especially fish and other seafood (Dallinger *et al.*, 2004; Björnberg *et al.*, 2005). For elemental mercury vapour, the most important source for the general human population in developed countries is dental amalgam. The WHO and several US federal and health and research agencies have confirmed that dental amalgam (i.e. an inexpensive alloy of silver, copper, tin and 50 percent mercury) is the largest source of human exposure to elemental mercury for those who have amalgam filings (ATSDR, 1999). The lungs rapidly absorb 75 to 85% of elemental mercury vapours coming from dental amalgam (Mackert and Berglund, 1997). Laboratory tests have shown that the average person with dental amalgam gets 10 times as much daily mercury exposure as the average person without amalgam fillings. Depending on the number of amalgam surfaces in a person's mouth, the average daily absorption of mercury is between 3 and 17 micrograms of Hg (ATSDR, 1999).

In most developing countries; exposure at work exceeds that of dental amalgam (Jack *et al.*, 2007). Weak enforcement of labour rights in developing countries places a disproportionate burden of mercury contamination on their people. Lenient environmental laws in much of the developing countries means that even in more formal industrial sectors, occupational exposure to mercury is a persistent threat to human health. For example, according to environmental and community groups in Kodaikanal, a hill station in southern India, 10 people died and dozens others were poisoned from mercury inhalation at the local thermometer factory run by a subsidiary of the multi-national giant Unilever. Mercury levels inside the plant were reportedly 600 times greater than internationally accepted safety limits (Rajgopal, 2003). A similar incident happened in a Thor chemicals facility in KwaZulu-Natal, South Africa. Workers were systematically exposed to elemental mercury for over a decade before the government finally stepped in and shut down the plant (Barratt *et al.*, 2002). Millions of people who are engaged in artisanal gold mining, where they use mercury to extract gold

from unwanted sediment, are also exposed to mercury (van Straaten, 2000). The use of skin-lightening creams and soaps that contain mercury and use of mercury for cultural/ritualistic purposes or in traditional medicine can also result in substantial exposures to inorganic or elemental mercury. For example, use of mercury containing soaps and cosmetics was reported to be the main exposure route for women in Kenya (Harada *et al.*, 2001) and Tanzania (Harada *et al.*, 1999).

When exposed to elemental mercury, either via water, air, or food, the human body absorbs only 0.1%. This contrasts with an absorption efficiency of 90% to 100% for methylmercury through the gastrointestinal tract. Mercury has an extended residence time in the human body, i.e. from a minimum of 50 days to more than 120 days (ASTDR, 1999). Acute mercury poisoning, such as that seen in the Minamata Bay (Japan) incident, can lead to paralysis and death (Klein and Goldberg, 1970). Chronic exposure to lower levels of Hg also results in debilitating health effects, including nervous system and kidney damage and genetic effects. The toxicological effects of Hg are now also considered as a contributing factor in some types of autism (Bernard, *et al.*, 2002). Children are impacted disproportionately when exposure occurs during foetal stage. Mercury readily passes the placental barrier and concentrates in the red blood cells of the foetus to levels 30% higher than those in the mother. A pregnant woman could be exposed to high levels of mercury and not demonstrate any symptoms. Yet, her child could suffer debilitating effects from the accumulated mercury exposure. As such, pregnant women who consume contaminated fish can compromise normal child development. After birth, infants are also susceptible to exposure to mercury from their mother through breast milk. Because of the extended residence period of mercury within the human body and its negative health effects on the foetus, both pregnant women and women of childbearing age are considered sensitive populations for possible mercury exposure (Davidson *et al.*, 2006; Desjardins *et al.*, 2004). Studies from one human population exposed to methylmercury in fish product also suggest an association with increased incidence of cardiovascular diseases (Salonen *et al.*, 2000; Virtanen *et al.*, 2007).

1.3.5.2. Ecotoxicological effect of mercury on the aquatic ecosystem

1.3.5.2.1. Effects on fish and aquatic invertebrates

In general, salts of mercury and its organic compounds are more toxic to aquatic organisms than salts of other heavy metals (Eisler and Hennekey, 1977). The highest Hg concentrations in fish occur in the blood, spleen, kidney and liver, and may exceed those in muscle by a factor of 2 to 10 (Robert *et al.*, 1996). The toxicity of Hg varies, depending on the fish's characteristics (e.g. species,

life stage, age and size), environmental factors (e.g. temperature, salinity, dissolved oxygen, water hardness and the presence of other chemicals) and the form of available mercury. In particular, early life stages exhibit greater sensitivity to elevated metal concentrations than later life stages. The toxicity of Hg compounds to fish tends to increase with temperature. Organo mercury compounds, such as methylmercury, generally are much more acutely toxic than Hg^{2+} to aquatic organisms (Niimi and Kissoon, 1994). General effects of Hg on fish include death, reduced reproduction, impaired growth and development, behavioural abnormalities, altered blood chemistry, impaired osmoregulation, reduced feeding rates and predatory success, and effects on oxygen exchange.

The inorganic mercury LC_{50} values for fish range from 30 µg/L for guppies to 1,000 µg/L for the Mozambique tilapia (US EPA, 1985). The symptoms of acute Hg^{2+} poisoning in fish include increased secretion of mucous, flaring of gill opercula, increased respiration rate, loss of equilibrium and sluggishness. Signs of chronic poisoning include emaciation, brain lesions, cataracts, inability to capture food, abnormal coordination and various erratic behaviours (e.g. altered feeding behaviour) (Weis and Weis, 1977). It has been shown that histological changes and effects on behaviour, reproduction and development can occur at water concentrations as low as 0.12 to 28 µg/L (Wiener and Spry, 1996). Methylmercury has been showed to affect growth; development and interfere with hormonal functioning of juvenile walleye at a concentration of 1.0 µg g^{-1}ww (Friedmann *et al.*, 1996). For Hg^{2+}, acute values (LC_{50}) for invertebrates range from 2.2 µg/L for the cladoceran *Daphnia pulex* to 2,000 µg/L for the larval stages of insects (US. EPA, 1985). Examples of some specific toxicity values for fish and aquatic invertebrates are provided in Table 1-2 and 1-3.

Table 1-2. Effects of inorganic mercury to selected species of aquatic organisms

Taxonomic group/Species	Life stages	Effect [a]	Concentration (µg Hg/L)	Ref. [b]
Protozoans				
Ciliate (*Uronema marinum*)	N.R	LC_{50} (24 h)	6.0	10
Crustaceans				
Crayfish (*Orconectes limosus*)	N.R	LC_{50} (30 d)	2.0	1
Daphnid (*Daphnia magna*)	N.R	LC_{50} (96 h)	5.0	1
Daphnid (*Daphnia magna*)	N.R	LC_{50} (life time)	1.3-1.8	1
Scud (*Gammarus pseudolimnaeus*)	N.R	LC_{50} (96 h)	10.0	1
Molluscs				
Rainbow mussel (*Villosa iris*)	N.R			
Glochidia	Juveniles (2 months, not fed)	LC_{50} (96 h)	99.0	2
Glochidia	Juveniles (2 months, fed)	No deaths in 21 d	114.0	2
Annelids				
Sandworm (*Nereis virens*)	Adults	LC_{50} (168 h)	60.0	8
Polychaete (*Capitella capitata*)	Larva	LC_{50} (96 h)	14.0	1
Echinoderms				
Starfish (*Asterias rubens*)	Adults	LC_{50} (96 h)	60.0	8
Coelenterates				
Coral (*Porites asteroids*)	Colonies	3 to 6 colonies dead in 72 h	180.0	9
Fish				
Zebrafish (Brachydanio rerio)	Embryo larvae	No deaths	<2.0	3
Goldfish (*Carassius auratus*)	-	LC_{50} (96 h)	122.0	4
Catfish (*Clarias lazera*)	Adults	LC_{50} (96 h)	720.0	5
Catfish (*Clarias lazera*)	Adults	LC_{50} (24 h)	960.0	5
Mosquitofish (*Gambusia affinis*)	Adults	LC_{77} (10 d)	1000.0	6
Largemouth bass (*Micropterus salmoides*)	Embryo larvae, static test	LC_{50} (8 d)	188.0	7
Largemouth bass (*Micropterus salmoides*)	Embryo larvae, (flow through test)	LC_{50} (8d)	5.3	7
Rainbow trout (*Oncorhynchus mykiss*)	Juveniles	LC_{50} (96 h)	155.0 – 200.0	1
Rainbow trout (*Oncorhynchus mykiss*)	Embryo larvae (static test)	LC_{50} (28 d)	4.7	7
Rainbow trout (*Oncorhynchus mykiss*)	Embryo larvae (flow through test)	LC_{50} (28 d)	<0.1	7
Brook trout (*Salvelinus fontinalis*)	N.R	LC_{50} (life time)	0.3-0.9	1

[a] Abbreviations: h = hours; d = days

[b] References: (1) USEPA, 1985; (2) Valent *et al.*, 2005; (3) Dave and Xiu, 1991; (4) Birge *et al.*, 2000; (5) Hilmy *et al.*, 1987; (6) Diamond *et al*, 1989; (7) Birge *et al.*, 1979; (8) Esler and Hennekey, 1977; (9) Bastidas and Garcia, 2004; (10) Parker, 1979.

N.R. Not reported

Table 1-3. Effects of organic mercury (MeHg) to selected species of aquatic organisms

Taxonomic group/Species	Life stages	Effect [a]	Concentration (µg Hg/L)	*Ref.*[b]
Planarians				
Flatworm (*Dugesia dorotocephala*)	Adult	LC_{50} (10 d)	500	1
Crustaceans				
Daphnid (*Daphnia magna*)	N.R.	LC_{50} (lifetime)	0.9-3.2	2
Midges				
Chironomus riparius	Larva	LC_{50} (48 h)	316-1800	3
	Larva	LC_{50} (96 h)	400-547	4
Fish				
Rainbow trout	Larva	LC_{50} (96 h)	24	2
	Juvenile	LC_{50} (96 h)	5.0-42.0	2
	Sub adult	LC_{50} (48 h)	34	5
Brook trout	Yearling	LC_{50} (96 h)	65	2
Air breathing catfish (*Clarias batrachus*)	Adults	LC_{50} (96 h)	430	6
Blue gourami (*Trichogaster* sp)	Adults	LC_{50} (96 h)	70	7

[a] Abbreviations: h = hours; d = days

[b] References: (1) Best *et al*, 1981; (2) USEPA, 1985; (3) Qureshi *et al*, 1980; (4) Rossaro *et al*, 1986; (5) Niimi and Kissoon, 1994; (6) Kirubagran and Joy, 1988; (7) Hamasaki *et al*.,1995

N.R. Not reported

1.3.6. Bioaccumulation and biomagnifications of Hg

Because of the long residence time of Hg in the body of biota, it has a tendency to bioaccumulate and biomagnify along the food chain. As MeHg is absorbed more efficiently than Hg^{2+} from water and food and is retained longer, it bioaccumulate more in organisms (Hill *et al.*, 1996). This ability of MeHg to biomagnify through a food chain is a primary cause for much of the concern with mercury. Concentration of MeHg at each level in a food chain, from bacteria to plankton to tiny crustacea, small fish, larger fish, and fish-eaters, organisms take in more mercury than they excrete thereby accumulating the excess in their organs. Thus the ultimate concentration in any organism is higher than the mercury concentration in its food. This results in elevated concentrations of mercury in higher trophic (feeding) levels of the food chain. Thus, Hg concentrations are comparatively elevated in fish eating fishes, birds and mammals (Langlois *et al.*, 2005). These concentrations can be harmful to the organism itself, or to predators of those organisms. Table 1-4 illustrates a hypothetical pattern of biomagnification, through which an infinitesimally low concentration of mercury (parts per trillion in water) can reach biologically dangerous concentrations (parts per million) in larger predators including humans. Three important factors which modify Hg uptake and influences its bioaccumulation in aquatic organisms are the age of the organism, water pH and the dissolved organic carbon content. In fish, for example, Hg tends to accumulate in muscle tissues of fish and to increase with age, weight, or length of the fish (Gorski *et al.*, 2003). It has also been

shown that Hg concentrations in fish tend to be higher in acidic lakes than in neutral or alkaline lakes (Grieb *et al.*, 1990). Mercury concentration in fish muscle also is positively correlated with dissolved organic carbon concentration (Sorensen *et al.*, 1990).

Table 1-4. Biomagnification of MeHg in a hypothetical aquatic system

Trophic level	Concentration
Water	1 ng/L = 1 ppt
Bacteria and phytoplankton	10 ng/kg of water = 10 ppt
Protozoan/zooplankton	100 ng/kg = 100 ppt
Insect larvae	1 µg/kg = 1 ppb
Fish fry	10 µg/kg =10 ppb
Minnows	100 µg/kg =100 ppb
Medium sized fish	1 mg/kg = 1 ppm
Large predators (fish, birds, humans)	10 mg/kg = 10 ppm

1.3.7. Human exposure assessment

Exposure assessment has been defined by the WHO as the qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agents via food as well as exposure from other sources if relevant (World Health Organization, 1997). The term 'exposure assessment' is often replaced by 'intake assessment' when considering only food as exposure route. Dietary intake of contaminants can be estimated in different ways. The most direct estimate is by measuring concentrations in duplicate diets collected over a certain time. This is a technique in which equivalent portions of all foods and beverages consumed by an individual are collected for direct analysis in order to estimate the individual's intake of energy, nutrients, and/or other food components (Cameron & Van Staveren, 1988). However, dietary intake is usually estimated by models combining data on food consumption with concentration data measured in foods and food groups (World Health Organization, 2007). The latter method involves three important steps:

1. The collection of food consumption data;
2. The collection of contaminant data; and
3. An appropriate method for combining both sources of data and assessing the intake

1.3.7.1 Consumption data

In principle, to assess food consumption four different types of data can be used:

1. Food supply data;
2. Data from household consumption surveys;
3. Data from dietary surveys of individuals;
4. The collection of duplicate diets (Kroes *et al.*, 2002b).

The dietary surveys can be conducted in four different four ways as follows; (1) Food records (also called dietary records or food diaries) are kept for a specified time period, usually 1 to 7 days. If total daily intake is required, the food records should include all foods and beverages consumed at meals and in between, in quantified amount (Kroes *et al.*, 2002b; Willett, 1998). (2) In the 24-hour recall method the subject is asked by a trained interviewer to recall and describe the kinds and amounts of all foods and beverages ingested during the immediate past, mostly a 24- or 48-hour period. Food quantities are usually assessed by using household measures, food models, or photographs (Kroes *et al.*, 2002b; Willett, 1998). (3) A food frequency questionnaire (FFQ) consists of a structured list of individual foods or food groups. The aim of the FFQ is to assess the frequency with which these items are consumed during a specified period (e.g. daily, weekly, monthly, yearly). FFQs may be qualitative, semi-quantitative or completely quantitative. Qualitative FFQs only obtain the usual number of times each food is eaten during a specified period. Semi-quantitative methods allow estimation of a standard portion or ask respondents to indicate how often they consume a specified common amount. A quantitative FFQ allows the respondent to indicate any amount of food typically consumed. The FFQ is often used to rank individuals by food or nutrient intakes and also by food group intakes so that high and low intakes may be studied (Kroes *et al.*, 2002b; Willett, 1998). (4) With the aid of a dietary history method, a trained interviewer assesses an individual's total usual food intake and meal pattern. The respondent is asked to provide information about his/her pattern of eating over an extended period of time (often a typical week) and also to recall the actual foods eaten during the preceding 24 hours. In addition, the interviewer completes a checklist of foods usually consumed. Finally as a cross-check, the respondent is often asked to complete a three-day estimated record (Kroes *et al.*, 2002b; Willett, 1998).

However, it should be noted that there is no single ideal method to assess food consumption. The choice depends on the objectives of the study, the foods of primary interest, the need for group versus individual data, the characteristics of the population, the time frame of interest, the level of specificity needed for describing foods, and available resources. Currently, most methods to assess

food consumption are not developed explicitly from the perspective of risk assessment and the available data are used for other purposes than the original ones as well (Kroes *et al.*, 2002b).

1.3.7.2 Contaminant concentration data

A key component in contaminant intake assessment is the collection of quantitative data being accurate (i.e. agreeing with the actual concentration) and representative (i.e. reflecting the concentration of the whole group). In order to collect data, two different strategies can be applied. First, contaminant concentrations can be measured in representative samples. Such an approach is scientifically preferable, but very expensive and laborious. Samples can be collected on the base of a representative sampling plan or by the application of a duplicate diet approach or a total diet study. The latter methodology is recommended by the FAO and WHO for estimating dietary exposure of the population. In total diet studies, representative samples of widely consumed foods are collected and analyzed for the constituents of interest (Kroes *et al.*, 2002b). Alternatively, data found in scientific literature, existing databases, and published reports can be used.

1.3.7.3 Methodologies for intake assessment

In its simplest form, the model to represent dietary intake/exposure can be considered as:

Consumption x (Concentration or Residue) = Dietary exposure.

There are, two main approaches for combining the consumption data with contaminant concentrations: deterministic modelling and probabilistic modelling.

1.3.7.3.1 Deterministic modelling

Deterministic modelling involves using a point estimate of each variable within the model (Vose, 1996). In the context of intake assessment, the term 'deterministic modelling' refers to a method whereby a fixed value for food consumption (such as the average or high level consumption value) is multiplied by a fixed value for the concentration and the intakes of all sources are then summed.

In a schematic way, it can be summarized as follows:

$\mu(X) \times \mu(C) = \mu(Y)$ ***Mean scenario***

$Max(X) \times Max(C) = Max(Y)$ ***Worst case scenario***

With X= consumption of a certain food item or food group, C= concentration of the considered compound in that food item, Y= exposure to the considered compound via the considered food item or food group.

Deterministic modelling is commonly used as a first step in exposure assessment because it is relatively simple and inexpensive to carry out. However, this approach does not provide insight into the range of possible exposures that may occur in a population and it also obscures the ability to determine which scenarios present a risk that is likely to occur (Kroes *et al.*, 2002b).

1.3.7.3.2. Probabilistic modelling

In contrast to the deterministic approach, probabilistic modelling involves incorporation of the variability and/or uncertainty for the different parameters. As such, it takes into account all the possible values that each variable could take and weights each possible model outcome by the probability of its occurrence (Vose, 1996). The probabilistic analysis for intake assessment, requiring appropriate modelling software, permits the exposure assessor to consider the whole distribution of exposure, from minimum to maximum, with all modes and percentiles (Kroes *et al.*, 2002b). More advantages and disadvantages of deterministic and probabilistic modeling in exposure assessments are given in Table 1-5.

Table 1-5. Advantages and disadvantages of deterministic and probabilistic risk assessments

Type of risk assessment method	Advantages	Disadvantages
Deterministic risk assessment	Uses upper-bound assumptions to ensure protection of human health	Results in a single-point estimate of risk
	Employs a consistence approach and standard reporting methods	Provides little insight into the range of risks
	Requires less time to complete	Lacks information about variability in the potentially exposed population
	Can be easily understood and communicated	Addresses uncertainty in a qualitative manner
	Is based on standard equations and exposure assumptions	
	Is consistent with historical risk assessment practices	
	Can be used as a screening tool	
Probabilistic risk assessment	Provides a range of risk estimates	Requires investment of time and resources for additional data collection review
	Provides quantitative information on variability and uncertainty	Requires good information on the probability density functions
	Identifies the drivers of risk and exposure by quantitative sensitivity analysis	Possesses less transparency and clarity than deterministic method unless distributions are accurately determined
	Provides more information to decision makers than deterministic method	Makes risk management decision more challenging
	Can help to identify data gaps	Possesses the potential for lack of consistency among different sites
	Provide confidence limits on the risk estimates	Requires extensive use of statistics, possibly limited by available software
	Uses a wide variety of site- specific information	Must conform to limitations on the interpretation and application of results
		More difficult to communicate the results to regulators, stakeholders, and risk managers

Source: USEPA, 1999

1.4. Conceptual framework of the study

1.4.1. Objectives of the study

The emissions, distribution, fate and eventual effects to human health and the environment of Hg emitted from artisanal gold mining activities in the Lake Victoria goldfields in Tanzania are complex and poorly understood. It is assumed that apart from Hg that is directly inhaled by miners during amalgam roasting, most Hg released from amalgamation and amalgam roasting eventually ends up in the agricultural lands and watersheds. In these agricultural areas, Hg accumulates and concentrates into food and pasture plants. As indicated earlier mercury that reaches the aquatic systems can undergo methylation and bioaccummulate in living organisms. High concentrations of Hg can affect the aquatic ecosystem and cause loss of biodiversity. In turn, human populations can be exposed to Hg that is accumulated in food crops, animal products and food of aquatic origin through the dietary route (Figure 1.2). The main objective of this study was therefore to assess the environmental and human health risk of Hg released from artisanal gold mining activities. Detailed descriptions of the specific objectives of this study are given below.

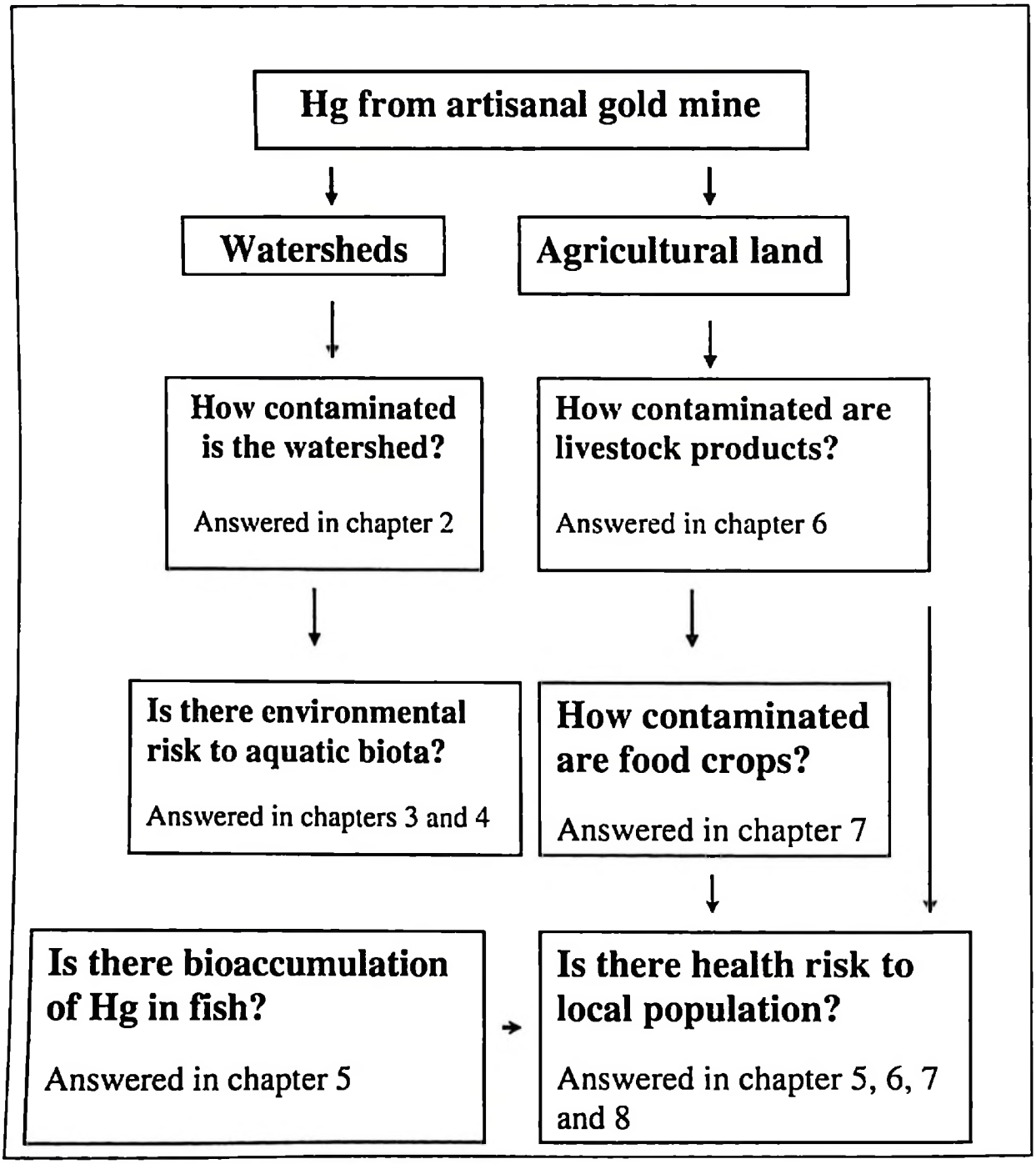

Figure 1.2. A simplified conceptual diagram illustrating environmental distribution and possible target systems of Hg from artisanal gold mines in Tanzania

- The efforts to understand and regulate the use of Hg in artisanal gold mines in Tanzania are hampered by lack of current information on mercury use and environmental contamination in the various mining centres. For most centres the available limited data were collected ten years ago and are exclusively about technical and/or contamination issues without an environmental and human risk dimension. These data also lack information about the miners' awareness and knowledge on environmental and human health issues. Thus, the first objective for this study was **to establish the current status of mercury use and environment contamination at Mugusu mine as a representative model (case) for artisanal mining in Tanzania. Additionally, we aimed at exploring the level of awareness and knowledge of miners about the effects of Hg on the environment and human health.**

- The available data on Hg contamination to watersheds draining artisanal gold mines in Tanzania show that Hg accumulates more in sediment than in the water column (Kondoro and Mikidadi, 1998; van Straaten, 2000a: Kahatano and Mnali, 1997). To date, there has been no study to establish whether the measured Hg concentrations in sediments can adversely affect sediment dwelling fauna. It remains unclear if sediments in these watersheds are adversely affected or simply contaminated with Hg. So, the second objective of this study was **to evaluate the toxicity of sediment bound mercury and establish the toxicity of sediment collected from Mugusu mine impacted watershed (river Mabubi) to the resident fauna.**

- Tanzania, like most other African countries does not have its own guidelines and environmental health standards or limits for the mercury contents in fish intended for human consumption. Instead, it uses standards formulated by the World Health Organisation (WHO) which proposes not to consume fish containing Hg levels $\geq 0.5\ \mu g\ g^{-1}$ ww. Previous studies which attempted to estimate the human health risk of consuming fish caught from watersheds located near gold mines in Tanzania were done by comparing the Hg concentration in fish and the WHO limits. These studies concluded that, there is no health risk in eating fish caught from these watersheds in Lake Victoria basin (Ikingura and Akagi, 1996; Machiwa, 2005). However, the human health risks associated with consuming fish contaminated with mercury are related to the fish consumption frequency, its duration and

the concentration of mercury in fish. This requires site-specific data which were not collected and accounted for in the previous tudies. Considering that the actual fish consumption patterns (type, frequency, quantity) of poor local communities which subsist on fishing and fish consumption in Tanzania are inadequately known, it is uncertain if the limits set by the WHO are protective to the health of such populations. The third objective of this study was therefore to **estimate the health risk of consuming Hg contaminated fish to local communities by using a deterministic risk analysis method.** For this study, Nungwe village which is located approximately 10 km from Mugusu mine was selected as a case study.

