ESTIMATION OF TOTAL ARSENIC IN MAIZE, FISH AND HUMAN URINE FROM SOME PARTS OF AMANSIE WEST DISTRICT IN THE ASHANTI REGION OF GHANA

By

NUWORDZRO, JOANITTA

A Thesis submitted to the Department of Chemistry, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi in partial fulfillment of the requirements for the award of degree MASTER OF PHILOSOPHY (Environmental Chemistry)

JUNE, 2013

DECLARATION

I hereby declare that this thesis is my own research work and that, to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any degree of the University, except where due acknowledgement has been made in the text.

…… ……

Joanitta Nuwordzro Date

(Student)

I declare that I supervised this student in undertaking this study; I hereby confirm that the student had my permission to present this thesis for assessment.

…… ……

Dr. Sylvestern K. Twumasi Date

(Supervisor)

…… ……

Dr. Ray B. Voegborlo Date

(Head of Department)

DEDICATION

This is dedicated to my best friend Isaac Annor who has supported me in every way in my academic pursuit and also to my mother Miss Monica Agbley and brother P. Y. Boateng who have been there for me.

ACKNOWLEDGEMENTS

Glory to God, the Father, the Son and the Holy Spirit. Great things He has done. I will always thank you Oh Lord for your grace and mercy.

My special thanks go to Dr. S.K Twumasi, my supervisor for his support, fatherly love and excellent supervision.

My sincerest appreciation goes to Mr. Nash Bentil of Ghana Atomic Energy Commission who led me in the use of the hydride generation atomic absorption spectrophotometerfor this work, Miss Ernestina Boapong of Keegan Resources Ghana Limited for her help during the sampling of fish along the river Offin and also Dr G. A. Koblah of St. Martins Catholic Hospital for his help in gathering information on Buruli ulcer from the district.

I would also like to thank Mr. Martin Owusu Asante, Mr. Andrews Agyemang Yirenkyi, Mr. Isaac Edzemadze, Miss Eunice Dzokpo and all those who in diverse ways helped me in carrying out this research work.

ABSTRACT

Amansie West District is a gold mining area in the Ashanti Region of Ghana and the method of mining used is surface mining. From the operation of mining activities (galamsey) arsenic is released into the atmosphere and water bodies in the environment. Consequently, the arsenic contaminates the water, soil, and foodstuffs like maize and fish leading to adverse effect on vegetation, animals and humans living in the area.

Levels of total arsenic concentration of maize, fish and human urine samples from the district were determined using Varian AA240FS (Fast Sequential AAS) Spectrophotometer and also some physicochemical properties of the samples were determined. The work was conducted between October 2011 and April 2012.

The mean total arsenic concentrations in maize ranged from 0.015 to 0.045 mg/kg dry weight. The fish from upstream had levels of arsenic ranging from 1.90 to 3.25 mg/kg dry weight. Mean total arsenic levels in fish from downstream samples ranged from 1.95 to 4.80 mg/kg and the mean total arsenic levels in fish samples from streams and ponds ranged from 1.70 to 3.95 mg/kg. The mean total arsenic in human urine samples ranged from 0.003 to 0.014 mg/l.

The pHs of maize, fish from upstream, fish from downstream and fish from streams and ponds ranged from 5.41 to 6.90, 5.34 to 6.87, 5.02 to 6.91 and 5.03 to 7.15respectively. The pH of the urine samples ranged from 5.54 to 8.20. Moisture content of fish from upstream, downstream and streams and ponds ranged from 49.20 to 66.75 %, 56.23 to 65.72 % and 49.43 to 65.98 % respectively Conductivity of the maize varied from 1508 to3371 μS/cm The conductivity of the urine samples ranged from 11691 to 37425 μS/cm. The ash content of maize varied from 15.87 to 20.73 % and for the fish from upstream, downstream and stream and ponds, their ash content

ranged from 9.98 to 16.27 %, 9.79 to 15.98 % and 10.68 to 15.92%. The arsenic levels in fish and maize are within the acceptable limits of consumption according to the WHO limit for foodstuffs of 10 mg/kg.

TABLE OF CONTENTS

DECLARATION.....ii
DEDICATION..... iii
ACKNOWLEDGEMENTS iv
ABSTRACT.....v
TABLE OF CONTENTS vii
LIST OF TABLES.....xi
LIST OF FIGURES.....xii
CHAPTER ONE 1
1. Introduction.....1
1.1 Background.....1
1.2 Problem Statement.....5
1.3 Objectives 6
1.3 Justification.....6
CHAPTER TWO 8
2. Literature Review.....8
2.1 Arsenic.....8
2.1.1 Sources and Occurrence of Arsenic in the Environment.....9
2.1.2 Levels of Arsenic in the Environment 10
2.1.3 Sources of Environmental Pollution of Arsenic 11
2.1.4 Arsenic Compounds in Foods.....12
2.1.4a Arsenic in Fish 13
2.1.5 Arsenic in Urine.....15
2.1.5a Composition of Urine.....15
2.1.5b Arsenic concentration in Urine 16
2.1.6 Chemistry and Analysis of Arsenic 17
2.1.6.1 Physical and Chemical Characteristics of Arsenic 17
2.1.6.2 Significant Chemical Behaviours of Arsenic.....26
2.1.6.2a Comparison of Arsenic to Phosphorus.....26
2.1.6.2b. Affinity of Arsenic for Sulphur.....27
2.1.6.2c Biomethylation of Arsenic 28
2.1.6.2d Geochemical Behaviours: Adsorption and Redox.....30
2.1.6.2e Microbial Activity and Arsenic Mobilization 31

2.1.6.2f Free Radical and Peroxy Species....31
2.1.7 Environmental Transport and Distribution....32
2.1.8 Biotransformation....35
2.1.9 Exposure Routes and Pathways....37
2.1.10 Metabolism and Disposition of Arsenic....40
2.1.10.1 Absorption....40
2.1.10.2 Transportation....41
2.1.10.3 Metabolic Transformation....43
2.1.10.4 Excretion....45
2.1.11 Mechanism of Toxicity....48
2.1.11.1 Oxidative Stress....49
2.1.12 Effects of Arsenic....50
2.1.12.1 Health Effects....50
2.1.12.2 Environmental Effects....53
2.1.13 Essentiality and Therapeutic Uses....54
2.1.13.1 Essentiality of Arsenic....54
2.1.13.2 Therapeutic Uses....55
2.1.14 Analysis of Arsenic Compounds....56
2.1.14.1 Hydride Generation Atomic Absorption Spectroscopy....56
2.1.14.1a Atomization....59
2.1.14.1b Atomization Mechanisms....60
2.1.14.1c Detection....60
2.1.14.1d Interferences....60
2.1.14.2 Quantitative Analysis by Atomic Absorption....61
2.2 Buruli Ulcer....64
2.3 Mining....65
CHAPTER THREE....67
3 Materials and Experimental Methods....67
3.1 Materials Used For the Analysis....67
3.1.1 Reagents....67
3.1.2 Glassware and Equipments....68
3.1.2.1 Cleaning of Glassware....68
3.2 Sampling Site, Sample Collection and Preparation....68
3.2.1 Sampling Site....69

3.2 .2 Sampling 72
3.2.2.1 Sampling of Maize 72
3.2.2.2 Sampling of Fish 72
3.2.2.3 Sampling of Human Urine 73
3.2.3 Sample Preparation 73
3.3 Preparation of reagents and Experimental Procedure for the Determination of Physicochemical Parameters and the Arsenic levels in the Samples 74
3.3.1 Reagents Preparation 74
3.3.2 pH Determinations on the Sampling Field 75
3.3.3 Determination of Conductivity of the Urine on the Field 76
3.4 Determination of Physicochemical Parameters 76
3.4.1 pH Determinations 76
3.4.1a Maize 76
3.4.1b Fish 77
3.4.2 Determination of Ash Content of Maize 77
3.4.3 Determination of the Moisture Content of Fish 77
3.5 Determination of Arsenic Concentration in Maize, Fish and Urine Samples 78
3.5.1 Digestion of Maize, Fish and Urine Samples 78
3.5.1a Fish and Maize 78
3.5.1bUrine 79
3.5.2 Determination of Levels of Arsenic Using Hydride Generation Atomic Absorption Spectroscopy 79
3.5.2a Instrumentation 80
3.6 Recovery 81
CHAPTER FOUR 82
4 Results and Discussions 82
4.1 Results obtained for the distilled water and blanks used for the Analysis 82
4.2 Maize 82
4.3 Fish, *Clarias agboyensis* 86
4.3.1 Fish from Upstream 86
4.3.2 Fish from the downstream 88
4.3.3 Fish from the Streams and Ponds 89
4.4 Human Urine 92
4.4.1 Physicochemical Analysis Results of Urine 92

4.4.2 Arsenic concentration in Urine 94
4.5 Recovery 99
CHAPTER FIVE 100
5 Summary, Conclusion and Recommendations 100
5.1 Summary of results 100
5.2 Conclusion 101
5.3 Recommendations 102
REFERENCES 103
APPENDICES 121

LIST OF TABLES

Table 2.1 Naturally occurring inorganic and organic As species 18

Table 2.2 Structures for R in Dimethylarsinoylribosides. 20

Table 2.3 Other As compounds of environmental significance referred to in the text 21

Table 3.1 Instrumental Parameters for the Atomic Absorption in the Varian AA240FS

(Fast Sequential AAS) Spectrophotometer for Arsenic……...80

Table 4.1 The pH, Conductivity, Ash Content and Arsenic Levels in Samples of Maize from Some parts of Amansie West District.....83

