KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY, KUMASI, GHANA

TOTAL MERCURY IN BLOOD, HAIR AND URINE OF ARTISANAL GOLD MINERS IN THE ASUTIFI DISTRICT OF BRONG-AHAFO REGION OF GHANA.

By

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DECLARATION

I hereby declare that this submission is my own work towards the MPHIL and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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ABSTRACT

Total mercury (THg) concentration in human blood, urine and hair samples were determined to ascertain if the miners in Asutifi district of Brong – Ahafo region of Ghana are at a high risk of mercury exposure. In all ninety four (94) samples comprising blood, urine and hair from sixty four (64) Artisanal gold miners and thirty non – miners were collected and analyzed for their total mercury levels using the cold vapor atomic absorption spectrometry. The blood total mercury concentrations ranged from 0.16 to 0.78 µg/L with a mean of 0.42 ± 0.25 µg/L for miners and 0.15 to 0.48 µg/L with a mean of 0.32 ± 0.13 µg/L for non – miners. For urine, THg levels ranged 0.82 – 30.88 µg/L with mean of 6.22 ± 6.42 µg/L for miner and 0.83 – 6.74 µg/L with mean of 3.67 ± 2.14 µg/L for non – miners. The hair total mercury concentration ranged from 1.58 to 32.92 µg/g with mean of 6.47 ± 7.56 µg/g for miners and 0.88 – 11.29 µg/g with mean of 3.85 ± 3.10 µg/g for non – miners. The total mercury concentration per creatinine was determined to correct the level of mercury in urine affected by dilution or concentration of the urine due to fluid intake. The results ranged from 0.54 to 17.67 µg/g, with an average value of 4.57 ± 4.20 µgHg/g per creatinine. Although the levels of mercury in blood, hair and urine of miners were higher than the non-miners, but the levels of exposures do not appear to pose a significant health threat according to the World Health Organization (WHO) safety limit.

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CHAPTER ONE

1. INTRODUCTION

For centuries, gold has been the world's recognizable form of economic exchange. The value placed on gold as a symbol for wealth had made gold mining, especially artisanal gold mining (AGM), one of the oldest and most reliable extractive industry. The first known usage of amalgamation in gold mining occurred in Spain as early as 700BC. Other records indicate that Artisanal Gold mining was practiced as early as the 4th century and the indigenous population of Ghana got more involved when the Europeans arrived in 1471 (Donkor *et al*, 2006).

Amalgamation technique has been used and is still being used to extract gold from the "crude" ores, despite the promulgation of mercury law in 1933 banning Ghanaian gold miners from using Hg in their operation. The practice continued (Akabzaa and Dramani, 2001) till its legalization in1989 by the Provisional National Defense Council (PNDC law 218). The legalization of small-scale gold mining has escalated AGM activities, thus providing direct and indirect employment to over one million people and playing a signification role in the economy of Ghana (Donkor *et al*, 2006).

Artisanal gold mining is the extraction of gold by miners working in small or medium sized operations, using rudimentary process to extract gold from secondary and primary ore bodies. The disaster in Minamata, Japan, where several mass poisonings involving mercury, attracted the general attention of the scientific community towards the end of the 1950s, is still fresh in memory.Subsequent investigations revealed contamination by industrial discharges of Hg into Minamata Bay (D'ltri and D'ltri, 1977). Other incidents of Hg poisoning were reported in Iraq, Pakistan, and Guatemala, with numerous deaths resulting from the eating of Hg-contaminated fish or consumption of food prepared from seed grain treated with mercurial fungicides (Zhrlich, 1990).

Mercury is used to separate and collect gold from rocks in which it is found. Mercury binds to the gold to form an amalgam which helps it to separate from rock, sand or other materials. The amalgam is then heated to vaporize the mercury leaving the gold behind. This practice produce atmospheric mercury emissions of around 300 metric tones per year world wide (GMP, 2006).

Mercury is a more effective, simple and very inexpensive reagent to extract gold (1 kg of Hg costs 1g of gold) than cyanide which requires a higher order of economic capital, training and organization (Lacerda and Salomons, 1998).

Mercury is a naturally occurring metal which has several forms. The metallic mercury is a shiny silver-white, odorless liquid. If heated, it is a colorless, odorless gas. It is also quite volatile and only slightly soluble in water. It is dispersed effectively through the atmosphere with long residence time of about 2 years and is normally transported from likely source of emission (Boening, 2000). Elemental Hg is now known to spread very effectively from diverse sources both terrestrial and aquatic systems.

. Mercury combines with other elements, such as chlorine, sulfur, or oxygen, to form inorganic mercury compounds or "salts" which are usually white powders or crystals. Mercury also combines with carbon to make organic mercury compounds. Mercury vapor is a danger not to the local population but can be carried long distances in the atmosphere and deposited into aquatic systems. Inorganic Hg may be converted by microbial activity in an organic-rich environment to organic forms of Hg, for example methyl-Hg (MeHg), which are many times more toxic to organisms(Beijer and Jernelov, 1979). Methylmercury is a potent neurotoxin, damages the central nervous system and especially toxic to fetus. It is also very soluble in lipids and therefore, crosses biological membranes with ease. Because of its protein binding properties, it readily bio-accumulates and bio-magnifies in aquatic food

chain. As a result, it poses a threat to humans and other fish eating animals(Lodenius and Malm, 1998).Once in the lungs Hg is oxidized forming Hg (II) complexes which are soluble in many body fluids. The ultimate effect of Hg and its related compounds are the inhibition of enzyme action.

The impairment of the blood-brain barrier together with the possible inhibition by Hg of certain associated enzymes will certainly affect the metabolism of the nervous system (UNIDO, 1997)

Artisanal gold mining has resulted in the use of an enormous amount of metallic mercury with little or no awareness of its risks. The mercury used by the miners is usually discharged in an abusive manner into the ecosystem (Pfeiffer *et al*, 1998).The major pathway of exposure of mercury by artisanal gold miners is through the inhalation of mercury vapor from burning mercury gold amalgam. Some mercury is also absorbed directly through the skin when amalgamation is done by hand, breathing contaminated air, ingestion of contaminated fish, food, and medical treatments (Ashton *et al*, 2007). The kidney is the affected organs in exposure of moderate duration to considerable levels while the brain is the dominant receptor. In long-term exposures to moderate levels (Suziki, 1979) total mercury elimination can take several years. Analysis of mercury in urine, blood or hair does not necessarily indicate or diagnose if a person is intoxicated. A person who has ceased burning amalgam for more than a year, but who has been exposed to mercury for a long time can measure low levels of mercury in blood or urine, but may still be suffering the effects of metallic mercury poisoning (GMP, 2006).The normal units of reporting mercury concentration in urine are µgHg/g creatinine. That is, mercury is adjusted or standardized according to the amount of creatinine (an amino acid) in the urine so that results are not affected by dilution. Blood is more difficult to analyze because specialized equipment is necessary (e.g. sterilized needles, special vials and containers containing chemicals to prevent coagulation) and personnel trained to collect

blood. For these reasons, measuring mercury in blood is less common than measuring in urine or hair.

Measuring mercury in hair provides an excellent record of exposure to mercury, especially from eating fish. Mercury becomes very concentrated in hair which is one of the routes that the body uses to rid itself of mercury. Mercury in hair is much easier to measure with no special equipment, or expertise required collecting the sample. In ASM communities, especially among burners of amalgam, mercury in hair comes mainly from fish consumption.

This study involved 64 miners and 30 non – miners where total amount of mercury in blood, urine and hair was determined by Cold-Vapor atomic absorption spectrometry (CV-AAS), using Automatic Mercury Analyzer HG-5000.

1.1 PROBLEM DEFINITION

Artisanal gold mining is largely a poverty-driven activity that constitutes an important source of livelihood for many rural communities, but it is also the world's fastest-growing source of mercury contamination (Swain *et al* 2007). The use of mercury to recover gold, by amalgamation, is a common and simple extraction process, but it is dangerous and contaminates air, soil, rivers, and lakes. Mercury is now commonly considered as an "invisible epidemic" because its impacts are usually not seen immediately but can accumulate and bioaccumulate in the food chain and create new dangers once transformed into methylmercury (Lacerda and Salomons, 1998). The health of people living in an area is negatively affected through inhalation of mercury vapor, direct contact with mercury, and the consumption of fish and other foods affected by mercury contamination (Castilhos *et al*, 2006). A health study conducted from 1986 to 1991 in Brazil showed elevated urinary and blood Hg concentration in miners with signs and symptoms of Hg intoxication (Branches *et al*, 1993).

In the last century, mercury (Hg) levels in the global environment have tripled as a result of increased pollution from industrial, occupation, medical and domestic uses (USEPA, 1997). There are both immediate and long-term effects of using mercury in the gold mining process for miners, mining families and communities. The major health concern of elemental mercury vapor poisoning is the effect on the central nervous system and kidney. Inhaling large amounts of metallic mercury vapor, such as when miners are burning amalgams in open pans cause acute exposure. This can cause many symptoms including difficulty and pain when breathing, chest pains, coughing, pneumonia and kidney failure (GMP, 2006).

Chronic exposure to smaller amounts of mercury over a long period of time come with many symptoms including, headache, metallic taste in mouth and bleeding gums, tremor of fingers and toes, poor coordination of movement of arms and legs, difficulty with writing, unsteady walking, slurred speech, blurred vision and long sightedness, dizziness, hearing loss, impotence in men, loss of coordination of hands and fingers, and inability to perform rapid alternating movements (WHO/UNEP, 2008).

Psychological symptoms include insomnia, irritability, fatigue, forgetfulness, difficulty concentrating, lack of energy, exaggerated emotional response, and loss of interest in sexual relations, melancholy and depression. These symptoms may not always be consistent and differ from person to person. Unborn and young children are the greatest risk when exposed to mercury because their organs, nervous tissue and brains are still developing (GMP, 2006).

1.2 OBJECTIVES OF THE STUDY:

The specific objectives of this study are to:

1. determine the levels of mercury in blood, hair and urine of artisanal gold miners in Asutifi District of Brong Ahafo Region.

2. determine the levels of mercury in blood, hair and urine of non – miners (control group) in the community and compare with the miners.
3. compare the levels with WHO/FAO standards to ascertain if the miners are at risk of mercury exposure.

1.3 JUSTIFICATION OF THE OBJECTIVE

Ghana has not paid the needed attention to Hg contamination due to AGM activities. Moreover there are no dependable data to assess the extent of Hg exposure and health condition of artisanal gold miners in Ghana. Literature available on Hg pollution in Ghana deals with survey data on some of the rivers draining the south-western gold belt (Donkor, 2006).

Adimado and Baah (2002) reported Hg concentration in human blood, urine, hair, nail and fish from Ankobra and Tano river basins in south-western Ghana. Kwaansa – Ansah, *et al* (2010) reported environmental and occupational exposures to mercury among indigenous people in Dunkwa – On – Offin, a small scale gold mining area in the south-west of Ghana. In addition, Voeborlo *et al,* (2009) also reported head hair total mercury level in some Ghanaian individuals. There is the need for further studies on the mercury level in biological samples of artisanal gold miners in order to predict the potential impacts of Hg poisoning in the miners and non – miners to ascertain if they are at risk of mercury exposure.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 MERCURY

Mercury, also called quicksilver, is a heavy, silver-white metal (substance) that exists as a liquid at ambient temperatures. Its chemical symbol, Hg, comes from the Latin word, hydrargyrum, meaning liquid silver. Mercury is the only metal that is present as a liquid at room temperature. It also has the lowest boiling point of all metals and that is why it evaporates or "burns" easily. There are three important types of mercury;

1. The pure element
2. Inorganic compounds(such as mercuric chloride)
3. Organic mercury compounds(such as methylmercury)

When river sediments are rich in humic substances (humic and fulvic acids), these acids can dissolve metallic mercury dumped by miners or condensed from atmosphere, forming Hg-organic soluble complexes (Troman *et al*, 1996). When dissolve organic matter is present at concentration higher than 1mg/l (ppm), the complex formed is more stable and predominant than any of the inorganic species. Biotic and abiotic methylations are possible processes to generate methylmercury. Particle of organic matter in suspension are substrates of bacteria that also favor methylation.

Elemental mercury is a liquid and gives off vapor which can be inhaled into the lungs and passed into the blood stream. Element mercury can also pass through the skin into the blood stream. If swallowed, this form of mercury is not absorbed out of the stomach, and usually passes out of the body without harm.

Inorganic mercury compound can also be inhaled and absorbed through the lung, and may pass through the skin. But the compound can also be absorbed through the stomach if swallowed. Many inorganic mercury compounds are irritating or corrosive to the skin, eyes and mucus membranes as well (ATSDR, 1999)

Methylmercury is easily absorbed by worms, snails and insects and become highly concentrated in fish. Eating fish contaminated by mercury can pose a great health risk to people living downstream of mining areas. Humans generally uptake mercury in two ways, as methylmercury (CH_3Hg^+) from fish consumption, or by breathing vaporous mercury (Hg^0) emitted from various sources such as metallic mercury, dental amalgams, and ambient air. All forms of mercury are toxic to humans, but methymercury is of special concern, because our bodies have a less well developed defense mechanism against this toxin. Mercury is mostly present in the atmosphere as a gaseous element. There are two main types of reactions in the mercury cycle that convert mercury through its various forms. These are oxidation-reduction and methylation-demethylation reactions. In oxidation-reduction reaction, mercury is either oxidized to a higher valence state (eg. form relatively inert Hg^0 to the more reactive Hg^{2+}) through the loss of electrons, or the reverse in which mercury is reduced to a lower valence state.