- People from communities residing around artisanal gold mining predominantly subsist on crop farming and livestock keeping (Kitula, 2006). In this area, domestic animals like cattle, fowl and ducks are allowed to graze/feed freely around mines and drink water from Hg contaminated rivers and ponds. To date, there is no information on the contribution of consuming animal products produced from animals reared in and around artisanal gold mines to the Hg exposure of residents. The fourth objective of this study was to **establish Hg residues in meat from cattle and domestic fowl reared in and around artisanal gold mines in the Geita district, and explore the possible public health implications.**

- It has been reported that food crops grown near artisanal gold mines or in swamps draining the artisanal gold mines accumulate mercury (LVEMP, 2002). Surprisingly, studies to estimate Hg exposure and health risk from consuming Hg contaminated foods in artisanal gold mines have been limited to exposure from consumption of Hg contaminated fish (Ikingura and Akagi, 1999; Machiwa, 2003). The World Health Organisation (WHO), however, recommends the use of Total Diet Studies (TDS) to assess contaminant total intake. This methodology takes into account different categories of consumed food and the effects of kitchen preparation on the levels of contaminants in the foods. The fifth objective of this study was **to estimate Hg exposure of residents of the Mugusu mining village by using TDS method.**

- The most recent information on the extent of Hg exposure to artisanal gold miners at Mugusu mine was collected about ten years ago (Ikingura and Akagi, 1996; Harada *et al*, 1999). It was deemed important to establish the current concentrations of mercury in the

body of these miners. The sixth objective of this study was therefore **to perform a monitoring study to establish the current level of Hg exposure to gold miners at Mugusu mine. This was accomplished using the largest head hair analysis study ever performed in this mine.**

1.3.2. Overview of the thesis

In addition to this introductory and literature review chapter this doctoral thesis consists of seven chapters followed by general conclusions, recommendations and future research needs.

In **Chapter 2** the current status of mercury use and environmental contamination at the Mugusu mine is explored and the level of awareness among miners on effects of mercury on the environment and human health is established.

In **Chapter 3** the toxicity of mercury to sediment dwelling organisms is studied, with the aim of predicting the toxicity of artisanal gold mining contaminated sediment to aquatic organisms. The benthic midge *Chironomus riparius* is used in an assay with artificial sediments spiked with Hg. Survival, growth and emergence of exposed organisms are used as end points. The results from this experiment are compared with Hg concentrations measured in artisanal gold mining impacted streams and rivers in Tanzania.

In **Chapter 4** the actual toxicity of Hg contaminated sediment from Mugusu artisanal gold mine impacted river (Mabubi) to the local fauna is established. Ecotoxicity test are performed with the early life stages of African catfish (*Clarias gariepinus*), a resident fish species in the study area. Embryo hatching success, larval survival and growth are used as endpoints.

In **Chapter 5** mercury exposure and the associated health risk to local human populations through consumption of Hg contaminated fish are estimated. Mercury intake is estimated as the product of Hg concentration in consumed species of fish and the respective quantity of fish eaten (fish intake). The health risk is estimated by comparing calculated weekly Hg intake with the WHO/FAO recommended Provisional Tolerable Weekly Intake (PTWI) of 1.6 µg/kg bwt/week.

In **Chapter 6** the contribution of consuming animal products, obtained from animals reared in or near artisanal gold mines to the overall Hg exposure is established. This chapter, reports on the concentration of mercury residues in free grazing cattle and domestic fowl reared in gold mining villages in the Geita district and discusses the public health implications.

In **Chapter 7** a Total Diet Study (TDS) approach is used to estimate mercury dietary intake from consuming food stuffs collected from the Mugusu mining village. Mercury intake was estimated as the product of Hg concentrations in all consumed food categories and food intakes.

In **Chapter 8** the current mercury body burden of gold miner's at Mugusu mine was measured using head hair monitoring study. The obtained Hg concentrations of miners were compared with previous findings and the associated human health risk evaluated

After chapter 8 there are sections for the general conclusion and the summary.

Chapter two

Mercury use and environmental contamination in the Mugusu artisanal gold mine in Tanzania

2.0. Mercury use and environmental contamination at the Mugusu artisanal gold mine in Tanzania

Abstract

Environmental mercury contamination associated with artisanal gold mining and processing causes environmental and human health problems in Tanzania. Studies performed ten years ago at Mugusu mine in the Geita district established that the uncontrolled use of Hg by miners leads to extensive contamination of the environment. In the present study we used a structured questionnaire and focussed group discussions to examine the miners' perception and understanding of the potential effects of mercury to human health and the environment. To assess the magnitude of environmental contamination, soil, water and sediment samples were collected and their mercury content was measured. Results from the present study indicate that, miners at Mugusu mine continue to use mercury carelessly resulting in an annual use of approximately 1460 kg of elemental mercury. Sediment and water samples collected within 3 km downstream from the gold mine contained up to 2.3 μg Hg g^{-1} dw and 1.05 μg Hg/L respectively. Soil samples collected near residential houses contained mercury concentration of 1.2 μg Hg g^{-1} dw. This study also established that, miners lacked proper knowledge about environmental and health effects associated with mercury pollution. It is recommended that to reduce mercury pollution from artisanal gold mining, miners should be educated on the effects of mercury on human health and the environment as well as safe mercury handling and disposal practices.

Key words: Artisanal gold mining, Mercury, Environmental contamination, Miners' perception, Human health

Redrafted from

R.T. Chibunda, and C.R. Janssen

Continued mercury use and environmental contamination at the Mugusu artisanal gold mine in Tanzania: Journal of Cleaner Production: To be submitted

2.1. Introduction

As explained in chapter one of this thesis, artisanal gold miners use mercury (Hg) amalgamation for extracting gold from the mined ore (van Straaten, 2000a). Not withstanding its economic benefits to poor people in Tanzania, artisanal gold mining has been the subject of strong opposition due to its potential adverse environmental and human health effects. The environmental and health effects of mercury use have been well documented in the literature (Ikingura *et al.*, 1997; Appleton, *et al.*, 2005; UNEP, 2002). The potential long-term effects on human health include impairment of the nervous system that can lead to neurobehavioral disturbances. Human exposure to mercury can occur through direct inhalation of mercury vapour or by eating mercury contaminated aquatic and terrestrial food (UNEP, 2002). Additionally, it is known that in artisanal gold mines, large quantities of noxious mercury vapour are released and inhaled by the miners during amalgamation and amalgam roasting (Hilson *et al.*, 2006).

In 2004, it was estimated that about 450,000 people were engaged in artisanal gold mining in Tanzania (UNIDO, 2004). One of the main areas with artisanal gold mining is the southern part of Lake Victoria. These mines are widespread in the goldfields of the Geita, Kahama, Musoma, Mugumu, Tarime, and Biharamulo districts. The Mugusu mine is one of the active artisanal gold mines in the Geita district. To the best of our knowledge the studies to assess the extent of mercury use and environmental contamination at the Mugusu mine were published ten years ago (Kondoro and Makundi, 1998; Ikingura and Akagi, 1996). Although, results from these studies provided insight into the extent and severity of mercury contamination in this area there have been no recent studies to establish the current magnitude of this problem. The objective of the study described in this chapter was to establish the current status of mercury use and environmental contamination in and around the Mugusu mine. We also explored the level of awareness among miners about mercury related effects on human health and the environment. To achieve these objectives, structured questionnaires and focussed group discussion were used to collect information about mining practices and community perception on the effects of mercury. To asses the magnitude of local environmental Hg contamination, soil, sediment and water samples were collected from the area surrounding Mugusu mine.

2.2. Material and methods

2.2.1. Description of the study area

Mugusu mine is found in the Geita district which is located south-west of Lake Victoria in Tanzania (Figure 2.1). It is situated between latitude 2° 28'- 3° 28' south and longitude 32° to 32° 45' east. Mugusu mine is located approximately 25 km north-west of Geita town, 10 km from the Geita–Biharamulo trunk road. It is located in the Geita forest reserve and on the edge of the Geita Gold Mine (GGM) concession. On the north of the mine lies the river Mabubi that is used for processing of ore from the gold mine and drawing water for domestic use. The river is very shallow, with depths varying between 0.5 meters to 3 meters depending on the season and level of precipitation. This river drains the mine into Nungwe Bay of Lake Victoria that is situated approximately 13 km downstream the mine. Oral history from village elders suggests that the present gold mining rush started in 1987 after the discovery of gold in the Saragura – Mugusu hill area. By 1989/90 the population of Mugusu mining village had grown from zero to about 10,000 inhabitants (Mwaipopo *et al.*, 2004). The present population is estimated to be around 6,500 persons.

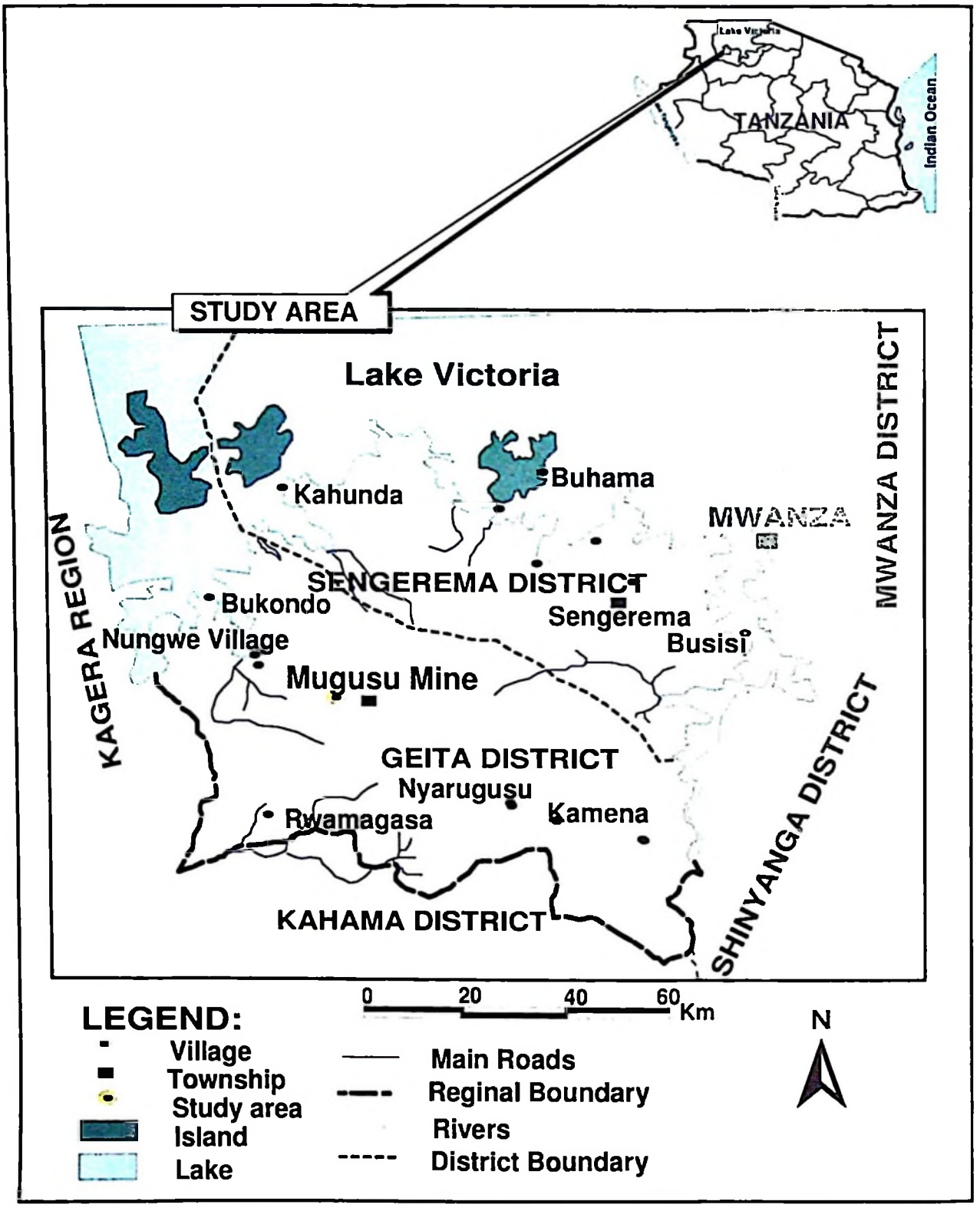

Figure 2.1. A map of the Geita district indicating the location of Mugusu mine

2.2.2. Collection of information on mining practices

For this study, structured questionnaire was used to collect information about the mining activity and knowledge on mercury effects on the environment and human health. The inclusion of persons into the survey was based on their age being above 18 years old. All selected respondents were involved in mining activities and were visited either at home or at work. The interviewer was able to speak both Swahili (national language) and the interviewee's vernacular languages (Sukuma and Zinza).

Additionally, focus group discussions were carried out to explore participant's insight perceptions of the effects of mercury on the environment and public health. Eight groups of six members each drawn from those involved in the survey above were involved in the focus group discussion. During the discussion a pair-wise matrix was drawn up to allow symptoms which respondents associated with Hg poisoning to be ranked according to their importance. This allowed the respondents to debate and if necessary vote by a show of hands which of the two symptoms being discussed was more diagnostic of Hg poisoning. For example, in Table 2-1 the symptom of chest pain was compared with fever, sneezing, hand tremor and headache. Chest pain ranked first as it was felt to be more diagnostic of Hg poisoning than others as indicated by its highest frequency (4) of its serial number '1' in the matrix where the column and row bisect. Similarly, hand tremor was felt more diagnostic than fever, sneezing or headache.

Table 2-1. An example of pair wise ranking matrix

	Symptoms	Chest pain	Hand tremor	Fever	Sneezing	Headache	**Rank**
1	Chest pain	x	x	x	x	x	**1st**
2	Hand tremor	1	x	x	x	x	**2nd**
3	Fever	1	2	x	x	x	**5th**
4	Sneezing	1	2	4	x	x	**3rd**
5	Headache	1	2	5	4	x	**4th**
	Frequency	**4**	**3**	**0**	**2**	**1**	

2.2.3. Collection of sediment, soil and water samples

Sediment and water samples were collected from five locations (points) along the river Mabubi, which flows along the mine and is intensively used for gold processing. The sampling points were arbitrary identified as A, B, C, D and E. Point A was located two kilometres upstream the gold mine and was used as a reference point. Points B, C and D were located at a distance of three, six and nine kilometres downstream from the gold mine, respectively. Point E was located at the river mouth i.e. where it enters Lake Victoria (Figure 2.2). At every sampling point, water sample was directly collected into 0.25 L 10 N nitric acid washed glass jars and acidified by HCl (0.4% v/v) before sampling sediment. About three kilograms of superficial sediment (~5cm) was collected by using a core sampler, and put into 10 N nitric acid washed glass jars, kept in cool boxes with ice and transferred to the laboratory. Additionally, two samples of soil were collected from areas near residential houses and two samples of water were collected from the amalgamation ponds.

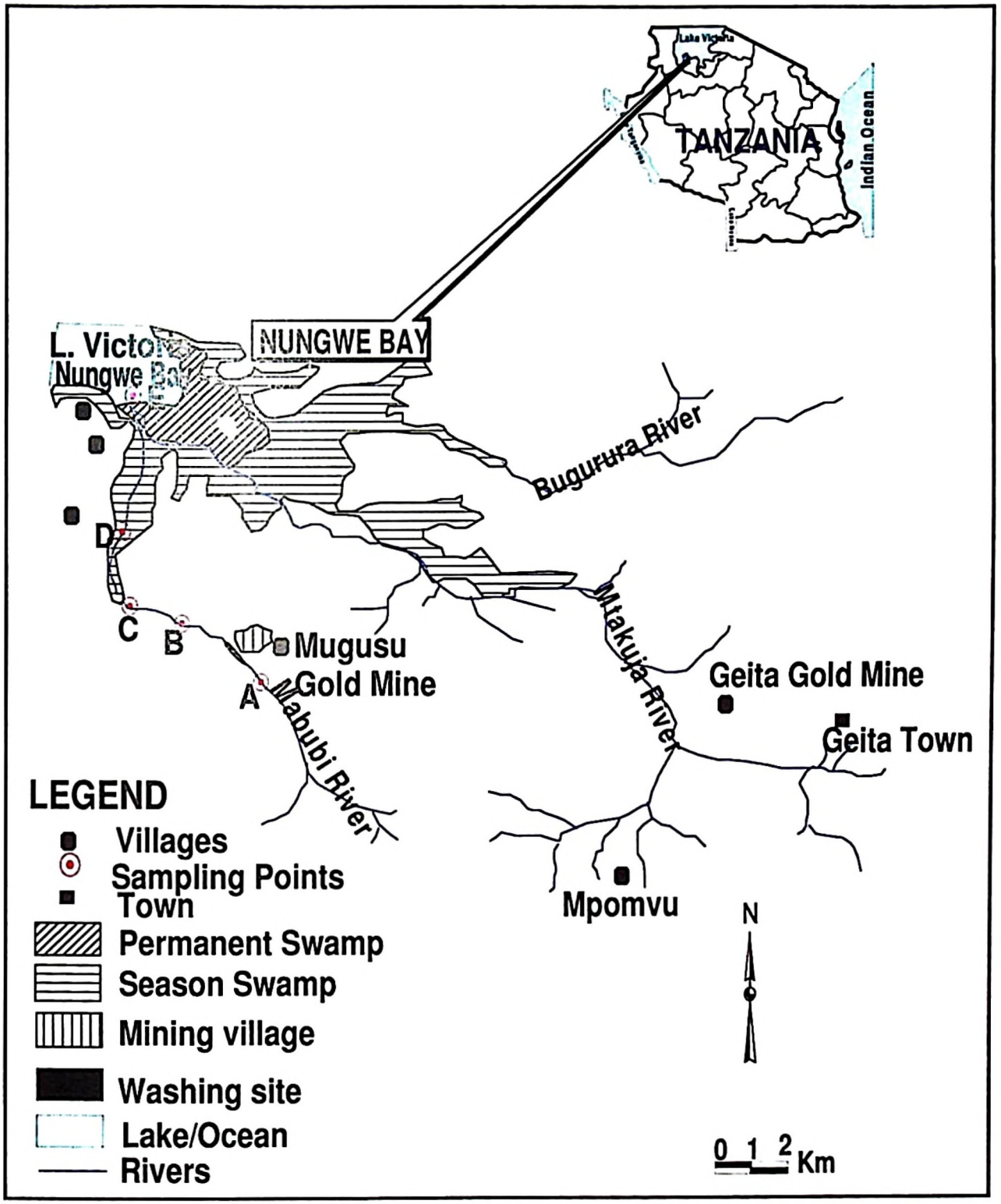

Figure 2.2. Map of Geita district indicating the sediment and water sampling points along river Mabubi

2.2.4. Chemical analysis of sediment, soil and water samples

Total mercury analysis in sediment, soil and water samples was performed at the Southern and Eastern Africa Mineral Centre (SEAMIC) in Dar es Salaam, Tanzania. Briefly, 10 ml of filtered water (0.45 µm) was mixed with 2 ml of 1:1 H_2SO_4 to 2% potassium permanganate solution. The mixture was allowed to stand for 15 minutes; subsequently 0.5 ml of 5% potassium sulphate was added. The mixture was heated in a water-bath at 95°C for 1 hour. After cooling, the 3% hydroxylamine solution was added drop wise, until the permanganate colour discharged completely. Five ml of the digested sample was acidified, using 5 ml 6M HCl. Samples were then analysed for total mercury by Atomic Absorption Spectrophotometer with cold vapour generation technique (ICP Ultima 2, Horiba Jobin Yvon, France). Sediment and soil samples were air dried in an air conditioned room set at 25°C and 65% relative humidity. Sediments were further dried in an oven at 45°C for 48 hours and then milled using an agate planetary micro-mill (Fritsh) from Laval lab - Canada. The resulting fine dried powder was used for digestion. 0.5 g of sediment was mixed with 4.5 ml concentrated HCl and 1.5 ml concentrated HNO_3 in a graduated test tube and digested on a water-bath at 70°C. Subsequently 5 ml of samples was mixed with 10 ml 6M HCl in a 50 ml test tube. After adding 1ml of KI-ascorbic acid solution, the mixture was vortexed before measuring. Acid washed glassware, analytical grade reagents and double distilled and deionised water were used in the analysis. In order to check purity of the chemicals used, one blank was run every 10 samples. There was no evidence of contamination in these blanks. Analytical quality control was ensured through the analysis of replicates of Hg standard solutions and the control reference material [BCR-580 for mercury with Hg 132 ± 3 µg g^{-1} from European Commission DG Joint Research Centre (IRMM)]. The recovery percentage was 84% the results were therefore not corrected for recovery. All samples were analysed in duplicates. The detection limit was 0.02 µg g^{-1} and 0.02 µg/L for sediment and filtered water respectively.

2.2.5. Statistical analysis

The questionnaire data were summarized in Microsoft spreadsheet files. Proportions and means for the various variables were computed by using Statistic for Social Studies Package (SSSP) software.

2.3. Results and discussion

2.3.1. Findings from the survey

2.3.1.1. Socio-economic characteristics of the respondents

Table 2-2 details the demographic information of the individuals interviewed in the survey. A total of 148 respondents participated in the survey. Sixty five percentage of the respondents were male. The small percentage of females interviewed (35%) is due to the fact that core mining activities are male-oriented (Kitula, 2006). Most females who reside in the mining villages engage in the activities like cooking, selling food and fetching water. The age of the respondents ranged from 18 to 65 years, with 40% clustering between 30 to 45. Most of the respondents (74%) had primary school education, 15% reported to have attained a secondary school and 11% with no formal education. The finding that 85% of the respondents had no/or only primary school education is an indication that artisanal mining, by its nature of being less profitable and dangerous, attracts mainly the less educated workers. Because of the low level of industrialization in Tanzania, individuals with low or no education have few job opportunities and are thus mainly employed in artisanal mining and peasantry agriculture. The respondents were involved in diverse mining activities, as follows; pitting (30%), crushing (10%), Sluicing (27.5%), amalgamation (17.5%), and amalgam roasting (15%).

Table 2-2. Demographic characteristics of respondents at Mugusu artisanal gold mine during a survey about the miners' perception on the effect of mercury on the environment and human health.

Variable	Number (percentages)
Gender	
Male	96 (65)
Female	52 (35)
Age category	
18-22	12 (8)
23-30	33 (22.3)
31-45	65 (44)
>45	38 (25.7)
Education	
No formal education	16 (11)
Primary education	109 (74)
Secondary education	23 (15)

2.3.1.2. Perception of the local community on the effect of mercury on human health and the environment

Seventy two percent of the respondents agreed that use of mercury could be harmful to human health. Forty three percent (43%) of the respondents were able to mention some symptoms associated with mercury poisoning, such as general body weakness and tremor. While 64% of male respondents mentioned effects associated with mercury, only 4% of the female did. The reason for this difference might be that most visitors/researchers to mining villages tend to talk more to male miners than to women. In addition, local leaders who are predominantly men after attending training seminars rarely share information with other miners, and when they do it is always with males (Kitula, 2006).

Many respondents (75%) mentioned mercury vapour inhaling during amalgam roasting as the common exposure route and only 15% of the respondents associated mercury exposure with eating mercury contaminated fish. Skin contact with mercury was not mentioned by the respondents as one of the route for mercury poisoning. It was surprising that much as large proportion of respondents mentioned mercury vapour inhalation as one of the exposure route they were not using protective gears during amalgam roasting. Many miners were encountered roasting amalgams on open air without a mask or any other protective device to reduce inhalation of mercury vapour (Figure 2.3). Furthermore, during focus group discussion the research team was informed that for security reasons many miners preferred roasting amalgam inside their respective houses (huts). In-house amalgam roasting exposes miners and their families to high levels of mercury vapour due to limited ventilation. van Straaten (2000a) measured mercury up to 324 $\mu g\ g^{-1}$dw in the walls of houses/huts where amalgam roasting was carried out at the Kantente mine, Kahama district. Mercury residues in these houses continue to pollute the in-house environment and poison people who use these houses long after the amalgam roasting. The small percentage of respondents who associated mercury exposure with eating contaminated fish is very worrying as it is well established that this is the major route of mercury exposure to human beings (JECFA, 2003). Results from this survey show that respondents had limited knowledge about possible negative effects of mercury on the environment, as no respondent linked mercury pollution to contamination of agricultural land or effects on aquatic and terrestrial biodiversity. The respondents obtained this information from a United Nation Industry Development Organization (UNIDO) project (4%), newspapers and radio

(11%), African Medical Research Foundation (AMREF) staff (12%), workshops and seminars (8%) and their friends (65%).

2.3.2. Findings from focus group discussion

2.3.2.1. General findings

During the in-depth discussion it was reported that the human population in the Mugusu mining settlement is currently estimated to be 6,423 living in 1,012 households. The mine is privately owned by the Chipaka family and the mining activities are carried out through sub-leasing small plots to pit owners who carry out mining activities in return for a share of the profit. The methods used for gold extraction and processing involve mainly manual methods, which consists of using simple and rudimentary equipment like hand hoes, chisels and shovels to open the soil and dig out the ore. Detailed description of the method is given in van Straaten (2000a). Briefly, after removing the mineralized bearing material (ore), the ore is finely crushed using diesel driven ball mills (Figure 2.4). The crushed ore is then concentrated in sluice boxes which are lined with sisal sacks or other fibre materials (Figure 2.5). The soil concentrate obtained is then amalgamated with mercury to trap fine gold from the ore pulp. A piece of cloth is used to squeeze off excess mercury by twisting it and leaving behind the amalgam. Once the amalgam is obtained it is burned in open air to evaporate mercury (Figure 2.3). To confine and prevent dispersion of Hg contaminated sediments and water into the river, amalgamation is carried out in concrete ponds (Figure 2.6).

Figure 2.3.Amalgam roasting in open air at Mugusu mine

Figure 2.4. Ball mill at Mugusu mine

Figure 2.5 Sluicing boxes at Mugusu mine

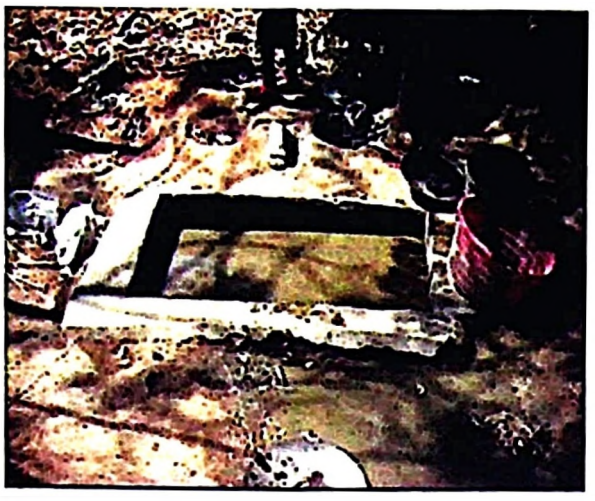

Figure 2.6. Amalgamation pond at Mugusu mine

2.3.2.2. Perception on the effect of mercury on human health and the environment

During pair wise ranking of symptoms linked to mercury poisoning, miners ranked the symptoms most associated with mercury use in the following order: chest pain, persistent coughing, hand tremor, general body weakness, sneezing and fever (Table 2-3). From these results it is interesting to note that symptoms like exaggerated emotion and coloration of the gums which are typical of mercury poisoning (ATSDR, 1999) were ranked last by respondents. Persistent coughing and chest pain which were ranked first and second respectively, appeared to be common illnesses among miners. Such symptoms may have high prevalence among miners due to lung related diseases rather than Hg poisoning. High incidences of lungs diseases in mining communities are somehow related to poor working and living conditions in the mines. Miners work in a dusty environment and live in over crowded houses which make them vulnerable to infections like tuberculosis (TB) and silicosis (Eisler, 2005). Similar findings have recently been reported in Ghana, where most of the interviewed artisanal miners reported experiencing frequent coughing and chest pain (Hilson *et al.*, 2006). These findings indicate that much as most miners perceive mercury as the major cause of their frequent illnesses, they lack knowledge of the actual symptoms of mercury poisoning.