Table 4.2 Physicochemical Properties and Arsenic Concentration Levels in the *Clarias agboyensi* from upstream of River Offin around Adobewura.....86

Table.4.3 Physicochemical properties and Arsenic Levels in the fish *Clarias agboyensi* from downstream of River Offin.....88

Table 4.4 Physicochemical Properties and Arsenic Levels of the fish *Clarias Agboyensis* from Some Streams. 89

Table.4.5 Field Test Result for the Physicochemical Properties of Human Urine Samples from Subject Living in some parts of the Amansie West District. 92

Table.4.6 Arsenic Concentration in Human Urine of Subject living in some part of the district. 94

Table.4.7. Recovery of Arsenic concentration of the HGAAS.....98

LIST OF FIGURES

Fig.2.1 The Eh-pH diagram for arsenic at 25 °C (from Ferguson et al, 1972).....25

Fig 2.2 Methylation of Arsenic (Cullen et al , 1989)……...28

Fig. 2.3 Arsenic methylation in mammals…..……44

Fig. 3.1 Map of Ghana showing Amansie West District in the Ashanti Region…..…71

Fig. 3.2 Map of Amansie West District showing some of the study areas……..…73

CHAPTER ONE

1. Introduction

1.1 Background

For centuries, man has been thriving on the generosity of the environment for which he is an integral part but has been an arch enemy of it. In man's quest to make life very simple and more luxurious, he has turned a blind eye to the damage caused to the environment. Our greed to get the most out of everything has made us contemptuously to neglect the environment, although we all know that our very existence depends on it.

The discrimination of our world into geographical entities based on colour, creed and language has resulted into comforting implications but the problematic aspect is that the refined status has come with damaging the environment. Population growth and poverty account a lot for the damage of the environment as a result of underlying social and economic problems.

Technological progress facilitated by super efficiency of capitalist business practices that is division of labour, cheaper production costs, overproduction, and overconsumption and over pollution had probably become one of the main causes of the serious deterioration of our natural environment (resources).

The rates, scales, kinds, and combinations of changes occurring now are fundamentally different from those that occurred at any other time in history; the Earth is being changed more rapidly than we understand it. In a very real sense, the world is in our hands and how we handle it will determine its composition, dynamics, and our fate.

Mining makes a large portion of the Gross Domestic Product GDP and plays a significant role in the economic recovery programme of ansy country including ours Ghana. However, the gains are achieved at a great environmental cost as the exploitation of gold puts stress on water, soil, vegetation and poses human health hazards (Amonoo-Neizer et al, 1993).

The main prospects in Ghana occur at Obuasi, Tarkwa, Prestea, Bibiani, Bogoso and Kenyasi, with the gold occurring in close association with sulphide minerals, especially arsenopyrite (Smedley, 1996). For gold mining, mercury, arsenic and cyanide are normally the reported cases of contamination according to Amofah et al, 2002. Studies have revealed high levels of urinary arsenic comparable to other arsenic-endemic areas of the world in urine samples of inhabitants of Tarkwa (Asante *et al*., 2009), and some villages near Obuasi (Smedley *et al*., 1996) in Ghana, while arsenic contamination has been reported in groundwater in Obuasi and Bolgatanga (Smedley, 1996).

The Amansie West District is located in northwest of Kumasi in the Ashanti region of Ghana and was once a major gold mining area and still illegal mining is in progress. Since the cessation of mining in the area, the mines remain closed and abandoned. Wuana et al, 2011, did a study where heavy metals such as mercury, arsenic and others located both in the mines and in waste ore on the surface were found to be under low pH conditions and can be transported by ground and surface waters. Moreover the illegal mining exposes the environment to these heavy metals especially arsenic as a result of the oxidation of arsenic-bearing minerals, occurring naturally in mineral deposits (WHO, 2000).

Buruli ulcer (BU) is a skin disease, which usually begins as a painless nodule or papule and may ulcerate the skin afterwards. If BU is not treated, it may lead to extensive soft tissue destruction, with soreness extending to deep fascia and mostly, marginal parts of the body are affected. Later complications may include contracture deformities. The main form of treatment is wide expurgation surgery, including amputation of limbs, which requires prolonged hospitalization and is thus a significant burden on hospital resources and budgets.

In recent years, there has been increased incidence of BU in West Africa (including Benin, Burkina Faso, Cote d'Ivoire, Ghana, Guinea, Liberia and Togo), Mexico, French Guyana, Papua New Guinea and Australia. The disease seems to affect mostly impoverished inhabitants in remote and rural areas; children are the most vulnerable, accounting for about 70% of the cases (Asiedu *et al*, 2000). The World Health Organization (WHO) has recognized BU as the third most prevalent mycobacterial disease after tuberculosis and leprosy and has called for urgent action to control it (Asiedu *et al*, 2000).

Buruli ulcer is a skin disease caused by *Mycobacterium ulcerans*. *The disease has some connection* to arsenic ingestion and exposure (Gorby, 1994). The high lipid solubility of arsenic helps it to bioaccumulate in fatty tissues of the skin (Mahieu *et al*, 1981), and this may provide a favourable environment for *Mycobacterium ulcerans* in the skin because arsenic is known to help microorganisms grow (Ahmann *et al*, 1994).

Buruli ulcer is most prevalent in the Amansie West District which is an old mining area and still illegal mining is in progress. In the Amansie West District, the arsenic-

enriched surface environments results from the oxidation of arsenic-bearing minerals, occurring naturally in mineral deposits (WHO, 2000).

The mineral deposit in the district is composed of pyrite, arsenopyrite, minor chalcopyrite, sphalerite, galena, native gold and secondary hematite which are associated with arsenic and sulphur (Robb *et al*, 1999).

The extraction of gold basically involves:

1. Crushing and grinding of the ore: During crushing and grinding, particulate matter is emitted and scattered into the atmosphere which reduces visibility, some particles settle later on building and vegetation which may impact some chemical behaviors.
2. Separation and floatation: There is amalgation and treatment with copper II sulphate and there are tailings which contain impurities that are discharged into rivers and stagnant water
3. Smelting, drying, calcinations and roasting: Here, chemically associated water and carbonates are removed into the atmosphere in the form of water vapour and carbon dioxide (CO_2). The extract is then roasted to expel arsenic trioxide and sulphur dioxide gas into atmosphere together with H_2S .
4. Cyanidation: This process consists of percolation or agitation leaching of gold ores with dilute cyanide solution, generally less than 0.3 percent sodium cyanide.
5. Electrowinnig: There is electrodeposition (precipitation) of metals (gold) from their ores in cyanide solution or liquefied, zinc powder is added to cyanide solution which reduced the oxidized gold. The impurities in the tailings are discharged into rivers.

The above mining processes release airborne particles and large quantities of arsenic pollutants into the atmosphere and water bodies in the environment. These may however have adverse effect on vegetation, animals and finally on human being living in the area

A proximity analysis carried out showed that there is a spatial relationship between Buruli ulcer-affected areas and arsenic-enriched farmlands and arsenic-enriched drainage channels in the Amansie West District (Duker *et al*, 2004).

1.2 Problem Statement

The arsenic cycle at Amansie West district has broadened as a consequence of mining a major anthropogenic activity, due to this, large amounts of arsenic end up in the environment and in living organisms contributing to arsenic contamination of air, water and soil which is widespread in the district. Foodstuffs taken by the inhabitants have become exposed to arsenic.

Arsenic exposure occurs through inhalation, ingestion, dermal or eye contact. Chronic exposure to arsenic can lead to dermatitis, mild pigmentation keratosis of the skin, vasospasticity, gross pigmentation with hyperkeratinization of exposed areas. Buruli ulcer is a skin disease which is being hypothesized to have its causative agent to be link to arsenic.

In Ghana, there have been more than 2000 reported cases of Buruli ulcer in the last ten years; outbreaks have occurred in at least 90 of its 110 administrative districts. In one of the worst affected districts, Amansie West, where there are arsenic-enriched surface environments resulting from the oxidation of arsenic-bearing minerals, occurring naturally in mineral deposits (Duker *et al*, 2004). Buruli ulcer is also

prevalent in the Amansie West District which may be caused by arsenic poisoning. This in a way suggests the fact that Arsenic has a role in the manifestation of BU. The increase in arsenic pollution and prevalence of Buruli Ulcer showed that arsenic has a role in the manifestation of BU.

1.3 Objectives

- To determine the levels of total arsenic in fish, maize and human urine from selected communities in the Amansie West District of Ghana.
- To determine the relationship between arsenic levels in fish, maize and urine.
- To determine pH and conductivity of the urine samples and find the relationship between these properties and the arsenic levels
- To check whether arsenic levels in the fish and maize samples are at levels of potential human health concern.
- To administer questionnaires as part of determining the arsenic content in the urine samples of people living in the district

1.3 Justification

The economic hardships of Ghana have made it such that people are not able to afford foodstuffs but rather depend on farm produces which are less expensive in their area. The inhabitants in the villages depend on the produce of farmlands maize and fish from the rivers. Humans have become exposed to arsenic through food of maize and fish taken by the people in the Amansie West district. A study by Luo et al, 2008 showed that the presence of arsenic in the environment is such that it binds to soil particles and move within short distances when water percolates down the soil and

these allowed it to be transported into plants, fishes and bioaccumulate through the food chain.

The effects of arsenic on the environment and the inhabitants are enormous and of great concern to the people of the Amansie West District and the socioeconomic hub of Ghana. Duker *et al*, 2005 did some study in parts of Amansie West District which showed that Buruli ulcer is common in settlements along arsenic-enriched drainage channels and farmlands.