Table 2.1: Physical and chemical properties of mercury

Property	**Value**
Atomic weight	200.59
Crystal system	Rhombohedral
CAS registry number	7439-97-6
Atomic number	80
Valences	1, 2
Outer electron configuration	$5d^{10}6s^2$
Ionization potentials, normal, eV	
1^{st} electron	10.43
2^{nd} electron	18.75
3^{rd} electron	34.20
Melting point, ^{0}C	-38.87
Boiling point, ^{0}C	356.9
Latent heat of fusion, J/g (cal/g)	11.80 (2.8)
Latent heat of vaporization, J/g (cal/g)	271.96 (65.0)
Specific heat, J/g (cal/g) solid.	
-75.6^{0}C	0.1335 (0.0319)
-40^{0}C	0.141 (0.1337)
-263.3^{0}C	0.0231 (0.00552)
Liquid	
-36.7^{0}C	0.1418 (0.0339)
210^{0}C	0.1335 (0.0319)
Electrical resistivity, Ω-cm, at 20^{0}C	$95x10^{-6}$
Density, g/cm^3	
At 20^{0}C	13.546
At melting point	14.43
At -38.8^{0}C (solid)	14.193
At 0^{0}C	13.595
Thermal conductivity, W/(cm^{2}k)	0.092
Vapor pressure, 25^{0}C	$2x10^{-3}$mm Hg
Solubility in water, 25^{0}C	20-30µg/L

(USEPA, 1997)

2.2 SOURCES OF MERCURY EXPOSURE

Populations living near waste sites, deforestation, reservoirs and mining areas are likely to be exposed to mercury. People can also be affected by mercury exposure through dental tooth filling, cultural and religious practices, cosmetic treatments, and as vaccinations.

2.2.1 WASTE SITES

Mercury – containing wastes generated through either industrial processes (pharmaceutical and car equipment plants, abandoned chlor-alkali plants, closed mining operation etc) or domestic use can be discarded improperly, resulting in contamination of the local area and creation of a "mercury waste site". People who live near these waste sites can be exposed to elevated levels of mercury due to releases to the soil, air and water bodies.

Mercury is present in a vast array of domestic products. In some cases, recycling programmes to retrieve these products before they are discarded with other refuse are cost-prohibitive, inefficient, or simply nonexistent in many communities. Many of these products are discarded to landfills or other disposal sites. Rain percolating through these dump piles can carry mercury resides into groundwater or downstream to rivers and lakes, resulting in contamination of water, sediment and fish. Sometimes significant releases can occur due to historical industrial or mining wastes. For examples, in many parts of Latin America, thousands of tons of mercury are still present in the environment, due to past gold mining operations. Parts of Mexico are still heavily contaminated from mercury that was brought from Spain in the 400 year period of Spanish rule (Population Probe, 2003).

In other types of mining operations mercury present in tailing piles as impurities can be leached by water infiltration to nearby watersheds. Similar situations also occur in gold mining operations using cyanide – leaching techniques instead of gold amalgamation. Here, dissolved cyanide reacts with traces of mercury in the tailing and acts as a carrier downstream (Boyle and Smith, 1994).Population consuming fish from the water bodies impacted by the presence of water sites could be at risk of higher mercury exposure.

2.2.2 DENTAL AMALGAMS

For more than a century, an inexpensive alloy of silver, tin and mercury has been used in dental practices as the preferred tooth-filling material, which mercury constitutes 50% of these materials. Mercury released from amalgam fillings can take several forms; elemental mercury vapor, metallic ions and fine particles of the mercury vapor. Some is exhaled before it further penetrates the respiratory tract, some is inhaled into the lungs and absorbed into the blood, and some is retained in the vapor form in the saliva and swallowed. Of that portion swallowed, only a small fraction is expected to be absorbed through the gastrointestinal tract.

Dental amalgams are the primary sources of exposure to inorganic mercury for most people who have mercury – containing dental fillings. Moreover, many workers in dental offices (such as dentists, dental hygienists) are exposed to mercury through the production and use of mercury fillings. There is clear evidence in the scientific literature of elevated body burden of mercury in dentists and dental hygienists. In fact, in a recent study Retchie *et al*. (2002) showed that dentists had, average urinary mercury concentration over four times that of the control subject.

2.2.3 CULTURAL / RELIGIONS / MEDICAL USE OF MERCURY

Historic records suggest signs of mercury use by ancient Chinese and Hindu Civilizations. Archaeologists found traces of mercury in an Egyptian tomb from 1500BC. Today, elemental and inorganic (oxidized) mercury are still used in some population for cultural, religious, or ritualistic purposes, in cosmetics or as folk medicine (UNEP, 2002).

2.2.4 COSMETIC TREATMENT

Examining 38 different skin lightening creams, Al-sahel and Al-Doush (1997) found that 45% contained mercury levels above the US Food and Drug Administration (US FDA) limit

of 1mg/kg: two of the products had mercury concentrations over 900mg/kg. Such uses have resulted in reports of toxicity in a number of cases (Kan - Yum and Oransky, 1992).

2.2.5 FOLK MEDICINE AND AYUVEDIC MEDICINE

Mercury is thought to exhibit healing properties and is sometimes used as an antiseptic, in herbal remedies, or even as a treatment for disease such as Syphilis. Mercuric chloride, mercuric oxide, mercuric iodide, mercurous acetate, and mercurous chloride are or have been used for their antiseptic, bactericidal, fungicidal, diuretic, and Cathartic properties.

Commercially produced herbal ball preparations used in traditional Chinese medicine can contain mercury. Adult dosage for traditional Chinese medicine is two balls daily, resulting in daily intake levels of up to 1.2ml of mercury (Kang - Yum and Oransky, 1992).

2.2.6 CULTURAL RELIGIOUS PRACTICES

In some cultures, mercury is believed to chase away evil spirits when placed on the walls of houses. Elsewhere, it is thought that mercury – based talisman can bring good luck.

Obscure religious practices involving the use of mercury are known, but the topic remains poorly documented (US EPA, 2002). Metallic mercury is sold in some regions, under the name "azogue" (sometimes called botanical). Some of the uses of this "azogue" include, among others, mixing the mercury in bath water or perfume, wearing it in vials as jewelers, placing it in devotional candles, sprinkling it on floors of houses or automobiles, applying it directly to the skin or in some cases injecting it. (WHO/UNEP, 2008)

2.2.7 DEFORESTATION

The impacts of large-scale deforestation on ecosystems are numerous. In tropical environments, the organic – rich layer of soil, naturally held in place by tree roots, is often eroded during seasonal rains. Mercury accumulated in these soils due to atmospheric

deposition can also be flushed to rivers and lakes. According to some authors (Roulet *et al*, 2002) this source of mercury loading to aquatic ecosystems might be of greater important in some region than ASM activities. For example, studies in the Brazilian Amazon identified situations of high mercury exposure through fish consumption for some populations living far from gold mining area in the Tapojos River drainage basin.

Trees and other vegetation contain mercury. For examples, in investigations in the U.S.A, the mercury content of letter and green vegetation from seven locations in the U.S.A ranged from 0.01 – 0.07 mgHg / kg dry weight (Friedly *et al*, 2001). This mercury in vegetation originates from both naturally present mercury and mercury deposited from anthropogenic emissions (COWI, 2002). Trees (especially needles and leaves) absorb mercury from the atmosphere over time (Friedly *et al*. 2001).

The amount of mercury emitted annually by deforestation in the Amazon has been estimated at between 0.78kg /km^2 and 1.76kg/km^2 (Lacerda, 1995). Through analysis of aerosol particles, it has been estimated that about 30% of the mercury in atmospheric particles in the Amazon region might be associated with biomass burning and 63% from gold mining (Veiga and Baker, 2004). Populations consuming fish from water bodies impacted by deforestation could be of higher mercury exposures.

2.2.8 VACCINES

Thimerosal is used as a preservation in vaccines (such as DTP, hepatitis B, and Hib), mostly in developing countries, to protect against bacterial contamination. This preservative contains nearly 50%ethylmercury. Thirmerosal has been used since 1930s in the manufacture of some vaccines and other medical products (WHO, 2006).

Once in the body, thimerosal is metabolized to ethylmercury. Half life of ethylmercury is only 6 days compared with 40-50 days for methylmercury. It is actively excreted into the intestinal tract and not accumulated in the body. It rapidly converts to inorganic mercury (that is less toxic to the brain than ethyl or methylmercury).

The United States and other industrialized countries have decreased or eliminated the use of thimerosal for many vaccines. However, thimerosal still exists in some vaccines used in various parts of the world, in particular where accessibility and cost require the availabilities of multidose vials of vaccines, such as in developing countries. Therefore, when assessing exposures to mercury for a population, this possible source of exposure should be considered (WHO, 2003).

2.2.9 RESERVOIRS

Most studies dealing with the environmental impacts of reservoir creation focus on the fact that flooding terrestrial ecosystems lead to increased mercury levels in fish species living in newly created reservoirs (Lucotte *et al*, 1999). In many cases, these increases result in mercury levels in fish that may be unsafe for regular human consumption.

Reservoirs also act as efficient incubators for mercury methylation. Population regularly consuming fish from young reservoirs could be exposed to elevated levels of mercury.

2.2.10 MINING PROCESS

For centuries, the capacity exhibited by mercury to form amalgamates with gold has led people to use mercury to extract the precious metal. Generally, this mining process involves the following: the wet ore (mud or ore concentrate) is mixed with metallic (liquid) mercury; the mercury chemically binds with the gold or silver in the mud; the remaining mud is washed away leaving a mercury-gold (or mercury-silver) amalgam and contaminated tailings. This liquorish mercury compound is squeezed through a cloth and a hard piece of amalgam is

the result. Sometimes the miner uses the mouth to hold the cloth whilst squeezing stronger (Boese-O'Reilly *et al,* 2004). The amalgam is then heated in various stages to release the mercury, with increasing levels of purity of the gold or silver.

Artisanal miners are generally the most directly exposed, either through direct handling or by breathing the mercury vapor generated during the burning of the gold-mercury amalgam. However in many cases, heat separation of gold is performed in houses, or in other locations close to family members and other people, exposing these other people to elevated levels of gaseous mercury. Vapor inhalation is generally the most important and dangerous pathway of exposure to metallic mercury for artisanal gold miners and their families. This is also true for gold dealers (and the people inhabiting in the vicinity of "gold shops"), who generally operate their business in more urban areas, purchasing amalgams from artisanal miners and refining gold pellets still containing appreciable amounts of mercury, often in closed rooms without proper ventilation.

Mercury releases by ASM occur as liquid mercury lost to aquatic environments during the amalgamation process or in waste discharges and vapors entering the atmosphere. It is difficult, even locally, to evaluate the amount of mercury emitted due to ASM. Mercury losses during ASM operations largely depend on the amalgamation technique used and on the way gold is separated from the amalgam, either through the use of nitric acid, retorting or burning in open pans. If retorts are not used with the heating process, the greatest part of the mercury introduced in the amalgamation process is released to the atmosphere. The most environmentally damaging approach to creating amalgamates is to place mercury on sluice boxes or spread it on the ground to be mixed which the raw ground ore to "attract" the gold, while losing a significant portion of this metallic mercury concentrates, enabling extraction of up to 90% of the initial gold content. Such amalgam has mercury contents of 20 – 40%.

Excess mercury can then be removed through centrifuging or using a piece of fabric (Hinton *et al*, 2003).

Mercury emissions from informal gold mining operations represent a serious environmental problem in developing countries. In the Amazon, from 70 to 170 t of Hg are discharged annually. Generally, it is considered that oxidation of mercury must occur to produce significant dissolution. Some authors (Meech *et al*, 1998) have examined the stability of mercury in the unoxidizedelemental mercury in aquatic environments.

Many of the available estimates on environmental mercury releases attributable to ASM are based on regional mercury sales, but these numbers do not take into account recycled mercury or mercury bought on alternative markets. Even given the uncertainties related to these estimates, ASM represents one of the most important sources of mercury emissions to the global environment attributable to human activities. Local increases in the mercury levels in fish tissues have been reported in various studies. These increased mercury levels in fish can lead to additional mercury exposures through fish consumption for people involved in ASM activities, as well as for other people who live in the vicinity of the ASM activities (Limbong *et al*, 2003)

Amalgam burning transforms some of the mercury-bound molecules into volatile elemental (reduced) mercury or reactive (oxidized) forms of mercury that are carried in the air, partially reaching the global atmospheric pool (long-range atmospheric transport). But part of this mercury might redeposit after short-range atmospheric transport and be readily available for methylation and bioaccumulation (Maxson, 2006).

2.3 TECHNICAL USES OF MERCURY

Metallic mercury is used to produce chlorine gas and caustic soda and also to extract gold from ores. It is also used in the thermometers, dental filling, and batteries. Some inorganic

mercury compounds are used in skin – lightening creams, as antiseptic cream and ointments, and as anti – mildew agents.