Table 2-3. Results of the pair wise ranking of the symptoms associated with mercury poisoning

	Symptoms	Fever	Persistence coughing	Sneezing	Chest pain	General body weakness	Hand tremor	Exaggerated emotions	Coloration of the gums	Rank
1	Fever	x	x	x	x	x	x	x	x	6th
2	Persistence coughing	2	x	x	x	x	x	x	x	2nd
3	Sneezing	3	2	x	x	x	x	x	x	5th
4	Chest pain	4	4	4	x	x	x	x	x	1st
5	General body weakness	5	2	4	4	x	x	x	x	4th
6	Hand tremor	6	2	6	6	5	x	x	x	3rd
7	Exaggerated emotions	1	2	3	4	5	6	x	x	7th
8	Coloration of the gums	1	2	3	4	5	6	7	x	8th
Frequency		2	6	3	7	4	5	1	0	

.When miners were asked if mercury exposure was possible through skin contact, many of them believed that mercury could not penetrate intact skin. This perception may be widespread among miners as many of them handle mercury with bare hands during panning, and some spent a long time standing in mercury contaminated water and sediments in the amalgamation ponds (Figure 2.7). Chemical analysis of water samples collected from two of these ponds indicated average mercury levels of 44 ± 5.3 µg/L. Contrary to local community perception, scientific evidence shows that mercury does penetrate the intact human skin at a rate of 0.01 to 0.04 ng Hg per cm^2 per min (Hursh *et al.*, 1989). It is possible that the amount of mercury penetrating the skin can be even higher when lesions/wounds are present (Hursh *et al.*, 1989). On the other hand, miners agreed that mercury from gold mining activities contaminates water sources which are vital for livestock and domestic use; however, similar to the results from the questionnaire survey miners did not link mercury pollution to contamination of agricultural land or effects on aquatic and terrestrial biodiversity. The lack of accurate knowledge and understanding on the ability of Hg to cause environmental effects in this study suggests that there has not been coordinated effort from the government and other stakeholders to educate the mining communities. It is also possible that,

results from previous scientific studies were not communicated to the local people. For example, Taylor *et al.* (2005) conducted and published a full assessment of mercury contamination in the Rwamagasa mine, but there was no indication if the findings were reported to the local population.

2.3.3. Estimation of the amount of mercury used at Mugusu mine

To estimate the amount of mercury used daily at the Mugusu mine, the amalgamation ponds were used as a unit of operation because it is at these ponds where the introduction of mercury into the process takes place. It was established that there are 25 amalgamation ponds on the mine, with each pond being used by an average of six miners. Miners measure and buy mercury in 350 ml Coca Cola bottle lids/caps (coke caps) (Figure 2.8). It was established that depending on the amount of gold extracted, miners use an average of one to three coke caps per day for each amalgamation pond. The quantity of mercury used was calculated by subtracting the weight of 30 coke caps weighed with and without mercury. The average mercury weight in one coke cap was 50.2 ± 3.2 g. Therefore, a total amount of 1255 to 3765 g of mercury per day was used for the 25 amalgamation ponds. To validate this information, an interview was conducted with the three major mercury sellers in the mine. It was established that in total they sell approximately 2500 g of mercury per day. Because mercury was sold very informally, it become clear that some more mercury is purchased from Geita town by individual miners or is brought in the mine by gold dealers and clandestinely sold to miners. This extra amount of mercury can explain the observed difference in amount between mercury used at the amalgamation ponds and the quantity sold by the main mercury dealers.

Figure 2.7. Panning of gold at Mugusu mine. Note: the miners'legs are resting in Hg contaminated water.

Figure 2.8. A coca cola bottle lid (coca cap) (arrow) for measuring elemental mercury.

2.3.4. Environmental contamination with mercury

Table 2-4 shows the measured Hg concentrations at the sampling points along the Mabubi river. Elevated levels of mercury in water and sediment were observed immediately downstream of the gold mine and decreased further downstream. Sediment samples collected from point A, B, C, D and E contained 0.03, 2.3, 1.6, 0.23 and 0.08 µg Hg g^{-1} dw respectively. Mercury concentration in the water samples collected from point A and E were below the detection limit of the analytical technique. Mercury in water samples collected at B, C, and D were 1.05, 0.67, and 0.05 respectively. Soil samples collected near the village houses contained a mercury concentration of 1.2 ± 0.87 µg g^{-1} dw.

Table 2-4. Average concentrations of total mercury in sediment and water (µg/L) ±SD collected along Mabubi river.

Sampling points	*A*	*B*	*C*	*D*	*E*
Sediment (µg g^{-1} dw) ± SD)	0.03 ± 0.00	2.3 ± 0.36	1.6 ± 0.02	0.23 ± 0.01	0.08 ± 0.00
Water (µg/L ± SD)	n.d	1.05 ± 0.07	0.67 ± 0.01	0.05 ± 0.00	n.d

n.d = Hg not detectable at the detection limit (0.02 µg/L)

The mercury concentrations in water sample collected from point B (1.05 µg/L) marginally exceeded the Tanzania standard for drinking water (1 µg/L) (TBS, 2003). The mercury levels in sediment at points B, C and D are higher than the recommended minimum toxic threshold proposed in the sediment quality criteria used in Canada of 0.13 to 0.14 µg Hg g^{-1} (Gaudet *et al.*, 1995; Garron *et al.*, 2005). The consensus sediment quality guideline recommended by Macdonald *et al.* (2000) of 1.06 µg Hg g^{-1} for sediments is also exceeded at points B and C. It is therefore suggested that sediments in the river Mabubi may have adverse effects on benthic organisms and the water immediately downstream of the mine can have adverse effect on human health if used for drinking.

Presence of high levels of Hg around Mugusu mine can be attributed to poor mercury management practices observed throughout the gold extraction process. During our field visits it was found that most amalgamation tanks were leaking (Figure 2.5) thus releasing mercury contaminated water into the river. Similarly, piles of mercury contaminated tailings were dumped close to the river bank, where they could easily be washed into the river during heavy rains and flooding. Mercury concentrations up to 1414 µg g^{-1} were measured in tailings collected at the Mwakitolya mine (van Straaten, (2000a). High mercury concentrations analyzed in the samples collected near the residential houses can be due to aerial deposition from mercury vapours emanating from amalgam burning.

2.4. Conclusion

In this study, information on artisanal gold miners' perception about the effect of mercury on human health and the environment at Mugusu mine was collected. Additionally, the current status of artisanal gold mining, mercury use and environmental contamination at the Mugusu mine was established. Results derived from this study are significant and in agreement with those reported by Hilson *et al.* (2006) for Ghana. They concluded that despite the large number of studies which have been conducted on mercury accumulation in the biota and the environment the use of Hg in artisanal gold mining has not declined. The present study has demonstrated that mercury is also still widely used at Mugusu mine, and that because of careless handling and poor disposal facilities it is contaminating the surrounding environment. Human beings, mostly miners inhale mercury vapour during amalgam roasting. It is assumed that the resistance of miners to reduce mercury use might be linked to their lack of understanding about long-term environmental and human health effects. It is therefore, recommended that interventions aimed at improving the knowledge of miners on (1)

effects of Hg on the environment and human health should be conducted and (2) skills on safe mercury handling and disposal practices should be taught.

Chapter three

Chronic toxicity of mercury to the benthic midge *Chironomus riparius*

3.0 Chronic toxicity of mercury to the benthic midge *Chironomus riparius*

Abstract

The results obtained in Chapter 2, indicate that in the Mabubi river Hg accumulates more in sediment. However, due to the limited information available on the ecotoxicological effects of mercury on sediment dwelling organisms it is difficult to predict the toxicity of these sediments. The present study evaluated the effects of inorganic mercury ($HgCl_2$) spiked sediment on the survival, growth and emergence of the midge *Chironomus riparius*. The obtained results were compared to environmental mercury concentrations reported in artisanal gold mining impacted streams and rivers in Tanzania. Mercury significantly reduced the larval survival and number of emerged midges at concentration of 3.84 $\mu g\ g^{-1}$ dw in comparison to the control sediment ($P<0.05$). The growth of the larvae was significantly inhibited at 2.42 μg Hg g^{-1} dw, while emergence of *C. riparius* midges was significantly delayed at 0.93 μg Hg g^{-1} dw. The results from the present study indicate that mercury delayed emergence of *C. riparius* at concentrations lower than those measured up to six kilometres downstream of the Mugusu mine in river Mabubi. Furthermore, mercury affected other life history characteristics of *C. riparius* such as survival and growth at lower concentrations than those which are occurring in sediments collected from other rivers and streams impacted by artisanal gold mining in Tanzania. It is concluded that *Chironomus* species and probably other benthic fauna living in these watersheds experience adverse effects from Hg exposure.

Key words: mercury, artificial sediment, *Chironomus riparius*, survival, growth and emergence

Redrafted from:

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Chronic toxicity of mercury to the benthic midge Chironomus riparius: Ecotoxicology: Submitted

3.1. Introduction

Mercury is a persistent, highly toxic element that bioaccumulates and biomagnifies in aquatic food webs (US EPA, 1997; Watras *et al.*, 1998). Since the beginning of the industrial era, the concentration of mercury in the global environment is estimated to have increased three fold (EU Report, 2004). Recently there have been numerous reports from developing countries on the contamination of rivers and natural streams by Hg released from artisanal gold mining (Kahatano and Mnali, 1997; LVEMP, 2002; Limbong *et al.*, 2003; Ikingura *et al.*, 2006). It has been reported that in most areas Hg accumulates in the sediment (Ikingura *et al.*, 2006), where it can easily bioaccummulate in benthic organisms or even biomagnify (Benoit *et al.*, 1998).

Surprisingly, the information on the distribution of Hg in the various environmental compartments exceeds by far the available information on its effects on the organisms residing in these compartments. Except for limited ecotoxicological information on pelagic organisms such as fish, (Grippo and Health, 2003; Virginia *et al.*,1982), daphnids (Qureshi *et al.*,1980) and rotifers (Teresa *et al.*, 2004), there is almost no ecotoxicological information on the effects of Hg to benthic organisms. Moreover, the available information is mainly reporting on the toxicity of mercury to benthic organisms exposed to field collected sediments (Winger *et al.*, 1993; Sferra *et al.*, 1999). Due to presence of multiple toxicants in natural sediments, results from such experiments fail to demonstrate the actual contribution of Hg to the sediment toxicity.

The present study evaluated the effect of inorganic mercury ($HgCl_2$) in artificial sediments on the survival, growth and emergence of the non biting midge *Chironomus riparius* (Diptera: Chironomidae) and compared the obtained effect concentrations to the Hg levels observed in sediments sampled from rivers and streams in artisanal gold mining impacted areas in Tanzania.

Chironomidae were chosen for this study as these organisms are important inhabitants of freshwater benthic habitats and have a world wide distribution in both lentic and lotic habitats (Péry *et al.*, 2002). They feed on sediment-deposited detritus (Rasmussen, 1984) and therefore play a key role in organic matter cycling in aquatic ecosystems. Their life cycle consists of an egg stage, four larval stages, and a pupal stage (all of which are aquatic) as well as a short-lived aerial adult stage. At 20°C, *C. riparius'* life cycle is typically completed within three to four weeks which makes this species convenient for full life-cycle testing. This species is recommended as model test organism

for sediment toxicity testing by various environmental regulatory bodies (ASTM, 1992; OECD, 2004).

3.2. Materials and methods

3.2.1. Sediment preparation

The artificial sediment was prepared according to the method described in the OECD (2004) document "Sediment-water chironomid toxicity testing using spiked sediment". The composition of the sediment was as follows (on a dry weight basis): 5% finely ground sphagnum moss peat (particle size ≤1 mm); 20% kaolin clay and 75% sand (< 200 µm). Deionized water was added to obtain a final moisture content in the range of 30-50%. $CaCO_3$ (obtained from Merck Darmstadt -Germany) was added to adjust the pH of the final mixture to 7.0 ± 0.5. Total Organic Carbon (TOC) was 2.5% of the dry weight and the concentration of Acid Volatile Sulphide (AVS) was below the detection limit (0.06 µmol/g dry weight).

3.2.2. Sediment spiking with $HgCl_2$

The mercury stock solution (0.7 g Hg/L) was prepared by using analytical reagent grade $HgCl_2$ (Merck-Germany) in double deionized water. For each individual concentration artificial sediment was thoroughly mixed with the appropriate amount of stock solution in acid washed plastic containers. Overlying water (distilled and double deoinized) was carefully poured on top of the sediment to achieve 1 sediment: 3 water ratio and then the samples were stored in the dark at 4°C for seven days, after which the overlying water was discarded. The spiked sediments were subsequently thoroughly mixed and aliquots of 100 g were dispensed into 1L test beakers and 175 ml of EPA-medium was carefully added in such a way that disruption of the sediment layer was minimal. The EPA-medium was prepared by adding 60 mg $CaSO_4.2H_2O$, 122.425 mg $MgSO_4.7H_2O$, 96 mg $NaHCO_3$ and 4 mg KCl (Merck –Germany) to 1L of deionized water, resulting in moderately hard water with a hardness of approximately 85 mg/L as $CaCO_3$ (US-EPA, 1985). Using the same procedure control sediments (without $HgCl_2$) also were prepared. The tested nominal Hg concentrations included 0 (control), 1, 1.8, 3.2, 5.6, 10 and 18 µg Hg g^{-1} dry weight (dw). The selection of the final test concentrations was based on results from our earlier range finding tests.

3.2.3. Pore water extraction

Pore water was extracted by centrifugation of the wet sediments at 2054g for 30 minutes by using a Sigma–3E-1 centrifuge (Hartz,. Germany).

3.2.4. Analysis of mercury in sediment and pore water

The total mercury concentration in the sediment and pore water was measured at the Southern and Eastern Africa Mineral Centre (SEAMIC) as described in chapter 2 (section 2.2.4).

3.2.5. Test organisms

Chironomus riparius egg ropes were obtained from a continuous culture held at the Laboratory of Veterinary Physiology and Toxicology at Sokoine University of Agriculture (Tanzania) and hatched in EPA-medium. The starter culture was obtained from the Laboratory of Environmental Toxicology, Ghent University (Belgium). The culture is kept at a controlled temperature of 20 ± 2°C and a 12:12 h light: dark photoperiod.

3.2.6. Experimental design

This test was conducted as described in OECD guideline 218. Eleven replicates per sediment test concentration were used: 5 of these were used to assess 14 day survival and growth, another 5 for the assessment of emergence and growth at day 28 while the last replicate was used for chemical analysis at the end of the test. Tests were initiated by placing the spiked sediments into 800 ml glass vessels and adding overlying water to produce a sediment-water volume ratio of 100 g sediment and 175 ml water. This was done one day prior to the introduction of the test organisms. A polystyrene plate was positioned over the sediment to minimize the disturbance of the sediment as the water was added. To each replicate, 10 larvae were introduced randomly into the overlying water below the air-water interface, using a glass pipette. Larvae were individually checked for viability (i.e. swimming motion) in the water column as they were added. Test larvae were taken from the synchronized culture and were 48 h old at the start of the test.

During the exposure period the overlying water was renewed three times a week (75% renewal). Organisms were fed daily with Tetramin® (Melle, Germany) at 0.5 mg per organism per day for the first 10 days and 1 mg per organism per day for the remaining 18 days. Experiments were conducted at 20 ± 2 C° and under a light regime of 12 h light and 12 h dark. Temperature, pH, dissolved

oxygen, hardness and ammonia were measured three times a week (i.e. before water renewal). Using a pipette water samples were taken from approximately 1 cm above the sediment surface without causing any disturbance (to avoid contamination of the overlying water with sediment particles).

3.2.7. Determination of survival and growth

On day 14, five of the replicate vessels per concentration were sieved through a 200 µm sieve to remove the surviving larva and allow assessment of survival and growth. Recovered organisms were placed overnight in EPA medium in order to empty their digestive tracts. All organisms from each replicate were subsequently placed into pre-weighed square boats of aluminium foil, dried at 60°C for 24 hours and weighed. The average weight of individual larvae was obtained by dividing the weight of pooled larva in each replicate by the number of dried larvae.

3.2.8. Determination of emergence

After the removal of the 5 replicates for survival and growth assessment, the remaining vessels were covered with emergence traps and checked daily for emerged midges until day 28 of testing i.e. when the test was terminated. Newly emerged adults were collected daily, counted and recorded. This practice was continued until 100% emergence was achieved in a given replicate. Only adults that had successfully broken free from the pupal skin were considered to have successfully emerged.

3.2.9. Data treatment

Data were arcsine square root transformed before analysis. Data for survival, growth and emergence were then tested for normality and homogeneity using the Shapiro-Wilkinson test. The differences between different treatments were determined with ANOVA (Fisher LSD test). The LC_{50} after 14 days were calculated using the probit analysis methods according to Finney (1971). Median emergence times (EmT50) were calculated using the Kaplain & Meier Method (Bland and Altman, 1998). Emergence times of each treatment were compared using the Mann-Whitney U test (Zar, 1999). All the statistical analysis was done with the Statistica™ 6.0 software.

3.3. Results and discussion

3.3.1. Physico-chemical conditions during exposure

Dry weight determination of the sediment revealed that the sediment contained 30% water. The monitoring results of the physico-chemical conditions during the 28 day exposure period are summarized in Table 3-1. Average temperature was 20°C ± 1.5°C and the oxygen level in the water column never dropped below 4.2 mg/L. The average pH ranged between 7.4 and 7.7 (except at the highest Hg treatment) and measured ammonia levels were always below 4.2 mg/L. The increase in levels of ammonia in the two highest Hg concentrations may be attributed to the accumulation and decomposition of food (Tetramin®) due to the high larva mortality which was observed in these treatments. Nevertheless, all the physico-chemical parameters were within the range recommended by OECD protocol (2004) for *Chironomus riparius* testing.

Table.3-1. Mean values (+/- SD) of the different physico-chemical parameters monitored during the 28 day exposure period.

Nominal Hg-conc. (µg Hg g^{-1} dry wt)	pH	Temperature (°C)	Ammonia (mg/L)	Hardness (mg/L $CaCO_3$)	Oxygen (mg/L)
Control	7.42 ± 0.12	20.2 ± 0.71	0.0	252.20 ± 49.61	5.18 ± 1.30
1	7.62 ± 0.12	19.9 ± 0.43	0.0	234.30 ± 50.87	4.90 ± 0.93
1.8	7.74 ± 0.12	19.7 ± 0.47	0.0	178.00 ± 47.70	5.08 ± 0.79
3.2	7.64 ± 0.23	20.4± 0.76	0.0	213.60 ± 29.25	5.17 ± 0.84
5.6	7.51 ± 0.16	20.1 ± 0.12	1.2 ± 1.10	240.30 ± 37.90	5.23 ± 0.79
10	7.69 ± 0.11	20.5 ± 0.43	3.0 ± 0.20	213.60 ± 46.70	5.28 ± 0.85
18	8.69 ± 0.11	20.5 ± 0.43	4.2 ± 0.20	215.63 ± 46.70	6.28 ± 0.85

3.3.2. Survival, growth, and emergence of *C. riparius*

The bioassay results on the survival, growth and emergence of *C. riparius* are presented in Table 3-2. There was no significant difference in mortality between organisms exposed to 0, 0.59 and 0.93 µg Hg g^{-1}dw (measured concentrations) of Hg and that of the control larvae. The control and 0.59 µg Hg g^{-1} dw treatment had the same high survival (98%), while organisms exposed to 0.93 and 2.42 µg Hg g^{-1} dw Hg exhibited a reduced survival (88% and 80%, respectively). The survival in the sediments containing 3.84 µg Hg g^{-1} dw and 7.2 µg Hg g^{-1} dw was significantly lower than that of the control group, i.e. 26% and 4%, respectively ($p<0.05$). Based on these results, a 14 day LC_{50}

value of 3.40 µg Hg g^{-1} dry wt (95% CL 2.6 - 4.2 µg g^{-1} dw) was calculated while the NOEC and LOEC values (for survival) were 2.42 to 3.84 µg Hg g^{-1} dw respectively.

No significant difference in dry body weight of larvae in the controls and those exposed for 14 days to 0.59 and 0.93 µg Hg g^{-1} dw was noted ($p>0.05$). Significant growth inhibition was observed in organisms exposed to 2.42 and 3.84 µg Hg g^{-1} dw. The mean dry body weight of larvae from the 7.2 µg Hg g^{-1} dw Hg spiked sediment is not reported as the small size of the recovered larvae did not allow accurate measurement and meaningful interpretation. Based on these results, the 14 day NOEC and LOEC values based on growth were 0.93 to 2.42 µg Hg g^{-1} dw respectively.

Emergence after 28 days of exposure in the control group (88%) was higher than the 70% validity criterion prescribed by the OECD (2004). No significant effects (compared to the control) were observed for the three lowest Hg treatments (i.e. 0.59, 0.93 and 2.42 µg Hg g^{-1} dw (Table 3-2). However, the emergence success of the organisms exposed to 3.84 µg Hg g^{-1} dry wt was significantly lower than that in the control sediment ($p<0.05$). No midges emerged from the sediment treated with 7.2 µg Hg g^{-1} dw. Furthermore, midges in the control and lowest Hg concentration emerged significantly earlier ($p<0.05$) than those exposed to the higher Hg levels (0.93, 2.42 and 3.84 µg Hg g^{-1} dw) as reflected by the EmT50 given in Table 3-2.

Table. 3-2. Average (± SD) for survival, growth and emergence parameters of *C. riparius* to Hg spiked sediment

Nominal conc. (µg Hg/ g dw)	Measured conc. in sediment (µg Hg/ g dw	Measured conc. in pore water (µg/L)	14 d-survival (%)	14d- growth (µg/organism)	28d-emergence (%)	28d-median emergence time – EmT50 (days)	day of first emergence
Control	<0.02	<0.02	98 ± 2	1,357.4 ± 47.6	88 ± 8	17.0 ± 1.95	15
1	0.59	<0.02	98 ± 2	1,359.75 ± 32.9	94 ± 4	17.0 ± 0.45	15
1.8	0.93	85	88 ± 9.7	1,219.8 ± 65.3	94 ± 6.8	22.0 ± 1.14*	17
3.2	2.42	142	80 ± 5.5	624.4 ± 25.88*	74 ± 7.5	24.8 ± 0.84*	21
5.6	3.84	316	26 ± 2.4*	304 ± 4.0*	8 ± 4*	27.0 ± 0.8*	26
10	7.20	512	4 ± 2.4*	n.a	0	n.a	n.a
18	12.68	802	0	n.a	0	n.a	n.a

n.a = not applicable, * significantly different from control ($p<0.05$)

Information on the effects of sediment-associated mercury to life history characteristics of benthic organisms is very scarce. The current study was aimed at investigating the toxicity of inorganic mercury ($HgCl_2$) to the freshwater midge *C. riparius*. The results show that no significant effects on mortality, growth and emergence of *C riparius* occur at or below 0.93 µg Hg g^{-1} dw. Median emergence time was the most sensitive of all endpoints evaluated. Our findings are consistent with results from the previous studies, which indicated that mercury is toxic to Chironomus larvae in the range of 316 to 1800 µg/L. Previous studies performed with *C. riparius* and using water only exposures, reported 24, 48, and 96 h LC_{50}s to be in the range of 316-1800 and 400-547 µg/L, respectively (Qureshi *et al.*, 1980; Rossaro *et al.*, 1986). Assuming that pore water was the main route of mercury exposure to *C. riparius* in the present study, the calculated LC_{50}s is 250 µg/L which is lower than ones calculated by Qureshi *et al.*, 1980 and Rossaro *et al.*, 1986. The difference between these results may be attributed to longer exposure period used in our study (14 days) compared to 24, 48 and 96 hours in the previous studies. Additionally, we used younger animals (48 hrs old) compared to 14 days old organisms used by the former authors. Young animals are mostly more sensitive to pollutants compared to older ones (Ristola, 2000). Alternatively, it can be urged that there was an additional exposure through the solid phase of the Hg contaminated sediment as *Chironomus riparius* is a benthic detritivore which can process large volume of contaminated sediment (Rasmussen, 1984; Armitage, 1995). Saouter *et al.* (1993) demonstrated that when mayfly larvae (benthic detritivore) were exposed to Hg via sediment the gut contributed more Hg than the gills, which was an indication that the sediment exposure through the gut is an important route.

Based on dry weight, larvae growth was significantly reduced at day 14 in a concentration dependent manner from 2.42 µg Hg g^{-1} dw onwards. Growth of *Chironomus* larva have been reported to be influenced by level of feeding (Sibley *et al.*, 1997; Ristola *et al.*, 1999; Péry *et al.*, 2002; Haas De *et al.*, 2006). In our study feeding was done uniformly for all tested Hg concentrations and at the recommended feeding rate (OECD, 2004). It can therefore be argued that growth reduction of the larva in higher Hg concentrations might have been caused by mercury toxicity. Alternatively, it has been reported that Hg is capable of causing mandible deformation in C. *riparius* larva (Vermeulen *et al.*, 2000). As mandibles are important apparatus for feeding such deformation is likely to reduce the feeding ability of the larva and result in poor growth.

The trend of midge emergence followed a similar trend as that observed for survival and growth, i.e. midges exposed to the three lowest Hg concentrations (0.59, 0.93 and 2.42 µg Hg g^{-1} dw) did not exhibit a significantly different emergence success compared to the control. There was, however, a drastic drop in the percentage of emerged midges from 74% at 2.42 µg Hg g^{-1} dw to 8% at 3.84 µg Hg g^{-1} dw and no midge emerged from 7.2 µg Hg g^{-1} dw treatment. It has been suggested that for *Chironomus* midges emergence, there is a minimum weight threshold to be attained by the larvae for emergence to occur (Hilsenhoff, 1966 and Ristola, 2000). It is possible that the larva exposed to 3.84 and 7.2 µg Hg g^{-1} dw spiked sediments did not attain the minimum weight to allow pupation and eventual emergence. For *C. tentans* a related species to *C. riparius* a minimum dry weight threshold for emergence was shown to be ranging from 500 to 800 µg/larvae (Sibley *et al.*, 1997). Based on the weight measurement obtained after 28 days in the current study dry body weight exhibited by the larva (304 ± 4.0 µg/larvae) from this treatment was much lower than the range suggested above.

Furthermore, these results indicate that emergence time (EmT50's) of the individual larvae was the most sensitive of all endpoints tested as it was the only parameter significantly affected at concentration of 0.93 µg Hg g^{-1} dw (Table 3-2). At this concentration there was a 2 day delay in emergence compared to the control. In general, our results are in agreement with other authors who showed that for *C. riparius*, larval dry weight and emergence time are more sensitive than survival and % emergence (Kimberly, 2004).

3.3.3. Comparison of laboratory toxicity test results with Hg field concentrations in artisanal gold mines in Tanzania

In the present study, Hg delayed the emergence of *C. riparius* at concentrations (0.93 µg Hg g^{-1} dw) lower than those measured up to six kilometres downstream of Mugusu mine in river Mabubi (1.6 µg Hg g^{-1} dw) (chapter 2). The initial concentration which inhibited growth (2.4 µg Hg g^{-1} dw) are far lower than Hg concentrations that have been reported occurring in several rivers and streams in artisanal gold mining impacted areas in Tanzania. For example, the mercury content observed in sediment from Bulyanhulu river and Isingile river in Tanzania was 5.35 µg Hg g^{-1} (van Straaten, 2000a) and 2.84 µg Hg g^{-1} dw (Taylor *et al.*, 2005) respectively. Ikingura *et al.* (1997) measured Hg concentrations in sediment collected in streams in Geita district Tanzania up to 136 µg Hg g^{-1} dw. It is therefore possible that Hg concentrations measured in sediment of these rivers and streams can cause adverse effects to the local fauna. However, it should be noted that in natural settings, mercury bioavailability varies depending on a variety of factors such as adsorption to particles, complexation by organic matter (e.g humic and fulmic acids) (Ankley *et al.*, 1994) presence of other cations (Driscoll *et al.*, 1994;) pH and sulfides (Di Toro *et al.*, 1990; Pak and Bartha, 1998). Therefore, effects to benthic organisms can be exhibited at higher Hg concentrations than the ones established in the present study. Indeed, Hg was more available to *C.riparius* in the present study due to low concentrations of competing cations as deionized water was used for sediment preparation and water renewal. Similarly, low concentrations of Hg binding ligands like sulfides (AVS), and organic matter in the sediment are the other factors which might have increased bioavailability.

3.4. Conclusion

Results from this study have contributed to our understanding of the effects of Hg to benthic macroinvertebrates at environmental realistic sediment concentrations. Concentrations at which adverse effects were observed in this study have been reported to occur in several aquatic ecosystems; for example, in rivers and streams occurring around artisanal gold mining impacted areas in Tanzania. It is therefore, possible that *Chironomus* spp and other fauna inhabiting artisanal gold mining contaminated water bodies are at risk.