The health effects of environmental arsenic is devastating, and over decades, can progress to various forms of cancer, so the knowledge of the amount of arsenic in fish from rivers streams and ponds in the area, maize grown in the community and urine of humans from the locality will inform us of the dangers humans in the area are exposed to.

CHAPTER TWO

2. Literature Review

This chapter deals with arsenic, its characteristics occurrence, biochemical processes and how it interacts with the environment, its toxicological effects and how it affects Buruli ulcer infections, how mining contributes to arsenic pollution as well as how arsenic is analyzed.

2.1 Arsenic

Arsenic (As) is a natural, ubiquitous element in the environment, cycling through land, water, air, and living organisms (ATSDR), 2005. The major anthropogenic sources of environmental pollution with arsenic are the burning of coal, industrial metal smelting, and more recently the semiconductor industry.

Arsenic has a colorful history of commercial and medicinal uses. Arsenic compounds have seen widespread use as pesticides and herbicides, wood preservatives, feed additives, medicines, constituents of organic and inorganic pigments, in a variety of electronic and industrial applications, and, of course, as a poison (Bissen et al, 2003).

Arsenic (As) is ranked as the first of hazardous element and has serious effects on plants, animals and human health (Baig et al.,2009, (IPCS), 2001; (ATSDR), 2005). According to epidemiological studies and clinical observations, arsenic toxicity is associated with increased risk of certain types of neurotoxin, carcinogenic and several other impacts arising from their consumption even at lower levels (Sathawara, *et al.*, 2004).

Arsenic is a toxic metalloid, whose trivalent arsenite, As(III), and pentavalent arsenate, As(V), ions can inhibit many biochemical processes. The solubility of As(III) oxide in water is fairly low, but high in either acid or alkali. In water, arsenic

is usually in the form of arsenate or arsenite. As (III) is systemically more poisonous than the As(V), and As(V) is reduced to the As(III) form without exerting any toxic effects (O'Neil, 2001; WHO, 2001).

2.1.1 Sources and Occurrence of Arsenic in the Environment

Arsenic is present in more than 200 mineral species, the most common of which is arsenopyrite. It has been estimated that about one-third of the atmospheric flux of arsenic is of natural origin. Volcanic action is the most important natural source of arsenic, followed by low-temperature volatilization.

Inorganic arsenic of geological origin is found in groundwater used as drinking-water in several parts of the world, for example Bangladesh.

Organic arsenic compounds such as arsenobetaine, arsenocholine, tetramethylarsonium salts, arsenosugars and arsenic-containing lipids are mainly found in marine organisms which are at higher levels although some of these compounds have also been found in terrestrial and freshwater species (WHO, 2001).

Elemental arsenic is produced by reduction of arsenic trioxide (As_2O_3) with charcoal. As_2O_3 is produced as a by-product of metal smelting operations. It has been estimated that 70% of the world arsenic production is used in timber treatment as copper chrome arsenate (CCA), 22% in agricultural chemicals, and the remainder in glass, pharmaceuticals and non-ferrous alloys.

Mining, smelting of non-ferrous metals and burning of fossil fuels are the major industrial processes that contribute to anthropogenic arsenic contamination of air, water and soil.

Historically, use of arsenic-containing pesticides has left large tracts of agricultural land contaminated. The use of arsenic in the preservation of timber has also led to contamination of the environment. Plants on land accumulate arsenic compounds via uptake from soil and/or deposition from air onto leaves.

2.1.2 Levels of Arsenic in the Environment

The mean total arsenic concentrations in air from remote and rural areas range from 0.02 to 4 ng/m^3. Mean total arsenic concentrations in urban areas range from 3 to about 200 ng/m^3; much higher concentrations (> 1000 ng/m^3) have been measured in the vicinity of industrial sources, although in some areas this is decreasing because of pollution abatement measures.

Concentrations of arsenic in open ocean seawater are typically 1 to 2 µg/litre. Arsenic is widely distributed in surface freshwaters, and concentrations in rivers and lakes which are generally below 10 µg/litre, although individual samples may range up to 5 mg/L near anthropogenic sources. Arsenic levels in groundwater average about 1 to 2 µg/litre except in areas with volcanic rock and sulfide mineral deposits where arsenic levels can range up to 3 mg/L.

Mean sediment arsenic concentrations range from 5 to 3000 mg/kg, with the higher levels occurring in areas of contamination. Background concentrations in soil range from 1 to 40 mg/kg, with mean values often around 5 mg/kg (Beyers et al, 1987). Naturally elevated levels of arsenic in soils may be associated with geological substrata such as sulfide ores. Anthropogenically, contaminated soils can have concentrations of arsenic up to several grams per 100 ml.

Marine organisms normally contain arsenic residues ranging from less than 1 to more than 100 mg/kg, predominantly in organic arsenic species such as arsenosugars

(macroalgae) and arsenobetaine (invertebrates and fish). Bioaccumulation of organic arsenic compounds, after their biogenesis from inorganic forms, occurs in aquatic organisms. Bioconcentration factors (BCFs) in freshwater invertebrates and fish for arsenic compounds are lower than for marine organisms. Biomagnification in aquatic food chains has not been observed.

Background arsenic concentrations in freshwater and terrestrial biota are usually less than 1 mg/kg (fresh weight). Terrestrial plants may accumulate arsenic by root uptake from the soil or by adsorption of airborne arsenic deposited on the leaves. Arsenic levels are higher in biota collected near anthropogenic sources or in areas with geothermal activity. Some species accumulate substantial levels, with mean concentrations of up to 3000 mg/kg at arsenical mine sites.

2.1.3 Sources of Environmental Pollution of Arsenic

Groundwater contamination by arsenic arises from sources of arsenopyrite, base metal sulfides, realgar and orpiment, arsenic-rich pyrite, and iron oxyhydroxide. Mechanisms by which arsenic is released from minerals are varied and are accounted for by many biogeochemical processes: oxidation of arsenic-bearing sulfides, desorption from oxides and hydroxides, reductive dissolution, evaporative concentration, leaching from sulfides by carbonate, and microbial mobilization (Garelick et al, 2008).

Tailings from metal-mining operations are a significant source of contamination, and can lead to contamination of the surrounding top soils, and, because of leaching, sometimes the groundwater too (Wewerka et al., 1978). As sulphur is often present in these tailings, exposure to the atmosphere in the presence of water leads to the production of an acid solution that can leach many elements including arsenic.

Arsenic is present in the rock phosphate used to manufacture fertilizers and detergents. (Hutton et al, 1986)

Arsenical pesticides were one of the largest classes of non-native biocontrol agents used in agricultural fields (Woolson, 1983). From the 1960s, there was a shift, in herbicide use, from inorganic compounds (including lead and calcium arsenate and copper acetoarsenite) to inorganic and organic compounds and also exposure to wood preserving arsenicals

The levels of arsenic in sewage sludge add significantly to arsenic-contaminated wastewater runoffs which are derived from sources including atmospherically deposited arsenic, residues from pesticide usage, phosphate detergents and industrial effluent, particularly from the metal-processing industry.

Arsenic enrichment also takes place in geothermally active areas; surface waters are more susceptible than groundwater to contamination in the vicinity of such geothermal systems, and evidence suggests that increased use of geothermal power may elevate risks of arsenic exposure in affected areas (Garelick et al, 2008). Mining activities continue to provide sources of environmental contamination by arsenic. All these add significantly to the arsenic pollution of the environment.

2.1.4 Arsenic Compounds in Foods

The actual total arsenic concentrations in foodstuffs from various countries vary widely depending on the food type, growing conditions (type of soil, water, geochemical activities, and use of arsenical pesticides) and processing techniques.

By far the highest concentrations of total arsenic are found in seafood. Meats and cereals have higher concentrations than vegetables, fruit and dairy products. On the basis of limited data, it has been estimated that the percentage of inorganic arsenic is about 75% in meats, 65% in poultry, 75% in dairy products, and 65% in cereals (Yost et al., 1998). Tao et al, (1998) estimated an inorganic arsenic intake for US men and women aged 60–65 years of 13 and 10 µg respectively. Other age groups had lower estimated daily intakes of inorganic arsenic, varying from 1.3 µg for infants to 9.9 µg for men aged 25–30 years.

In fruits, and vegetables and seafood the organic species predominate, with inorganic arsenic contributing 10%, 5% and 0–10% respectively. On the basis of these preliminary data it has been estimated that approximately 25% of the daily intake of dietary arsenic is inorganic (Yost et al., 1998).

2.1.4a Arsenic in Fish

Barrows et al. (1980) exposed bluegill sunfish (*Lepomis macrochirus*) to 130 µg As(III)/litre of As_2O_3 for 28 days. The maximum BCF was found to be 4, with a half-life in tissues of 1 day. Nichols et al. (1984) found no accumulation of arsenic in a 6-month study on coho salmon (*Oncorhynchus kisutch*) exposed to As_2O_3 concentrations of < 300 µg As(III)/litre. Whole-body residues had arsenic concentrations which were below 0.4 mg/kg (wet weight) and were not dose dependent.

Sorensen (1976) found that green sunfish (*Lepomis cyanellus*) exposed to higher arsenic concentrations of 100, 500 and 1000 mg As(V)/litre (as arsenate) accumulated whole-body arsenic concentrations of 33.4, 541.2 and 581.6 mg/kg (BCFs ranging from 0.3 to 1.1). Green sunfish exposed to 60 mg As(V)/litre for 6 days accumulated mean arsenic residues of 158.7, 47.7, 18.9 and 14.2 mg/kg in the gallbladder (plus bile), liver, spleen and kidney respectively (BCFs ranging from 0.2 to 2.6) (Sorensen et al., 1979).