2.3.1 GOLD EXTRACTION WITH MERCURY – AMALGAMATION PROCESS

Mercury is consumed during gold extraction with the amalgamation process – typically applied in artisanal gold mining. The main steps of this extraction process are:

1. Ore concentrated by sedimentation is mixed with metallic (liquid mercury, during which the most easily extractable gold is dissolved in the mercury "amalgamation").
2. The mercury – gold amalgam is separated from the solids and heated until most of the mercury has evaporated, and the gold can be collected. This process is often done with no effort to capture the volatilized mercury, which is consequently emitted to the atmosphere, although semi – closed "retorts" are sometimes used to capture some of the mercury (COWI, 2002). Green Peace (1994) estimated the total world – wide consumption of mercury for gold mining was 400 – 500 tonnes / year in 1993 – 94.

The most comprehensive recent analysis lost for each kilogram of gold recovered (Veiga, 2002), and suggested that AGM activities use 500 to 1000 tonnes of mercury annually.

2.3.2 CHLOR – ALKALI PRODUCTION WITH MERCURY – TECHNOLOGY

Mercury is used as a fluid cathode in one of the three main types of electrolytic process used for production of chlorine and sodium hydroxide (NaOH) from brine. A single mercury cell chlor – alkali plant may have many tones of mercury in use (COWI, 2002). Other well known mercury – free processes are widely used, and it is generally accepted that these alternative processes will replace the mercury process over time.

2.3.3 DENTAL MERCURY AMALGAM FILLINGS

Dental amalgam fillings consist of an alloy of mercury (typically 44-51% mercury by weight), silver, copper and tin. It is mostly supplied to dentists. Depending on the size and type of filling, sometimes 0.4g and 1.0g of mercury is normally consumed per filling. Dental amalgam is a large global consumer of Hg, after chlor – alkali, artisanal gold mining and batteries.

RPA (2002) has estimated a significant decrease in mercury use for fillings in the EU, from 110 tonnes in 1990 to about 70 tonnes in 2000.

2.3.4 MEASURING AND CONTROL DEVICES

Mercury thermometers have traditionally been used for most medium temperature range measurements. Mercury thermometers may contain between about one and several milligrams per unit, depending on the use (COWI, 2002).

Medical blood pressures gauges (sphygmonometer) are used widely in hospitals and private medical practices, which normally contain70g mercury per unit.

Barometers containing mercury are commonly used for meteorological purposes. The quantities of mercury per unit in these instrument are between 70 – 140 mercury per item. Pressure valves contain 100 – 600g mercury per unit, sometime more.

2.3.5 OTHER PRODUCTS AND PROCESSES CONTAINING MERCURY

Mercury is present in a large number of other products and processes both within the EU and in third World countries, including pesticides and fungicides (seed dressing, sugarcane bedding plant treatment etc), paints (mercury preservative in latex and marine paints), laboratory use (chemical, electrodes and specialized equipment in limited numbers), pharmaceuticals (vaccines, eye drops, some herbal medicines), catalysts (for production of

PUR elastomers and other chemicals and polymers), Cosmetics (skin lightening cream, preservatives in some eye cosmetics), gyroscope for marine and aviation use, lighthouses (lens and light source unit rest on mercury in a common design), pigments, cultural and religious rituals, explosives etc (COWI, 2002).

2.5 EFFECTS OF MERCURY IN HUMAN

The impairment of the blood-brain barrier, together with the possible inhibition by Hg of certain associated enzyme will certainly affect the metabolism of the nervous system (UNIDO, 1997). The Kidneys are the effected organs in exposures of moderate duration to considerable levels while the brain is the dominant receptor in long-term exposure to moderate levels (Suzuki, 1979). Total mercury elimination can take several years. In Japan, workers with peak urinary Hg concentration of 600ug/l showed neurobehavioral disturbances 20 to 35 years after the mercury vapor exposure (Satoh, 1994). The common manifestation of chronic exposure to excessive level of Hg vapor is metallic taste and gum diseases, such as gingivitis, ulcers and formation of a blue line at gum margins. A person suffering from a mild case of poisoning can be unaware because the symptoms are psycho-pathological.

Symptoms of mercury poisoning have been detected in miners, gold dealers and citizens living near emission sources (Schulz-Garban, 1995). Samples of urine have shown high levels (as high as 400ug/L) for workers burning amalgam daily. Some of these individuals show signs of mercurialism (UNIDO, 1999). It has been observed that when female's intake of the poison is large and she becomes ill, sterility occurs. One major problem of mercury is a known adverse effect on the growing fetus and baby due to a high maternal burden and a cross of mercury through the placenta or to the breast-milk. High numbers of miscarriages, still births and birth defects have been reported as consequence of the mass intoxicities with mercury in Minamata Japan, (Drasch *et al*, 2004).

Because methylmercury- cysteine conjugate readily passes both the placental barrier and the blood –brain barrier and the developing fetus are especially sensitive to the toxic effects of methlmercury, exposures during pregnancy are of highest concern. Offsprings born of women exposed to high levels of methylmercury during pregnancy have exhibited a variety of developmental neurological abnormalities similar to cerebral palsy, including the following: delayed onset of walking, delayed onset of talking, altered muscle tone and deep tendon reflexes, and reduced neurological test scores (UNEP, 2002).

Smaller dose permits conception and live birth, but the baby will have severe neurological symptoms. A dosage too small to cause noticeable neurological symptoms in the child may cause congenital mental deficiency. But in any of these cases, the mother's symptoms are relatively mild.

Even in low doses, methylmercury poisoning causes neurological problems and is especially dangerous for women of child-bearing age. The critical target for methylmercury toxicity is the nervous system, especially during its development stage. Neurotoxicity is the most sensitive endpoint. In humans, the indices of neurotoxicity include neurobehavioral deficits, neuronal loss, ataxia, visual disturbances, impaired hearing, paralysis and death (WHO, 2004).

From the methylmercury poisoning empoisoning episodes in Japan and Iraq, it is known that severe effects take place in the development of the brain and nervous system of the unborn child (the fetus), but severe effects on adults were also observed. The most common clinical signs observed in adults were par aesthesia, ataxia, sensory disturbances, tremors, impairment of hearing, constriction of the visual field and difficulty in walking. Both the central and peripheral nervous systems show signs and damage (Eto *et al*, 2002). Developmental delays were significantly associated with the methylmercury exposures, even including the children

whose mothers had higher hair mercury levels (above 10 µg/g) (Grandjean *et al*, 1997). Within the low exposure range, doubling of the prenatal methylmercury exposure level was associated with a developmental delay of 1-2 months (UNEP, 2002). Various studies also suggested effects on the cardiovascular system, including increased risk of acute mynocardial infarction and elevated blood pressure (Virtanen *et al*, 2005). These studies suggest that even small increases in methylmercury exposures may cause adverse effects on the cardiovascular system, thereby leading to increased mortality (UNEP, 2002).

Elemental (metallic) mercury causes primary health effects when it is breathed as a vapor. Inhaled mercury vapor may cause headaches, cough, chest pains, chest tightness, and difficulty in breathing. It may also cause chemical pneumonitis. Other symptoms include, tremors, emotional changes (e.g. mood swings, irritability, nervousness, excessive shyness); insomnia, neuromuscular changes, such as weakness, muscle atrophy, twitching, disturbances in sensations, changes in nerve responses, performances deficits on test of cognitive function (NIOH, 2007).

With inorganic mercury high exposure may result in damage to the gastrointestinal tract, the nervous system, and the kidneys. Symptoms of high exposure to inorganic mercury include; skin rashes and dermatitis, mood swing, memory loss, mental disturbances, and muscle weakness (NIOH, 2007).

2.6 HEALTH QUESTIONNAIRE AND ASSESSMENT ON MERCURY EXPOSURE

A health assessment study can provide important information about the health status of the study subjects and the population and help determine whether or not there is an association between some health conditions and mercury exposures. A health assessment can also provide valuable insights for developing site-appropriate interventions (behavioral, medical, environmental and economic). A medical examination can be performed that includes the

health history of each individual, a physical examination, and a neurological examination. A health assessment questionnaire (including a dietary survey) can also be used to gather information about possible exposures to mercury and other health information. An excellent example of such a health assessment questionnaire and content to be covered in a medical examination is cited in the Protocols for Environmental and Health Assessment (Veiga and Baker 2004). The questionnaire covers the following data:

- Personal data (such as age, gender, address, etc);
- General questions related to work exposure to mercury;
- Other means of exposure to mercury (use of traditional medicines, use of mercury for ritualistic or religious purposes, known spills such as a broken thermometer) presence of dental amalgam;
- Diet issues (frequency and type of food, particularly fish);
- Health problems (based on symptoms described by the patient);
- Alcohol consumption habits (frequency, amount and type);
- Other possible confounding factors (use of drugs; smoking; malaria, handling of gasoline, kerosene, pesticides)

Specific health questions related to mercurialism (metallic taste, salivation, loss of weight, etc.); Physical examination (blood pressure, signs of gingivitis, tremor, reflexes, number of dental fillings, etc.); Specific neuropsychological tests (memory, coordination, etc.);

Exposures to elemental, inorganic, or methylmercury, depending on their magnitude, can result in a continuum of health effects by severity from subtle responses to very frank adverse outcomes. Subjective symptoms include numbness, dizziness, trembling, and motor disturbance, and irritability, loss of memory, insomnia, and metallic taste. Objective symptoms include gingivitis, bluish discoloration of gums, sensory disturbance, disturbance

in balance, abnormal gait, altered reflexes, and disturbance in coordination, tremor, and dysarthria. If mercury intoxication is apparent, the volunteer should be informed about ways to reduce further exposure (Veiga and Baker, 2004).

A series of specific neurophysiologic tests (digit span test, match box test, Frosting score, pencil tapping, etc) can be used to detect the effects of mercury poisoning. These tests are simple, but local health care professionals need to be trained to administer them. These tests do not demand special equipment and when associated with analysis of human tissues (such as urine, blood, cord blood, hair, and human milk) that reflect the extent of exposure, can provide information on potential effects of mercury exposure (UNIDO, 2003)

Confounding factors should be investigated to exclude from the statistical analysis other explanations for any symptom found. Many factors can cause symptoms (such as fatigue, dizziness, and tremors) and can introduce false diagnosis to the clinical examination and neuropsychological tests (Veiga and Baker, 2004). Volunteers must be fully informed by the interviewers about the project and how the data generated by the study can help them and their community. Brochures can be useful to provide this preliminary information about the hazards related to mercury exposure and a simplified diet advisory (UNIDO, 2003). It is important to identify dietary sources of mercury exposure. Inclusion of fish in the diet varies with geographical location, season of the year, ethnicity, economics, and personal food preferences.

It is also important to try to identify non-dietary exposures to mercury, including occupational (artisanal gold mining and processing, dentistry, etc.), nearby industrial releases, use of traditional medicines, ritualistic purposes, spills, etc. Exposures to various contaminated environmental media should be quantified when possible (IPCS, 2000).

2.7. MERCURY IN HAIR, URINE AND BLOOD

Mercury in biological samples including; hair, blood, urine or umbilical cord is measured in order to evaluate the level of human exposure and body burden.

HAIR

The mercury concentration in hair is often used as a biomarker for methylmercury exposure. It reflects the concentrations in the blood at the time the hair was formed. At the same time, a hair sample provides simple and noninvasive sampling method as well as a storage method offering good sample preservation. Since the hair grows at a rate of roughly 1 cm per month, evaluation of part exposure is possible (IPCS, 2000). However, the mercury concentration in hair can increase as a result of adhesion of external mercury vapor and inorganic mercury.

In cases of no exposure to external inorganic mercury vapor, almost all mercury in hair is in the form of methylmercury, therefore the level of methylmercury exposure from diet can be evaluated by measuring total mercury.

However since people in gold mining and gold refining have a high risk of contamination from metallic mercury and mercury vapor, evaluation of actual methylmercury exposure is possible only by measuring methylmercury as well as total mercury in hair. Hair sample collection usually involves cutting a bundle of hair, approximately 100 – 150 strands (which is a bundle approximately 0.75 – 1.0 centimeter in diameter) about 2cm in length, from the occipital region of the head. It is very important to cut the bundle as close to the scalp as possible and retain the orientation of the hair stands whenever possible as the distance from the scalp is directly proportional to duration of time since exposure. Caution must be exercised to avoid contamination during sample collection. Blunt-tipped, clean stainless steel scissors can be used for cutting the hair and a cleaning step involving rinsing the hair with 70% isopropyl alcohol is recommended prior to sample collection. Once cut, the hair bundle

in wrapped closed to the scalp end with a small Post-it note or a clean piece of paper (approximately 3.5 cm x 5 cm) and held together with a plastic clip. A clean, sealable bag (such as zip closable bag) can be used to store the collected samples.

However, some authors (Trace elements laboratory, 2005) discourage the use of plastic bags because of the generation of static electricity that makes the handling of hair very difficult and their weight unreliable, therefore, they recommend to place the hair sample in a marked paper envelope. If the hair is too short to be cut and clipped together, hair can be cut directly into the storage bag (or envelope) using scissors or thinning shears. Collected samples can be stored in properly labeled zip-closable bags (or envelopes) and shipped to the analysis laboratory at ambient temperature.

If a hair mercury concentration is used as a dose parameter, then such factors should be taken into account. Hair color also seems to play a role and permanent wave treatment removes mercury from the hair. The normal level of mercury in hair is 1-2 ppm (1-2 μg/g), but people who eat fish one or more times per day have mercury levels in hair exceeding 10 ppm (WHO, 2004).