Chapter four

Ecotoxicity of mercury contaminated sediment collected from the Mabubi river (Geita district, Tanzania) to the early life stages of African catfish (*Clarias gariepinus*)

4.0. Ecotoxicity of mercury contaminated sediment collected from Mabubi river (Geita district, Tanzania) to the early life stages of African catfish (*Clarias gariepinus*)

Abstract

The quality of Hg contaminated sediments in artisanal gold mining areas of Tanzania have to date only been assessed through bulk chemical analysis and subsequent comparison with reference values from uncontaminated areas. However, measurement of contaminant levels alone has a limited ability to predict adverse effects on living resources. In this study we investigated the possible effects of Hg contaminated sediments from the river Mabubi on the hatching success, larval survival and growth of the African catfish (*Clarias gariepinus*), a resident species whose demersal behaviour keeps it in frequent contact with the sediment. To control for the effects of other contaminants, additional experiments were conducted using artificial sediments spiked with mercury chloride. Natural sediments collected downstream from the Mugusu mine (Geita, Tanzania) decreased survival and impaired growth of this species but did not affect its hatching success. These effects were observed up to six kilometres downstream of the mining activity. The calculated 5 d-LC_{50} value (larval survival) was 1.75 µg Hg g^{-1} dw (95% CL of 0.72 – 2.53), the 5d-NOEC for hatching was >2.3 µg Hg g^{-1} dw and that for larval survival and growth was 0.23 µg Hg g^{-1} dw. In the additional assays with artificial sediment, the 5 d-LC_{50} (larval survival) was 3.1 µg Hg g^{-1} dw (95% CL of 2.2 - 4.0) and the NOEC values for hatching, larval survival and growth were 3.23, 1.22 and 0.59 µg Hg g^{-1} dw, respectively. In conclusion, chemistry and ecotoxicity results from this study suggest that sediments collected in the Mabubi river downstream of the Mugusu mine adversely affect catfish and probably other fauna and as such present a considerable local environmental risk.

Key words: artisanal gold mine, mercury, sediment, *Clarias gariepinus*, hatching, survival and growth

Redrafted from

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Ecotoxicity of mercury contaminated sediment collected from Mugusu gold mine (Geita district, Tanzania) impacted river to the early life stages of African catfish (Clarias gariepinus). Archives of Environmental Contamination and Toxicology: Submitted

4. 1. Introduction

Within an ecological risk assessment framework, it has been recommended that multiple lines of evidence are evaluated to fully characterize the risks of contaminants in sediments (Fairbrother, 2003). The sediment quality triad system represents such a multidisciplinary approach as it includes evaluation of sediment chemistry, ecotoxicity and invertebrate community structure (Canfield *et al.*, 1994). In artisanal gold mining impacted areas of Tanzania, sediment contamination has to date only been assessed by analysing bulk chemical contamination and comparing the results with reference values from uncontaminated areas (Ikingura and Akagi, 1996, 1999; Kondoro and Makundi, 1998; LVEMP, 2002). Mercury sediment concentrations up to 136 mg kg^{-1} dw have been reported by Ikingura *et al.*, (1997) and sediments collected in the Mabubi river downstream of Mugusu mine contain up to 2.3 mg Hg kg^{-1}dw (Chapter 2). Chemical analysis of contaminants levels, however, has a limited ability to predict adverse effects on living resources. Thus, in order to provide information on the ecological impact of sediment contamination on aquatic biota, sediment toxicity bioassays are recommended. In addition, sediment toxicity testing is considered to be a more appropriate method of assessing sediment quality, as sediment chemistry measurements do not always integrate contaminant fluctuations over time (Canfield *et al.*, 1994).

Results obtained from the toxicity evaluation reported in chapter 3 suggested that current levels of Hg in artisanal gold mine impacted watersheds in the Lake Victoria basin may adversely affect local ecosystems. However, the extent to which these sediments are actually toxic to the local fauna has not been studied. Results in this chapter provide information on the toxicity to the African catfish (*Clarias gariepinus*) of sediments collected from the Mugusu mine impacted river. The ecotoxicological endpoints used in this study included hatching success, larvae survival and growth of *C. gariepinus*. To control for the effects of other contaminants, additional experiments were conducted using artificial sediments spiked with mercury chloride.

C. gariepinus occurs in most rivers and all streams in the Lake Victoria basin (David, 1992). It has a demersal life style that makes it vulnerable to exposure to contaminated sediments (Bezuidenhout *et al.*, 1990; Adeyeye *et al.*, 1996). Like many other organisms early developmental stages of *C. gariepinus* are probably the most sensitive stages of its life cycle. These can be affected by mercury through the disruption of several processes such as the inhibition of metabolic pathways leading to failure of the energetic balance, cellular division and differentiation, and cellular migration (Itow *et*

al., 1998). It has been demonstrated that fathead minnow (*Pimephales promelas*) foraging ability and capture speed is affected by mercury due to alterations in neurotransmitter levels (Grippo and Heath, 2003). Chronic Hg exposure of *Clarias batrachus* larvae results in a progressive degeneration of the kidneys and eventually vacuolation, atrophy and extensive damage of the tubules (Kirubagaran and Joy, 1988). Similarly, exposure of Atlantic salmon (*Salmo solar*) to dietary mercury led to peroxidative brain injury, severe brain pathology and appearance of neurological toxicosis (Berntssen *et al.*, 2003).

4.2. Material and methods

4.2.1. Sediment preparation

4.2.1.1. Natural sediment sampling

Sediment samples which were collected for the metal analysis described in chapter 2 (section 2.2.3) were used for this study. Briefly, sediment samples were collected from five points along river Mabubi identified as A, B, C, D and E (Figure 2.2, chapter 2). Point A was located two kilometres upstream of the gold processing area and was used as a reference point. Points B, C and D were located at a distance of three, six and nine kilometres downstream of the gold processing area. Point E was located at the river mouth, i.e. where it enters Lake Victoria. About three kilograms of superficial sediment (~5 cm) was collected using a core sampler, put into 10 N nitric acid washed glass jars, kept in cool boxes with ice and transferred to the laboratory. The sediment was used in the toxicity tests within one month of collection. Sediments were also analyzed for heavy metal content (described below), % organic carbon, Acid Volatile Sulphide (AVS), Simultaneously Extracted Metals (SEM) and grain size. Samples for AVS and SEM analysis were simultaneously collected from each sampling point and were transferred into nitrogen gas purged glass containers (0.25 L). The containers were completely filled with sediment to reduce oxygenation. These samples were stored at 4°C until they were transferred to Belgium for AVS and SEM analysis.

4.2.1.2. Artificial sediment preparation and spiking with $HgCl_2$

The artificial sediment was prepared following the procedure described in chapter 3 (section 3. 2.2). This procedure was done according to the guideline for sediment-water testing using spiked sediment recommended by the OECD (2004). Mercury stock solution for spiking was also prepared following the procedure described in section 3.2.2 of chapter 3. The sediment was spiked with 0 (control), 0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 µg Hg g^{-1} dw. The selection of the concentrations to be tested was based on results from earlier range finding assays. Overlying water (deoinized,) was

carefully poured on top of each spiked sample to achieve a ratio of 1 (sediment): 3 (water), and the sample was stored in the dark at 4°C for seven days. On the seventh day, the overlying water was discarded and the sediment transferred to the test containers.

4.2.2. Sediment physical and chemical properties measurements

The sediment particle sizes of the natural sediment were measured by wet sieving according to the method described by Gee and Bauder (1986) in the Laboratory of Soil Science, Sokoine University of Agriculture-Tanzania. Percentage organic carbon (%OC) was measured by using the volatile solids technique which involved drying sediments and burning off organic matter in a furnace for 16 hours at 550°C. The % OC was calculated based on the change in sediment weight before and after ignition (ASTM, 1992). AVS was determined according to the modified diffusion method (Leonard *et al.*, 1996) (10 g sediment extracted for 1 h in 60 ml of 1 N HCl), at the Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Belgium. The container with sediment collected from point B broke during transport and it was not analysed for AVS and SEM.

Both the natural and artificial sediments were sampled for chemical analysis on day 0 of the experiment. Natural sediments were analysed for Cu, Pb, Cr, Zn, Cd, Hg and As; only Hg was analyzed in the artificial sediment. The analysis of Cu, Pb, Zn, Cr and Cd was performed by using Atomic Absorption Spectrometer (GBC 906) (USA) in the flame mode. Briefly, water was decanted from the sediment samples and the sediments were air-dried in an air-conditioned room set at 25°C and 65% relative humidity. Sediments were further dried in an oven at 40°C for 48 hours and then milled using an agate planetary micro-mill (Fritsh) from Laval lab - Canada. The resulting fine dry powder was used for digestion, which was done by using an aqua regia digestion system (3:1 parts of HCl to HNO_3). Dry powdered sediment (0.5 g) was mixed with 1.5 ml concentrated HCl and 0.5 ml concentrated HNO_3 in a graduated test tube and digested on a hotplate at 95°C. The digest was cooled to room temperature and diluted with deionized water to 10 ml. After settling overnight, samples were ready for analysis. Total mercury was analyzed by using AAS under cold vapour generation technique as described in chapter 2 (section 2.2.4).

4.2.3. Toxicity testing

4.2.3.1. Test organisms

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

4.2.3.2. Sediment- embryo test

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

4.2.3.3. Sediment – larva test

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

the test vessels, counted and preserved in 10% carbonate-buffered formalin. Total length (mm) measurements were done by using a microscale calliper.

4.2.4. Data treatment

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

4.3. Results and discussion

4.3.1. Sediment physical and chemical characteristics

4.3.1.1. Natural sediment

The physical characteristics of the natural sediments are presented in Table 4-1. The sediment collected upstream of the gold mine (point A) was relatively coarser than the sediment samples taken downstream of the mine. The clay percentage ranged from 62.5% at point B to 54.3% at point D. Organic carbon (OC) percentage varied from 5% before the gold mine to 1.2% at point D. Point E which was located at the river mouth (in the lake) had the highest percentage of OC (38.2%). The high % OC at point E can be due to a large amount of detritus and vegetation remains which are washed into the lake during heavy rains and flooding and poor microbial degradation found in sub-oxic marshes that surround the river mouth (Gichuki *et al.*, 2005).

Table. 4-1. Physical characteristics of the natural sediments sampled from different points along the Mabubi river (Geita, Tanzania)

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

Concentrations of metals and AVS measured in the samples collected from the Mabubi river are shown in Table 4-2. Sediment sampled from point B had the highest concentrations of As, Hg, Cr and Zn with values of 72, 2.3, 55.2 and 51.04 $\mu g\ g^{-1}$ dw respectively. These concentrations were 76 and 6 times higher than those found at point A for Hg and As, respectively. From these results it appears that high levels of metals are found near the gold washing site and decrease downstream. Tanzania lacks it own sediment quality guideline for metals. Measured metal concentrations in sediment were therefore compared with the consensus-based sediment quality guideline values referred to as the probable effect concentration (PEC) proposed by MacDonald *et al.* (2000). The PEC guideline was selected for comparison because various evaluations have demonstrated that this guidelines provide a unifying synthesis of the existing sediment quality guidelines and reflect causal rather than correlative effects (MacDonald *et al.*, 2000). In the present study, the concentrations of As at point B and Hg at point B and C exceeded the PEC. Similarly, the concentrations of Hg in

sediment collected from point B and C exceeded the sediment guidelines proposed for Ecuador (0.45 µg Hg g^{-1} dw), Washington State-USA (0.41 µg Hg g^{-1} dw) and Canada (0.14 µg Hg g^{-1} dw) (Eisler, 2005).These guidelines provide concentrations of metals above which adverse effects are expected to occur. Therefore sediments collected from these two points are likely to cause adverse effects to local fauna.

As discussed in chapter 2, the presence of higher Hg levels in sediments sampled downstream of the mine compared to those taken from upstream locations is the result of the use of mercury in the gold extraction process. The occurrence of other metals like As, Cu, Pb and Cr in the river sediment sampled downstream of the mine can be explained by their natural presence in the mined ore. It has been shown that, complexes of these metals like arsenopyrite ($FeAsS_2$), CuS_2 and $FeO.Cr_2O_3$ are abundant in tropical gold bearing soils (Lacerda, 1997; Oyarzun *et al.*, 2004). These metals are released into the tailings during gold processing.

Concentrations of AVS in sediments collected from points A, C, D and E were 1.06, 0.03, 0.65 and 2.02 µmol g^{-1} dw, respectively (Table 4-2). The concentration of AVS in sediments is determined both by the rate at which AVS is produced and by the rate at which it is lost through oxidation or diffusion. AVS production is favoured by the presence of anoxic conditions in the aquatic system (Howard and Evans, 1993). The section of the Mabubi river that lies along and immediately downstream of the mine is very shallow due to the large amount of tailings and powdered ore that is washed into the river at the gold washing sites. Because of this shallow depth sediments in this section of the river are probably well-oxygenated thus diminishing AVS formation. In contrast, points A and E were located at deeper sites with high organic carbon concentrations which favour AVS production (Zehl and Einax, 2005).

Table 4-2. Concentrations of metals, acid-volatile sulfide (AVS), total simultaneously extracted metals (SEM) to AVS molar ratio in sediments collected along the Mabubi river (Geita district, Tanzania)

Sampling points	Metal concentrations (μg g^{-1} dry weight)							∑SEM (μmol/g dw)	AVS (μmol/g dw)	∑SEM/AVS ratio
	Cu	Pb	Cr	Zn	Cd	As	Hg			
A	1.09	21	31	26	0.62	12	0.03	0.87	1.06	0.82
B	2.41	34	55.2	51.04	0.86	72*	2.3*	n.a	n.a	n.a
C	1.15	36	43	43.06	0.43	28	1.6*	1.85	0.03	59.27
D	3.35	17	19	25.2	0.86	22	0.23	0.98	0.65	1.49
E	3.17	13	6.7	26.56	0.85	8	0.08	0.67	2.02	0.33
PEC	149	128	111	459	4.98	33	1.06	n.a	n.a	n.a

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

4.3.2. Toxicity testing with artificial sediment

4.3.2.1. Physical-chemical conditions during testing

The artificial sediment contained 20% clay, 75% sand (< 200 micron) and 2.5% organic carbon (TOC). The physico-chemical conditions of the sediments during the exposure period are summarized in Table 4-3. All the measured parameters were within the recommended values for testing with fish early life stages (OECD guideline, 210). However, the increase in levels of ammonia in the three highest Hg concentrations may be attributed to the decomposition of dead embryo/larva before removal.

Table 4-3. The mean values (+/- SD) for different physico-chemical parameters monitored in the assay with artificial sediment

Nominal Hg (µg g^{-1} dw)	pH	Temperature °C	Ammonia (mg/L)	Hardness (mg/L $CaCO_3$)	Oxygen (mg/L)
0	7.4 ± 0.12	20.2 ± 0.71	0.0	252.0 ± 49.61	5.2 ± 1.30
0.125	7.6 ± 0.12	19.9 ± 0.43	0.0	234.3 ± 0.87	5.0 ± 0.93
0.25	7.7 ± 0.12	19.7 ± 0.47	0.0	178.0 ± 7.70	5.0 ± 0.79
0.5	7.6 ± 0.23	20.4 ± 0.76	0.0	213.6 ± 9.25	5.2 ± 0.84
1	7.5 ± 0.16	20.1 ± 0.12	1.2 ± 1.10	240.3 ± 37.90	5.2 ± 0.79
2	7.7 ± 0.11	20.5 ± 0.43	3.0 ± 0.20	213.6 ± 46.70	5.3 ± 0.85
4	8.7 ± 0.11	20.5 ± 0.43	4.2 ± 0.20	215.6 ± 46.70	6.3 ± 0.85
8	7.8 ± 0.2	21 ± 0.02	3.6 ± 0.12	178.0 ± 7.70	5.0 ± 0.79
16	8.0 ± 0.01	20.6 ±0.2	4.3 ± 0.30	213.6 ± 9.25	5.0 ± 0.84

4.3.2.2. Measured levels of Hg and effects on *C. gariepinus* hatching, survival and growth

Measured values of Hg in the artificial sediments and overlying water are presented in Table 4-4. The measured Hg concentrations in sediments were lower than the nominal values, with spiking efficiency (i.e. measured/nominal concentration) ranging from 54% to 82%. This can be expected as there is always a fraction of the spiked Hg that is not bound to the sediment but that remains in solution (i.e. the fraction measured in pore water and in the overlying water). Also, Hg is lost during sediment preparation through adsorption to the walls of the mixing containers.

In this test, there was a significant reduction in the hatching success ($p<0.05$) of eggs exposed to 6.42 µg H g^{-1} (compared to the control eggs). Larval survival and total mean length were significantly reduced at Hg concentrations of 3.23 and 1.22 µg Hg g^{-1} dw, respectively (Table. 4-4). Based on these results, a 5 day LC_{50} value of 3.1 Hg µg Hg g^{-1} dw (95% CL of 2.2 - 4.0) was calculated and the NOEC value for survival, hatching, and growth were 1.22, 3.23 and 0.59 µg Hg g^{-1} dw, respectively.

Table 4-4. Survival, hatching and growth of *C.gariepinus* exposed to Hg spiked artificial sediment

Nominal Hg ($\mu g\ g^{-1}$ dw) sediment	0	0.125	0.25	0.5	1	2	4	8	16
Measured Hg ($\mu g\ g^{-1}$ dw) sediment	n.d	n.d	0.09	0.26	0.59	1.22	3 23	6.42	13.2
Measured Hg ($\mu g/L$) overlying water	n.d	n.d	n.d	0.14	0.43	2.2	5.1	8.4	22.6
Eggs hatching %	63	66.6	60	63.3	56.7	60	53	30*	10*
Larvae survival %	80	73	83	80	73	63	54*	23*	0
Larvae mean length (mm) ± SD	6.6±0.8	7±0.7	6.7±0.5	6.5±0.97	6±0.5	5.7±0.7*	5.5±0.7*	5.4±0.9*	n.a

n.d = below detection limit; *significantly different from the control; n.a = not applicable

The results demonstrate that the early life stages of *C. gariepinus* are affected with sediment associated with Hg in a concentration dependent manner. Survival and growth were impacted at concentrations $\geq$ 1.22 μg Hg g^{-1} dw, while hatching was shown to be less sensitive. Indeed, effects on survival and growth were observed at concentrations a factor of two and five lower than ones that affected embryo hatching, respectively. This difference in sensitivity may be explained by the hardening of chorion layer which takes place after fertilization, thus providing a barrier against the entrance of the contaminants (Somasundaram *et al.*, 1984; Rombough, 1985). Nguyen *et al.* (2002) postulated that the chorion of *C. gariepinus* started its hardening process 3 h after fertilization. Mekkawy and Osman (2006), using electron microscopy confirmed the occurrence of chorion hardening for *C. gariepinus* embryos, however, they showed that hardening starts within the first one hour after fertilization. Therefore, in the present study chorion hardening may have played a role as the embryos were exposed to test sediments two hours after fertilization. Similar findings have been reported by Nguyen and Janssen (2001).

4.3.3. Toxicity testing with natural sediment

4.3.3.1. Physico-chemical parameters

Physico-chemical conditions in the exposure assays with natural sediments are summarized in Table 4-5. All measured parameters were within the recommended values for chemical testing with fish early life stages (OECD guideline 210).

Table 4-5. Mean values (± SD) for different physico-chemical parameters monitored in the assay with natural sediment

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

4.3.3.2. Effects on *C. gariepinus* hatching, survival and growth

The effect of the sediments collected from the river Mabubi on *C. gariepinus* are summarised in Table 4-6. Compared to the reference sediment, in general sediments collected downstream from the mine adversely affected the survival and growth of the *C.gariepinus* larvae, but not the hatching success of the embryos. Sediments collected at point B significantly reduced survival and inhibited growth of the catfish larvae and the samples collected from point C impaired growth ($p<0.05$). Sediments collected from points D and E did not affect the survival, growth or embryo hatching success. Based on these results, the calculated 5 day LC_{50} (larval survival) was 1.75 µg Hg g^{-1} dw (95% CL of 0.72 – 2.53) and the NOEC for hatching was >2.3 µg Hg g^{-1} dw and that for larval survival and growth was 0.23 µg Hg g^{-1} dw. The biological effects observed at point B and C corresponded with Hg and As levels that exceed the probable effective concentrations of the sediment quality guidelines proposed by MacDonald *et al.*, 2000 of 1.06 µg g^{-1} dw for Hg and 33 µg g^{-1} dw for As (Table 4-2). Similarly, the concentrations of Hg in sediment collected from point B and C exceeded the sediment guidelines proposed for Ecuador, Washington State-USA and Canada. Because these guidelines propose limits of metal concentrations above which effects are likely to occur; it is very likely that the elevated concentrations of Hg and As at these points caused the observed adverse effects on survival and growth of *C.gariepinus* larvae.

Table 4-6. Survival, hatching and growth of *C.gariepinus* exposed to field collected sediment.

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

* Significantly different from the reference sediment (A)

Like other metals the toxicity of mercury to aquatic organisms depends on species sensitivity differences the concentration of mercury and its bioavailability. In natural settings, metal bioavailability depends on a variety of factors such as adsorption to particles, complexation by organic matter (e.g humic and fulmic acids), presence of other cations (Driscoll *et al.*, 1998), and pH (Klinck *et al.*, 2005). It has been established that mercury can react with sulfide (generated in sediments by sulfate-reducing bacteria) and render it biologically unavailable (Di Toro *et al.*, 1990; Miller *et al.*, 2007). Based on these observations, it may be expected that mercury bioavailability and subsequent toxicity in same natural sediments is lower than that in spiked artificial sediments. However, in the present study, we found that natural sediment was toxic at Hg concentrations lower than those measured in artificial sediment (i.e. the calculated LC_{50} for larvae survival of 3.1 in artificial sediment is 1.7 times higher than the one calculated for natural sediment of 1.75 µg Hg g^{-1} dw). The observed high toxicity of natural sediment may be the result of low concentrations of metal binding substances in some of the natural sediment samples which reduce bioavailability of metals. Indeed, metal toxicity in sediment occurs only when the concentrations of metals are in excess of the sulfides or organic carbon content and/or other metal binding substances (Macdonald *et al.*, 2000). In fact, it has been shown that there is no toxicity if the SEM to AVS ratio is one or less because of the available free metal will be bound to the AVS phase (Ankley, 1996; Burton *et al.*, 2005). In the present study, the ratio of the sum of SEM to AVS concentrations was less than one only for sediments collected from points A and E (Table 4-2). These findings suggest that metals were readily bioavailable in the sediments sampled from points C and D. In addition, artificial sediment contained 2.5% of organic carbon which is higher than that measured in the field sediments collected from points B (0.56%), C (0.91%) and D (1.2%). This difference in organic carbon content may partly explain the observed difference in toxicity to *C. gariepinus* early life stages.

An alternative, but plausible explanation for the observed high toxicity in the natural sediment is the additional toxicity of the other metals that were measured in the natural sediments i.e. arsenic, cadmium, copper, chromium and lead. Indeed, mercury has been shown to have additive and possibly synergistic interactions with Cu, Cd and Pb (Fernàndez and Beiras, 2001). No chemical analysis of organic pollutants was performed as in this area there is no known polluting organic chemicals industry, either do farmers in the neighbourhood use chemical fertilizers, herbicides or pesticides (Kitula, 2006).

Given that the values of LC_{50} (1.75 µg Hg g^{-1} dw) for survival of catfish larvae exposed to sediments collected from the Mabubi river is lower than the Hg concentrations recorded in other artisanal gold mining impacted watersheds in Tanzania, it is possible that Hg may have already affected the ecology and biodiversity of these rivers and streams. For example, the mercury content in sediments from the Bulyanhulu river and Isingile river in Tanzania is reported to be 5.35 µg Hg g^{-1}dw (Kahatano and Mnali, 1997) and 2.84 µg Hg g^{-1} dw (Taylor *et al.*, 2005). It is thus suggested that more investigations on the effect of mercury originating from artisanal gold mining are needed to evaluate the impact of this practice on most sensitive species living in the Lake Victoria watershed.

4.4. Conclusion

To the best of our knowledge this is the first study evaluating the ecotoxicity of sediment contaminated by artisanal gold mine practices in the Lake Victoria basin. The Hg levels were found to be higher in sediments collected downstream of the mine than those at an upstream location. Sediments collected up to six kilometres downstream from the mine were toxic to the early life stages of catfish. The finding that 5d LC_{50} of Hg in artificial sediment was higher than that obtained with naturally Hg contaminated sediment suggests that, next to Hg, other pollutants or factors present in the river may contribute to the observed toxicity. Overall, the chemistry and ecotoxicity results from this study suggest that sediments downstream of the mine in the Mabubi river adversely affects the local biota. In addition, given that concentrations of mercury at which adverse effect were observed in this study are lower than levels reported occurring in other artisanal gold mining impacted watersheds in Tanzania it is suggested that the aquatic biodiversity in these watersheds are at risk.

Chapter five

Estimation of mercury intake through fish consumption of women living in a subsistence fishing community near the Mugusu artisanal gold mine – Tanzania

5.0 Estimation of mercury intake through fish consumption of women living in a subsistence fishing community near Mugusu artisanal gold mine - Tanzania

Abstract

In this study we determined mercury exposure of women living in a subsistence fishing community and eating fish from an area suspected to be contaminated with mercury. Estimation of mercury intake was achieved by combining information on fish consumption patterns and the mercury concentration in frequently consumed species of fish. To asses their health risk, the calculated Hg intake was compared with WHO/FAO recommended Provisional Tolerable Weekly Intake (PTWI) of 1.6 µg/kg body wt/week. The mean total mercury concentrations in fish were below the WHO safety limit (0.5 µg Hg g^{-1}ww). The calculated fish intake of 144 g/person/ day in the studied subsistence community exceeds the Tanzania average fish intake (17 g/day/person) and those of women in European countries: e.g. Greece (18.2 g/day), Spain (26.4 to 46.2 g/day), the UK (15.7 g/day) and France (20.6 to 27.9 g/day). The estimated average weekly Hg intake of women was 4 µg/kg body weight, which was found to be above the PTWI limit. It is concluded that, based on the prevailing fish consumption pattern in Nungwe village and probably also in neighbouring communities current Hg concentrations in fish pose a health risk to newborns in these communities.

Key words: Fish consumption, Mercury exposure, Women, Artisanal gold mining, Tanzania

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5.1. Introduction

It is recognized that mercury and its compounds pose a significant threat to human health. Mercury is found in the environment in its metallic, inorganic and organic forms. Most of the Hg in the atmosphere is elemental mercury vapour and inorganic mercury, while most of the mercury in water, soil, plants and animals occurs in its inorganic and organic forms (WHO 1985). In oceans and lakes inorganic mercury is transformed by micro-organisms to methylmercury which bioaccumulates in the aquatic food chain. Methylmercury is the most toxic form of mercury to man and other mammals (WHO, 1985). Since the beginning of the industrial era the concentrations of mercury in the global environment has increased three-fold (Mason and Sheu, 2002). Mercury emissions have recently decreased in the industrialized countries due to stringent regulatory laws. Conversely, emissions in developing countries have been increasing; this is mainly due to increased industrial activities, urbanization, intensive use of mercury in artisanal gold mining and the absence of effective legislation (UNIDO, 2004).