Cockell et al (1988) fed rainbow trout (*O. mykiss*) on diets containing As_2O_3 with arsenic concentrations of(180–1477 mg/kg of diet), disodium arsenate heptahydrate (DSA) 137– 1053 mg/kg diet), DMA (163–1497 mg/kg diet) or arsanilic acid (193–1503 mg/kg) for 8 weeks. For each of the arsenicals investigated, carcass arsenic concentration showed a dose–response relationship to dietary arsenic concentration and exposure rate. At lower levels of exposure (137 mg/kg diet), dietary DSA yielded the highest mean carcass arsenic concentrations (6.9 mg/kg), but at higher levels, dietary As_2O_3 (1477 mg/kg diet) yielded the highest mean residues (21.6 mg/kg). Inorganic arsenicals were accumulated from the diet to a greater degree than the organic forms. In a 16-week study, dietary DSA (8–174 mg/kg diet) accumulated in the carcass (0.25–5.7 mg/kg), liver (0.7–34.4 mg/kg) and kidney (1.1–31.9 mg/kg) in a dose-related manner (Cockell et al., 1991).

Oral administration of sodium arsenate to estuary catfish (*Cnidoglanis macrocephalus*) and school whiting (*Sillago bassensis*) resulted in an accumulation of trimethylarsine oxide in their tissues (Edmonds et al, 1987). Yelloweye mullet (*Aldrichetta forsteri*) fed the organic arsenicals such as 2-dimethylarsinylethanol, 2-dimethylarsinylacetic acid or 2-dimethyllarsinothioylethanol showed no arsenic accumulation in their tissues; fish fed arsenate-contaminated food showed a small but

significant increase in arsenic concentration (muscle tissue = 1 mg/kg wet weight). However, administering arsenobetaine or arsenocholine in the diet led to muscle arsenic concentrations of around 24 mg/kg (wet weight) (Francesconi et al., 1989).

Oladimeji et al. (1984) fed rainbow trout (*O. mykiss*) on a diet containing 10, 20 or 30 mg As(III)/kg (as sodium arsenite) (equivalent to 0.2, 0.4 and 0.6 mg/kg fish wet weight per day) for up to 8 weeks. Arsenic accumulation was dose related, with residues ranging from 1.28 to 1.52 mg/kg (dry weight) for muscle, 1.55 to 5.21 mg/kg for liver, 0.84 to 1.88 mg/kg for gills and 1.21 to 1.98 mg/kg for skin tissue.

2.1.5 Arsenic in Urine

2.1.5a Composition of Urine

Urine is typically a sterile liquid by-product of the body that is secreted by the kidneys through a process called urination and excreted through the urethra. Cellular metabolism generates numerous by-products, many rich in nitrogen, which requires elimination from the bloodstream. These by-products are eventually expelled from the body in a process known as excretion, the primary method for excreting water-soluble chemicals from the body. These chemicals can be detected and analyzed by urinalysis. Urine is a transparent solution that can range from colorless to amber but is usually a pale yellow. In the urine of a healthy individual, the color comes primarily from the presence of urobilin. Dark yellow urine is often indicative of dehydration. Yellowing/light orange may be caused by removal of excess B vitamins from the bloodstream.

The pH of normal urine is generally in the range 4.6 - 8, a typical average being around 6.0. Much of the variation is due to diet.

The actual quantity of urine per person per day depends on numerous factors including level of hydration, activities, environmental factors, weight of individual, and the individual's health. In adult humans the average production is about 1-2 litres per 24 hours.

Urine is approximately 95% water. The other 5% consists of solutes (chemicals that are dissolved in the water). Some of these solutes are the results of normal biochemical activity within the cells of the body. Other solutes may be due to chemicals that originated outside of the body, such as pharmaceutical drugs. Solutes found in urine may be classified as ions such as Na^{+}, K^{+}, Cl^{-}, Mg^{2+}, Ca^{2+}, NH_4^{+}, SO_4^{+} and the phosphates or organic molecules are urea, creatinine and uric acid.

Normal urine density or specific gravity values vary between 1.003–1.035 gcm^{-3} and any deviations may be associated with urinary disorders.

2.1.5b Arsenic concentration in Urine

Levels of arsenic or its metabolites in hair, nail and urine are used as biomarkers of arsenic exposure (IPCS, 2001). The concentration of total arsenic in urine has often been used as an indicator of recent exposure because urine is the main route of excretion of most arsenic species (Buchet et al. 1981; Vahter 1994). The halftime of inorganic arsenic in human subjects is about 4 days. However, the total urinary arsenic concentration does not provide information on the form of arsenic absorbed.

Some foods, especially those of marine origin, often have high concentrations of arsenic mainly in the form arsenobetaine, which is not metabolized in the body but is rapidly excreted in the urine (Vahter 1994). Thus, ingestion of such foods results in a rapid increase in the concentration of total arsenic in the urine; that increase would invalidate urinary arsenic as an indicator of exposure to inorganic arsenic. One

serving of seafood might give rise to urinary arsenic concentrations of more than 1,000 µg/L (Norin et al, 1981). In comparison, concentrations of 5-50 µg/L are found in the urine of subjects with no intake of seafood arsenic or excessive exposure to inorganic arsenic in drinking water or in the working environment. Navas-Acien et al, 2009, did a study on some adults in the US and found the median total arsenic concentration to be 0.0034 mg/L. Certain other foods (e.g., chicken) also might contain arsenobetaine if fish meal is used as a source of protein in the feed.

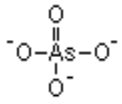
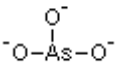
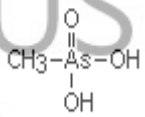
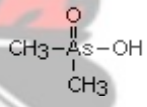
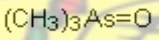
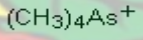
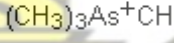
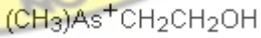
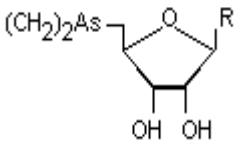
2.1.6 Chemistry and Analysis of Arsenic

2.1.6.1 Physical and Chemical Characteristics of Arsenic

Elemental arsenic is an odorless, tasteless semi metallic compound, which appears steel gray and a crystalline material characterized by atomic number 33, atomic weight of 74.92, density of 5.727, melting point of 817 °C, sublimation at 613 °C, and chemical properties similar to those of phosphorus (EPA 1985).

The symbol is As and is the third element in Group VA of the periodic table. As the 20th most abundant element in the earth's crust, arsenic has an average soil concentration of 5–13 mg/kg. In regions of volcanic activity, the average arsenic content of soils is higher, around 20 mg/kg. There are many arsenic compounds of environmental importance. Representative marine arsenic-containing compounds, of which some are found in terrestrial systems. Table 2. 1shows the various forms of arsenic.

Table 2.1 Naturally occurring inorganic and organic As species

CAS No.	**Name**	**Structure**
1303-28-2	Arsenate	
1327-53-3	arsenite	
124-58-3	methylarsonic acid	
75-60-5	dimethylarsinic acid	
4964-14-1	trimethylarsine oxide	
27742-38-7	tetramethylarsonium ion	
64436-13-1	Arsenobetaine	
39895-81-3	Arsenocholine	
	Dimethylarsinoylribosides	 R= structure 1-11

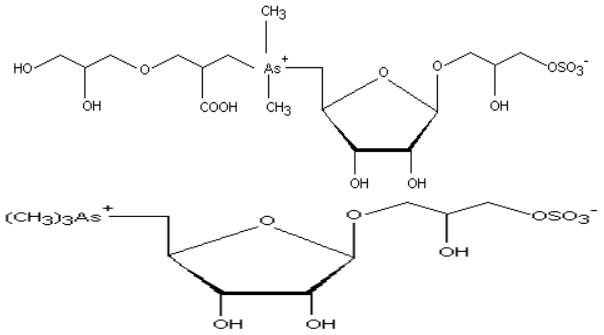
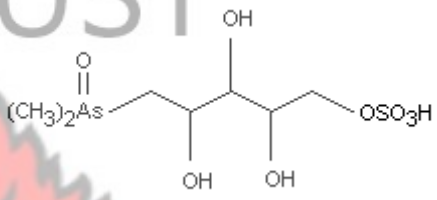
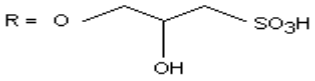
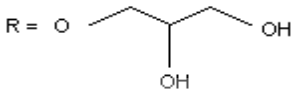
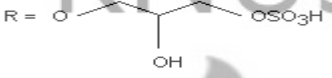
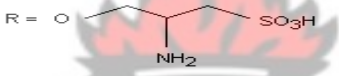
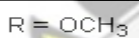
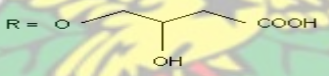
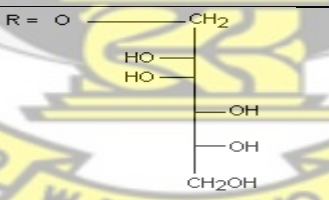
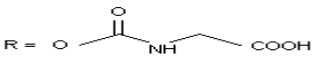
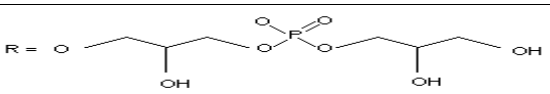
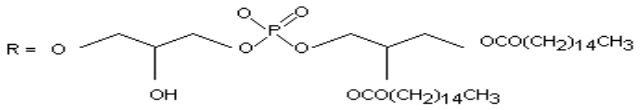
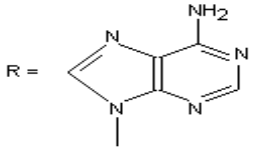
	Trialkylarsonioribosides	
	dimethylarsinoylribitol sulfate	

Table 2.2 Structures for R in Dimethylarsinoylribosides.