The USEPA reference dose corresponds to approximately 1ppm mercury in hair for people who have low fish consumption. Among the general population in Japan, the mercury concentration in hair is in the range of 1-5ppm and seldom exceeds 10 ppm. Hair is not a good indicator of mercury vapor exposure as urine is (UNIDO, 2003). Measurements of mercury levels in hair allow sequential analysis, and help in the identification of peak exposures (such as due to seasonal consumption variations). Peak exposures, in a chronic exposure setting, have been identified in some studies as an important contributing factor to adverse health effects (McDowell *et al*, 2004).

Numerous analytical methods are available for analysis of hair for total mercury. CVAAS is one of the most widely used analytical methods for the hair mercury analysis. CVAAS has adequate sensitivity to measure mercury at sub-ppm levels and has a low per-sample cost compared to some newly developed methods. Neutron activation analysis (NAA) can also be used to measure mercury in hair sample; however, the detection limits are not as good as cold vapor generation methods.

BLOOD

For people who eat quantities of fish and shellfish, the mercury concentration ratio of red blood cell to plasma (serum is as approximately 10:1, and most mercury contained in the red blood cells is in the form of methylmercury): Therefore, the methylmercury explosive can be evaluated by measuring total mercury in blood. It is believed that 50% of inorganic mercury is present in the plasma and the mercury concentration in the plasma increases in relation to the amount of inorganic mercury accumulated in the kidney (Suzuki *et al*, 2004). Thus, the exposure to inorganic mercury and mercury vapor can be evaluated by measuring the total mercury in plasma.

Usually blood methylmercury concentration reaches a maximum within 4 to 14 hours and undergoes clearance from the blood to other body tissues after 20 to 30 hours. WHO considers the normal mean concentration of total mercury in blood to be between 5 and 10 µg/l in individuals with no consumption of contaminated fish (UNIDO, 2003). The NRC identifies 2µg/l as the normal mean concentration for populations with little or no fish consumption in the US (NRC, 2000). Collection, storage, and shipping of blood samples can be resource intensive.

Also, blood samples are collected safely and properly (IPCS, 2000), and necessitates subject consent. The standard metric for mercury in blood is for whole blood (Stern, 2003).

A blood sample in the range of several milliliters is collected as usual from a vein into an injection tube already containing an anticoagulant (heparin) and transferred into a sealed container. The sample is then centrifuged at 3,000rpm for 10 minutes to separate the red blood cell from plasma. Sample to be stored for a long period of time should be frozen. While the mercury concentration in the blood of the general population in Japan does not exceed 40ng/g, people whose diet is rich in fish sometimes have higher value (Suzuki *et al*, 2004)

Blood mercury concentrations can be determined by a variety of analytical techniques. Often blood samples are digested with high purity mineral acids and oxidants prior to instrumental analysis. Sample preparation and digestion procedures play an important role in blood sample analysis as the sample matrix can interfere with analysis and lead to inaccurate result. Cold vapor atomic absorption spectrometry (CVAAS) is widely used for determination of mercury in blood (Oskarsson *et al*, 1996). CVAAS has adequate sensitivity to measure total mercury in blood at low parts-per-billion (ppb) levels, and the method is relatively easy to perform in a standard laboratory.

Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) with or without cold vapor generation can also be used for measurement of mercury in blood at low ppb levels (Buneaux *et al*, 1992).

Cold vapor atomic fluorescence spectrometry (CVAFS) is one of the most sensitive methods available with detection limits ranging down to sub parts-per-trillion levels (ppt) (Pellizzari *et al*, 1999). The method is also very specific and less prone to matrix-related interferences. The increased sensitivity of the method can be highly advantageous in situations where sample amount is very limited.

URINE

Most mercury present in the urine is in the form of inorganic mercury. The mercury concentration in the urine increases in relation to the level of inorganic mercury accumulated in the kidneys. Accordingly, the total mercury value in the urine is an important biomarker for evaluating inorganic mercury and mercury vapor exposure (Clarkson *et al*, 1988). On the other hand, leaking of methyl mercury into urine may occur in those with renal disease. Since the concentration of waste products in urine can vary significantly due to amount of dilution with water, tests for contaminants in urine are often expressed in units of µg contaminant per gram creatinine. Creatinine is a breakdown product of creatine, which is an important part of the muscle. Over time, the creatine molecule gradually degrades to creatinine. Creatinine is a waste product, that is, it cannot be used by cells for any constructive purpose. The daily production of creatine and subsequently creatinine, depends on muscle mass, which fluctuates little in most normal people over long period of time. Creatinine is excreted from the body entirely by the kidneys. With normal kidney function, the serum (blood) creatinine level should remain relatively constant and in the normal range. Therefore, measuring µg mercury per gram creatinine is a useful measure of mercury levels in urine.

The presence of mercury in urine generally represents exposure to inorganic mercury and considered to be the best measure of recent exposures to inorganic mercury or elemental mercury vapors because urinary mercury is thought to indicate most closely the mercury levels present in the kidneys (Clarkson *et al*, 1988). The preference is to collect the 50 – 100 ml urine sample in the morning (first –morning void). This can be easily achieved by providing each participant with an instruction sheet describing how to collect and store the sample until pick-up.

However, spontaneous urine collection does not dramatically affect the result. In general, new sterile plastic containers (100 or 150 ml size) are used for collection of samples, and the

containers are kept closed until ready for analysis. It is important that participants wash their hands before collection, open the container just before collection and close it immediately after collection, avoid touching inside the container or cap. The sample must be placed inside a secondary container such as a sealable bag to avoid potential contamination. Steps must be taken to ensure that microorganisms do not proliferate, as they may cause inorganic mercury to reduce to mercury vapor, which will be lost.

The sample should be frozen shortly after collection and kept frozen during transportation to the laboratory (Pellizzar*et al*, 1995). Acidifying the urine sample has been suggested as a means of stabilization prior to storage in a frozen condition.

Drinking large amounts of water a few hours before sample collection should be avoided because this dilutes the urine samples. Once the samples are received at the laboratory, an aliquot should be transferred into a clean, sealed glass container as a precaution to avoid any potential vapor phase contamination of mercury (UNIDO, 2003). Since urine contains many organic salts, even fresh urine may generate precipitate. Thus, the sample must be homogenized by shaking before analysis. A method also exists where the solubility of the salt is increased by lowering the pH of the urine sample by adding a small amount of hydrochloric acid. However, as previously explained, inorganic mercury accumulates in the kidney and is slowly excreted through the urine. Therefore urine mercury levels can also represent exposures to elemental mercury and inorganic mercury that occurred sometime in the past.

A strong correlation between elemental mercury levels in inhaled air and urine levels at medium and high concentrations has been reported. The maximum urine mercury concentration set by WHO (1991) is 50 µg/g creatinine. Mercury urine levels rarely exceed 5µg/g creatinine in persons who are not occupationally exposed to mercury (UNIDO, 2003).

It is believed that the average mercury level in the urine of the general population in a region without any particular mercury exposure is less then 10 ng/m1.

Mercury levels exceeding 20µg/L urine have been found in urine sample from miners who frequently burn gold-mercury amalgams in open pans. Very high mercury concentrations in urine (as high as 1, 168 µg/l) were reported in workers of gold shops in Amazonian villages (Malm, 1991). The gold shop workers (who work in confined environments) had higher concentrations of mercury in urine than miners burning amalgam outdoors. In Alta Floresta, Mato Grosso, Brazil, the urine of employees in gold shops (where gold was melted in fume hoods with no filters) was analyzed; the results showed mercury urine levels greater than 20 µg/L for at least 13 of 17 workers samples (Veiga and Baker, 2004)

A variety of analytical techniques are available for urinary mercury analysis. Usually samples are prepared by treating with mineral acids and oxidants or just diluted with an appropriate solvent prior to analysis. CVAAS is often used for determination of mercury in urine. CVAAS has adequate sensitivity to measure mercury in urine at low ppb levels, and the method is relatively low-cost.

2.8 DETERMINATION OF TOTAL MERCURY BYTHE WET DIGESTION/REDUCTION/COLD VAPOR ATOMIC ABSORPTION SPECTROMETRY (CVAAS) (CIRCULATION-OPEN AIR FLOW SYSTEM).

The present method involving reduction and cold vapor atomic absorption spectrometry (CVAAS) (circulation –open air flow system) is in principle, similar to the conventional circulation system in which the method includes the following: reduction of Hg^{2+} ions in the sample test solution with stannous chloride to generate elemental mercury vapor (Hg^{o}), and the introduction of mercury vapor into the photo-absorption cell for the measurement of absorbance at 253.7 nm.

However, unlike the conventional closed system in which the elemental mercury vapor generated is continuously circulated with a diaphragm pump through a reaction vessel, a U-shaped tube packed with a drying agent, and the photo-sorption cell, the present method uses a circulation-open air flow system. The apparatus constitutes a closed system and comprises a diaphragm pump, reaction vessel, acid gas trap, moisture trap (ice bath), and a 4-way cock. During its operation, the elemental vapor generated by the addition of stannous chloride is circulated via the 4-way cock at a flow rate of 1-1.5 L/min for 30 seconds to homogenize the concentration in the gas phase.

The 4-way cock is then rotated by 90^{o} to introduce the gas phase into the photo-absorption cell all at once. The measurement is completed within one minute per sample with this apparatus, which can measure even 0.1ng in the sample test solution using the present method, the conventional wet digestion method is improved by the use of a 50-ml flask with a long neck (at least 10cm), such as a thick-walled volumetric flask with a ground glass stopper, as well as a mixed acid system with an increased level of sulfuric acid, HNO_2-$HClO_4$-H_2SO_4 (1 + 1 + 5), that already contains perchloric acid, for the sample digestion. This is innovative in that the sample digestion can be completed in a relatively short time without loss of mercury. It is a simple method where the sample is subjected to wet digestion on a hot plate at 200-230^{o}C for 30 minutes and cooled followed by topping up to a fixed volume with water. This method can be applied directly to the digestion of biological samples including hair, blood, and fish as well as various solid samples such as sediment and soil. A reflux condenser is not required during heating (Suzuki et al, 2004)

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 SAMPLING

A great deal of difficulty was encountered during the collection of the samples. This was due to traditional beliefs, superstition and other untoward religious practices among the various Artisanal Gold Mining (AGM) communities in the Asutifi district of Brong-Ahafo region of Ghana.

Fig. 3.1: Map of Brong-Ahafo Region showing the study area. The shaded area is Asutifi District.

The study area (Fig. 3.1) is part of the Asutifi district (population of 120,567 and a land area of 1,100 km^2) of Brong-Ahafo Region of Ghana where various AGM sites, such as, Mahame Nkwanta, Ataneata, Nkesem and Kanyasi are found. The communities in the Asutifi area where small scale mining activities take place were visited. Majority of the population were farmers. At the community, individual miners and non – miners from the community were briefed shortly about the need and the importance of the study. Those who agreed and volunteered had their samples taken. Ninety – four (94) participants, comprising sixty four

(64) miners from various AGM sites and thirty (30) non – miners from the community volunteered. Each participant was asked to fill a questionnaire after it was translated into the Twi language. The samples were transported to the laboratory at the Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana for analysis.

3.2 APPARATUS AND EQUIPMENT

All glass-ware used were soaked in detergent solution overnight; rinsed and soaked in 10% (v/v) HNO_3 overnight. Prior to use, laboratory glass ware and sample containers were thoroughly washed with 0.5% $KMnO_4$ solution.

Automatic Mercury Analyzer model HG – 5000 (Sanso Seisakusho Co., Ltd, Japan), equipped with mercury lamp operated at a wavelength of 253.7nm and connected to a personal computer was used for mercury determination. Digestion apparatus for mercury with thick walled long neck 50ml made of Pyrex and a Clifton hot plate capable of attaining a surface temperature of 250^{o} C.

3.3 REAGENTS

All reagents used were of analytical reagent grade. Mercury standard solution (1000 mg/L) was prepared by dissolving 0.0677g of $HgCl_2$ in the acid mixture HNO_3:H_2SO_4:$HClO_4$, (1:5:1) in a 50ml volumetric digestion flask with heating on a hot plate at temperature of 200^{0}C for thirty (30) minutes. The clear solution was cooled and diluted to 50ml with distilled water. Blank solution was also prepared alongside. Stannous chloride solution (10% w/v) was prepared by dissolving 10g of the salt in 10ml of 1M HCl.

3.4 SAMPLING AND SAMPLE PREPARATION.

3.4.1 HAIR

About 20mg – 200mg of hair samples were collected from participant by cutting with a pair of clean stainless steel scissors. From each subject, a hair sample of approximately 2cm was taken near the root just above the neck (occipital area). During the collection of the hair sample each individual was asked to complete a questionnaire determine the sex, age, dietary habits, occupation, and work history. A total of thirty (30) hair samples were obtained from Artisanal gold miners and fourteen (14) from non - miners from the community. The hair samples were stored in sealed polyethylene bags and sent to Chemistry Department of the Kwame Nkrumah University of Science and Technology Laboratory. The hair samples were washed with neutral detergent (shampoo) and rinsed well with distilled water. The hair samples were also washed with acetone and air dried under reduced pressure. The samples were then cut into fine pieces in the vial using a pair of dissection scissors and stored in desiccators until analysis.

3.4.2 BLOOD

A qualified health worker was contracted to take the blood samples. Blood samples ranging from 4 to 5 milliliters were collected from the veins of six (6) Artisanal Gold Miners and five (5) non – miners in the community using a syringe and transferred into a seperate sealed container (EDTA coated veiled container) with the age, sex, dietary habit, rate and fish species consumed. To avoid degradation, all blood samples were stored in refrigeration at 4^{o}C until analyses.