The threat of environmental Hg exposure to human health has been known for over five decades following widely reported poisoning incidents such as the consumption of Hg contaminated fish in Japan (Harada, 1995) and use of contaminated grain in Iraq (Bakir *et al.*, 1973). Exposed individuals suffered neurological related diseases, which included (among others) blindness, deafness and deficiencies in memory (Harada, 1995). Since then, Hg levels in fish have been thoroughly investigated in the context of its risk to human health. Different national and international organizations have developed guidelines and standards for the mercury contents in fish intended for human consumption. In 1979, the Food and Drug Administration of the United States of America (US FDA) established an action level of 1 μg Hg g^{-1} wet weight (ww) to regulate the Hg content in commercial fish. Other guidelines include those proposed in Canada and Brazil (0.5 μg Hg g^{-1}ww) (Evans *et al.*, 2005); Finland, Sweden and Japan (1 μg Hg g^{-1}ww) and the European community (1 μg Hg g^{-1}ww for predatory fish and 0.5 μg Hg g^{-1}ww for non-predatory fish) (EU, 2005). The World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) adopted an action limit of 0.5 μg Hg g^{-1}ww except for predatory fish for which the allowed level is 1 μg Hg g^{-1} ww (FAO/WHO, 1991). It is always recommended that the human population restrains itself from consuming, on a regular basis, species exceeding these values. In extreme cases even advisory warnings against fishing from highly contaminated waterways are given.

In the last decade there has been considerable research on the Hg contamination of fish in Lake Victoria, Tanzania (van Straaten, 2000a; LVEMP, 2002; Campbell, *et al.*, 2003a; Machiwa, 2005). Many of these studies were triggered by concerns about the gold ore processing practices in artisanal gold mines that use mercury amalgamation for gold extraction. Most of these studies were mainly focused on measuring Hg concentration in fish and assessing its possible effects on human health (Ikingura and Akagi, 1996; LVEMP, 2002; Campbell *et al.*, 2003a; Machiwa *et al.*, 2005). Because Tanzania lacks its own safety limits for mercury in fish, results obtained from these analyses were compared to the WHO/FAO standard. The obtained results indicated that the concentrations of mercury in the analysed fish were elevated but still below this safety limit of (0.5 to1 μg g^{-1}ww). Thus, it was concluded that these fish are safe for human consumption.

However, it remains unclear if consumption of fish from Lake Victoria is safe for the subsistence fishing communities which reside in Lake Victoria basin. These poor communities do not have a wide variety of food items to choose from. Consequently, they depend largely on fish consumption supplemented with either corn or root crops (José *et al.*, 2004; Kinabo *et al.*, 2006). As a result these communities may be disproportionately exposed to higher levels of mercury from fish consumption than the general public. To date, it is not known if their current Hg intake through fish consumption can probably result in deleterious outcomes. This study was aimed at collecting information on specific exposure factors which were not collected and accounted for in the previous studies, but which are important for estimating the Hg exposure and the health risk to these communities. We established the frequently consumed fish species, their frequency of ingestion, total mercury levels in their edible tissues and estimated the total Hg weekly intake among women living in a subsistence fishing village near the Mugusu mine. To estimate the health risk, the calculated weekly Hg intake was compared with the Provisional Tolerable Weekly Intake (PTWI) of 1.6 μg/kg bwt/week, that was recommended by the Joint FAO and WHO Committee on Food Additives and Contaminants (JECFA) in 2003). We tested the null hypothesis: there is no health risk to local subsistence fishing communities which consume fish caught from areas near artisanal gold mines in the Lake Victoria basin.

5.2. Material and methods

5.2.1. Study area

This study was conducted at Nungwe Village of the Geita District, Mwanza region, Tanzania (Figure 5.1) in July through November 2005. The local population residing in Nungwe village may be at risk of mercury exposure through consuming fish caught from Nungwe Bay. Nungwe Bay is situated approximately 13 km downstream from the Mugusu artisanal gold mine and is a draining area for the mines' effluents through the Mabubi river. The main activity of the villagers throughout the year is fishing, with very limited participation in artisanal gold mining and crop farming.

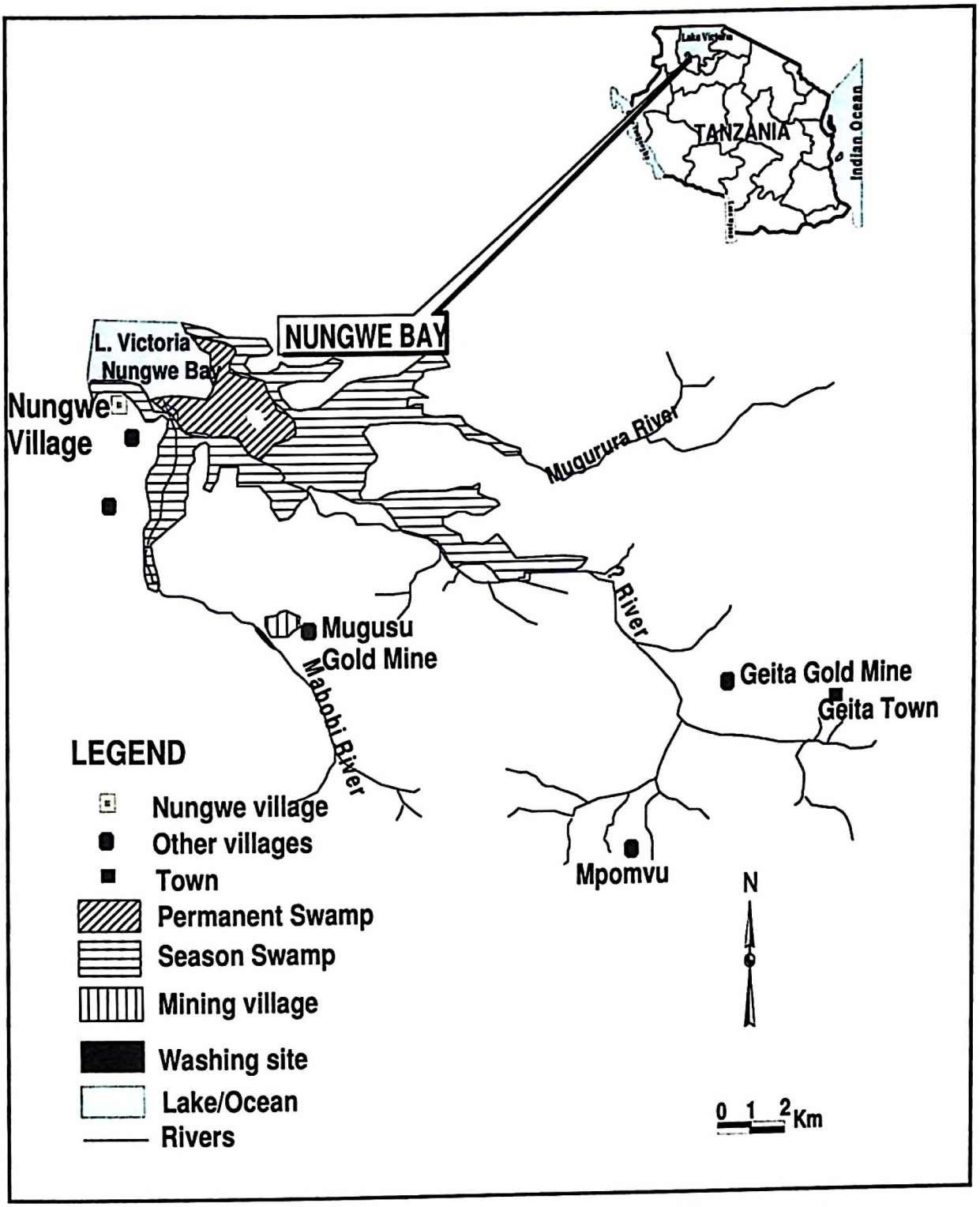

Figure 5.1 Map of Geita district indicating the location of Nungwe village

5.2.2. Collection of information on fish consumption patterns

Structured questionnaires and focus group discussions were used to collect information on the preferred species of fish, frequency of consumption and amount ingested. Before the definitive study, the questionnaires were pilot tested on fishing community representatives and were found to be suitable and culturally acceptable. The interviewer was able to speak both Swahili (national language) and the interviewee's vernacular languages (Sukuma and Zinza). Three hundred and twenty women were interviewed. The women were selected based on the findings that prenatal exposure to Hg leads to significant behavioural effects during infant development. Women at reproductive age are thus considered to be the most vulnerable group to Hg poisoning (Desjardins, 2004; Davidson *et al.*, 2006). The inclusion of a woman in the interview was based on her willingness to participate, her age being between 18 to 38 years, having a member or members of her family participating in the fishing activities in Nungwe Bay and the family regularly eating self-caught fish. Each household was visited and one or two women from a household were interviewed. When we encountered two women in one household we interviewed them separately. All interviewed women were weighed during the visits and their average weight was used as default adult body weight for calculating mercury intake. The interviewees were asked to recall and identify different fish species which they had consumed during the past six month by using a pre-determined list of fish which included nile perch (*Lates niloticus*), tilapia (*Oreochromis niloticus*, furu (*Haplochromines* spp), catfish (*Clarias gariepinus*), sardine (*Rastrineobola argentea*), lung fish (*Protopterus aethiopicus*), ningu (*Labeo victorianus*), soga (*Brycinus spp*), gogogo (*Barbus spp*) and others. The list of fish was determined based on a pilot survey on the landing sites in Nungwe bay and information collected from Nyegezi Fisheries Research Institute (TAFIRI) in Mwanza.

From the same list of fish species, the interviewees were requested to recall how many fish meals they normally ate in a day. Additionally, the interviewees were requested to recall how many days they ate fish meals in a week for each type of fish on the list. Because of high illiteracy and the low ability to recall past events among the respondents which was observed during the pilot survey, the possible answers were limited to five. These answers were: not at all, one day, two days, three days or more than three days. Using the same list of fish, the interviewees were also requested to give information on the size of the portions of fish they consumed in a day. The possible answers for the size of the portions included: whole, half, quarter or less of the fish. In addition, to avoid undue emphasis on fish the respondents answered questions on the frequency of eating meat or other

sources of proteins in their diets. For the focus group discussions, eight groups of six women each were involved. The women were drawn from those who participated in the questionnaire survey.

5.2.3. Quantifying the edible portion of fish

The fish that were found in houses during interviews and at landing sites (intended for home consumption) were weighed [i.e. nile perch (n=27), tilapia (n=46), catfish (n=15), lungfish (n = 8) and sardines were measured in 26 households]. To quantify the edible part of different species of fish, the percentage of the edible flesh of the respective species of fish was; tilapia (37%) (FAO, 1989), nile perch (42%) (Ogunja *et al.*, 1990), catfish (70.6%) (Oyedapo and Mosunmola, 2005). The percentage edible part for catfish was assumed to also apply for lungfish and 100% was considered for sardines. The weight of the consumed portion was derived according to the following formulae:

Weight of the consumed portion (g) = average weight of fish (g) x % of edible part x fraction of the fish consumed

5.2.4. Collection of fish samples

Species of fish within the size and classes determined in the fish consumption interview were purchased at two landing sites in Nungwe Bay. Seventy nine specimens of five fish species were collected. These included nile perch (n=24), tilapia (n=26), catfish (n=9), sardine (n=10) and lungfish (n=7). The fish were identified and weighed and approximately 100 g of muscle tissue was removed from the dorsal area for nile perch, tilapia, catfish and lungfish. The whole fish was used for sardines. The sampled tissues were individually transferred in nitric acid washed glass containers with tight caps. The containers were then transferred in a cooler box to the laboratory in Mwanza city where the samples were frozen.

5.2.5. Analysis of total mercury

Total mercury analysis in fish was performed at the Southern and Eastern Africa Mineral Centre (SEAMIC) in Dar es Salaam, Tanzania. Homogenized samples of the tissues (2 g ww) were digested to a transparent solution with 15 ml of the mixture HNO_3:H_2SO4 (1:2) mixture under reflux. The resultant solutions were then diluted to 20 ml with deionised water and the total Hg concentrations were analysed by Atomic Absorption Spectrophotometer with cold vapour generation technique (ICP Ultima 2, Horiba Jobin Yvon, France) after reduction with $SnCl_2$. Acid washed glassware, analytical grade reagents and double distilled then deionised water were used in the tissue analysis.

In order to check the purity of the chemicals used, one chemical blank was run every 10 samples. There was no evidence of contaminations in these blanks. Analytical quality control was ensured through the analysis of certified reference material DORM-2 (Dogfish muscle) obtained from the National Research Council Canada (4.64 ± 0.26 μg Hg g^{-1} certified level of mercury). All data are expressed as μg g^{-1} ± standard deviation (SD) on a wet weight basis.

5.2.6. Estimation of exposure to mercury

We assumed that 87.8% of the Hg in the fish caught from Nungwe Bay was methylmercury (Ikingura and Akagi, 1996; US EPA, 2001). Exposure to MeHg was calculated using the dietary exposure approach proposed by Chien *et al.*, (2007) using the following equation:

$$\text{Exposure dose } (\mu g\ kg^{-1}\ day^{-1}) = \frac{[\text{MeHg concentration in fish } (\mu g\ g^{-1} \text{ fresh weight}) \times \text{consumption } (g\ day^{-1})]}{\text{Average body weight (kg)}}$$

Consumption was calculated as a product of average number of fish meals consumed per day and the average weight of portion of fish consumed. The average body weight of the interviewed women (55 ± 8.2 kg) was used in the above equation. Using the obtained data, three plausible exposure scenarios were constructed: (1) women that eat a diet with meals composed of a single species of fish for either one, two, three or seven days in a week and eat other species of fish during the remaining days; (2) women that eat a diet with meals composed of two or three specific species of fish for either one, two, three or seven days in a week and eat other species of fish during the remaining days; (3) women that eat a diet composed of all five species of fish for either one, two, three or seven days in a week and eat fish meals composed of single species of fish during the rest of the week. Risk was examined by comparing the calculated MeHg intake to the WHO/FAO PTWI of 1.6 μg MeHg/kg body weight/ week.

5.2.7. Data analysis

The questionnaire data were summarized into spreadsheet database files and combined with fish mercury data. Mean values and graphing of average % were used to characterize the patterns of fish consumption. These were done by using the Statistical Package for the Social Sciences (SPSS). Comparison of Hg concentrations in different fish species was done using one way ANOVA (STATISTICA version 6.0 software).

5.3. Results and discussion

5.3.1. Fish consumption pattern

Table 5-1 provides the weekly frequency of consumption of different species of fish. The results indicate that species which are frequently consumed are tilapia, sardine, nile perch and catfish. Lungfish was consumed by only 15% of the respondents. Tilapia was the most consumed fish in the study area, with 38% and 32% of the respondents consuming it three and two days a week, respectively.

Table 5-1. Weekly frequency of consumption of different fish species by women in Nungwe village, Geita district, Tanzania. (% of respondents)

Days/week	Nile perch (%)	Tilapia (%)	Sardine %)	Catfish (%)	Lungfish (%)
Not at all	0	0	3	43	85
One day	46	7	59	48	9
Two days	38	32	21	6	5
Three days	9	38	16	2	0.5
More than 3 days	7	23	1	1	0.5

The relatively few species of fish (5) which were available to the studied community can be explained by the widely reported decline of fish biodiversity in Lake Victoria (Balirwa *et al.*, 2003; Ogutu-Obwayo, 2004). It is estimated that some 200 endemic species of haplochromines and other non haplochromine species which previously comprised 90% of the fish biomass and formed the main part of diet for native communities has declined to near extinction (Witte *et al.*, 2007). The present fishery is dominated by only three species: nile perch, tilapia and sardine (Getabu *et al.*, 2003). Lungfish inhabit mainly shallow inshore waters; the extensive destruction of wetlands for agricultural use (Hongo and Masikini, 2003) has lead to the decline in their catch (Kees *et al.*, 2002) as is reflected also in the small percentages of respondents (15%) who reported eating this species. In general, eighty two percentage of the interviewed women reported eating two fish meals every day. While, 15 and 3 percentages of the interviewed women reported eating one and >2 fish meals a day, respectively.

The average weight (g) (± SD) of nile perch, tilapia, catfish and lungfish, which were either found at home or at landing sites (earmarked for home consumption), were 1336 ± 213; 859 ± 78; 2234 ± 372 and 2010 ± 342 g, respectively. It appears that, except for tilapia, moderately small sized fish are the ones selected for home consumption. Our discussions with fishermen at the landing sites

established that they prefer selling large sized nile perch to commercial traders because they fetch a good price. On the other hand it also become clear that the number of large sized catfish and lungfish that are caught in Nungwe bay has been decreasing during the last decade.

Table 5-2 shows the % of respondents consuming different portions of the various species of fish in a day. For nile perch, 2%, 86% and 12% of respondents eat half, quarter and one sixth of the fish, respectively. For catfish and lungfish, the majority of respondents (i.e. 89% and 92%, respectively) ate approximately one tenth of the fish in a day. For tilapia, 72%, 20% and 8% of the respondents ate half, quarter and whole fish, respectively. Because of their small size, sardines are always eaten in bulk of approximately 120 g in a day. The calculated daily average fish intakes were 140, 159, 121, 158 and 142 g/day for nile perch, tilapia, sardines, lungfish and catfish respectively. The calculated total average (± SD) daily fish intake in the studied community was 144 ± 15 g/person/day.

Table 5-2. Percentage of respondents consuming different sized portions of different species of fish per day in Nungwe village, Geita district, Tanzania.

Days/week	Nile perch (%)	Tilapia (%)	Sardine (%)	Catfish (%)	Lungfish (%)
Whole fish	0	8	0	0	0
Half fish	2	72	0	0	0
Quarter fish	86	20	0	11	8
Others*	12	0	100	89	92

* sardine = eaten in bulk of ~ 120 g, nile perch =one sixth of the fish, lungfish and catfish = one tenth of the fish

The average daily fish intake calculated in the present study exceeds the Tanzanian average fish intake of 17g/day/person (FAO, 2005) and those documented for women in Greece (18.2g/day), Spain (26.4 to 46.2 g/day), the UK (15.7 g/day), Germany (5.6 to 7.1 g/day), Sweden (9.7 to 14.9 g/day) and France (20.6 to 27.9 g/day) (Welch *et al.*, 2002). The fish intake of women in Nungwe village is also higher than the documented fish intake for the general public in the UK (21 g/day), Spain (75 g/day), Norway (53 to 80 g/day), Greece (38 g/day) (Byrd-Bredbenner *et al.*, 2000), Canada (22.3 to 50 g/day) (Legrand *et al.*, 2005) and Chile (33.1 g/day; Muñoz *et al.*, 2005). The high consumption of fish in the studied community is probably due to lack of access to other food stuff. In general, populations in rural Sub-Saharan Africa depend on maize, rice and tuber crops as their staple food (Kinabo *et al.*, 2006). For communities residing in the vicinity of lakes and oceans,

however, fish consumption complements these main food items. Lack of access to a variety of food items was also evident in the studied community as only 10% of the respondents reported eating meat at least once a week. In a fish consumption survey in a community living near Mindu dam in the Morogoro district (Tanzania), Mdegela (2006) found that most families were eating one to three fish meals in each day. A study in Lubindu village near the shore of Lake Nyasa, (south west Tanzania) reported a fish consumption rate of 300 to 600 g of fish per day (Winnicki *et al.*, 2002). Similar high fish consumption patterns have been reported in the Seychelles, where women were reported eating an average of 12 fish meals a week (Davidson *et al.*, 2006).

5.3.2. Estimated methylmercury intake

5.3.2.1. Concentration of mercury in fish

The concentration of Hg in fish was species-specific and varied according to the feeding habits and size of the fish. The mean (± SD) mercury levels were 0.41 ± 0.04, 0.24 ± 0.02, and 0.51 ± 0.17 µg Hg g^{-1}ww for nile perch, tilapia and catfish, respectively. Samples with Hg levels below the detection limit were registered in 40%, 46% and 33% of nile perch, tilapia and catfish samples, respectively. Also, all collected specimens of sardine and lungfish had Hg concentration below the limit of detection. For the purpose of estimating the MeHg intake, these samples were assigned a value of 0.01 µg Hg g^{-1} which is half the value of the limit of detection (0.02 µg Hg g^{-1}) (Govaerts *et al.*, 2005). The observed variation of mercury levels among the different species is related to the uptake process of this metal in fish and the interaction of numerous parameters, either abiotic (water and sediments) or biotic (size, sex, longevity, growth rate, feeding habits, trophic position, habitat). It has been documented that benthic feeders show higher total mercury levels in their muscle tissues than pelagic species (Campbell *et al.*, 2003b). In this context, catfish a benthic feeder that lives in close proximity to the sediment showed higher levels of mercury than the more pelagic species. Mercury concentrations in catfish similar to the levels found in this study have been reported elsewhere in the Lake Victoria basin. For example, catfish collected from some ponds in the Rwamagasa and Isanga river (which are areas also impacted with Hg from gold mines) had mercury concentrations ranging from 0.3 to 2.5 µg Hg g^{-1} ww (Taylor *et al.*, 2005; van Straaten, 2000b). The high level of mercury found in nile perch reflects the higher trophic position of this species. It has been shown that in Lake Victoria nile perch occupy the highest trophic position (Campbell *et al.*, 2003b) making it more vulnerable to Hg accumulation. The present study showed higher Hg concentrations in nile perch caught in Nungwe Bay compared to the Hg levels that were found in the

same species by Ikingura and Akagi (1996) and Machiwa *et al.* (2003). Assuming that fish of nearly equal size were sampled, our findings indicate that with time there is a progressive increase of mercury body burden in nile perch. The absence of mercury in sardine and tilapia can be explained by their planktivous feeding nature.

5.3.2.2. Exposure of MeHg to women

The total mercury concentration analysed in fish from this study are below the mercury quality guidelines proposed in most countries and recognized by international organisations. These include the guidelines for Canada and Brazil (0.5 µg Hg g^{-1}ww); Finland, Sweden, and Japan (1 µg Hg g^{-1}ww); and by the European Community (1 µg Hg g^{-1}ww for predatory fish and 0.5 µg Hg g^{-1}ww for non-predatory fish) (EU, 2005). The predatory fish (nile perch and catfish) contained mercury levels which are lower than the recommended safe limit of 1 µg Hg g^{-1}ww, while sardine, tilapia and lungfish (i.e. the non-predatory fish) had mercury concentrations lower than the accepted limit of 0.5 µg Hg g^{-1}ww. Based on the above mercury quality guidelines, all fish analyzed in this study would be considered safe for human consumption. However, because of the high consumption rate of fish in the studied community Hg intake may be higher. Table 5-3 provides comparison of estimated weekly MeHg intake for women eating a single species of fish daily at varying frequencies and PTWI. These results show that consumption of nile perch and catfish for two or more days and tilapia for three or more days a week would result in a MeHg intake leading to exceedence of the PTWI of 1.6 µg/kg body weight per week. If we consider a situation, where a woman will consume a mixed meal of all species every day of the week, the average weekly MeHg intake would be 4 µg/kg body weight, which is well above the PTWI.

Table 5-3. Comparison of estimated MeHg intake (µg/Kg body weight/week) from meals with single species of fish and the WHO/FAO Provisional Tolerable Weekly Intake (PTWI) of 1.6 µg/kg body weight/week for women in Nungwe village

Fish type	Average fish intake (g/kg bwt/day)	MeHg concentration (µg MeHg/g) in fish	Consumption frequency (days/week)			
			One day	Two days	Three days	> three days†
Nile perch	2.55	0.36	0.92	1.84*	2.75*	6.43*
Tilapia	2.89	0.21	0.61	1.21	1.82*	4.25*
Sardine	2.20	0.01	0.02	0.04	0.07	0.15
Lungfish	2.58	0.01	0.03	0.05	0.08	0.18
Catfish	2.87	0.45	1.30	2.58*	3.87*	9.03*
Average	**2.62**	**0.21**	**0.57**	**1.15**	**1.72***	**4.00***

† Assuming a consumption frequency of seven days/week

* Exceeds the PTWI (1.6 µg MeHg/kg bwt/week)

The results provided in Table 5-4, show a comparison of the estimated weekly MeHg intake for women eating a combination of either two or three species of fish daily at varying frequencies a week and the PTWI. Consumption for two or more days a week of catfish in combination with either nile perch or tilapia will lead to MeHg intake exceeding the PTWI. Similarly, PTWI is exceeded if a meal comprising of catfish with either sardine or lungfish is eaten for three or more days a week. When nile perch and tilapia are eaten in combination for three or more days a week the MeHg intake will also exceed the PTWI. A combination of the three most consumed species of fish in the studied community (nile perch, tilapia and sardine) for seven days a week also results in a MeHg intake that exceeds the PTWI.

Table 5-4. Comparison of estimated MeHg intake (µg/Kg body weight/week) from meals consisting of a combination of fish species and the WHO/FAO Provisional Tolerable Weekly Intake (PTWI) of 1.6 µg/kg body weight/week.

Combination of fish meals	Average fish intake (g/kg bwt/day)	MeHg concentration (µg MeHg/g) in fish	Consumption frequency (days/week)			
			One day	Two days	Three days	> three days†
Nile perch + tilapia	2.72	0.29	0.78	1.55	2.33*	5.43*
Nile perch + sardine	2.37	0.19	0.44	0.88	1.32	3.07*
Nile perch + lungfish	2.57	0.19	0.48	0.95	1.43	3.33*
Nile perch + catfish	2.71	0.41	1.2	2.2*	3.3*	7.68*
Tilapia + sardine	2.55	0.11	0.29	0.56	0.84	1.96*
Tilapia + lungfish	2.74	0.11	0.30	0.60	0.91	2.11*
Tilapia + catfish	2.88	0.33	0.95	1.90*	2.85*	6.65*
Sardine + lungfish	2.39	0.01	0.02	0.05	0.07	0.17
Sardine + catfish	2.54	0.23	0.58	1.17	1.75*	4.09*
Lungfish + catfish	2.73	0.23	0.63	1.26	1.88*	4.4*
Nile perch + tilapia + sardine	2.55	0.19	0.49	0.97	1.45	3.39*

† Assuming a consumption frequency of seven days/week

* Exceeds the PTWI (1.6 µg MeHg/kg bw/week)

Contrary to other authors who have analysed fish samples collected from various points around Lake Victoria and who concluded that there is no health risk in eating fish caught from Lake Victoria (Ikingura and Akagi, 1996; Machiwa, 2005), we suggest that at least in the studied community there is a clear health risk. Indeed, the results from our survey and mercury analysis point to a potential health risk for the local communities consuming fish caught from areas near artisanal gold mines. These results show that at the current fish consumption pattern and Hg concentrations in fish the unborn children from women who eat nile perch and catfish for two or more days in a week are at the risk of Hg poisoning. Furthermore, consumption of tilapia for three or more days would also results in a risk. Nile perch and tilapia can be safely consumed up to three days a week, if each of them is combined with either sardine or lungfish. Eating a meal that is combining either tilapia or nile perch with catfish for two or more days in a week however, will results in Hg intake above the PTWI. In general, our results show that children born to 61% of the interviewed women are at risk of suffering Hg related illnesses because they reported eating fish at a weekly frequency that results in an Hg intake above the PTWI. Possibly, the previous studies were

largely aimed at evaluating the suitability of fish caught in Lake Victoria for export to the European market, with less consideration to the health of local consumers. As a result, their conclusions are only based on the premise that analysed mercury concentrations are below the internationally accepted limits. Because these international limits are based on fish consumption patterns found mainly in developed countries (JECFA, 2003) they might not protect consumers in African subsistence populations. This study has demonstrated that the fish consumption pattern in the studied community differs from those usually considered in the derivation of safety limits. For instance, while the frequency of fish consumption in developed countries ranges from one to three meals a week (Panagiotakos *et al.*, 2007; Welch *et al.*, 2002), families in the studied community eat fish meals twice a day. This high fish consumption enhances MeHg intake even when the concentration of Hg in the fish are below the international safety limits. These findings are in agreement with the results obtained in a survey among recreational anglers and native tribes in the United States of America (USA) (Mariën and Patric, 2001). In that survey, anglers and members of the native tribes had a MeHg intake that was higher than the national average due to high fish consumption. Similar findings have also been reported in Canada among ice anglers (Flaherty *et al.*, 2003) and in a coastal community on the bay of Fundy (Legrand *et al.*, 2005). Differences in fish consumption among communities in the USA has been also reported by Burger *et al.*, 1999.