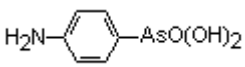
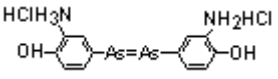
R	**Structure**
1	
2	
3	
4	
5	
6	
7	
8	
9	

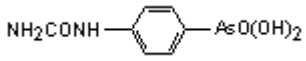
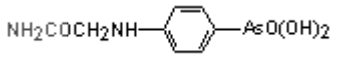
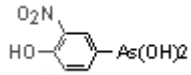
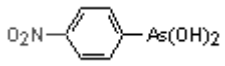
10	
11	

From http://www.inchem.org/documents/ehc/ehc/ehc224.htm

Table 2.3 Other As compounds of environmental significance referred to in the text

CAS No.	**Name**	**Synonyms**	**Formula**
	Inorganic As, trivalent		
1327-53-3	As(III) oxide	As trioxide, arsenous oxide, white As	As_2O_3 (or As_4O_6)
13768-07-5	arsenenous acid	arsenious acid	$HAsO_2$
7784-34-1	As(III) chloride	As trichloride, arsenous trichloride	$AsCl_3$
1303-33-9	As(III) sulfide	As trisulfide orpiment,	As_2S_3

		auripigment	
	Inorganic As, pentavalent		
1303-28-2	As(V) oxide	As pentoxide	As_2O_5
7778-39-4	arsenic acid	*ortho*-arsenic acid	H_3AsO_4
10102-53-1	arsenenic acid	*meta*-arsenic acid	$HAsO_3$
	arsenates, salts of *ortho*-arsenic acid		$H_2AsO_4^-$, $HAsO_4^{2-}$, AsO_4^{3-}
	Organic As		
593-52-2	Methylarsine		CH_3AsH_2
593-57-7	Dimethylarsine		$(CH_3)_2AsH$
593-88-4	Trimethylarsine		$(CH_3)_3As$
98-50-0	(4-aminophenyl)-arsonic acid	arsanilic acid, *p*-aminobenzene-arsonic acid	
139-93-5	4,4-arsenobis(2-	arsphenamine,	

	aminophenol) dihydrochloride	salvarsan	
121-59-5	[4-[aminocarbonyl-amino]phenyl] arsonic acid	carbarsone, *N*-carbamoylarsanilic acid	
554-72-3	[4-[2-amino-2-oxoethyl)amino]-phenyl] arsonic acid	Tryparsamide	
121-19-7	3-nitro-4-hydroxy-phenylarsonic acid		
98-72-6	4-nitrophenylarsonic acid	*p*-nitrophenylarsonic acid	
	Dialkylchloroarsine		R_2AsCl
	Alkyldichloroarsine		$RasCl_2$

From http://www.inchem.org/documents/ehc/ehc/ehc224.htm

Arsenic is concentrated in magmetic sulfides and iron ores, most commonly associated with sulfide and oxide complexes in soil and rock formations. Some of the more prominent arsenic-bearing minerals include orpiment (As_2S_3), realgar (AsS), arsenopyrite (FeAsS), and scorodite ($FeAsO_4.\ 2H_2O$) (Bissen et al, 2003). In nature,

arsenic is found in oxidation states of +V (arsenate), +III (arsenite), 0 (arsenic), and -III (arsine) (Bissen et al, 2003). In the aqueous environment, the oxy-anions consisting of arsenite species (H_3AsO_3, H_2AsO_3, $HAsO_3^{2-}$ and AsO_3^{3-}) and arsenate species (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ and AsO_4^{3-}) predominate (Melliker et al. 2008).

Arsenite species tend to predominate in groundwater under reducing conditions, whereas arsenate species are more frequently found under oxidizing conditions (Haswell et al., 1985). Exceptions, however, are common in the natural environment, where arsenate and arsenite species are found in both reducing and oxidizing waters (O'Neil, 2001; WHO, 2001). Bacteria, fungi, yeasts, and animals can methylate inorganic arsenic species to form monomethyl arsenic (MMA) and dimethyl arsenic (DMA).

The mobility of arsenic compounds in soils depends on the pH value, the redox potential, organic matter, clay and sand content, and other elements in the soil (Bissen et al, 2003). Arsenic in soil has the potential to be transported in wind or in runoff, or can leach into the subsurface soil; however, many arsenic compounds strongly partition to soil or sediment under oxidizing conditions, resulting in limited mobility. Under reducing conditions, which can occur during flooding or in underwater sediment, arsenic absorbed to iron and manganese oxides may be released. Microbes can also play a role in arsenic dissolution from sediment. Under oxidizing and aerated conditions, the predominant form of arsenic in water and soil is arsenate. Under reducing and waterlogged conditions (< 200 mV), arsenites should be the predominant arsenic compounds. The rate of conversion is dependent on the reduction potential, Eh and pH of the soil as well as on other physical, chemical and biological factors. In brief, at moderate or high Eh, arsenic can be stabilized as a series of pentavalent (arsenate) oxyanions, H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ and AsO_4^{3-}. However,

under most reducing (acid and mildly alkaline) conditions, arsenite predominates (Ferguson et al, 1972). At one atmosphere with total arsenic of 10^{-5} mol/litre and total sulphur of 10^{-5} mol/litre, solid species are enclosed in parentheses in cross hatched area, which indicates solubility less than 10^{-3} mol/litre as observed in Figure 2.1 in the next page.

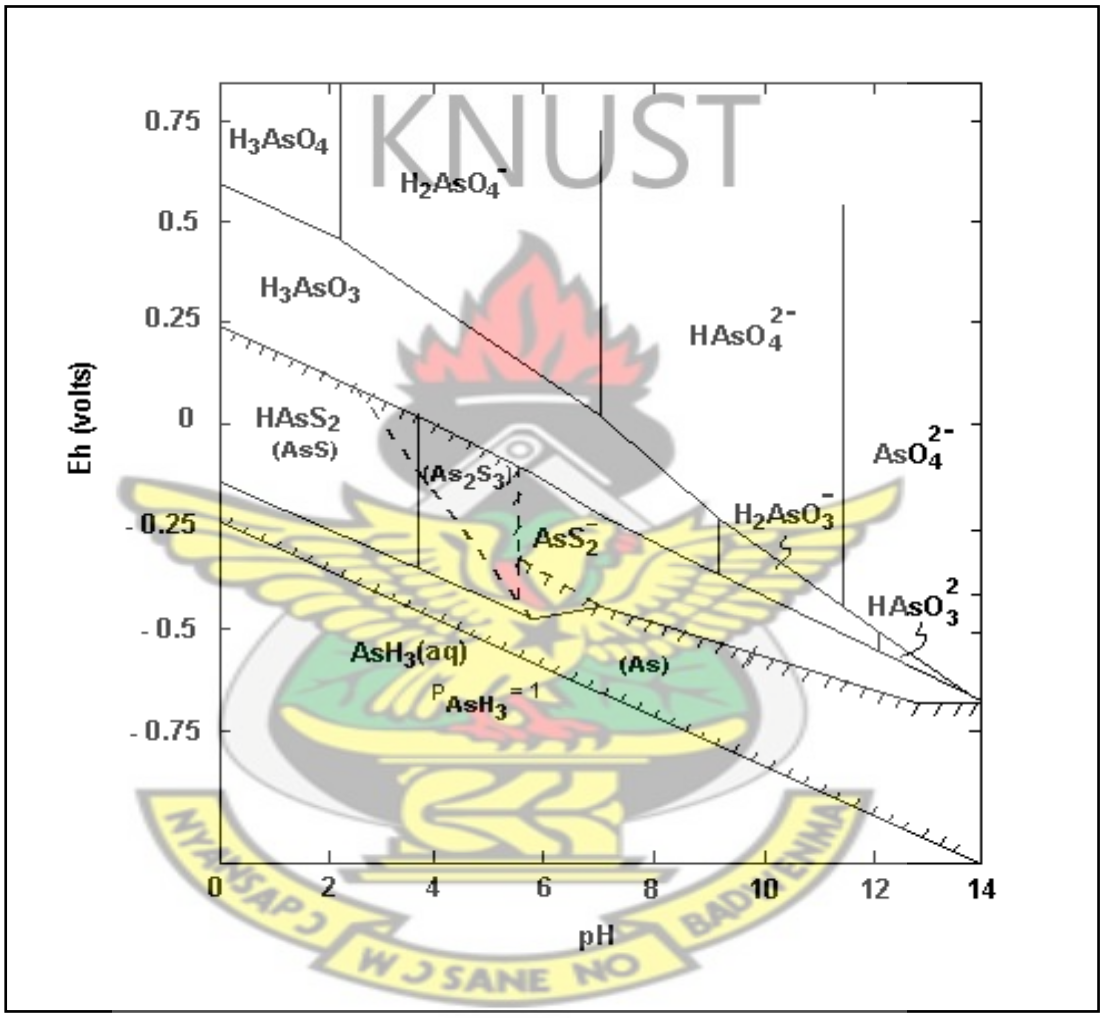


Fig.2.1 The Eh-pH diagram for arsenic at 25 °C (from Ferguson et al, 1972)

2.1.6.2 Significant Chemical Behaviours of Arsenic

2.1.6.2a Comparison of Arsenic to Phosphorus

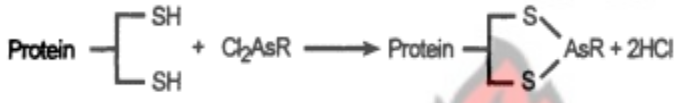
Arsenic and phosphorus are situated in the Periodic Table in Group VA. The oxidation state of arsenic and phosphorus in compounds found in the environment is either III or V but the chemistry of arsenic compounds results from the easy conversion between these two states. The two-electron reduction of arsenate As(V) to arsenite As(III) is favored in acidic solution (E°, standard reduction potential = 0.56 volts), whereas the reverse is true in basic solution (E^o = -0.67 volts) (Latimer et al 1951). In contrast, phosphorus (V) compounds are difficult to reduce.