3.4.2 URINE

About 50 to 100ml of urine samples were collected from 32 miners and 14 non-miners into plastic containers between 6 and 7am. This was achieved by providing each participant with an instruction sheet describing how to collect and store the sample until pick-up.

The sample was placed inside a container to avoid potential contamination. Steps were also taken to ensure that microorganisms do not proliferate, as they may cause inorganic mercury to reduce to mercury vapor, which would be lost. To avoid degradation, all urine samples were stored under refrigeration at 4^{o}c until analyses.

3.5 DIGESTION OF SAMPLES FOR THE DETERMINATION OF TOTAL MERCURY.

For total mercury determination, samples were digested by the highly sensitive and reliable method that was recently developed by Voegborlo and Akagi (2007). In the procedure, 0.05g of hair or 1ml of whole blood or 1ml of urine was digested in a 50ml digestion tube using 1ml water, 2ml mixture of nitric acid and perchloric acid (1+1) and 5ml sulphuric acid added in turns; and heated at a temperature of 200^{0}C for thirty (30) minutes. The solutions in the tubes were allowed to cool to room temperature and made up to 50ml mark with distilled water (chart 3.1).

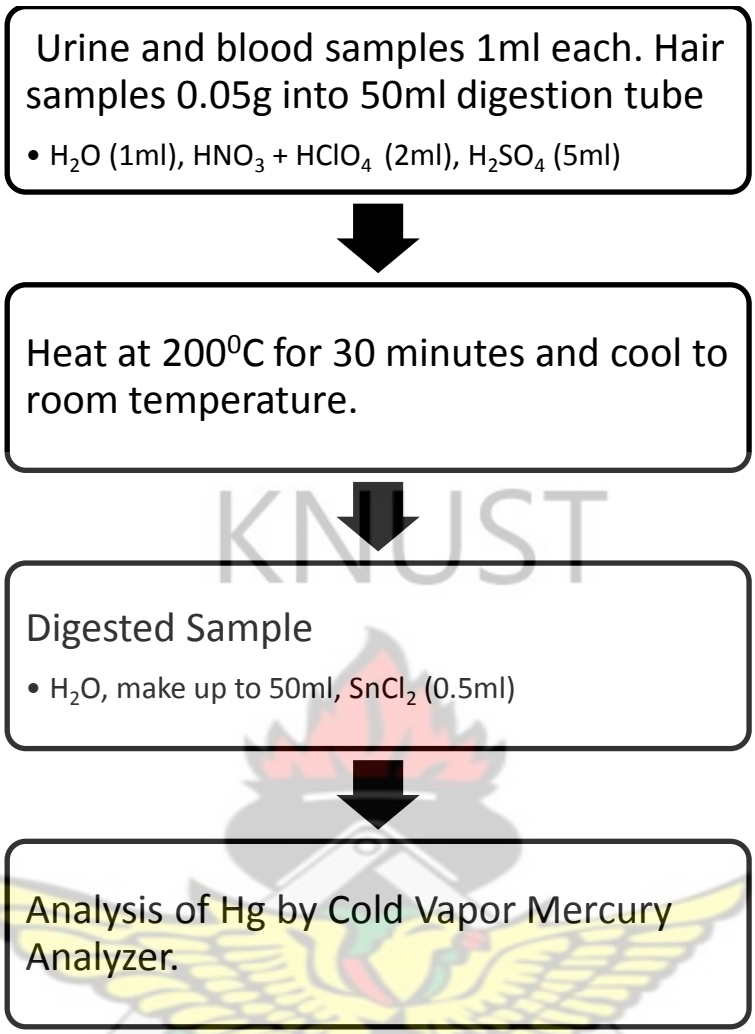

Chart 3.1: Flow chart of the procedure for the determination of total mercury in urine, blood and hair.

3.6 DETERMINATION OF MERCURY IN THE DIGESTS

Determination of mercury in all the digests were carried out by cold vapor atomic absorption spectrophotometry using an Automatic Mercury Analyser Model HG-500 (Sanso Seisakusho Co., Ltd, Japan) developed at National Institute for Minamata Disease (NIMD) in Japan. The analyzer consists of an air circulation pump, a reaction vessel, tin (II) chloride dispenser, an acidic gas trap and a four stop-cock with tygon tubes to which is attached a ball valve. The operations of the ball valve and the air circulation pump are controlled by a microprocessor. During the determination, a known volume of the sample solution normally 5ml is introduced into the reaction vessel using a micropipette (1-5ml). The reaction vessel was stoppered

tightly and 0.5ml of 10% (w/v) tin (II) chloride in 1M HCl was added from a dispenser for the reduction reaction. During this time, air is circulated through the four-way stop-cock to allow the mercury vapor to come to equilibrium and the acidic gases produced by the reaction also swept into the sodium hydroxide solution. After 30sec the four-way stop-cock is rotated through 90^{o} and the mercury vapor is swept into the absorption cell (fig 3.1)

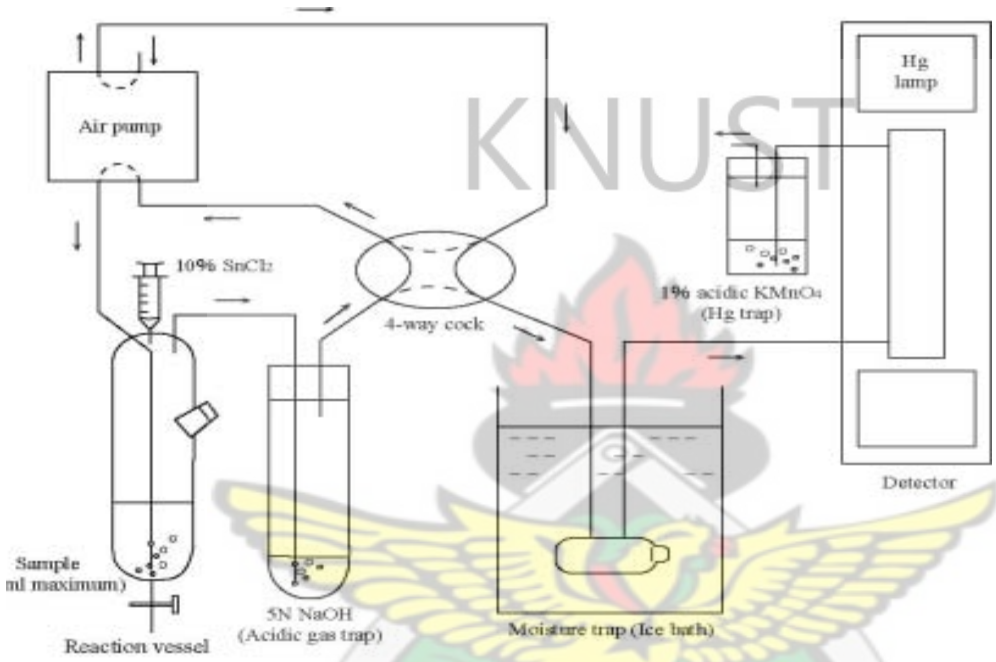

Fig 3.2: Schematic Diagram of Cold Vapor Atomic Absorption Spectrometry (CVAAS)

3.7 Quality Assurance

Quality assurance samples analyzed included digestion blanks, replicate samples, pre-digestion spike and post-digestion spikes. The percentage recovery was also carried out. Hair and urine samples were spiked with various concentrations of mercury for recovery and repeatability test and for verifying the analytical methodology. For each run, triplicate sample, spiked samples and blanks were carried through the same digestion procedure. They were then analyzed for THg. Statistical analysis of data was carried out using ANOVA.

3.8 Determination of creatinine in urine.

The level of mercury in urine is affected by dilution or concentration of the urine, as may occur with a high or low fluid intake respectively. To minimize this effect, mercury concentrations should be corrected for creatinine content of the urine and expressed as ug/g creatinine. Exactly 1 ml of urine sample was pipetted into GMP Biochemical Analyzer which already contains certain reagents such as picric acid and alkaline buffer solution (sodium hydroxide buffered with sodium borate and sodium phosphate). The samples were analyzed according to the instrument manual. The results were obtained by converting from µmol to mg/dL then to g/L. This result is then used in conjunction with the total mercury obtained as follows:

$$\mu g\ mercury/g\ creatinine = \frac{\boldsymbol{Total\ mercury\ ug/L}}{\boldsymbol{Creatinine\ g/L}}$$

CHAPTER FOUR

4. RESULTS AND DISCUSSION

A total of 94 samples were analyzed for total mercury levels. Precision and accuracy of the analytical methods used were evaluated by repeated analysis of some samples. Quality assurance samples analyzed included digestion blanks, replicate samples, pre-digestion spike and post-digestion spikes. Hair and urine samples were spiked with various concentrations of mercury for recovery and for verifying the analytical methodology. For each run, triplicate sample, spiked samples and blanks were carried through the same digestion procedure. They were then analyzed for total mercury.

The percentage recovery of Hg added to the samples were greater than 90%. Results of the study on assessment of the concentrations of total Hg level in human blood, urine and hair of the miners are summarized in tables 4.1 and 4.2. The concentrations of total mercury in human blood, urine and hair of non – miners are also summarized in tables 4.3 and 4.4 respectively.

In this study, the occupationally exposed people (mining workers) had the highest mean levels of total mercury in blood, urine and hair. These clearly indicate substantial mercury contamination in the miners than the non – miners who were used as a control group.

Table 4.1: T-Hg levels in hair, urine and blood of Female miners

	T-Hg in Blood (µg/L)	T-Hg in urine (µg/L)	T-Hg in hair (µg/g)
Mean	0.35	4.70	4.27
Maximum	0.47	9.14	6.94
Minimum	0.22	1.03	1.60
Standard deviation	± 0.31	± 2.88	± 1.95

Table 4.2: T-Hg levels in hair, urine and blood of male miners

	T-Hg in Blood (µg/L)	T-Hg in urine (µg/L)	T-Hg in hair (µg/g)
Mean	0.46	6.67	7.05
Maximum	0.78	30.88	32.92
Minimum	0.16	0.82	1.58
Standard deviation	± 0.31	± 7.15	± 8.34

Table 4.3: T-Hg levels in hair, urine and blood of female non – miners

	T-Hg in Blood (µg/L)	T-Hg in urine (µg/L)	T-Hg in hair (µg/g)
Mean	0.19	2.30	3.62
Maximum	0.23	4.81	5.86
Minimum	0.15	0.83	0.92
Standard deviation	± 0.06	± 1.78	± 2.37

Table 4.4: T-Hg levels in hair, urine and blood of male non - miners

	T-Hg in Blood (µg/L)	T-Hg in urine (µg/L)	T-Hg in hair (µg/g)
Mean	0.40	4.23	4.42
Maximum	0.48	6.74	11.29
Minimum	0.32	1.12	0.88
Standard deviation	± 0.08	± 2.25	± 3.74

T-Hg in blood

Result for blood samples collected from the miners in the vicinity of the mining sites and from non – miners in the community show a total range (miners: 0.16 – 0.78µg/L and non – miners 0.15 – 0.48µg/L). The mean of total mercury in the blood samples is 0.42 ± 0.25µg/L for miners and 0.32 ± 0.13µg/L for non – miners. Comparing the result of this study to other results, high total mercury level of 96µg/L, was found in blood from people living in Dumasi in Ghana (Babut *et al*, 2003). Drasch *et al* (2001) reported result for blood samples taken from miners in Philippines with a range of 0.25 – 107.6 µg/L with 8.2 µg/L as the mean. Boese – O'Reilly *et al* (2004) also reported Hg in blood sample of miners in both Zimbabwe (0.2 – 100.8µg/L, mean = 12.55µg/L) and Indonesia (1.45 – 429µg/L, mean = 16.5µg/L).

The total mercury concentration in blood of the male miners were higher than male non – miners. Female miners also had their T-Hg higher than the female non – miners. An average total mercury level in blood of 0.46 µg/L and 0.35 µg/L for both male miners and non – miners and 0.19 µg/L and 0.40 µg/L were also the average for female miners and non – miners respectively.

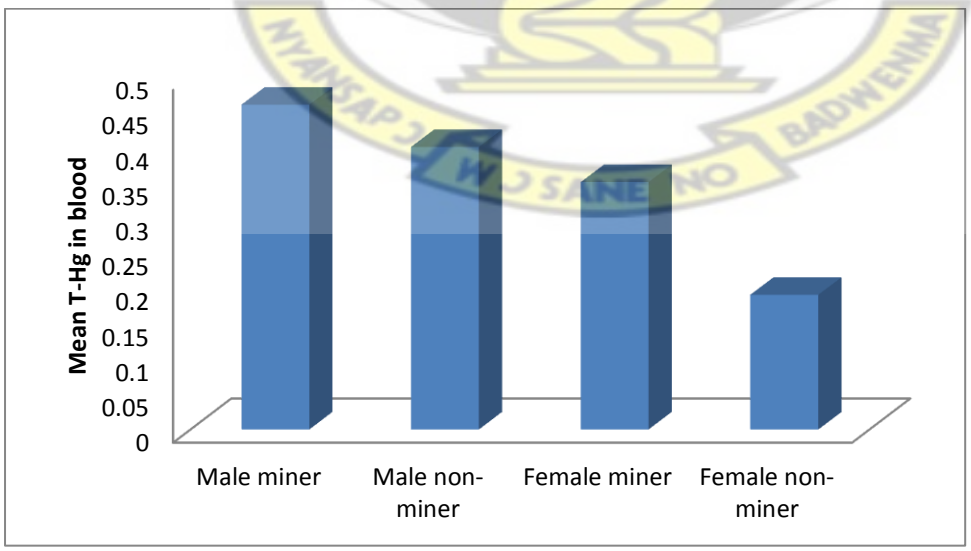

Fig 4.1: Mean total mercury in blood

From fig 4.1 the levels of T-Hg in samples from male miners were higher than those from female miners. Moreover the male non – miners recorded higher T-Hg levels than female non – miners. This may due to the more frequent consumption of fish by the males than females in both miners and non – miners as revealed by the interview administered questionnaire.