The MeHg intake estimated in this study may even be higher if the consumption pattern of fish changes and is dominated by large fish or exclusively catfish. It should also be considered that during the rain season when the rivers are flooded catfish moves upriver to spawn making them easy to catch and thus more available for consumption. As such, the high rate of fish consumption observed for women living in Nungwe Bay may also apply to other communities residing in the vicinity of other lakes in Africa. It is important that collectively or individually, sub-Saharan African countries formulate safety limits for mercury in fish based on realistic subsistence consumption patterns occurring in the sub-continent.

5.4. Conclusions

Results from the present study indicate that nile perch, tilapia, catfish, sardine and lungfish are the most frequently consumed fish in Nungwe village and probably also in neighbouring villages. Mercury concentrations in these fish were below the mercury concentration guidelines proposed in the WHO/FAO codex alimentarius of 1 μg Hg g^{-1} for predatory fish and 0.5 μg Hg g^{-1}ww for non-

predatory fish. However, the calculated average fish intake of 144 g/person/ day among the women in the Nungwe village exceeds by far the Tanzanian national average fish intake (17g/day) and that of women in Greece (18.2 g/day), Spain (26.4 to 46.2 g/day) the UK (15.7 g/day) and France (20.6 to 27.9 g/day). Consequently, the calculated weekly MeHg intake of women in this village exceeds the WHO/FAO established PTWI levels. It is recommended that measures to reduce mercury input in Nungwe bay should be taken and that MeHg intake of women and children in that area should be monitored. As a long term strategy for public health protection, safety guidelines based on the local fish consumption patterns should be developed. Where possible, site-specific guidelines for fish consumption in areas known to be contaminated with mercury should be formulated.

Chapter six

Mercury residues in free grazing cattle and domestic fowl living in the artisanal gold mining area of Geita district, Tanzania

6. Mercury residues in free grazing cattle and domestic fowl living in artisanal gold mining area of Geita district, Tanzania

Abstract

Environmental contamination with mercury from artisanal gold mines in the Lake Victoria basin is wide spread. People living in or around mining villages keep domestic animals which are allowed to feed freely in mercury contaminated areas. This study investigated Hg accumulation in the liver and muscle tissue of cattle and domestic fowl reared in and around mining villages. Data from mining villages were compared with the results collected in a reference area. Total mercury levels up to 436 µg/kg and 820 µg/kg wet weight were found in liver samples taken from cattle and domestic fowl, respectively. Significantly higher mercury concentrations were found in liver samples collected at the mining villages ($p<0.05$) than those taken from the reference area. While mercury concentrations in liver samples exceeded the acceptable maximum concentrations for human consumption set in the some European countries, the Hg concentrations in muscle were below human consumption limits of most countries. However, due to low consumption of meat and chicken in the studied community, there is no immediate health risk to local consumers. But, for long term public health protection it is recommended that the keeping of freely grazing cattle and domestic fowl in or around artisanal gold mines should be avoided.

Key words: artisanal gold mining, mercury, liver, muscle tissue, cattle and domestic fowl

Redrafted from:

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Mercury residues in free grazing cattle and domestic fowl living in artisanal gold mining area of Geita–Tanzania: Food Additives and Contaminants. Submitted

6.1. Introduction

Concerns about the effects of mercury pollution on human health increased considerably after the Minamata disease event in Japan during 1960s (Harada, 1995). Since then, much information has been gathered on mercury concentrations in fish and seafood (Machiwa *et al.* 2003; Levine *et al.*, 2005 and Seixas *et al.*, 2005) as these were the main mercury source in this poisoning incident. In contrast, the amount of data on mercury concentrations in terrestrial livestock, meat and milk products is limited. The use of mercury in gold recovery by artisanal gold miners in the Lake Victoria basin, Tanzania has been widely documented (van Straaten, 2000a; Ikingura and Akagi, 1996, Kondoro and Makundi, 1998; Taylor *et al.*, 2005; Ikingura *et al.*, 2006). One of the most affected districts is Geita which is situated in the south west of Lake Victoria. The Geita district has the largest number of mining centres in the Lake Victoria Gold Fields (LVGF) among which are the four major centres: Nyarugusu, Nyakagwe, Mgusu and Rwamagasa (Tesha, 2003). Chemical analysis of water, sediment, soil and discarded tailings around these mines have been shown to contain elevated concentrations of mercury (see chapter 2). Soils and sediments collected around Mugusu mine by Ikingura *et al.* (1997) measured up to 28.3 and 136 mg Hg kg^{-1} dw, respectively. Concentrations up to 2.84 and 56.5 mg Hg kg^{-1} dw were also measured in sediments and tailings at Rwamagasa (Taylor *et al.*, 2005).

People from communities residing around gold mining areas predominantly subsist on crop farming and livestock keeping (Kitula, 2006). In this area, cattle are allowed to graze freely around the mine sites and drink water from Hg contaminated rivers and ponds. In some villages, during the dry season the mercury contaminated rivers and riverbanks around the mines are the only available watering points and source of green pastures for ruminant animals (Chibunda, personal observation). Previous studies indicated that crops grown in the vicinity of gold ore sluice/washing areas had elevated levels of mercury. For example, up to 0.092 and 5.1 mg Hg kg^{-1} dw of Hg was found in yams and rice, respectively (Taylor *et al.*, 2005). High concentrations of mercury in vegetables and wetland plants have also been reported by the Lake Victoria Environmental Management Project (LVEMP) team (2002). Therefore, it is possible that natural pastures found in such areas are similarly contaminated. In addition, domestic fowl reared within the mining villages are free ranging and thus able to scavenge around the compounds including the amalgamation ponds. They have also been observed to exhibit scratching and pecking behaviour in the discarded tailings (Lyatuu, 2002).

The aim of the present study was to establish the accumulation of Hg in the liver and muscle tissues of cattle and domestic fowl reared in and around mining villages. For comparison, the Katunguru ward of the adjacent Sengerema district which has no history of gold mining was selected as a reference area. In the present chapter, results of Hg accumulation in cattle and fowl and possible public health implications are described and discussed.

6.2. Materials and Methods

6.2.1. Animal and area of study

Liver and muscle samples from cattle (*Bos indicus*) and domestic fowl (*Gallus domesticus*) were collected in July through November 2005. Samples were collected from three mining villages (Mugusu, Rwamagasa and Nyarugusu) in the Geita district and from the Katunguru ward in the Sengerema district (Figure 6.1). For the Geita district, liver and muscle samples from cattle were collected from the district council slaughter house. Information about the origin and history of animals slaughtered and subsequently sampled were provided by local meat inspectors and butchermen based on the information contained in the movement permit for each respective animal. Movement permits are documents which are issued by the veterinary department of the village/location from where the animal originates. These permits contain information that specifically identifies the animal, village of origin, owner, health status, reason for movement and destination. For the present study, only animals with authentic movement permits from Mugusu, Rwamagasa and Nyarugusu mining centres were sampled. A total of 18, 13 and 17 animals were sampled from Mugusu, Nyarugusu and Rwamagasa, respectively. For comparison purposes, 11 animals from the Katunguru ward in the Sengerema district were also sampled. Only animals aged above two years were sampled. For domestic fowl, 16 and 5 birds of different age were bought from different households in the Mugusu mining village and Katunguru ward, respectively. All birds were weighed before being sacrificed.

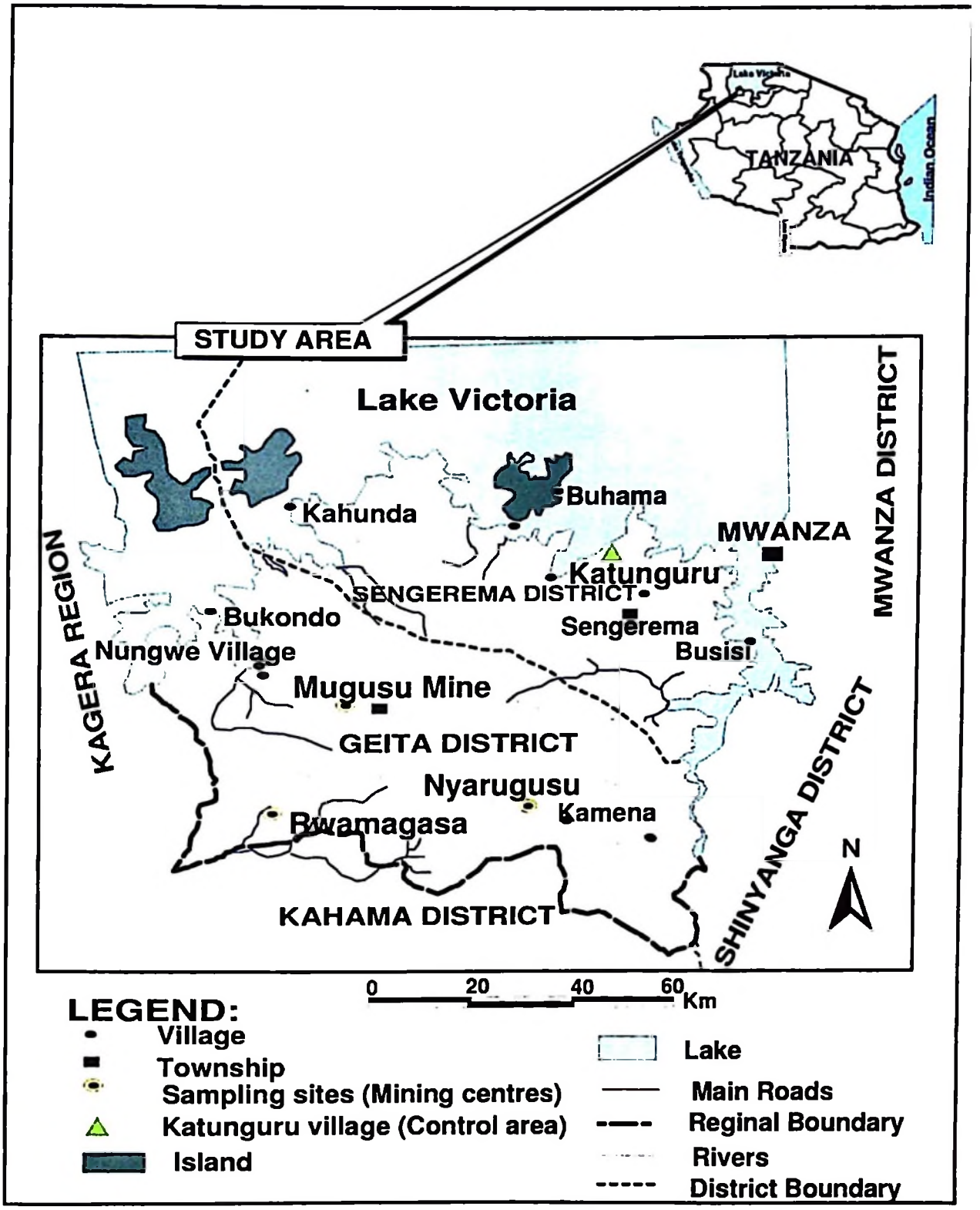

Figure 6.1. A map of Tanzania showing the study areas (● mining villages, ▲ Katunguru ward)

6.2.2. Tissue collection

Samples of at least 200 g were taken from the *Lobus caudatus* of the liver immediately after opening the abdomen cavity. A similar size of the *Longissimus lumborum* muscle was simultenously cut from the cattle carcass. This internal muscle was selected because it is not easily contaminated during skinning. Liver and pectoral muscle tissue (200 g) was sampled from domestic fowl. Each sample was carefully cut by using a disposable stainless steel surgical blade. Samples were individually preserved in nitric acid washed glass containers with tight caps and packed in self zipping plastic bags. The bags were then kept in a cooler box and transported to the laboratory in Mwanza city. In the laboratory, visible fat, connective tissue and major blood vessels were removed from the samples. The samples were subsequently homogenised and stored at -20°C until analysis.

6.2.3. Mercury determination in samples

Samples of liver and muscle (2 g) were acid-digested by using diluted nitric acid (HNO_3:H_2O = 2:1) at 130°C for 2 hours. Undissolved particles were filtered off and the solution diluted to 25 mL with deionized water. The concentration of mercury was determined using Atomic Absorption Spectrophotometer with cold vapour generation technique (ICP Ultima 2, Horiba Jobin Yvon, France) Acid washed glassware, analytical grade reagents and double distilled water were used in the tissue analysis. In order to check purity of the chemicals used, one chemical blank was run with every five samples. There was no evidence of contamination in the blanks. Analytical quality control was ensured through the analysis of certified reference material CRM (Bovine muscle) obtained from Promochem, Welwlyn Garden City, UK (2.6 µg/kg certified level of mercury). The calculated recovery ranged from 96 to 100%. Therefore results of the analysed samples were not corrected for recovery.

6.2.4. Data treatment

Mercury concentrations in liver and muscles from the mining villages and the control village were tested for normality and homogeneity using the Shapiro-Wilkinson test. The differences between mercury concentration in the organs (liver vs muscles) and between the mining villages and the control village were determined by one-way analysis of variance (ANOVA) (STATISTICA version 6.0). Results were considered significant with $p<0.05$. Relationships between mercury concentration in domestic fowl liver samples and body weight were explored using linear regression. Data were normal log-transformed before analysis to meet the underlying assumptions of the tests. The average

values reported are the geometric mean ± SD wet weight. For statistical purposes, samples below the Level of Detection (LOD) of Hg (20 µg/kg) were assigned half of the LOD (Govaerts *et al.*, 2005).

6. 3. Results and discusion

6.3.1. Cattle

Liver samples taken from cattle living in/near the mining villages exhibited the highest levels of mercury. The maximum concentration in the liver samples was 436 µg/kg ww. All muscle samples and 45% (5) of the liver samples taken from animals slaughtered in the Katunguru ward did not contain detectable mercury residues. In contrast, 24% (11) of muscle samples obtained from animals in the mining villages contained detectable levels of mercury ranging from 22 to 81 µg/kg ww. The observed mercury accumulation pattern in cattle studied in the present study are similar to patterns reported for cattle from mercury contaminated areas in Spain (Alonso *et al.*, 2002 and Alonso *et al.*, 2003a) and for pigs in Czech Republic (Ullrich *et al.*, 2001). Like in the present study, the above studies demonstrated that the liver contained higher concentration of mercury than the muscles for both cattle and pigs. These differences in concentrations highlight the difference in the physiological function of the two tissues and reflect the vital role of the liver in the storage and detoxification of contaminants (Simpson *et al.*, 1997; Rudolfs *et al.*, 2000). Levels of mercury in both muscle and liver tissue of cattle from the Katunguru wards were similar to or lower than those reported in cattle from unpolluted areas in some European countries. For example, analysis of liver samples taken from cattle kept in North West Spain (Alonso *et al.*, 2003a, 2003b), Sweden (Jorhem *et al.*, 1991), and Poland (Falandysz, 1993) contained mercury concentration of 1.01, 6, 4.2 and 57 µg/kg ww, respectively. Corresponding muscle samples had values of 0.42, 5 and 1.2 µg/kg.

Mercury levels in cattle liver samples from the Rwamugasa, Mugusu and Nyarugusu mining villages were significantly higher than those in samples from Katunguru ward ($p<0.05$) (Figure 6.2). The high mercury residues in samples originating from the mining villages can be explained by the indiscriminate use of mercury for gold amalgamation (Veiga and Baker, 2004) which has resulted in natural pasture and water contamination (Ikingura *et al.*, 2006). The relatively low mercury concentrations observed in the liver samples from Rwamagasa (49.0 µg/kg) compared to Mugusu (84.0 µg/kg) and Nyarugusu (112 µg/kg) mining areas can be attributed to the decrease in active artisanal mining at Rwamagasa village. Indeed, here the number of artisanal miners has decreased in the last four years, following privatisation of gold rich plots and the eviction of artisanal miners (Manaku, personal communication). Therefore, the decrease in active gold mining might have reduced mercury contamination to pasture and water.

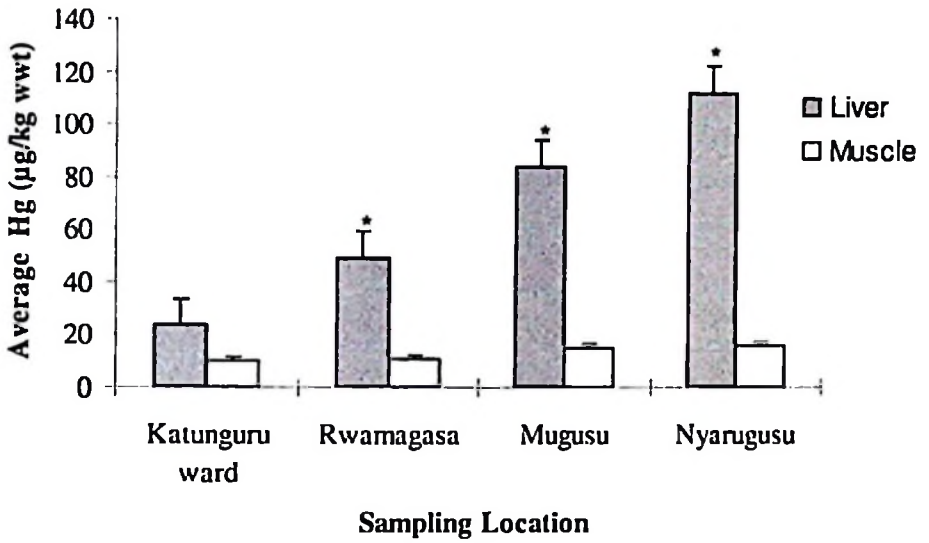

Figure 6.2. Mean concentrations (± SD) of Hg in the muscle and liver samples collected from cattle in artisanal mining villages and a control area (Katunguru ward)

(*= significantly different from Katunguru ward)

Only a generalized comparison is possible between mercury residues observed in cattle living in the mining villages of our study area with data reported in literature. This is because there is considerable variation between published studies in their limits of detection, the value assigned to non-detection concentrations (zero, half the limit of detection (like in this study), or limit of detection value) and the way the average values are reported (arithmetic means, geometric means or medians). Nevertheless, it is clear from our results that artisanal gold mining has a significant effect on the mercury levels in cattle. Concentrations of mercury measured in liver sample collected from mining villages are higher than levels reported for cattle living in other mercury contaminated areas. Ullrich *et al.* (2007) measured an average of 5.74 µg/kg (ww) from a chlor-alkali plant contaminated area in Northern Kazakhstan, and Miranda *et al.*, (2003) reported up to 8 µg/kg of mercury in the kidney tissue taken from calves grazing in an industrial area in Northern Spain. It is clear that average mercury concentration in liver samples from Mugusu (84.0 µg/kg) and Nyarugusu (112.6 µg/kg) mining villages are well above the concentrations reported in the above-cited studies. It is also evident that the mercury levels in our samples from the mining villages are above established regulatory limits for cattle in the Netherlands and Poland (Table 6-1). Tanzania has no established regulatory limit for mercury levels in meat and meat products (Tanzania Bureau of Standards (TBS), 2002). However, comparison of the commonly used acceptable maximum concentration for mercury

of 50 µg/kg for liver (Alonso *et al.*, 2003a) with the average mercury concentration in liver samples indicates that this acceptable maximum concentration is attained at Rwamugasa and exceeded in the Mugusu and Nyarugusu samples. Considering the average Tanzanian daily meat consumption rate of 18 g/person/day (FAO, 2005) in the relatively unlikely situation where an individual would be eating liver only meal such a person will consume 0.9, 1.5 and 2.0 µg Hg/day for cattle from Mugusu, Nyarugusu and Rwamagasa, respectively. These values are well below the WHO and FAO recommended daily Hg intake of 49 µg/person (JECFA, 2003) thus, there is no immediate health risk to the local consumers.

Table 6-1. Regulatory limits for total mercury in cattle products in various countries and levels measured in artisanal gold mining villages in Tanzania.

Regulatory limits	Liver (µg/kg ww)	Muscles (µg/kg ww)	Reference
Canada	500	500	Salisbury *et al.*, (1991)
Poland	30	30	Zarski *et al.*, (1997)
The Netherlands	50	50	Vos *et al.*, (1992)
Slovac Republic	100	10	Kottferovà and Korènekovà (1995)
Egypt	500	500	EOSQ, 1993
Hg levels. in cattle from mining villages			
Mugusu	84	15	Present study
Nyarugusu	112	16	Present study
Rwamagasa	49	11	Present study

6.3.2. Domestic fowl

In the present study mercury concentrations in domestic fowl exhibited the similar patterns as the ones observed in cattle. Fowl liver samples contained higher mercury concentrations than the muscle samples ($p<0.05$) (Figure 6.3). This is in agreement with several other studies which demonstrated that liver samples obtained from birds living in mercury contaminated environments have higher mercury concentrations in liver than in muscle. For example, for domestic ducks reared in Mugusu mining village, Lyatuu (2002) reported mercury concentrations ranging from 19 µg/kg to 373 µg/kg and from 4 µg/kg to 32 µg/kg in liver and lung samples, respectively. Wayland *et al.* (2005) observed the same Hg concentration patterns in Canadian arctic ducks. In our study, liver samples collected from Mugusu mining village contained mercury concentrations which were six times higher than those observed in fowl from the Katunguru ward ($p<0.05$) (Figure 6.3). Mercury residues in poultry meat and offal have always been associated with animals feeding on mercury

contaminated feed (Sayo and Iromidayo, 2003). In both Katunguru ward and Mugusu mining village, domestic fowl are free ranging where birds are allowed to scavenge around the homesteads. Lyatuu (2002) reported seeing domestic ducks and chicken at the Mugusu mining village feeding from amalgamation ponds and abandoned tailings. It is therefore possible that these contaminated feeding grounds (Kondoro and Makundi, 1998; Taylor *et al.*, 2005) are the main source of the observed mercury residues in the analysed samples.

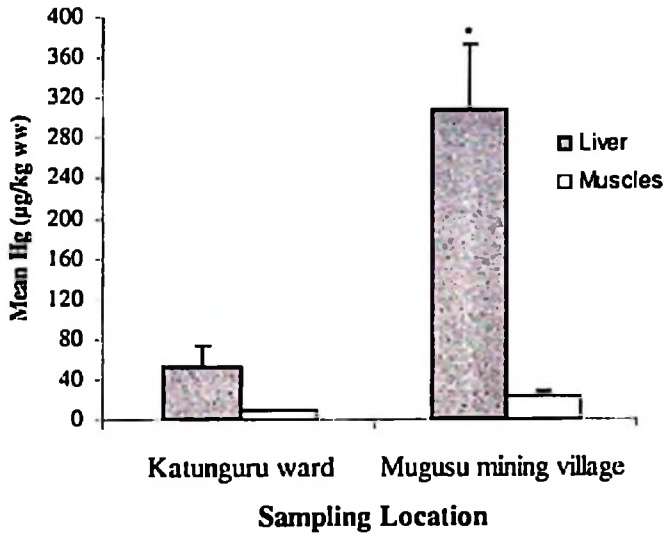

Figure 6.3. Mean concentrations of Hg (±SD) in the muscle and liver samples of domestic fowl collected from Mugusu mining village and from Katunguru ward (control village)

(*= significantly different from Katunguru ward)

We established that the mercury concentrations observed in the liver samples increased with the body weight of the fowl. A positive and significant relationship (R^2 = 0.68, F =32.54 and p = 0.0005) between body weight of the domestic fowl and mercury concentration in the liver (Figure 6.4) was observed. This finding indicates that mercury accumulates with age, probably due to increased exposure period. Domestic fowl (*G. domesticus)* in Tanzania generally have a low growth rate. Katule (1990) observed that these birds attain an average weight of 1000 g after 20 weeks. In a similar study, Msoffe *et al,* (2004) reported that domestic fowl of different ecotypes attained body weights ranging between 541 – 827 g after 16 weeks. It is possible that due to low growth rate, domestic fowl in the mining villages take a long time to attain slaughter weight which results in longer exposure periods and consequently higher Hg accumulation.

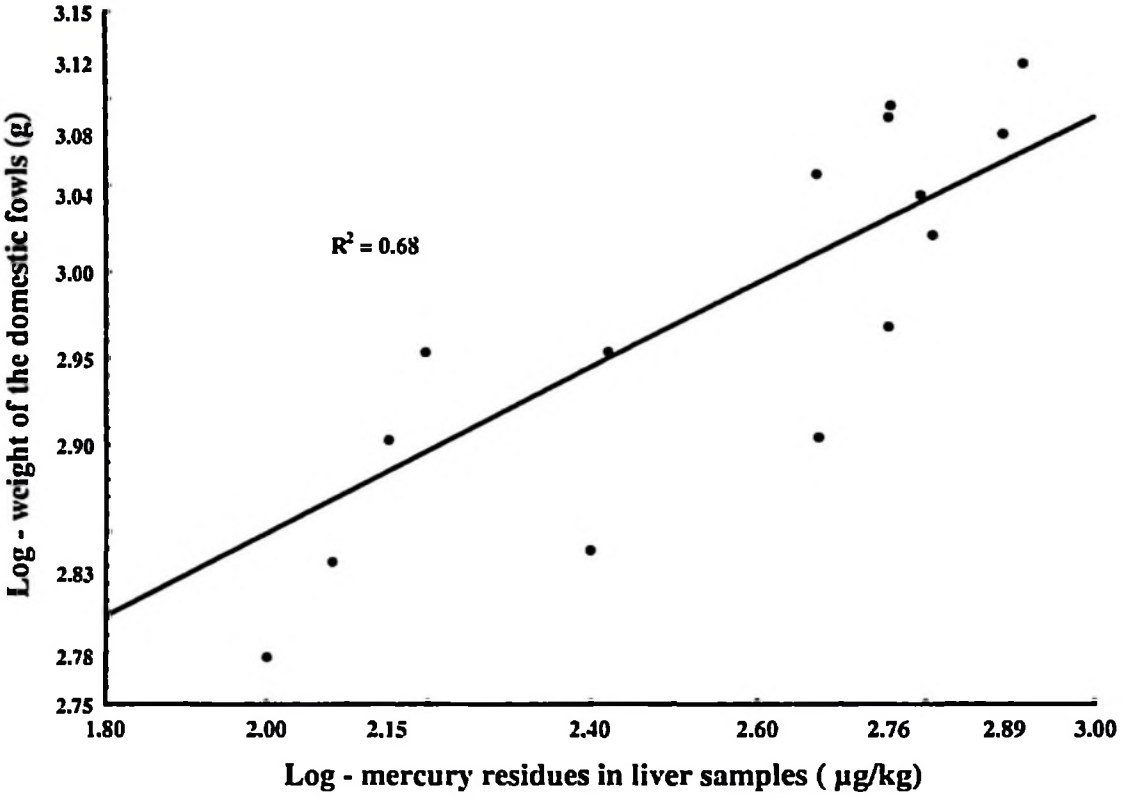

Figure 6.4. Relationship between the weight of domestic fowl and the total Hg levels in liver samples collected from birds in Mugusu mining village

Tanzania has not formulated regulatory limits for Hg contamination in poultry (TBS, 2002). Assuming that the regulatory limits for cattle products applies to poultry products, the mercury concentrations in the fowl liver samples exceeded regulatory limits established in the Netherlands, Poland and the Slovac Republic (Table 6.1). However, because of the low meat consumption frequency that was observed in this area during our food consumption survey (see chapter 5) and that the Tanzanian average poultry products daily consumption is only 3 g/day/person (FAO, 2005). It is unlikely that the current Hg concentration in chicken will have a significant health effect on the wider population in the studied community. However, health risk can be anticipated in case domestic fowl from gold mining villages are sold and consumed in areas with high meat consumption rate, such as urban centres.

6.4. Conclusion

In the present study total mercury levels up to 436 µg/kg and 820 µg/kg ww were found in liver samples taken from cattle and domestic fowl living in or near gold mining villages in Tanzania, respectively. Average mercury concentrations in liver samples exceeded the acceptable maximum concentration for human consumption proposed by different European countries. However, considering that the consumption of beef and chicken in the studied community is very low, there is no immediate health risk to the local consumers. As animals from the mining centres may be sold in urban areas of the country where meat consumption is high it is recommended that the keeping of freely grazing cattle and domestic fowl in or around artisanal gold mines should be avoided.