Another major difference between arsenic and phosphorus is the stability of the esters of phosphoric acid to hydrolysis, allowing the existence of DNA and adenosine 5'-triphosphate (ATP). Esters of As(V) acids are easily hydrolyzed; the half-life in neutral pH is about 30 min. If As(V)OR has a good leaving group such as -P(V) or C(O)R', the half-life falls to seconds. Enzymes can accept arsenate to incorporate into other compounds, such as ATP, but the analogues formed hydrolyze immediately. Thus, arsenate uncouples oxidative metabolism from ATP biosynthesis. This phenomenon is believed to account for some of the toxicity of arsenate (Dixon 1997).

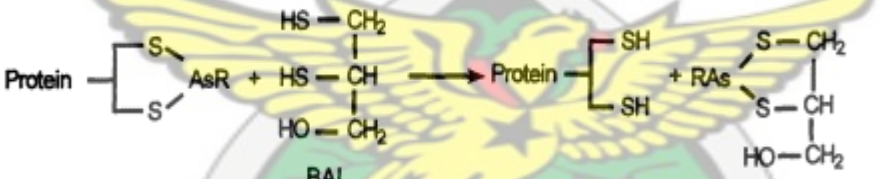
Many As(III) compounds are formulated as RAsX or $(R_2As)_2X$ (X = 0, S). In the solid state, some of these compounds can be polymeric (e.g., $(CH_3AsO)_3$ and $(CH_3AsS)_3$), but $CH_3As(OH)_2$ and $(CH_3)_2AsOH$ seem to exist in dilute aqueous solution (Hasegawa, 1997).

2.1.6.2b. Affinity of Arsenic for Sulphur

The affinity of arsenic for sulphur is revealed in any list of natural arsenic-containing minerals. Many are sulfides and include As_4S_4 (realgar), As_4S_6 (orpiment), and FeAsS (arsenical pyrites, mispickel). This affinity has invoke the toxicity of As(III) compounds through its interaction with protein sulfhydrals, as shown in Equation 2.1. Such binding to proteins may inhibit the function of the enzymes such as pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase (Dixon 1997).



Equation 2.1



Equation 2.2

The action of dimercaprol, British Anti-Lewisite (BAL), in aiding the elimination of arsenic species from humans, is believed to be as a result from the displacement of bound arsenic from a protein because of the formation of a more stable complex as in equation 2.2. The reduction of As(V) compounds by sulfhydrals has been well documented (Cullen et al. 1984), but sulfhydryl groups in enzymes do not always affect the reduction (Dixon, 1997), presumably because this reductive interaction with sulfhydryl groups requires that more than one sulfhydryl group reach the same arsenic atom hence the reduction is a two-electron process.

The As(III)-sulphur bond is much more resistant to hydrolysis than an As(III)-oxygen moiety (Sagan et al. 1972; Zingaro and Thomson 1973).

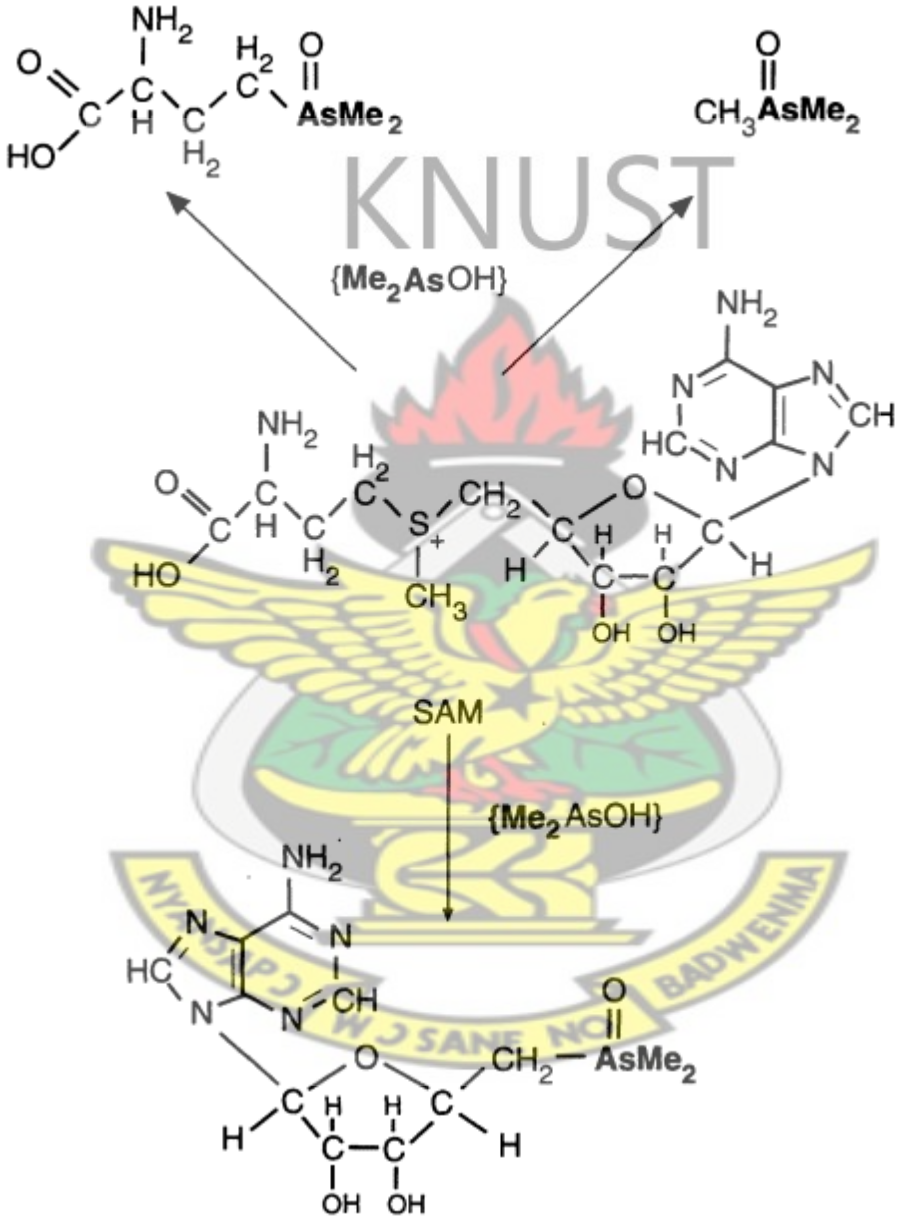
2.1.6.2c Biomethylation of Arsenic

Endogenous sulfhydrals probably play a critical role in the metabolic conversion of As(III) and As(V) species. It is likely that glutathione (GSH) acts as a reducing agent for As(V) species; the resulting As(III) species can then accept a methyl group from S-adenosylmethionine (SAM) to produce the methylarsenic(V) species in an oxidative-addition reaction (Cullen et al, 1989). This cycle of reduction followed by oxidative addition of a methyl group as shown in Figure 2.3 can be continued, and the end product may depend on the organism. This cycle is based on the pioneering studies of Challenger (1951). The end products can be trimethylarsine oxide or trimethylarsine for fungi, the tetramethylarsonium ion for clams, and probably DMA for humans (Cullen et al. 1994).

Fig 2.2 Methylation of Arsenic (Cullen et al , 1989)

Methylation of arsenic: SAM as methyldonor, GSH as reducing agent. GSH, glutathione.

SAM is probably the source of the adenosyl group that is found in the arsenosugars (Francesconi et al, 1997). The As(III) derivatives seem to have the unique ability to accept all three groups that are attached to sulphur in SAM, as illustrated in Equation 2.3.



Equation 2.3

The As(III) species that are intermediates in the biotransformation of arsenic might well be toxic (Cullen et al. 1989). For instance, glutathione reductase (GR) is a key enzyme in the metabolism of GSH and is inhibited by the methylarsenic(III) and As(III) species (Delnomdedieu et al. 1994). The action of GR is critical in maintaining the redox status of cells.

2.1.6.2d Geochemical Behaviours: Adsorption and Redox

As(III) exists in most natural water as $As(OH)_3$ with pKa of 9.2 and is more mobile than As(V) because it is less strongly adsorbed on most mineral surfaces than the negatively charged As(V) oxyanions (H_3AsO_4; pKa = 2.22, 6.98, 11.53). Iron(III) oxy species are well known to have a high affinity for As(V) (Waychunas et al. 1993; Lumsdon et al. 1984), and As(III) also adsorbed on some iron(III) surfaces (Sun et al, 1996).