These clearly indicate substantial mercury contamination among female miners, male miners and male non-miners, because a person with no record of exposure to mercury would probably have a blood mercury level of 0 to 0.2 µg/L and level above 0.28 µg/L means the person is exposed (WHO/UNEP, 2008). From the results of table 4.3, it appears that the female non-miners are not exposed because they had mean total mercury of 0.19 µg/L.

The levels of total mercury in blood of miners and non-miners in these results may be low because, usually total mercury concentration in blood reaching a range 4 to 14ug/L undergoes clearance from the blood to other tissues after 20 to 30 hours when the person is not frequently exposed (WHO/UNEP, 2008).

Mercury exposure in terms of blood sample of the Artisanal miners in the Asutifi district is low because the duration in terms of mining activities is relatively short. Blood mercury levels do not provide information regarding historical exposure. However variation in it can be used to document methylmercury short – term exposure and provide different information than hair (WHO / UNEP 2008). This result demonstrates that it is not all that useful to measure these levels for suspected cases of elemental or inorganic mercury poisoning because the short half-life of mercury in blood (Ibrahim *et al*, 2006). Levels of mercury in whole blood below 0.2µg/L would be considered to be related to a low occupational exposure and equivalent to a high non – occupational exposure (WHO/UNEP 2008). Two females (miner and non – miners) had their total mercury levels below what is described as low occupational exposure. The NRC also identifies 2 µg/L as the normal mean concentration for

population in the US (NRC, 2000). Miners and burners of amalgam, although exposed to mercury vapor showed lower concentration values in their blood samples.

Fig. 4.2 represents the age distribution among miners and non – miners in the community. The range, 20 – 30years shows the highest frequency, which is the average working age in the area. The male miners do the digging, washing of the mud that contains the mineral and burning of the amalgam. While the female miners do the carrying and washing of the mud that contains the mineral. The male non – miners are mostly farmers and the females are traders.

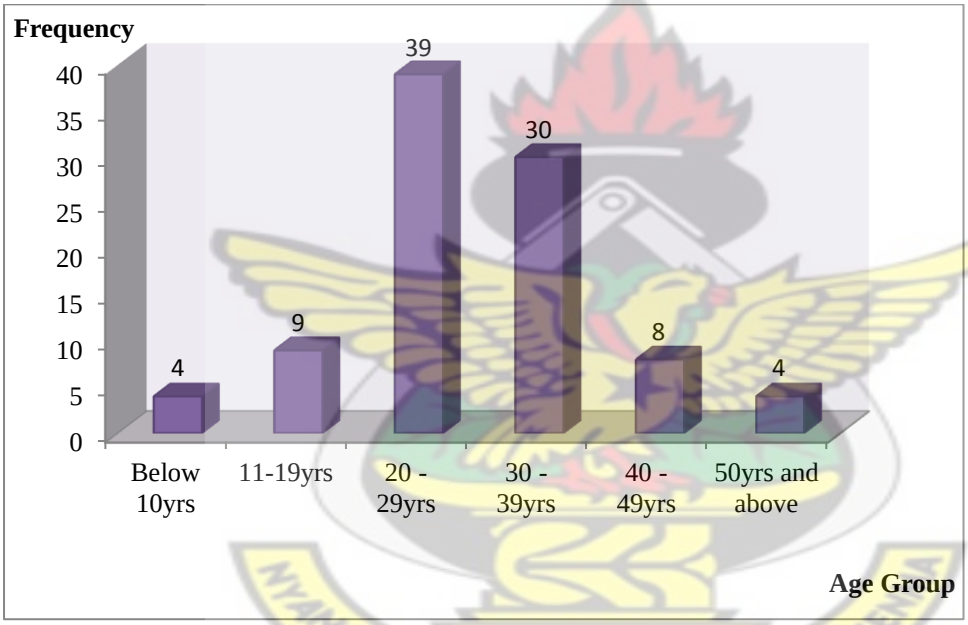

Fig. 4.2: Age distribution among miners and non – miners in the community.

T-Hg in urine

In the Asutifi mining communities, urinary mercury is considered to be the most valid bioindicator for exposure from inhalation of elemental vapor from the assessment. In this region, gold mercury amalgam burning has contributed to substantial mercury exposure, with urinary Hg values ranging from 0.82 to 30.88 μg/L for male miners, 1.03 to 9.14 μg/L for female miners, 0.83 to 4.81 μg/L for female non – miners, and 1.12 to 6.74 μg/L for male non

– miners. The results indicate that the women in both miners and non – miners category had low exposure than the male. (fig. 4.3)

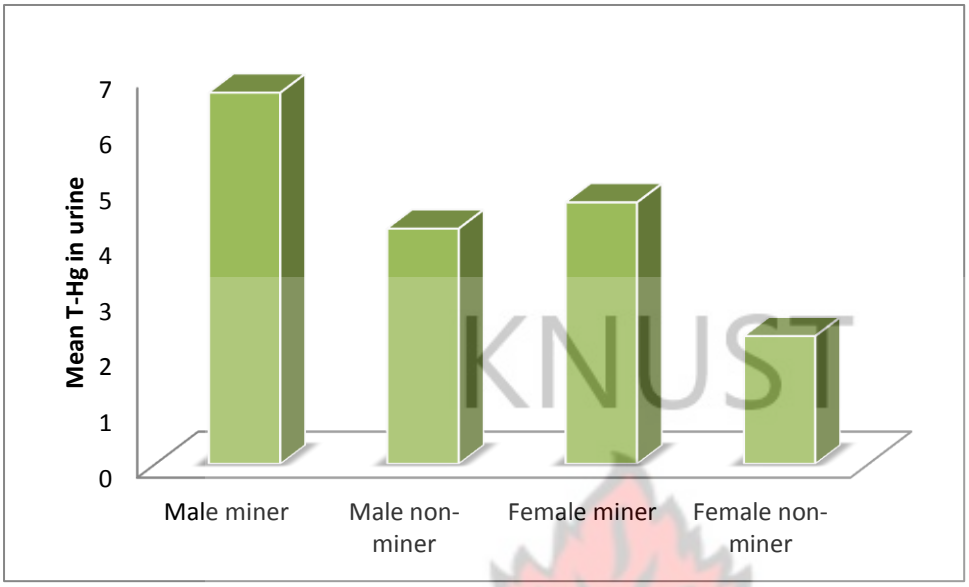

Fig 4.3: Mean total mercury in urine

Levels of mercury in urine ranging between 0.82 µg/L to 30.88mµg/L with mean of 6.62 µg/L is an indication that the person might here had an occupational exposure to mercury and therefore work practices have to may be modified to reduce exposure levels. Mercury levels in urine is the best indicator of recent inorganic (metallic) mercury exposure, especially from burning of mercury (WHO, 1991). In this study, a gold shop worker (who works in confined shop without a retort) had the highest concentrations of mercury (30.88 µg/L) in his urine. This was higher than levels detected for miners that burn amalgam outdoors. In this research the total mercury in urine in female miners were higher than male non-miners. Significant total mercury levels have been reported in urine samples from populations living in artisanal gold mining areas in Philippines (0.25 – 294 µg/L, mean =18.08 µg/L). Babut *et al* (2003) also reported total mercury level among miners in Dumasi in Ghana as ranging from 1.1 to 252 µg/L, with mean of 23.85 µg/L. The values obtained from these two areas were generally higher than values obtained in this study. Total mercury concentration in human urine obtained from miners in Dunkwa – on – offin, a small scale gold mining area in the south –

west of Ghana showed a mean value of 1.23 ± 0.86 µg/L and range of 0.32 – 3.62 µg/L (Kwaansa – Ansah *et al*, 2010). The results indicate that the total mercury levels were lower than the values obtained from miners in this study. The World Health Organization considers 4 ug/L of total mercury in urine to be normal (GMP, 2006). The average total mercury levels in the urine of miners in this study was above normal according to the guideline of the World Health Organisation and these values are also greater than the values obtained from non – miners from the community. These studies suggest that mercury exposure which was identified in the miners could result in mild and minor adverse effects.

CREATININE

In urine since the concentration of waste products in urine can vary significantly due to amount of dilution with water, test for contaminants in urine are often expressed in units of µg contaminate per gram creatinine (WHO, 2008). Creatinine concentration was therefore determined in the urine samples and the concentration of Hg expressed per gram of creatinine. The mean creatinine level in the urine samples was 1.38 g/L (4.57 ± 4.20 µg Hg/g creatinine) and a range of 0.89 to 2.40 g/L (0.54to 17.67 µg/g creatinine) for miners and mean of 1.28g/L (2.95 ± 1.76 µgHg/g creatinine) and range of 1.2 to 1.5 g/L (0.64 to 5.65µgHg/g creatinine) for non – miners. According to WHO, the normal concentration of creatinine is between 0.5 to 3.5 g/L. In all only two miners had their creatinine levels above 3.5 g/L. Artisanal gold miners in Asutifi are more exposed than non – miners as shown from the results of the study.

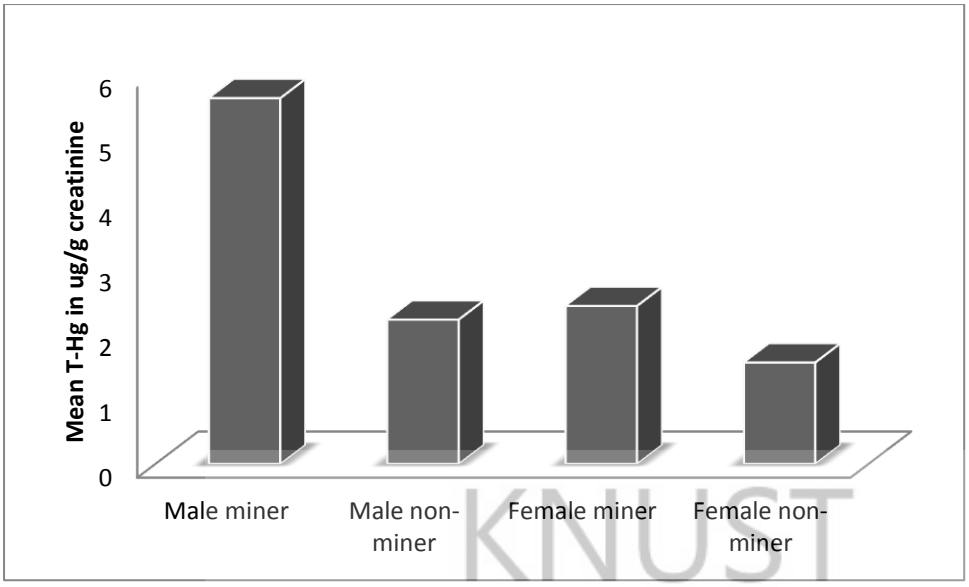

Fig. 4.4: Mean T-Hg per creatinine in urine

There is evidence of mercury exposure among the Asutifi population even though mercury concentrations in these biological samples do not reach levels where exposure symptoms usually appear. Akagi and Naganuma (2000) found mercury concentration in urine from two separate areas. In Alta Floresta (mean = 165.7 ± 96.5 µg/g creatinine, range = 20.8 – 449.5 µg/g creatinine) and Minamata Japan (mean = 22.5 ± 14.9 µg/g creatinine, range = 2.6 – 80.3 µg/g creatinine).

Evidence from these two studies show that miners and burners of amalgam, although exposed to mercury vapor show low concentration values in their urine. A concentration higher than 5 µgHg/g creatinine means risking health effects. At concentrations above 20 µgHg/g creatinine, there is definite cause for concern and probable risk of mercury intoxication (WHO/UNEP 2008). Classes of Hg concentration were selected to highlight the results. About 30.76% of the sampled individuals had mercury levels in urine above the alert level of 5 µg/g creatinine. Nobody had Hg levels above the action level of 20 µg/g creatinine and critical level of 199 µg/g creatinine which is the level where neurological symptoms should

be evident (WHO, 1991). Considering the non – miners only 7.69% had Hg in urine above the alert level and nobody had levels above the critical level.

High levels of total mercury of 17 µg/g creatinine were found in urine of a miner who burn amalgam in an enclosed area. While miners are more exposed than non-miners, people in Asutifi generally present moderate concentration of mercury in urine per creatinine levels. From this trend, it is worth to note that mercury concentration in urine did not exceed the maximum level of 50 µg/L as described by WHO, (2004)

T-Hg in hair

A total of 44 head hair samples were analyzed for total mercury levels. The average level of total mercury in the hair samples was 6.49µg/g for miners and 3.85 µg/g for non – miners, which were all below the World Health Organization (WHO) safety limit of l0 µg/g above which adverse effects on brain development are likely to occur (WHO, 1990). Total mercury concentration of 32.92 µg/g was found in the sample of hair from a gold miner who is a commercial amalgam burner with ten years exposure and eats a lot of fish. Hair mercury is generally considered to be a good indicator of mercury exposure through fish consumption.