Chapter seven

Dietary exposure to mercury of the population residing in Mugusu artisanal gold mining village in Tanzania: a total diet approach

7.0. Dietary exposure to mercury of the population residing in Mugusu artisanal gold mining village in Tanzania: a total diet approach

Abstract

To date, estimates of dietary human exposure to mercury in artisanal gold mining areas in Tanzania have been limited to assessment of the contribution of consumption of Hg contaminated fish. However, WHO recommends the use of Total Diet Studies (TDS) which account for the contribution of all the representative food items in the diet. In this study the dietary Hg intake of people living in Mugusu gold mining village was determined using a total diet study approach. A 24 hour dietary recall indicated that 37 types of foods are consumed in that area. These foods were grouped into 11 food categories according to their chemical composition. Rice, maize, tubers and fish were the most consumed food categories with an average daily consumption of 134, 270, 113 and 97 g, respectively. The fish category contained the highest average content of total Hg (0.16 µg g^{-1} wet weight). Based on the total dietary approach the calculated average dietary intake of Hg was 32.2 µg/day, which is two times higher than the provisional tolerable daily intake established by FAO/WHO (15.5 µg/day) for protection of the foetus and young children. Based on the results from the present study it is concluded that the current total dietary intake of Hg in the Mugusu artisanal gold mining village is a risk to children born to women living in this village.

Key words: dietary exposure, mercury, total diet study, population in gold mining village, Tanzania

Redrafted from:

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Dietary exposure to mercury of the population residing in Mugusu artisanal gold mining village in Tanzania: a total diet approach. Food Additives and Contaminants: Submitted

7.1. Introduction

Many reports have been published on environmental Hg contamination associated with the artisanal gold mining in Tanzania. The build up of mercury in soils, sediments, fish, pastures and some root crops have been reported by various authors (Ikingura and Akagi 1996, 1999; Kondoro and Makundi 1998; LVEMP, 2002). For humans there is both a potential for direct exposure (i.e. persons handling Hg) and indirect exposure through consumption of Hg contaminated food. Until now, the studies that have been conducted to estimate exposure of humans to mercury in artisanal gold mining areas in Tanzania have been confined to examining exposure through fish consumption (Ikingura and Akagi, 1999; Machiwa, 2003). The World Health Organisation (WHO), however, recommends the use of Total Diet Studies (TDS) to assess total Hg intake. The TDS approach has the advantage of yielding more refined exposure data since it consists of analysing a representative 'market basket' of foods. This methodology takes into account the effects of kitchen preparation on the levels of contaminants in foods (WHO, 1985; Reilly, 2002; Kroes, 2002a). The food products are bought or collected from the normal retail channels and prepared as if for consumptions: edible parts from different food stuffs are analysed for the contaminant of interest (Hg-in this study). The combination of the metal concentrations measured in the food products analysed and the consumption information permits estimation of dietary exposure.

The objective of the present study was to assess the dietary mercury intake by the people living in the Mugusu artisanal gold mining village using the TDS approach. To evaluate the health risk, the estimated dietary daily Hg intake was compared with the Provisional Tolerable Intake recommended by the joint FAO/WHO Expert Committee for Food Additives (JECFA) for Hg for the protection of the general public (49 µg/day) and that for the protection of developing foetus (15.5 µg/day) (JECFA, 2003).

7.2. Materials and Methods

7.2.1. Total diet study

A 24 hours recall food survey was carried out with a representative sample (n = 217) of the adult population (aged 15-63) taken from the Mugusu mining village. All participants were visited at home and a food frequency and amount questionnaire combined with household containers of different sizes was used to estimate intake. The 24 hours recall indicated consumption of up to 37 food items, which were grouped into 11 food categories according to similarity in chemical composition. Food stuffs which were reported to be consumed in large quantities, e.g maize and rice, were considered as independent food categories (Table 7-1). Food items in the established categories were purchased from different sellers at a local market, and others were obtained free of charge from households in the surveyed area. The samples were individually packed in self zipping air tight bags and kept in a cooler box until prepared and cooked. The samples were subsequently prepared according to the most typical form of consumption (raw, washed and/or peeled). The most representative cooking method of boiling was employed. After cooking, the foods were allowed to cool, homogenized, aliquoted in 100 g amounts and stored frozen at -20°C in nitric acid washed glass bottles until chemical analysis.

7.2.2. Mercury analysis

Total mercury analysis was done at the chemical laboratory of Southern and Eastern Africa Mineral Centre (SEAMIC) in Dar es Salaam - Tanzania. Homogenized samples of (1-2 g ww) were digested to a transparent solution with 15 ml of the mixture HNO_3:H_2SO4 (1:2) under reflux. The resultant solutions were then diluted to 20 ml with deionised water and the total Hg concentrations were measured by atomic absorption spectrophotometer cold vapour generation technique (ICP Ultima 2, Horiba Jobin Yvon, France). Acid washed glassware, analytical grade reagents and deionised water were used in the tissue analysis. In order to check the purity of the chemicals used, one chemical blank was run every 10 samples. There was no evidence of contamination in these blanks. Analytical quality control was ensured through the analysis of certified reference material DORM-2 (Dogfish muscle) from the National Research Counsel Canada (4.64 ± 0.26 $\mu g\ g^{-1}$ certified level of mercury) and analysis of mercury standards. All data were computed on a $\mu g\ g^{-1}$ wet weight basis.

7.2.3 Estimation of dietary exposure to mercury

Dietary exposure to mercury from an individual food category was determined by multiplying the concentration of mercury in the food by its intake. Dietary exposures from individual food categories were expressed on a body weight basis by dividing the total dietary exposure by the average body weight of the population according to Lee *et al.*, (2006).

Daily intake of Hg (μg/day) = ∑[mean conc. of Hg in each food category (μg/g) x mean food intake(g/person/day)]

Samples below the Level of Detection (LOD) (0.02 μg/g) were assigned half value of LOD (0.01μg/g) (Govaerts *et al.*, 2005).

7.3. Results and discussion

Table 7-1 shows the list of food types, their respective quantity consumed (g/day) and mean Hg content. A total of 37 food items were mentioned during the food survey. The number of food stuffs mentioned in this study is smaller than ones reported in similar studies performed in Chile (300; Muñoz *et al.*, 2005) and Japan (116; Maitani, 2004). This discrepancy is explained by the poor access in sub-Saharan Africa to a diversity of food stuffs. Populations in rural sub Saharan Africa depend mainly on maize, rice and tuber crops (Kinabo *et al.*, 2006). It is therefore not surprising that rice, maize, tuber crops and fish were the principal food categories in the present study and made up 84 % of the total food weight (Table 7-2). However, the consumption of fish reported in this study (97 g/day) is higher than one reported in the UK (21 g/day), Spain (75 g/day), Norway (53 to 80 g/day), Greece (38 g/day) (Byrd-Bredbenner *et al.*, 2000), Canada (22 to 50 g/day) (Legrand *et al.*, 2005) and Chile (33 g/day; Muñoz *et al.*, 2005) The consumption of meat (24 g/day) is very low in this study compared to values reported in Chile (178 g/day; Muñoz *et al.*, 2005). These differences in daily consumption for each of the food items should be taken into account when establishing the maximum mercury content in food, as standards used in developed countries may not be suitable for implementation in sub Saharan African countries.

Table 7-1. Food types, quantity consumed and Hg concentrations in food items included in the total diet study performed at the Mugusu mining village

Category	Food type	Consumption (g/day)	Hg concentration (µg/g)
Rice	Rice	134	0.026
Maize	Maize	270	0.022
Other cereals	Bulrush Millet	23	< 0.02
	Finger millet	17	< 0.02
	Simsim	8	< 0.02
Tubers/roots	Cassava	134	0.036
	Sweet potatoes	163	0.023
	Yams	41.2	0.054
Legumes/nuts	Bambala nuts	12	< 0.02
	Groundnuts	7	< 0.02
	Beans	34	0.022
Fish	Sardine	176	< 0.02
	Catfish	69	0.335
	Nile perch	65	0.37
	Lungfish	54	0.245
	Furu	23.5	< 0.02
	Soga	34	< 0.02
	Tilapia	260	0.123
Beverage	Gongo (local beer)	10	< 0.02
	Lubisi (local beer)	5	< 0.02
	Kangara (local beer)	37	0.021
	Western beer	18	< 0.02
	Milk	0.8	< 0.02
Fruits	Sugarcane	3	0.068
	Pawpaw	4	< 0.02
	Mangoes	4	< 0.02
	Banana	7	< 0.02
	Pineapples	1.2	< 0.02
Meat	Beef	30	< 0.02
	Chicken	23	< 0.02
	Mutton	18	< 0.02
Vegetables	Tomato	21	< 0.02
	Cabbage	19	0.123
	Amaranths	17	< 0.02
Sugar and salt	Sugar	34	< 0.02
	Table salt	12	< 0.02

Table 7-2 presents the daily mercury intake for each food category. In this study the highest mercury concentrations were found in fish (0.16 µg g^{-1}) with a concentration three and four times higher than that in vegetables and tuber crops, respectively. In the fish category, nile perch exhibited the highest Hg concentration (0.37 µg g^{-1}) which reflects the higher trophic level of this species in Lake Victoria and its carnivorous feeding behaviour (Campbell *et al.*, 2003b). Presence of relatively high Hg concentrations in cassava (0.036 µg g^{-1}), sweet potatoes (0.023 µg g^{-1}), sugarcane (0.068 µg g^{-1}),

yams (0.054 μg g^{-1}), rice (0.026 μg g^{-1}) and cabbage (0.123 μg g^{-1}) is probably due to the cultivation of these crops on Hg contaminated soils. Evidence for this was obtained during the field survey, where yams, cabbage, sugarcane and rice were found growing in swampy areas which are draining areas for the Mugusu mine through river Mabubi. In our previous study we analysed up to 2.3 μg Hg g^{-1} dw in sediment collected from the Mabubi river (Chapter 2). It is worth noting that the Hg concentration in fish and other food categories do not exceed the maximum limit of 0.5 μg g^{-1} established by WHO (1985).

Table 7-2. Average (± SD) Hg concentrations and % intakes for different food categories

Group	food intake (g/day)	% of total food intake	Hg concentration (μg/g wt weight)	Hg intake from each food category (μg/day)	% of total Hg intake for each food category
Rice	134	18	0.03	4.0	12
Maize	270	37	0.02	6	19
Cereals	16 ± 7.64	2	0.01	0.2 ± 0.08	0.5
Tubers/roots	113 ± 63	15	0.04 ± 0.02	4.35 ± 1.37	14
Regumes/nuts	18 ± 14	2	0.02 ± 0.01	0.4 ± 0.4	1
Fish	97 ± 87	14	0.16 ± 0.16	15.6 ±13	48
Beverage	12 ± 11	2	0.01	0.1 ± 0.1	0.37
Fruits	4 ± 2	0.54	0.02 ± 0.03	0.1 ± 0.08	0.25
Meat	24 ± 6	3.25	0.01	0.25 ± 0.06	0.74
Vegetables	19 ± 2	3	0.05 ± 0.06	1.0 ± 0.2	3
Sugar and salt	23 ± 16	3	0.01	0.2 ± 0.16	1
Total	730	100	0.38	32.2	100

The estimated average Hg intake per person in this study is 32.2 μg/day, which is much higher than intakes previously estimated using similar approaches in Korea (1.16 μg/day; Lee *et al.*, 2006), the UK (4 μg/day;Ysart *et al.*, 1999), Chile (5 μg/day; Muñoz *et al.*, 2005), Japan (9 μg/day; Maitani, 2004), China (10.3μg/day; Chen and Gao 1993), USA (1.25 μg/day; Egan *et al.*, 2002) and Lebanon (3.0 μg/day; Nasreddine *et al.*, 2006). This difference can probably be explained by difference in fish intake as it was the food category that contributed most to the mercury intake (48 %). The calculated mercury intake from fish in the present study (15.6 μg/day) is higher than that reported in Spain (13.2 μg/day), which is considered to have a high fish consumption among the developed countries (Urieta *et al.*, 1996). Although the Hg concentration in maize (0.02 μg.g^{-1}ww) was low, it contributed 19 % to the total mercury intake because it is the major staple food in the studied community. Other food categories which are consumed in large quantities and contributed 26 % of the total mercury intake are rice and tuber crops (cassava, sweet potatoes and yams).

The maximum daily Hg intake recommended by WHO/FAO for the protection of general public is 49 µg/day for an adult of 68 kg (JECFA, 2003). This value is 1.5 times higher than the estimated Hg intake of residents living in the Mugusu mining village. Based on the findings that prenatal exposure to MeHg leads to significant effects during infant development, the World Health Organization (WHO) reduced the mercury daily intake safe limit by half, setting it at 15.5 µg/day. This value is already equivalent to the Hg intake from the fish category in the present study, assuming that all the Hg in fish is found as methylmercury (US EPA, 2001). When all the food categories included in this study are consumed the WHO limit is exceeded by a factor of two. Thus, results from the present study indicate that there is a health risk for children born to women living in the Mugusu gold mining village. Due to limited information on dietary mercury intake in Tanzania it is unclear if the dietary Hg intake observed in this study is only due to Hg pollution caused by artisanal gold mining. The presence of elevated Hg levels in tuber crops that are grown around swampy areas near Mugusu mine does, however, indicate that a significant contribution of gold mining activities can be expected. It is important to take into consideration some inherent drawbacks associated with TDS approach when interpreting these results. This study combined mean concentrations of mercury with mean food intakes; therefore the results do not represent the extremes of the population. Also, this study was like many exposure assessments conducted around the world limited to the adult fraction of the population (Chen and Gao, 1993; WHO 1985; Nasreddine *et al.*, 2006). However, without excluding the possibility that the daily intakes calculated in the current study may be not fully representative of the entire village population, results from this study do provide a first estimate of the Hg dietary exposure of people residing in artisanal gold mining areas in Tanzania.

7.4. Conclusion

To our knowledge this is the first study to use a total diet approach to estimate mercury intake by people living in artisanal gold mining areas in Tanzania. The estimated Hg intake of 32.2 µg/day is two times higher than the safe limit for the protection of the developing foetus and young children (15.5µg/day) and is above reported Hg intakes in Korea, the UK, Chile, Japan, China, the USA and Lebanon. It is concluded that a health risk exist for people residing in Mugusu gold mining village and probably other artisanal mining settlements in the country. It is therefore recommended that, mercury intake in artisanal gold mining settlements should be monitored regularly. Also, people living in these settlements should be educated on the potential health risks associated with consuming Hg contaminated food.

Chapter eight

Monitoring of human exposure to mercury by head hair analysis in an artisanal gold mining community – Geita district, Tanzania

8.0 Monitoring of human exposure to mercury by head hair analysis in an artisanal gold mining community – Geita district, Tanzania

Abstract

Hair analysis is a very useful method to assess human exposure to mercury. In the present study head hair analysis was used to asses mercury exposure in a population of miners at Mugusu mine. Samples were acid–digested and levels of mercury determined by using atomic absorption spectrometer technique under cold vapour technique. The mean value of total mercury was 1.55 ± 1.17 μg g^{-1}dry weight, with people involved in amalgam roasting exhibiting a significantly higher mean value (4.6 ± 0.57 μg g^{-1} dry weight) than those involved in pitting, sluicing and other mining activities. Compared to the results from previous monitoring studies, data from the present study indicate that the average Hg exposure of the mining population at Mugusu artisanal gold mine has increased but has not exceeded the WHO limit of 10 μg g^{-1} established for pregnant women. It is recommended that attempts should be made to minimize mercury vapour exposure to miners through providing of the necessary education and the use of retorts.

Redrafted from

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Monitoring of human exposure to mercury by head hair analysis in an artisanal gold mining community – Geita district, Tanzania: Human and Ecological Risk Assessment: To be submitted

8.1. Introduction

Gold mining and subsequent processing of gold ores using amalgamation techniques with mercury (Hg) are known to contaminate the environment and potential human health risks implications. Acute poisoning from Hg vapour causes erosive bronchitis and bronchiolitis with interstitial pneumonitis which may be combined with tremors (UNEP, 2002). Chronic exposure to inorganic Hg is characterized by weakness, forgetfulness and loss of weight. Neurological damage such as severe cerebral palsy was evident among those exposed to methylmercury (MeHg) in Japan and Iraq in the early 1970s (Eisler, 2005).

Many reports have been published on the environmental Hg contamination associated with artisanal gold mining activities in the Lake Victoria basin of Tanzania. Artisanal miners extensively use mercury amalgamation to recover gold (Ikingura and Akagi, 1996, Kondoro and Makundi, 1998; LVEMP, 2002). A potential risk of Hg exposure of persons directly involved in handling Hg exists. Furthermore, inorganic mercury released into river systems during gold ore-processing is likely to be transformed into methylmercury which can biomagnify through the aquatic food chain and get into fish consumed by the local population (van Straaten, 2000b). Results obtained in chapter 2 of this thesis indicate that the Mugusu mine and its surrounding areas are contaminated with mercury resulting from the local gold mining activities as gold miners continue to carelessly use and dispose off Hg. On the other hand, results from chapters 5 and 7 indicate that fish caught from watersheds near the mine and crops which are cultivated in nearby swamps are contaminated with Hg and thus pose a risk to local consumers. However, studies to assess human exposure to mercury for people living and working in the Mugusu mine were performed ten years ago (Ikingura and Akagi, 1996; Harada *et al.*, 1999). There is no current information on the extent of Hg exposure of this community. The current study was therefore designed to use head hair analysis to establish the current status of human exposure to mercury in the population working and residing in the Mugusu mining village.

The Hg content of hair represents the cumulative exposure from the occupational environment and/or the daily diet (Barbosa *et al.*, 1997). Although the levels of total mercury in hair of a normal non exposed person are in the range of 0.4 to 6.0 $\mu g\ g^{-1}$, a concentration up to 10 $\mu g\ g^{-1}$ is considered to be safe for pregnant women (WHO, 1990). Above this limit, deleterious effects might occur to the foetus (Barbosa *et al.*, 1998). There are several reasons why hair analysis for mercury

biomonitoring is preferred to other biomakers. In adults, the Hg concentrations in hair are approximately 250 times higher than that in blood (Cernichiari *et al.*, 1995). In addition, mercury levels in hair correlate with levels in the brain (Legrand *et al.*, 2005) and hair samples are easily obtained and are less invasive compared to blood sampling (Cernichiari *et al.*, 1995).

8.2. Material and methods

8.2.1. Hair sampling

Hair samples were collected from inhabitants of the Mugusu artisanal gold mining village. To overcome the strong superstitious beliefs which exist among gold miners (Ikingura and Akagi, 1996) we conducted an educative seminar to precisely explain to the community that the sole intention of the head hair sampling was to analyse for mercury. We also promised to communicate the results and explain the health implication to the community. A total of 52 people volunteered for head hair sampling. For comparison purposes, samples were also collected from eight people at Geita town who were not involved in mining activities. In the present study, hair was not sampled from women as it has previously been shown that women exhibit high levels of Hg in their hair because of using mercury containing cosmetics (Harada *et al.*, 1999). Hair samples were collected by cutting approximately 60 mg of hair as close to the scalp as possible. Each sample was preserved in a labelled paper envelope and kept in an air tight self zipping plastic bag. Record forms were utilized and archived indicating the name, age, place and activity of the donor in the mine.

8.2.2. Hg analysis

For total mercury analysis, 12 mg of hair samples were digested with 2 ml HNO_3-$HCLO_3$ (1:1) , 5 ml H_2SO_4 and 1 ml distilled H_2O at 250°C for 20 min. The concentration of total mercury (THg) was determined using atomic absorption spectrometry under cold vapour technique after reduction with $SnCl_2$ at the Institute of Minamata Disease in Japan. All data are computed on a $\mu g\ g^{-1}$ dry weight basis.

8.2.3. Statistics

Proportions and means were computed for various variables by using excel spread sheets. Analysis of variance (ANOVA) was used to test statistical differences among different activity categories. Correlation between total mercury levels in hair and age (years) of their donors was tested by using regression analysis. Variables were considered different if $p < 0.05$.

8.3. Results and discussion

The mean value of total mercury was 1.55 ± 1.17 μg g^{-1}. The distribution of this result was as follows: 49% between 0.1 and 0.5 μg g^{-1}, 23.5% between 1 and 1.7 μg g^{-1}, 21.6% between 2.0 and 4.8 μg g^{-1} and 5.9% between 6 and 12 μg g^{-1}. The low Hg concentrations that were observed in the present hair survey are consistent with the findings of two earlier monitoring surveys carried out at Mugusu mine. Ikingura and Akagi (1996) reported Hg concentrations in hair of miners ranging from 0.16 to 5.43 μg g^{-1} with a mean value of 0.95 μg g^{-1}. In the hair survey conducted by Harada *et al.* (1999) the Hg concentration in hair samples from the Mugusu mine (Chipaka) ranged from 0.38 to 7.0 μg g^{-1} with an average of 1.31 μg g^{-1}. Assuming that hair was sampled from the same population, an increase of the average Hg levels in hair was observed, i.e. from 0.95 μg g^{-1} in 1996 to 1.31 μg g^{-1} in 1999 and 1.55 μg g^{-1} in the present study. This trend may indicate a progressive increase of the mercury body burden, probably due to the continued use of mercury by miners. However, the observed trend in hair Hg concentrations might be biased by the frequent movement of miners from one mining centre to another (Jesper *et al.*, 2006; unpublished results). Due to the high mobility many of the miners who were sampled during the previous studies might have moved to other mining centres.

Mercury hair levels close to 50 μg g^{-1} are considered to be high enough to cause Minamata disease (Harada, 1995). Considering that none of the analysed hair samples in the present study contained such levels it could be concluded that mercury exposure to the inhabitants of the Mugusu mine does not affect their health. However, these results indicate that hair samples collected from individuals involved in amalgam roasting contained significantly higher average mercury levels (4.6 ± 0.57 μg g^{-1}) compared to those involved in pitting (1.08 ± 0.06 μg g^{-1}), sluicing (0.92 ± 0.021 μg g^{-1}) and crushing (0.75 ± 0.01 μg g^{-1}). Mercury concentrations in the control population at Geita town (0.3 ± 0.012 μg g^{-1}) was significantly lower than the levels analysed in hair samples taken from the mining population ($p<0.05$). These results are in agreement with the descriptions of van Straaten (2000b) who found elevated mercury in hair samples collected from miners who were involved in amalgam roasting in Zimbabwe. Similar findings were reported by Akagi *et al.* (1995) in Brazil. Presence of high mercury levels in individuals involved in mercury amalgam roasting is probably due to inhalation of mercury vapours which are released during amalgam roasting. van Straaten (2000a) observed that 70 to 80% of mercury introduced in gold processing is lost during amalgam roasting.

Also, results obtained during the field survey at Mugusu mine (reported in chapter 2) indicated that most miners still roast amalgam in open air without any protective gears (Figure 2.6).

As illustrated in Figure 8-1, there was no statistically significant correlation (P>0.05) between mercury concentration and the age of the subjects (r^2 = 0.0.035) These results may indicate that, apart from dietary intake, the main source of mercury to the surveyed community is direct uptake of inorganic mercury from mining related activities, and is dependent on the type and duration of activity one is involved in at the mine rather than the age. Mercury exposure from eating mercury contaminated fish is usually characterized by gradual accumulation and thus it's concentration in hair depends on the duration one has been consuming fish. These findings have been reported in several studies performed in the Amazon region of Brazil, where mercury exposure through consumption of contaminated fish has been widely reported (Brado *et al.*, 2000; Harada *et al.*, 2001).

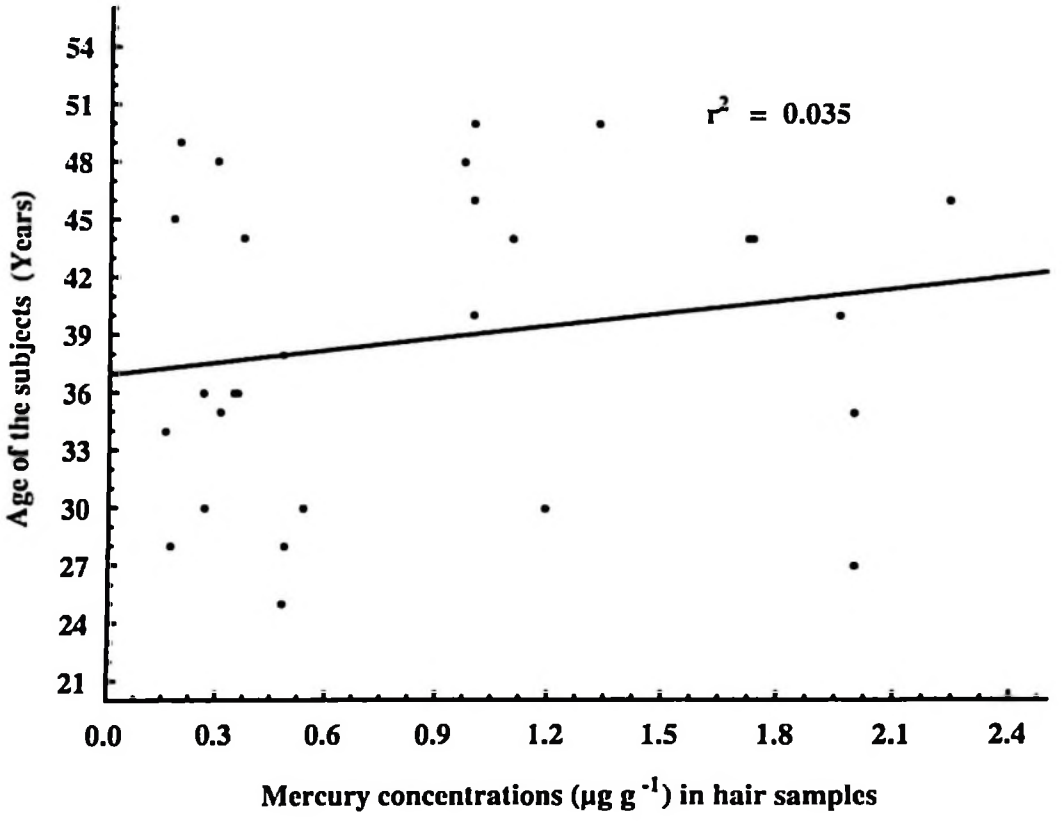

Figure 8-1. Relationship between mercury levels in hair samples and the age of the subjects from Mugusu mining village

8.4. Conclusion

This study was conducted as a follow up to recommendations made by Ikungura and Akagi (1996) and Harada *et al.* (1999). They suggested that although mercury exposure in communities living in the Mugusu mining village was low at the time of their studies, continued monitoring was necessary. Results from the present study show that the average mercury exposure to people living in the studied community has increased from 0.95 $\mu g\ g^{-1}$ in 1996 to 1.31 $\mu g\ g^{-1}$ in 1999 and to 1.55 $\mu g\ g^{-1}$ in the present study. The average total mercury measured in the present study is still far below the WHO warning limit for pregnant women (10 $\mu g\ g^{-1}$). According to our results, mercury exposure in the community living in Mugusu artisanal gold mine and probably other similar mines in Tanzania has not reached the levels which induce Minamata disease. However, the increase of the mercury body burden in this community during the last decade is worrying. This is especially true for individuals engaged in mercury–gold amalgam roasting who exhibited the highest levels of total hair mercury. Mercury levels similar to ones analysed in individuals involved in amalgam roasting in the present study have been associated with reduction in fertility (Dickman *et al.*, 1998). It is therefore recommended that attempts should be made to minimize mercury vapour exposure to miners through providing the necessary education and the use of retorts.