Little is known about the adsorption of As(III) on the terrestrially abundant aluminum oxides and aluminosilicate minerals. From Ghosh et al, 1987 activated alumina has a twofold higher affinity for As(V) than for As(III) at pH 7; negligible removal of As(III) from drinking water is achieved by coagulation with alum (Hering et al. 1997). Kaolinite and montmorillonite also have higher affinities for As(V) than for As(III) (Frost et al 1977). In the presence of the clay minerals kaolinite and illite abiotic oxidation of As(III) is enhanced, a process that results in strongly bound As(V) species (Manning et al 1997; Scott et al, 995). Consequently, long-term modeling of arsenic mobility in soils and aquifers considers the effects of pH and mineral conditions, which will influence both adsorption and abiotic oxidation of As(III).

The adsorption behavior of the organic arsenic species in spite of the use of the methylarsenicals MMA and DMA and their salts as pesticides, herbicides, and defoliants are yet to be discovered.(Nriagu et al. 1990).

2.1.6.2e Microbial Activity and Arsenic Mobilization

Direct microbial reduction of arsenate to the more mobile arsenite is known for bacterial, algal, and fungal species (Cullen et al, 1989; Silver et al. 1993). Microbial activity has been implicated in arsenic mobilization from sediments. Iron-reducing bacteria might cause arsenate dissociation from sediment that is solid as a consequence of iron oxide dissolution (Lovley et al. 1991). Sulphate-reducing bacteria produce hydrogen sulfide, which might promote arsenate reduction. In recent years, some arsenate-respiring bacteria have been isolated; those include the dissimilatory arsenate-reducer strain MIT-13 (Ahmann et al. 1994) and *Chrysiogenes arsenatis* native to gold-mine waters (Diorio et al. 1995). Ahmann et al. (1997) showed that arsenic-rich hypoxic sediments from the Halls Brook storage area are mobilized by native microbial activity and it was found that microbial activity catalyzed rapid dissolution of arsenic, as As(III), from Fe(II) and Fe(III) arsenates.

2.1.6.2f Free Radical and Peroxy Species

The observed tumor promotional activity of DMA has been suggested to be due to the action of active oxygen-containing species, such as the dimethylarsenic peroxyl radical $(CH_3)_2As\text{-}O\text{-}O$ (Rin et al. 1995; Yamanaka et al. 1996). Peroxy species of arsenic are not known, but the proposed As(III) species does not seem to be a plausible entity. The intermediate peroxy acid $C_6H_5AsO(OH)(OOH)$ can be formed as a result of phenylarsonic acid, $C_6H_5AsO(OH)_2$ which acts as an oxygen-transfer

agent reacting with peroxide(Jacobson et al. 1979). One compound formulated as $(CH_3)_2As\text{-}S\text{-}S\text{-}As(CH_3)_2$, was close in structure to the proposed peroxyl radical, turned out to have the structure $(CH_3)_2As(S)\text{-}S\text{-}As(CH_3)_2$, which has arsenic in two oxidation states (Camermana et al, 1964). On that basis and because of the ease of oxidatin of DMA(III), $(CH_3)_2As(O)\text{-}O$ or even $(CH_3)_2As(O)\text{-}O\text{-}O$ might be more likely candidates for active oxygen-containing species (Dodd et al. 1992).

2.1.7 Environmental Transport and Distribution

Arsenic is primarily emitted into the atmosphere by high-temperature processes such as coal-fired power generation, smelting, burning vegetation and vulcanism. Also natural low-temperature biomethylation and microbial reduction also release arsenic into the atmosphere; microorganisms can form volatile methylated derivatives of arsenic under both aerobic and anaerobic conditions, and can reduce arsenic compounds to release arsine gas (Tamaki et al, 1992).

Arsenic is released into the atmosphere primarily as As_2O_3 or, less frequently, as one of several volatile organic compounds. Arsenic released to air exists mainly in the form of particulate matter (Coles et al, 1979). These particles are dispersed by the wind to a varying extent, depending on their size, and the particles are returned to the earth by wet or dry deposition. Arsines that are released from microbial sources in soils or sediments undergo oxidation in the air, reconverting the arsenic to less volatile forms that settle back to the ground (Parris et al, 1976).

Total atmospheric arsenic emissions from both natural and anthropogenic sources have been estimated to be 31×10^9 g/year, and total atmospheric arsenic removal was estimated to be 30 to 50×10^9 g/year. The global tropospheric residence time of arsenic appears to be about 9 days (Walsh et al., 1979). Nakamura et al. (1990)

estimated global atmospheric emissions into the atmosphere and deposition of arsenic. Total emissions were estimated at 36×10^9 g/year, with the major source of atmospheric arsenic being anthropogenic emissions; the major natural source of arsenic was volcanic activity. Emissions from anthropogenic sources were estimated at 24×10^9 g/year, representing 64% of total arsenic influxes. Depositions from the atmosphere to the land and the oceans were estimated at 24×10^9 g/year and 9×10^9 g/year respectively. Akeredolu et al. (1994) calculated the total annual transport of arsenic into the Arctic atmosphere at 285 t (285×10^6 g) on the basis of a chemical transport modelling approach previously used for sulphur. Arsenic in the atmosphere exists primarily adsorbed to particulate matter and mostly to particles < 2 μm in diameter (Coles et al., 1979).

The dissolved forms of arsenic in the water column include arsenate, arsenite, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Braman et al, 1973). Some As(III) and As(V) species can interchange oxidation states depending on Eh, pH and biological processes (Ferguson et al, 1972). Some arsenic species have an affinity for clay mineral surfaces and organic matter, and this can affect their environmental behaviour. Methylation and demethylation reactions are also important transformations which control the mobilization and subsequent distribution of arsenicals (Mok et al, 1994). Transport and partitioning of arsenic in water depends on the chemical form of the arsenic and on interactions with other materials present. Arsenic may be adsorbed from water on to clays, iron oxides, aluminium hydroxides, manganese compounds and organic material (Welch et al., 1988). The distribution and transport of arsenic in sediment is a complex process that depends on water quality, native biota and sediment type. There is a potential for arsenic release when there is

fluctuation in Eh, pH, soluble arsenic concentration and sediment organic content (Abdelghani et al., 1981).

Sanders (1980) found that the major inputs to the marine environment were river runoff and atmospheric deposition. Biological uptake caused changes in arsenic speciation resulting in measurable concentrations of reduced and methylated arsenic species. The overall cycle is similar to the phosphate cycle, but the regeneration time for arsenic is much slower. Arsenic flows into the estuary as arsenate and arsenite from river water and mine adits. There is oxidation of arsenite to arsenate, microbiological reduction of arsenate to arsenite and removal of arsenic by dilution with seawater and subsequent transport out of the estuary. Inorganic arsenic can be adsorbed on to charged particles of iron oxyhydroxides and manganese oxides and deposited as flocculated particles to sediment. There is subsequent release of dissolved arsenite and arsenate following the reduction and dissolution of the iron and manganese carrier phases in the anoxic sediments. Arsenate can be reduced, either microbially or chemically, to arsenite within the anoxic sediment, and arsenic (as arsenate or arsenite) can enter by sediment resuspension (Sanders, 1980; Knox et al., 1984). Studies on the pH dependence of arsenate and arsenite adsorption to soils and sediments and to minerals are not consistent.

Arsenic from weathered rock and soil may be transported by wind or water erosion. However, because many arsenic compounds tend to adsorb to soils, leaching usually results in transportation over only short distances in soil (Welch et al., 1988). However, rainwater or snowmelt may leach soluble forms into surface water or groundwater, and soil microorganisms may reduce a small amount to volatile forms (arsines) (Turpeinen et al., 1999).

Under reducing conditions, arsenite dominates in soil (Haswell et al., 1985) but elemental arsenic and arsine can also be present (Walsh et al, 1975). Arsenic would be present in well-drained soils as $H_2AsO_4^-$ if the soil was acidic or as $HAsO_4^{2-}$ if the soil was alkaline. Oxidation, reduction, adsorption, dissolution, precipitation and volatilization of arsenic reactions commonly occur in soil (Bhumbla et al, 1994). In the porewater of aerobic soils arsenate is the dominant arsenic species, with small quantities of arsenite and MMA in mineralized areas.

2.1.8 Biotransformation

Most environmental transformations of arsenic appear to occur in the soil, in sediments, in plants and animals, and in zones of biological activity in the oceans. Biomethylation and bioreduction are probably the most important environmental transformations of the element, since they can produce organometallic species that are sufficiently stable to be mobile in air and water. However, the biomethylated forms of arsenic are subject to oxidation and bacterial demethylation back to inorganic forms (IPCS, 1981).

Three major modes of biotransformation of arsenic species have been found to occur in the environment: redox transformation between arsenite and arsenate, the reduction and methylation of arsenic, and the biosynthesis of organoarsenic compounds. There is biogeochemical cycling of compounds formed by these processes (Andreae, 1983).

Arsenic is released into the atmosphere primarily as As_2O_3 or, less frequently, in one of several volatile organic compounds, mainly arsines (US EPA, 1982). Trivalent arsenic and methyl arsines in the atmosphere undergo oxidation to the pentavalent

state, and arsenic in the atmosphere is usually a mixture of the trivalent and pentavalent forms (Scudlark & Church, 1988).

Arsenic can undergo a complex series of transformations, including redox reactions, ligand exchange and biotransformation (Welch et al., 1988). Factors affecting fate processes in water include the Eh, pH, metal sulfide and sulfide ion concentrations, iron concentrations, temperature, salinity, and distribution and composition of the biota (Wakao et al., 1988).