Some mercury levels have been reported in some individuals living in artisanal gold mining areas in Ghana. For example, Voegborlo *et al* (2009) reported hair total mercury levels for some Ghanaian individual with range of 0.119 to 4.140 µg/g, with a mean of 0.843µg/g. Kwaansa – Ansah *et al* (2010) also reported total mercury in hair among miners in Dunkwa – On – Offin, a small scale gold mining area in the south – west of Ghana showing range of 0.32 – 3.62 µg/g, with mean value of 1.23 µg/g.Adimado and Baah (2002) also reported hair total mercury levels for artisanal gold miners in the Ankobra (0.15-5.86 µg/g, mean, 2.65 µg/g) and Tano basins (0.06 - 28.3 µg/g, mean, 3.45 µg/g). All these values were lower than the values obtained in this study.

Considering hair samples analyzed in this study, only 13.63% had mercury levels over 10 µg/g, which the World Health Organisation (WHO) considers as safety limit, above which adverse effects on brain development are likely to occur. Recent evaluation considers 5 ug/g Hg in hair as safety guideline for pregnant women (Yagev, 2002), and 6µg/g as the limit of biological tolerance (LBT) for a general population (WHO, 1990). From the analysis, 50% of females had their hair samples showing values above the safety guideline for pregnant women. However, none of the women were pregnant. The normal level of mercury in hair is, 1 – 2 µg/g. The USEPA reference dose corresponds to approximately 1µg/g mercury in hair for people who have low fish consumption. Results from the samples analysed and the responses to questionnaire administered testify that the miners are really exposed to mercury. Some of the male miners and non – miners have high consumption of fish. Methylmercury usually constitutes at least 80% of the total mercury analyzed in hair among fish consumers (McDowell *et al*, 2004). Total mercury levels in hair were generally higher in both miners and non – miners who consume extensive amount of fish as compared to Akagi and Naganuma (2000) who reported 0.9 – 3.1µg/g, with mean, 1.7 µg/g for non-regular fish – eaters and 1.5 – 13.0 µg/g, mean, 5.4 µg/g for frequent fish – eaters. In all the hair samples, only nine (9) subjects yielded values equal to or above the WHO/FAO hair mercury reference dose of 7 µg/g and only five (5) exceeded the 10µg/g value proposed by Barbosa *et al* (1995). The levels of total mercury in hair from males were about two (2) times higher than those in females.

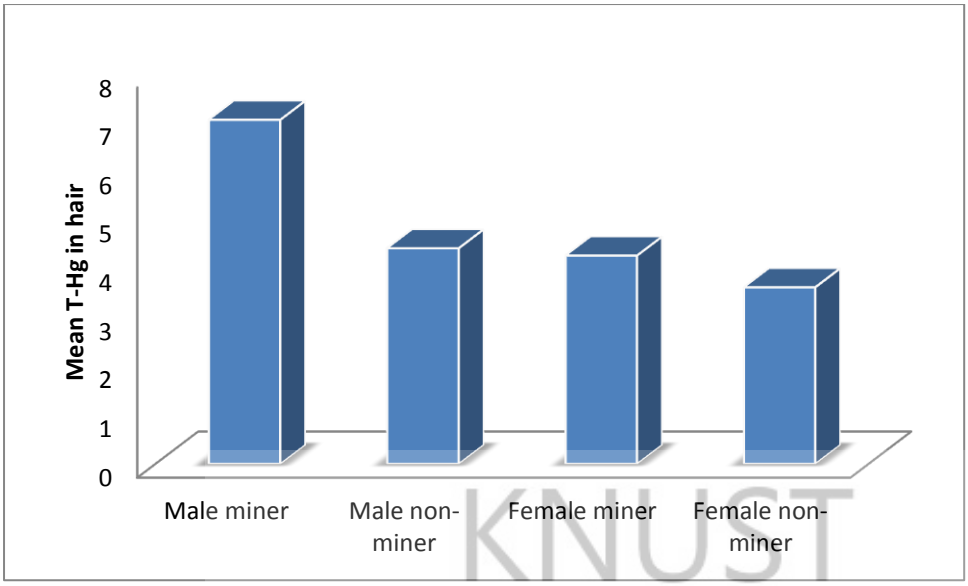

Fig. 4.5: Mean T-Hg in hair

From fig 4.5, the mean T-Hg in the hair of male miners was higher than that of the male non – miners. For the female counterparts, the miners had their T-Hg higher than the non – miners. But the T-Hg in the hair of male non – miners and female miners are almost the same. Female non – miners recorded the lowest T-Hg value.

From fig 4.6 and 4.7, the occupationally exposed people (mining workers) had the higher mean levels of total mercury in blood, urine and hair than the non-exposed people. This clearly indicates substantial mercury contamination in the miners than the non – miners who were used as a control group.

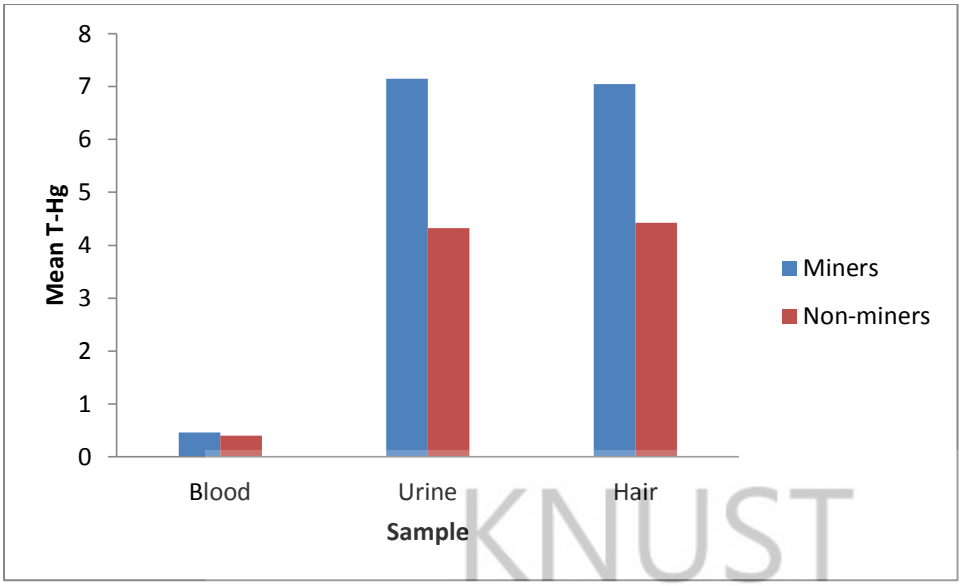

Fig 4.6: Average T-Hg in biological samples from male miners and non – miners.

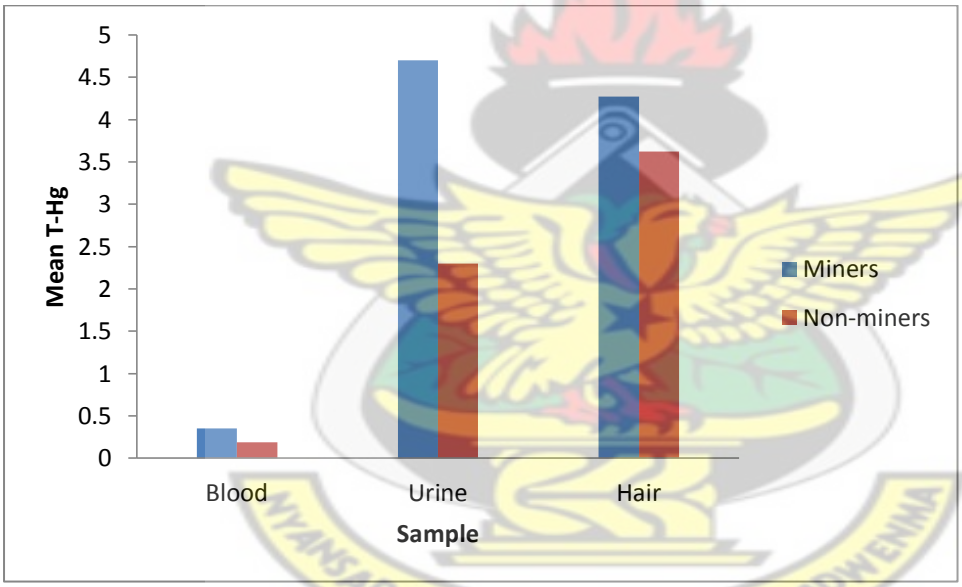

Fig 4.7: Average T-Hg in biological samples from female miners and non – miners

From WHO (1991) situation line up states that total mercury levels in hair less than 10µg/g means the person is unexposed, 10 – 50 µg/g means quarterly exam needed and greater than 50 µg/g means the victim must be removed from mercury source. For 100 – 500 µg/g,

clinical symptoms are likely to appear and for greater than 500µg/g, clinical symptoms are visible.

The results of this study were subjected to regression analysis to determine any relationships. The miners had a significantly positive linear correlation between urine T-Hg and blood T-Hg (R^2 = 0.921) as shown in fig 4.8. Levels of total mercury in blood in miners show low correlation with those in hair (R^2=0.142) as shown in fig 4.9. From figs 4.10 and 4.11 there is a strong positive linear correlation between T-Hg in urine and T-Hg per creatinine in urine with (R^2=0.971) and (R^2=0.941) for T-Hg in blood and T-Hg per creatinine in urine respectivily. These results were similar to what were reported by Ohno *et al*, (2007) and Kwaansa – Ansah *et al*, (2010). There were similar results for non – miners.