General conclusion, recommendations and future research needs

General conclusion, recommendations and future research needs

The **first goal** of this study was to establish the current status of mercury use and environmental pollution at the Mugusu artisanal gold mine, and to assess the level of awareness among miners on effects of mercury on the environment and human health. The results of current status of mercury use and environmental pollution at Mugusu mine and the extent of Hg dispersion towards Lake Victoria waters via river Mabubi are reported in Chapter 2. This chapter also describes the local people's perception and knowledge on the effects of Hg on the environment and human health.

The **second goal** of this study was to assess the effect of Hg resulting from artisanal gold mining to living resources in the Lake Victoria basin. The effects of Hg spiked sediments to the benthic midge (*Chironomus riparius*) and the discussion on its implication to the artisanal gold mining impacted watersheds in Tanzania are described in Chapter 3. Chapter 4 describes the effect of sediment collected from river Mabubi to the early life stages of the African catfish (*Clarias gariepinus*).

The **third goal** of this study was to establish mercury concentrations in locally available food stuffs, estimate dietary mercury intake and asses the associated health risk to the local human population. Chapter 5, reports the estimated Hg intake and health risk associated with consuming Hg contaminated fish by women residing in a subsistence fishing village near the Mugusu mine. Chapter 6 examined the possible health risk of consuming meat obtained from cattle and domestic fowl freely feeding/grazing in and around artisanal gold mines. Chapter 7 reports on the dietary mercury intake and health risk implication by the people living in Mugusu mine through a total diet study.

The **fourth goal** of this study was to assess the current status of mercury exposure to individuals working in the Mugusu mine. Chapter 8 reports and discusses on the results of mercury analysis of head hair collected from gold miners.

Based on the results and discussion of the respective chapters, general conclusions about four issues were drawn:

(1) The current status of gold mining, mercury use and environmental pollution at the Mugusu mine;

(2) The toxicity of sediments collected from watersheds impacted by artisanal gold mining activities;

(3) The mercury exposure to communities residing within and around Mugusu mine through the dietary route;

(4) The current Hg exposure of gold miners at Mugusu mine.

Current status of gold mining, mercury use and environmental pollution at Mugusu mine

Site visits to the Mugusu mine showed that gold mining was ongoing and mercury was still used unabated. Mercury was continuing to pollute the environment as evidenced by high Hg concentrations measured in soil, sediment and water samples collected in the vicinity of the Mugusu mine (as compared to background levels). The continued mercury use and environmental contamination at the Mugusu mine was related to the finding that miners lack proper understanding of the ability of Hg to cause harm to man and the environment. The miners who handle Hg without using protective gear during Hg-Au amalgamation and amalgam roasting are at risk of Hg intoxication. It is therefore recommended that in order to reduce mercury pollution, miners and neighbouring communities should be educated on the negative effects of Hg on the environment and human health. Additionally, miners should be trained on the safe use, handling and disposal of Hg. To improve awareness about effects associated with Hg contamination among miners and local communities it is recommended that findings from various research projects should also be disseminated locally.

Ecotoxicity of sediments collected from artisanal gold mining impacted watershed

Chemical analysis of environmental samples from areas around the Mugusu mine indicated that heavy metals accumulate more in the sediments than in any other abiotic compartment. Given that, chemistry measurements alone have a limited ability to predict adverse effects on living resources; toxicity bioassays were performed to assess sediment quality. Ecotoxicity tests with Hg spiked sediment and a benthic midge (*Chironomus riparius*) showed that Hg was toxic at levels lower than those measured in the Mabubi and other rivers located in the artisanal gold mining areas of

Tanzania. Similarly, sediment collected up to six kilometers downstream of the Mugusu mine reduced survival and inhibited growth of *Clarias gariepinus*. Generally, chemistry and ecotoxicity testing indicated that sediments from the river Mabubi pose a risk to living resources and can alter its biodiversity. It is therefore recommended that efforts should be made to reduce effluents discharge from the artisanal gold mines.

Mercury dietary exposure of communities residing within and around the Mugusu mine

While muscle tissues obtained from cattle grazing around the gold mine and chicken feeding on Hg contaminated tailings exhibited low Hg levels, liver samples did contain levels above the safe limits proposed by most developed countries. Consumption of liver obtained from animals known to be reared within or around artisanal gold mines should be discouraged.

Except for catfish which contained Hg concentrations slightly above the WHO (Codex alimentarius) recommended safe limit (0.5 $\mu g\ g^{-1}$ ww), analysis of edible tissues taken from other frequently consumed fish in the Nungwe village contained Hg levels below this limit. The calculated fish intake for women in Nungwe bay was however, far higher than the Tanzanian average fish intake and those documented in developed countries. Consequently, their mercury intake was higher than the WHO/FAO recommended Provisional Tolerable Weekly Intake (PTWI) of 1.6 µg/kg bwt/week. It is therefore advised that women of reproductive age should reduce the consumption of fish known to contain high mercury levels. As a long term strategy for protecting public health, safety guidelines based on the local fish consumption patterns should be developed and consumption guidelines issued. However, any advice against fish consumption should take into consideration the nutritional benefit of consuming fish such as dietary intake of long chain omega-3 fatty acids and the unavailability of alternative food.

A total diet study was conducted to estimate mercury intake by people living at the Mugusu mine site. The results indicated that total mercury intake was two times higher than the PTWI for protecting the developing foetus and young children. Next to fish, food crops which are grown in wetlands near Mugusu mine (rice, sugarcane, cabbage, yams) also exhibited high levels of Hg concentration and thus contributed to the Hg intake. Cultivation of crops on Hg contaminated wetlands should be discouraged.

Current status of mercury exposure of gold miners at the Mugusu mine

Results from the analysis of head hair obtained from individuals engaged in different mining activities at the Mugusu mine showed an increase of hair Hg from 0.95 $\mu g\ g^{-1}$ in 1996 to 1.55 $\mu g\ g^{-1}$ in the present study. The current average total mercury measured in hair is still below the WHO warning limit for pregnant women of 10 $\mu g\ g^{-1}$ and far below levels that can induce Minamata disease (50 $\mu g\ g^{-1}$). However, the trend of progressively increasing mercury body burden with time is worrying as with time these values can achieve health threatening levels. It is therefore recommended that efforts should be done to minimize mercury vapour exposure to miners through providing the necessary education and the use of retorts.

Recommendation for policy makers

- Research findings should be disseminated and communicated to local communities
- Regulations and laws governing safe mining practices should be enforced in artisanal gold mining areas
- Importation, distribution and use of toxic substances like mercury in Tanzania should be monitored
- Food safety guidelines based on the local fish consumption patterns should be developed
- Promote environmental awareness (use and handling of mercury) and environmental rehabilitation of the sites after mining
- Promote training on mining and processing methods that are environmentally acceptable in order to improve skills and competency and reduce health risks to miners and their dependants

Future research needs

Based on the findings from this study, future studies should focus on:

- Understanding of the socio-economic factors which influence the community's altitude and behaviour towards mercury use and environmental contamination
- Innovating and introduction of safe methods of gold extraction for artisanal gold miners in Tanzania
- Understanding the physical and chemical factors influencing mercury speciation, dispersion and bioavailability in Lake Victoria basin

- Exploring the ecotoxicity of gold mining wastes to additional local species selected from different levels of the food web in the Lake Victoria basin.
- Exploring the role of arsenic and other metals found in large quantities in mined ore during gold mining in the ecotoxicity of mining waste from gold mines
- Monitoring of mercury in animals found in higher levels of the food web (birds, mammals)
- Considering that the deterministic method which was used in this study to estimate Hg intake does not incorporate the variability and uncertainty found in different categories of fish consumers and Hg concentration in different sizes and species of fish, it is recommended that future studies should use the probabilistic modelling
- Formulating safety guidelines for pollutant concentrations in food staffs based on local consumption patterns
- Monitoring of mercury body burden in miners during a long monitoring period and a medical based investigation in the mining villages to establish clinical and sub-clinical symptoms which may be linked to Hg poisoning should be considered

Summary

Summary

Artisanal gold mining is one of the major sources of mercury (Hg) contamination in Tanzania. Whilst the gold extraction process (known as amalgamation) is a simple technology, it is potentially very harmful to the environment and can contaminate air, soil, rivers and lakes with mercury. High levels of environmental mercury can cause harm to organisms living in the various environmental compartments. Also the health of the miners and other people living within the contaminated area may be adversely affected through inhalation of mercury vapour, direct contact with mercury, through eating Hg contaminated fish and other food items, or through ingesting Hg contaminated water. The Mugusu mine, which is privately owned by Chipaka family, is one of the active artisanal gold mines in Geita district, Tanzania. The current status of mercury use, environmental contamination and the potential risks of Hg to humans and the environment at the Mugusu mine are not well understood.

This doctoral study aimed at using the Mugusu mine as a model to asses the risks of Hg use in artisanal gold mining in Tanzania. In this thesis, the current status of mercury use and environmental contamination at Mugusu mine was established. The knowledge and perception of miners about the possible effects of mercury to the environment and human health were explored using structured questionnaire and focussed group discussion. Based on chemical analysis and ecotoxicity testing the possible ecological effects to the aquatic ecosystem of wastes released from artisanal mine was studied. The potential health risk to humans was assessed by estimating mercury exposure of women residing in a fishing village near Mugusu mine through fish consumption. Concentrations of mercury in muscle and liver tissues of free grazing cattle and scavenging domestic fowl in gold mining centres were determined and used to assess the potential contribution of this exposure route to the Hg intake. Additionally, a total diet study approach was used to estimate the dietary mercury intake of people living and working in the Mugusu village. Finally, through head hair monitoring we established the current exposure of mercury to artisanal gold miners at the Mugusu mine.

This study identified that intensive use of mercury in gold extraction and the associated environmental pollution is occurring at Mugusu mine. The current mining practice involves excavation of gold rich ore by using hand hoes, chisels and sledge hammers. After removing the mineralized bearing material, the ore is grinded in the ball mills and soil concentrate is recovered in sluice boxes which are lined with sisal sacks or any other fibre materials. The soil concentrate

obtained is subsequently amalgamated with mercury to trap fine gold from the ore pulp. A piece of cloth is used to squeeze off excess mercury by twisting it and leaving behind the amalgam. Once the amalgam is obtained it is burned in open air to release Hg. Retorts are not used, neither is any other form of protection against mercury contamination and/or exposure. Most miners lack knowledge on the possible environmental and human health problems associated with mercury release and exposure. Amalgamation mainly takes place adjacent to the river Mabubi that in turn drains into Lake Victoria, thus potentially contaminating Lake Victoria resources.

Results from the present study show that use of mercury in gold mining contaminates nearby water resources and soils. To perform an exploratory environmental risk assessment, selected environmental compartments were studied. Analysis of water and sediment samples collected from the river Mabubi demonstrated that Hg concentrations in sediment and water decreased towards the river mouth. The highest concentrations of Hg in sediment (2.3 μg g^{-1} dw) were measured three kilometres downstream of the mine. As in previous studies in the Lake Victoria goldfields, we showed that the distribution of Hg in river sediments away from the mine is relatively restricted. Probably Fe-rich laterites and seasonal swamps act as natural barriers restricting the movement of Hg downstream into the Lake Victoria.

The soil samples collected near the mine contained up to 1.2 μg g^{-1} dw. In the ecotoxicity assessment, sediments collected up to six kilometres downstream of Mugusu mine significantly reduced survival and growth of the African catfish (*Clarias gariepinus*). The calculated 5 day-LC_{50} value (larval survival) was 1.75 μg Hg g^{-1} dw (95% CL of 0.72 – 2.53), the 5 day-NOEC for hatching was >2.3 μg Hg g^{-1} dw and that for larval survival and growth was 0.23 μg Hg g^{-1} dw. These results were in agreement with results obtained from laboratory toxicity evaluations using mercury spiked artificial sediments and the benthic invertebrate *Chironomus riparius*. Indeed, the emergence of *C. riparius* midges was significantly delayed at Hg concentrations of 0.93 μg Hg g^{-1} dw. Mercury affected some life characteristics of *C. riparius* at concentrations lower than the levels of mercury which have been measured in different artisanal gold mining impacted watersheds in Tanzania. For example, in our assays Hg delayed emergence at concentrations that was 2.5 times lower than those measured three kilometres downstream of the mine in the Mabubi river. Overall, the chemical analysis and the ecotoxicity results from this study indicate that sediments downstream

of the mine in the Mabubi river and probably in other artisanal gold mining impacted watersheds in Tanzania pose a risk to aquatic fauna.

To assess the risk associated with Hg to the local human population the Hg intake through consumption of Hg contaminated fish, meat and other food types was estimated. Additionally, head hair analysis was done to establish the concentrations of Hg in the body of people living and working in Mugusu gold mining village. Results from the fish consumption survey indicate that, the main fish caught and consumed by the people residing in the Nungwe village that borders the Mugusu mine included nile perch (*Lates niloticus*), tilapia (*Oreochromis niloticus*, catfish (*Clarias gariepinus*), sardine (*Rastrineobola argentea*), and lung fish (*Protopterus aethiopicus*). Catfish contained marginally (0.51 ± 0.17 μg g^{-1} ww) higher concentration of mercury than the WHO safety limit of 0.5 μg Hg g^{-1} ww; the four other species had Hg levels below this safety limit. Because of high fish intake (144 g/day), the calculated weekly MeHg intake in this village (4.0 μg/kg bwt/day) was above the Provisional Tolerable Weekly Intake (PTWI) recommended by WHO/FAO for protection of the unborn child and young children (1.6 μg/kg bwt/week).

On the other hand, the total diet study showed that daily Hg dietary intake for people living and working within the Mugusu mining village exceeded the provisional tolerable daily intake recommended by WHO/FAO for protecting unborn children (15.5 μg/day) by a factor of two. Thus, children born to mothers living in this village are at risk of intrauterine mercury poisoning. The contribution to overall Hg exposure of meat obtained from cattle and domestic fowl reared in the mining villages was low. Indeed through analysis of muscles obtained from these animals we established that Hg levels in these products were rather low and ranged from 22 to 81 μg/kg ww. These Hg concentrations are far lower than the international safety limits of 500 μg/kg ww.

In general, chemical analysis of Hg in head hair collected from people working and living in the Mugusu mining village indicated low exposure. The mean concentration (1.55 ± 2.2 μg g^{-1} dw) was lower than the warning limit (10 μg Hg g^{-1}dw) established by WHO for pregnant women. However, hair obtained from individuals involved in amalgam roasting contained significantly more Hg than those involved in other mining activities. Compared with studies performed ten years ago, the mercury concentrations in the hair of miners at Mugusu mine have slightly increased.

In conclusion, this doctoral study has demonstrated that, artisanal gold mining at the Mugusu mine causes contamination of all the environmental compartments which lead to increased risks to the environment and human health. The current level of Hg concentrations in the rivers and streams draining the Mugusu mine into Lake Victoria pose risk to aquatic biota. Based on the survey conducted in this study it is clear that miners lack clear knowledge and understanding of the possible effects of Hg on the environment and human health. Furthermore, based on the current local food consumption pattern and Hg concentrations in different food items it is concluded that there is no risk to the general public but there is a health risk to unborn children. It is therefore recommended that more efforts are made (1) to reduce the use of Hg in gold mining and (2) to educate the miners and surrounding communities on the effects of Hg on the environment and human health.

Samenvatting

Samenvatting

Artisanale goudwinning is één van de belangrijkste bronnen van kwikverontreiniging in Tanzania. Het goudextractieproces (bekend als amalgamatie) is een eenvoudige techniek, maar is potentieel zeer schadelijk voor het milieu omdat bij het extractieproces kwik (Hg) gebruikt wordt dat lucht, bodem en water kan contamineren. Hoge milieuconcentraties van dit metaal kunnen schade toebrengen aan organismen uit de verschillende milieucompartimenten, maar ook de gezondheid van de mijnwerkers en de inwoners van gecontamineerde gebieden kan negatief beïnvloed worden door inhalatie van kwikdampen, direct contact met kwik, het eten van met kwik verontreinigde vis of ander voedsel, of door het drinken van met kwik verontreinigd water. De Mugusu mijn, welke privaat eigendom is van de Chipaka familie, is één van de actieve artisanale goudmijnen in het Geita district van Tanzania. Over de omvang van het gebruik van kwik en de resulterende milieuverontreiniging rondom de Mugusu mijn is weinig geweten, en de potentiële gevaren ervan voor de mens en het milieu zijn onduidelijk.

Deze doctoraatsstudie had als doel de Mugusu mijn als model te gebruiken om de risico's geassocieerd met het gebruik van kwik in de artisanale goudwinning in Tanzania in te schatten. In dit werk werd de omvang van het kwikgebruik en de milieuverontreiniging door kwik onderzocht. De kennis en de perceptie van de mijnwerkers inzake de mogelijke effecten van kwik voor het milieu en de menselijke gezondheid werden geëxploreerd aan de hand van gestructureerde vragenlijsten en gerichte groepsdiscussies. De mogelijke ecologische effecten van de lozing van afvalwater van de mijn in het aquatische milieu werden bestudeerd door middel van chemische analyse en ecotoxiciteitstesten. Het potentiële risico voor de menselijke gezondheid werd bepaald door schatting van de blootstelling aan kwik via visconsumptie van vrouwen uit een vissersdorp nabij Mugusu. De kwikconcentraties in spier- en leverweefsel van runderen en pluimvee gekweekt nabij de goudmijn centra werden gemeten en gebruikt om de potentiële bijdrage van deze blootstellingsroute aan de totale kwikopname in te schatten. Daarnaast werd een dieetstudie uitgevoerd om de kwikopname via voedsel in te schatten bij de mensen die leven en werken in en rond de Mugusu mijn. Tenslotte werd, door monitoring van hoofdhaar, de huidige Hg blootstelling van de mijnwerkers van de Mugusu mijn onderzocht.

Deze studie toonde aan dat kwik intensief gebruikt wordt tijdens de goudextractie en dat dit resulteert in ernstige milieuverontreiniging in en rondom de Mugusu mijn. De huidige ontginningspraktijken omvatten het delven van goudrijke ertsen met behulp van handschoffels,

beitels en voorhamers. Na het verwijderen van het gemineraliseerde dragermateriaal wordt het resterende erts tot fijn poeder vermalen in een kogelmolen. Vervolgens wordt het gouderts gerecupereerd in wasgoten die bekleed zijn met zakken uit sisal of een ander vezelachtig materiaal. Het verkregen bodemconcentraat wordt vervolgens geamalgameerd met kwik om het goud van het verpulverde erts te scheiden. Het overtollige kwik wordt verwijderd door deze oplossing handmatig door een katoenen doek te persen. Eens het amalgaam verkregen is, wordt het in open lucht verhit om het kwik te laten verdampen. Er wordt geen gebruik gemaakt van distilleerkolven, noch van enige andere vorm van bescherming tegen kwik contaminatie en/of blootstelling. De meeste mijnwerkers hebben onvoldoende kennis over de mogelijke milieu- en gezondheidsproblemen geassocieerd met het vrijkomen van en de blootstelling aan kwik. Amalgamatie vindt hoofdzakelijk plaats nabij de Mabubi rivier die uitmondt in het Victoriameer. Hierdoor kunnen ook de hulpbronnen van dit meer gecontamineerd worden.

De resultaten van deze studie tonen aan dat het gebruik van kwik in dit model-goudwinningsgebied de nabijgelegen oppervlaktewateren en (water)bodems contamineert. Om een verkennende risico-evaluatie uit te voeren, werden geselecteerde milieucompartimenten onderzocht. Analyse van water- en sedimentstalen die werden verzameld in de Mabubi rivier, toonde dat de kwikconcentraties in sediment en water afnamen naar de riviermonding toe. De hoogste kwikconcentraties in sediment (2.3 µg Hg g^{-1} drooggewicht) werden drie kilometer stroomafwaarts van de mijn gemeten. Zoals eerder aangetoond in de goudvelden van het Victoriameer, werd ook in onze studie geobserveerd dat de distributie van kwik in riviersedimenten buiten het mijngebied relatief beperkt is. Dit is waarschijnlijk te wijten aan ijzerrijke laterieten en seizoenale moerassen die optreden als natuurlijke barrières en zo de stroomafwaartse beweging van kwik beperken. In de (terrestrische) bodemstalen die verzameld werden nabij de mijn werd tot 1.2 µg kwik g^{-1} drooggewicht gemeten.

Uit de ecotoxicologische beoordeling bleek dat sedimenten die verzameld werden tot zes kilometer stroomafwaarts van de Mugusu mijn, de overleving en groei van de Afrikaanse meerval (*Clarias gariepinus*) significant reduceerde. De LC50 waarde bepaald door middel van de 5-dagen larvale overlevingstest bedroeg 1.75 µg Hg g^{-1} drooggewicht (95% betrouwbaarheidslimieten van 0.72 – 2.53), de 5-dagen NOEC voor het ontluiken van de larven was >2.3 µg Hg g^{-1} drooggewicht, en deze voor larvale overleving en groei was 0.23 µg Hg g^{-1} drooggewicht. Deze resultaten waren in overeenstemming met deze bekomen met laboratoriumtoxiciteitstesten op de benthische invertebraat

Chironomus riparius die werden uitgevoerd met artificiële sedimenten waaraan kwik toegevoegd werd. Het uitvliegen van *C. riparius* muggen werd significant vertraagd bij een concentratie van 0.93 µg Hg g^{-1} drooggewicht. Kwik beïnvloedde bepaalde levenskarakteristieken van *C. riparius* bij concentraties lager dan deze gemeten in verschillende waterbekkens die gecontamineerd zijn door goudwinningsactiviteiten. Zo bleek uit analyses uitgevoerd in deze studie dat kwik het moment van uitvliegen significant vertraagde bij concentraties die 2.5 keer lager waren dan deze gemeten drie kilometer stroomafwaarts van de mijn (Mabubi rivier). Algemeen tonen de chemische en ecotoxicologische analyses uit deze studie aan dat sedimenten in de Mabubi rivier stroomafwaarts van de mijn en waarschijnlijk ook in andere waterbekkens belast door de artisanale goudwinning in Tanzania, een risico vormen voor de aquatische fauna.

Om het risico van de kwikvervuiling voor de plaatselijke bevolking te evalueren, werd de kwikopname door consumptie van vis, vlees en ander voedsel ingeschat. Bovendien werden chemische analyses van hoofdhaar uitgevoerd om de lichaamsconcentraties van kwik vast te stellen bij mensen die leven en werken rondom de Mugusu goudmijn. De resultaten van het onderzoek naar visconsumptie tonen dat hoofdzakelijk vijf vissoorten gevangen en geconsumeerd worden door de bevolking van Nungwe, een dorp dat aan de Mugusu mijn grenst. Dit zijn de nijlbaars of Victoriabaars (*Lates niloticus*), de tilapia (*Oreochromis niloticus*), de Afrikaanse meerval (*Clarias gariepinus*), de dagaa (*Rastrineobola argentea*) en de longvis (*Protopterus aethiopicus*). De weefselconcentraties in de Afrikaanse meerval (0.51 ± 0.17 Hg µg g^{-1} natgewicht) lagen iets hoger dan de WHO veiligheidslimiet van 0.5 µg Hg g^{-1} natgewicht; de vier andere vissoorten hadden kwikconcentraties die lager lagen dan deze limiet. Omwille van de hoge visconsumptie (144 g/dag), lag de berekende wekelijkse kwikopname in dit dorp (4.0 µg/kg lichaamsgewicht/dag) boven de 'voorlopig toelaatbare wekelijkse inname' (PTWI) aangeraden door WHO/FAO voor de bescherming van ongeboren en jonge kinderen (1.6 µg/kg lichaamsgewicht/week).

De (volledige) dieetstudie toonde daarentegen aan dat de dagelijkse kwikopname van mijnwerkers en de bewoners van het Mugusu mijndorp de 'voorlopig toelaatbare dagelijkse inname' aangeraden door WHO/FAO voor de bescherming van het ongeboren kind (15.5 µg Hg/dag) overschreed met een factor van twee. Dus, ongeboren kinderen van vrouwen uit dit dorp lopen een risico op intra-uteriene kwikvergiftiging. De bijdrage van de consumptie van vlees afkomstig van runderen en pluimvee (dat in de mijndorpen gekweekt wordt) tot de algemene kwikblootstelling was laag.

Analyse van spierweefsel van deze dieren bevestigde dat de kwikgehaltes in deze producten eerder laag waren en varieerden van 22 tot 81 µg Hg/kg natgewicht. Deze kwikconcentraties zijn veel lager dan de internationale veiligheidslimieten van 500 µg Hg/kg natgewicht.

De chemische analyses van kwik in hoofdhaar van de mijnwerkers en inwoners van het Mugusu mijndorp duidden – in het algemeen - op een lage blootstelling. De gemiddelde concentratie (1.55 ± 2.2 µg g^{-1} drooggewicht) was lager dan de waarschuwingslimiet (10 µg Hg g^{-1}drooggewicht) vastgesteld door WHO voor zwangere vrouwen. Haar van individuen betrokken bij het roosteren van het kwikamalgaam, bevatte daarentegen significant meer kwik dan dit van individuen betrokken bij andere mijn-activiteiten. Vergeleken met studies van tien jaar geleden, zijn de kwikconcentraties in het haar van de Mugusu mijnwerkers licht toegenomen.

Algemeen kan gesteld worden dat deze doctoraatsstudie aangetoond heeft dat de artisanale goudwinning in de Mugusu mijn kwikverontreiniging in alle milieucompartimenten veroorzaakt en dat deze aanwezige concentraties leiden tot een verhoogd risico voor het milieu en de menselijke gezondheid. Het huidige niveau van de kwikconcentraties in rivieren en stromen die via de Mugusu mijn in het Victoriameer terechtkomen vormen een risico voor de aquatische biota. Uit de enquêtes die in deze studie werden uitgevoerd, is het duidelijk dat de mijnwerkers onvoldoende kennis hebben van de mogelijke effecten van kwik op het milieu en de menselijke gezondheid. Bovendien kan besloten worden, op basis van de huidige locale voedselconsumptiepatronen en kwikconcentraties in de verschillende eetwaren, dat er geen risico is voor de algemene bevolking, maar dat er wel een gezondheidsrisico is voor ongeboren kinderen. Het is daarom aan te raden dat inspanningen geleverd worden om (1) het gebruik van kwik in het goudwinningsproces te reduceren en (2) de mijnwerkers en de omringende gemeenschappen kennis bij te brengen over de effecten van kwik op het milieu en de volksgezondheid.

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Curriculum Vitae

Raphael Tihelwa Chibunda was born in 1966 in Sengerema district–Tanzania. He obtained his bachelor degree in Veterinary Medicine at the Sokoine University of Agriculture (SUA) in Tanzania, 1995. In 1998 he obtained a Masters degree in biological sciences from the University of Nairobi (Kenya). From 1998 to mid-2000 he worked as a self employed veterinarian in Mwanza city, Tanzania. In late 2000 he was employed as an assistant lecturer at SUA and in 2003 he was promoted to a rank of lecturer, he lecturers to undergraduate students on animal physiology, environmental toxicology and health hazards. In November, 2003, he was admitted at the Laboratory of Environmental Toxicology and Aquatic Ecology (Ghent University-Belgium) as a pre-doctoral student. In 2004 he was registered as a PhD student at the same laboratory under the guidance of Prof.dr. C.R. Janssen. Raphael is married to Grace Tumbo and has two sons (Fransis and Dalton). Raphael has co-authored over eight scientific publications in both local and international peer reviewed journals. He has made over 16 presentations to local and international conferences. His permanent address is P.O Box 3017, SUA, MOROGORO, TANZANIA. Email: chibu@suanet.ac.tz or Chibunda@yahoo.com

Publications

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Recent poster presentation

Chibunda R. T, Pereka A.E, Tungaraza C and Janssen C.R. E Ecotoxicity of mercury contaminated sediment collected from Mugusu gold mine (Geita district – Tanzania) impacted river to the early life stages of African catfish (*Clarias gariepinus*). 17th Annual meeting of SETAC-Europe, 20-24 May 2007, Porto, Portugal

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Membership in professional organization

Member of the Society of Environmental Toxicology and Chemistry (SETAC)–African branch

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