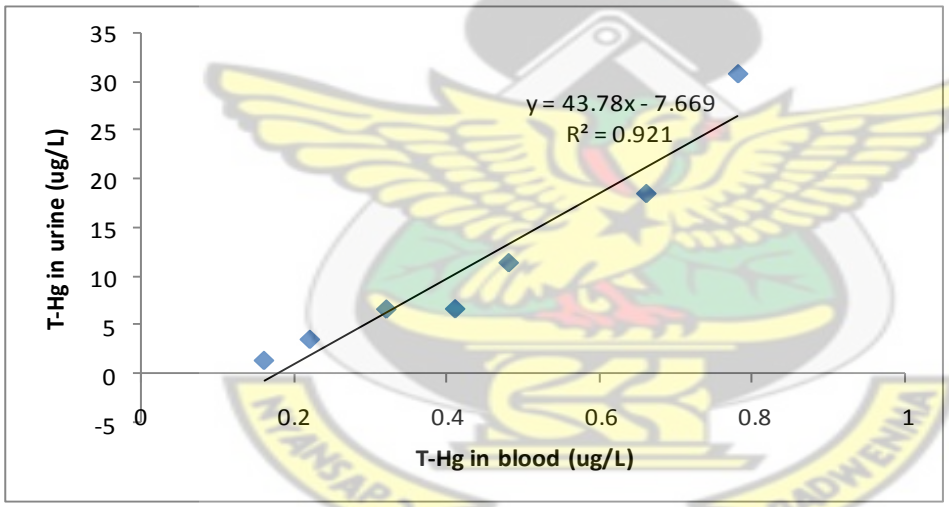

Fig 4.8: Plot of T-Hg in blood against T-Hg in urine

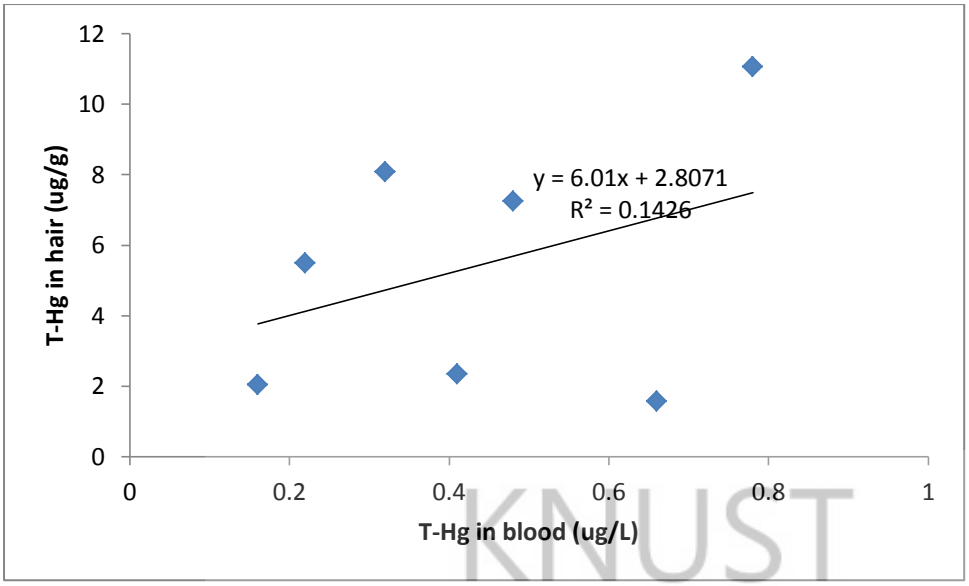

Fig 4.9: Plot of T-Hg in blood against T-Hg in hair

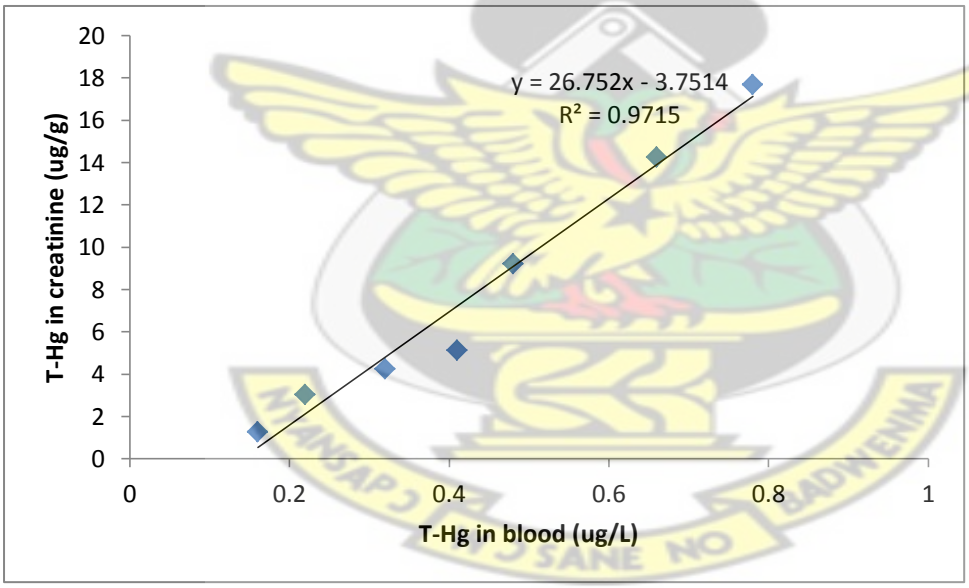

Fig 4.10: Plot of T-Hg in blood against T-Hg per creatinine in urine

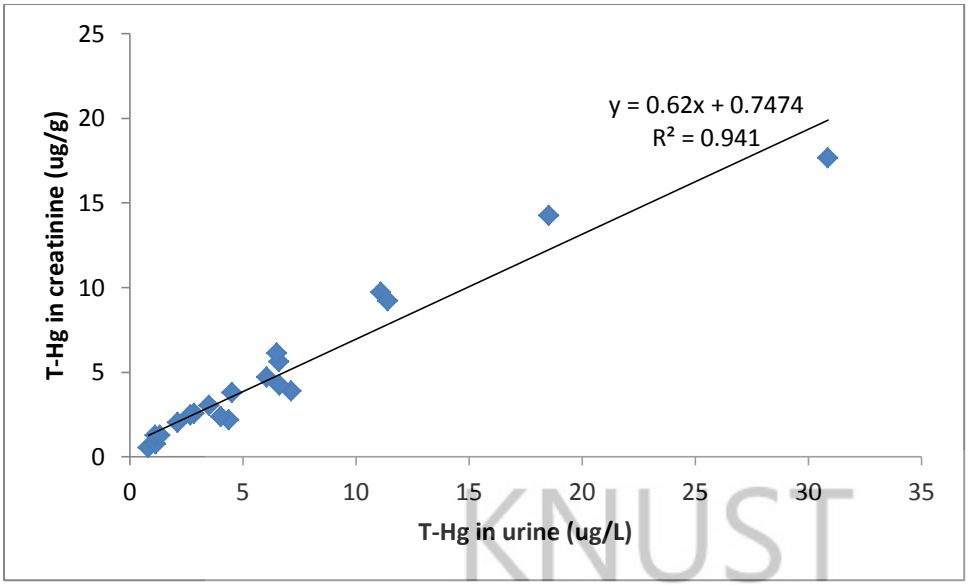

Fig 4.11: Plot of T-Hg per creatinine against T-Hg in urine

Factors such as age, place of residence, alcohol consumption, smoking, and medicines usually used were considered in the interview administered questionnaire. None of the factors were found to influence mercury concentration in the hair, urine and blood of the individuals according to the statistical analysis using SPSS statistical package. This could be attributed to the scattered nature of the sample population being individuals with varied background, resident in different mining sites and villages. Miners are exposed to mercury vapor at considerable rates, depending on the frequency of burning of amalgam in their operations.

Almost all mercury exposure in the miners in Asutifi was derived from gold mining activities and fish eating habits. The percentage of total mercury in the samples of the miners is obviously higher than in the samples of the non - miners living in the community. This means that most of the higher mercury burden in the control group was derived from nutritional methylmercury. In this region, gold mercury amalgam burning has contributed to substantial mercury exposure.

In conclusion, it is difficult to gather accurate data from mercury contamination at gold mining sites in Asutifi, but some of the data obtained in this study show that there is indeed a wider spread and substantial contamination of mercury in the mining environment. There is also a strong evidence of mercury exposure among Asutifi miners through mercury concentrations in biological samples.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

According to the results of this study, the hair total mercury concentrations obtained from the non – miners and the miners cannot be considered background not high enough for high mercury exposures. The levels do not appear to pose a significant health threat to the people. This is because the mean T-Hg for miners and non-miners (6.49 µg/g and 3.85 µg/g) were below the WHO safety limit of 10 µg/g which cause health effects.

The analysis of blood also shows mercury exposure for male miners, female miners and male non-miners. Female non-miners with T-Hg in blood of 0.19 µg/L indicate no exposure because UNEP/WHO indicate a person with no exposure to mercury would have a blood mercury level of 0.2 µg/L and a level above 0.28µg/L means the person is exposed.

The World Health Organization considers 4 µg/L of total mercury in urine to be normal. The average total mercury levels in the urine of male miners (6.67 µg/L), female miners (4.70 µg/L) and male non-miners (4.23 µg/L) in this study were above normal according to the guideline (4 µg/L) of the WHO.

The results also indicated higher levels of exposure to total mercury in Artisanal Gold miners than the non – miners who are mainly farmers and traders.

5.2 RECOMMENDATION

Since this is the first study to estimate exposure of mercury among miners and non – miners living in Asutifi, there should be implementation of an interdisciplinary strategy to educate workers and residents to reduce and avoid mercury vapor exposure. This is because miners in Asutifi do not believe in mercury pollution and keep burning amalgams in pans and shovels which in many cases is conducted in a closed environment.

The use of retorts, provision of adequate ventilation and efficient exhaustion systems to avoid mercury vapor pollution is strongly recommended.

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APPENDICES

APPENDIX A

Concentrations of total mercury in blood, urine, creatinine and hair of Artisanal Gold Miners in Asutifi District of Bonog-Ahafo region of Ghana. (N=64)

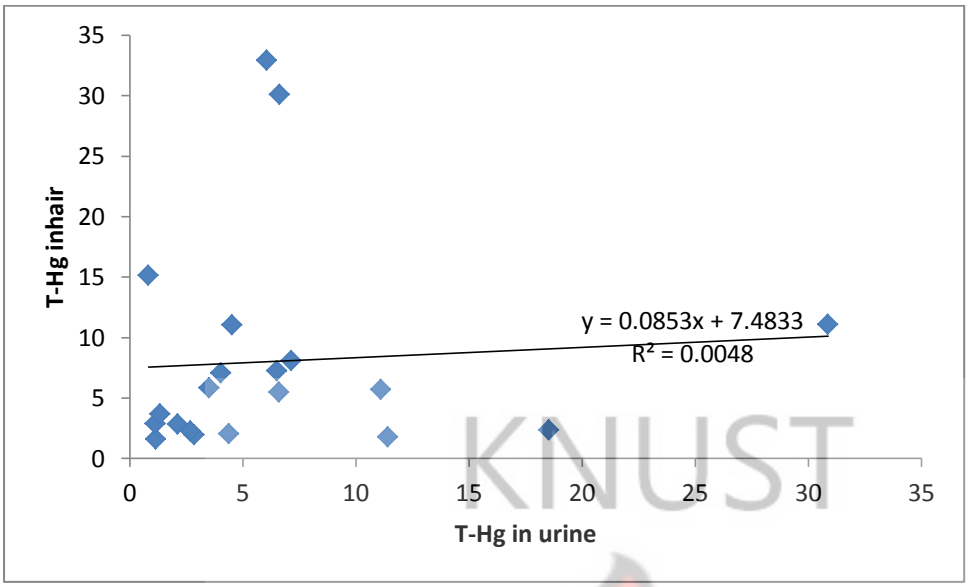
BLOOD (µg/L)	URINE (µg/L)	CREATININE (µg/g)	HAIR (µg/g)
Male	Male	Male	2.04 (Male)
0.66	4.38	2.19	5.50
0.16	6.59	5.62	8.08
0.78	7.15	3.89	11.08
0.22	30.88	17.67	15.16
	0.82	0.54	1.58
Female	1.16	0.75	7.06
0.22	4.04	2.37	2.86
0.47	1.12	1.26	2.35
	18.54	14.26	5.70
	11.10	9.71	3.69
	1.34	1.27	11.06
	4.53	3.78	5.83
	3.51	3.02	1.76
	11.41	9.21	1.93
	2.85	2.56	7.25
	6.51	6.13	2.83
	2.11	2.02	30.10
	6.63	4.24	32.92
	6.05	4.70	2.25
	2.68	2.46	1.58
	Female	Female	2.91
	6.85	6.13	2.83
	3.42	1.42	2.45
	9.14	6.85	3.90
	3.24	2.15	5.50 (Female)
	1.03	0.85	1.60
	4.52	3.05	2.74
			6.94, 5.12, 3.47

APPENDIX B

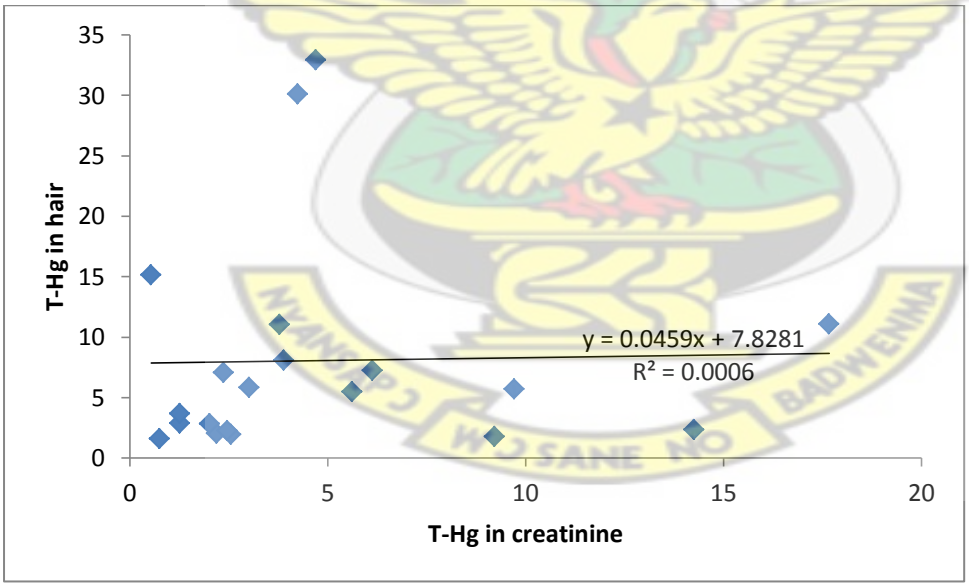
Concentrations of total mercury in blood, urine, creatinine and hair of Non-Miners in a community in Asutifi District of Bonog-Ahafo region of Ghana. (N=30)

BLOOD (µg/L)	URINE (µg/L)	CREATININE (µg/g)	HAIR (µg/g)
Male	Male	Male	11.29 (Male)
0.48	6.66	5.13	6.43
0.32	1.92	1.28	1.76
0.41	6.74	5.67	2.82
	3.62	2.88	2.32
Female	3.71	2.91	1.79
0.23	5.83	4.99	8.10
0.15	1.12	1.00	0.88
	Female	Female	3.56
	3.47	2.68	4.12
	0.83	0.65	5.34 (Female)
	4.81	3.81	0.92
	1.68	1.48	5.86
			2.34

APPENDIX C



Plot of T-Hg in hair against T-Hg in urine



Plot of T-Hg in creatinine against T-Hg in hair

APPENDIX D

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF CHEMISTRY

QUESTIONNAIRE FOR ARTISANAL GOLD MINERS AND NON-MINERS IN THE ASUTIFI DISTRICT OF BRONG AHAFO

(1) Questionnaire No……

(2) Village Name……..

(3) **Name…**……

(4) Date of Birth…….Age……. (years)

(5) **Telephone number**……...

(6) **Occupation…**…… (Miner/Non-miners)

(7) **Have you ever worked in the……. Area?** Yes/No

If yes, for how many years……

(8) **Have you ever worked as a miner with direct contact with mercury?** Yes/No

If yes, from when to when……..

(9) **Have you ever stored mercury containers or flask and where?** Never/At work/At home

(10) **For how many years have you been working with mercury**?

…….year(s)

Not applicable (have not worked directly with mercury)

DIET ISSUES

11. How frequently do you eat fish?

Never

At least once a month

At least once a week

At least once a day

……grams

12. Do you know where the fish come from?

Don't know the origin of the fish (buy from the market)

From areas distance from mining

From areas impacted by mining

13. Do you smoke

Never

Rarely (0 – 10 cigarettes per day)

Medium (10 – 20 cigarettes per day)

Lot (more then 20 cigarettes per day)

14. **Do you drink alcohol**?

Never

At least once a month

At least once a week

At least once a day

15. **Have you ever had neurological disorders**? Yes/No

Which diseases (problems)…….