The rates of photochemical decomposition of arsenite, DMA, MMA and arsenobetaine have been studied in both distilled water and seawater. All species were found to degrade rapidly in aerated distilled water. In deaerated solutions the rate of oxidation of arsenite was almost two orders of magnitude slower. Half-lives for the degradation of DMA, MMA and arsenite were 9.2, 11.5 and 0.9 min respectively for aerated distilled water and 25, 19 and 8 minute for deaerated distilled water. In seawater, the rates of photochemical decomposition were slower. For example, in seawater only 20% of DMA was converted to MMA after 300 min with no other products detected, whereas in distilled water DMA was completely degraded within 100 min (Brockbank et al., 1988).

The predominant form of arsenic in water is usually arsenate (Callahan et al., 1979; Wakao et al., 1988), but aquatic microorganisms may reduce the arsenate to arsenite and a variety of methylated arsenicals.

Marine organisms tend to contain much higher levels of arsenic than terrestrial organisms; this is because of the high arsenate/phosphate ratio in oceans, which is a consequence of the very low phosphate concentration. Most of the arsenic

accumulated in marine organisms is in a water-soluble form of arsenic, namely arsenobetaine.

Bioconcentration of arsenic under laboratory conditions occurs in aquatic organisms, primarily in algae and lower invertebrates. Bioconcentration factors (BCFs) measured in freshwater invertebrates for several arsenic compounds generally ranged up to 20; bioconcentration factors in fish were < 5; higher concentration factors have been observed in algae. Biomagnification in aquatic food chains does not appear to be significant (Callahan et al., 1979). Terrestrial plants may accumulate arsenic by root uptake from the soil or by adsorption of airborne arsenic deposited on the leaves, some species accumulating substantial levels.

2.1.9 Exposure Routes and Pathways

Human inhalation exposure to inorganic arsenic can occur as a consequence of industrial activity (e.g. smelting of ores) and energy production (e.g. coal-fired power plants), and during cigarette smoking. Pulmonary exposure may contribute up to approximately 10 µg/day in a smoker and about 1 µg/day in a non-smoker, and more in polluted areas.

Non-occupational human exposure to arsenic in the environment is primarily through the ingestion of food and water. Of these, food is generally the principal contributor to the daily intake of total arsenic. In some areas arsenic in drinking-water is a significant source of exposure to inorganic arsenic. In these cases, arsenic in drinking-water often constitutes the principal contributor to the daily arsenic intake. The daily uptake with food is estimated to be between 0.04 (without fish) and 0.19 mg arsenic (with fish). (The MAK-Collection, 2005)Limited data indicate that approximately 25% of the arsenic present in food is inorganic, but this depends highly on the type of

food ingested. Inorganic arsenic levels in fish and shellfish are low (< 1%). Foodstuffs such as meat, poultry, dairy products and cereals have higher levels of inorganic arsenic. Reported average food intakes of arsenic vary from 17 to 291 mg/day for representative adults from different countries (Delgado-Andrade et al., 2003). Differential seafood consumption between populations helps explain this variation, as seafood accounts for 60–96% of the total dietary intake of arsenic. Most of the arsenic in seafood, however, is in the form of organic arsenic compounds (arsenobetaine and arsenosugars), believed to be a relatively nontoxic form of arsenic. The concentration of metabolites of inorganic arsenic in urine (inorganic arsenic, MMA and DMA) reflects the absorbed dose of inorganic arsenic on an individual level. Generally, it ranges from 5 to 20 µg As/litre, but may even exceed 1000 µg/litre.

In workplaces with up-to-date occupational hygiene practices, exposure generally does not exceed 10 µg/m^3. However, in some places workroom atmospheric arsenic concentrations as high as several milligrams per cubic metre have been reported

Humans may be exposed to arsenic through food, water and air. Exposure may also occur through skin contact with soil or water that contains arsenic. Contaminated soils such as mine tailings are also a potential source of arsenic exposure.

Although anthropogenic arsenic contamination is widespread, it is mainly a cause for human health concern in occupational settings and local communities faced with industrial contaminations. The primary sources of exposure for the majority of the world's population remain food and groundwater. Due to widespread natural contamination of aquifers around the world, groundwater is the predominant source of elevated inorganic arsenic exposure for most individuals. Arsenic is mobilized from geologic deposits into groundwater via oxidation, reduction, and/or carbonation

mechanisms, although precise mechanisms of arsenic mobilization are not well understood.

There is more concern toxicologically from ingesting inorganic forms of arsenic (and subsequent methylation of these inorganic forms once in the body), which are believed to account for 10 to 40 % of the arsenic in food. Reports have established that some of our foodstuffs are contaminated with arsenic (Huq *et al*., 2003; Das *et al*., 2004) for instance vegetables, mushrooms, grains, roots, milk, chicken, beef, and pork which are believed to be the primary dietary sources of inorganic arsenic (Schoof et al., 1999). In arsenic-endemic regions, crops irrigated with arsenic-rich water accumulate inorganic and other forms of arsenic. In non arsenic-endemic regions, rice and chicken appear to be the principal sources of inorganic arsenic in the diet. Natural uptake of arsenic by rice results in accumulation of inorganic arsenic (55–97 ng/g) and methylation of arsenic compounds. Arsenic accumulates in chicken when arsenic compounds are added to chicken feed to control intestinal parasites.

Herbal medicines and some forms of seaweed have also been identified as important sources of inorganic arsenic.

Arsenic exposure may be higher for people that work with arsenic, for people that live in houses that contain conserved wood of any kind and for those who live on farmlands where arsenic-containing pesticides have been applied in the past. Reported systemic toxicity in persons having extensive acute dermal contact with solutions of inorganic arsenic indicates that skin can be a route of exposure (Hostynek et al. 1993).

2.1.10 Metabolism and Disposition of Arsenic

Humans are exposed to many different forms of inorganic and organic arsenic species (arsenicals) in food, water and other media. The metabolism and disposition of arsenic may depend on the form in which it is thus inorganic or organic, its valence state, particularly at high doses. Arsenic metabolism is also characterized by relatively large qualitative and quantitative interspecies differences.

In general, organoarsenicals are less extensively metabolized than inorganic arsenic and more rapidly eliminated in both laboratory animals and humans.

2.1.10.1 Absorption

Arsenic in air exists on particulate matter and thus respiratory absorption of arsenic is a two-stage process, involving deposition of the particles on to airway and lung surfaces, followed by absorption of arsenic from deposited particulates. The extent of deposition of inhaled arsenic will depend largely on the size of the inhaled particulates, and absorption of deposited arsenic is highly dependent on the solubility of the chemical form of arsenic.

Arsenic can be absorbed from the gastrointestinal tract after ingestion of arsenic-containing food, water, beverages or medicines, or as a result of inhalation and subsequent mucociliary clearance. The bioavailability of ingested inorganic arsenic will vary depending on the matrix in which it is ingested (e.g. food, water, beverages, soil), the solubility of the arsenical compound itself and the presence of other food constituents and nutrients in the gastrointestinal tract.

When arsenic in the dissolved form is ingested, inorganic arsenic is readily absorbed. About 80 to 90 % of a single dose of arsenite As (III) or arsenate As(V) is absorbed from the gastrointestinal tract of humans and experimental animals(Vahter and Norin

1980; Freeman et al. 1995). Also, less soluble arsenic compounds such as arsenic selenide, arsenic trisulfide and lead arsenate, and gallium arsenide are absorbed much less efficiently than is dissolved arsenic. Arsenic appears to be poorly absorbed through intact human skin but can bind externally to skin and hair (Hostynek et al. 1993).

2.1.10.2 Transportation

In the body, As(III) is mainly bound to SH groups. In particular, As(III) forms high-affinity bonds with vicinal sulfhydryl (SH) groups in proteins and low-molecular-weight compounds such as glutathione (GSH), cysteine, lipoic acid and DMSA (Delnomdedieu et al. 1993) to the organs in the body.

Formation of complexes between trivalent arsenicals and GSH, probably mainly in the form of $As(GS)_3$, has been demonstrated in water solutions (Scott et al. 1993; Delnomdedieu et al. 1994) However, As(III) can be transferred easily from the $As(GS)_3$ complex to binding sites of higher affinity. Recently, inorganic arsenic was reported to be the main form of arsenic bound to serum proteins in patients on continuous ambulatory peritoneal dialysis, and transferrin was the main carrier (Zhang et al. 1998).

Most of the arsenic in blood is rapidly cleared, following a three-exponential clearance curve . (Pomroy et al. 1980). The majority of arsenic in blood is cleared with a half-time of about 1 hr. The half-times of the second and third phases are about 30 and 200 hr, respectively. Experimental data on animals and data on patients with uremia indicate that the concentration of arsenic in red blood cells is severalfold that in plasma at low or background exposure concentrations but is close to onefold at increased blood concentrations (De Kimpe et al. 1993).

Studies on blood arsenic concentrations in hemodialysis patients indicate that part of arsenic is bound to transferrin (De Kimpe et al. 1993). The extent of the binding in healthy individuals is not known. Most of the arsenic in blood is cleared with a half-time of about 1 hr. The whole-body half-time of ingested arsenite is about 4 days, urine being the major excretory pathway.

In humans, inorganic arsenic is methylated to MMA(V) and DMA(V), which are less reactive with tissue constituents, less acutely toxic, and more readily excreted in the urine than inorganic arsenic. The methylation involves addition of methyl groups from S-adenosylmethionine to arsenic in its trivalent oxidation state. A major part of absorbed pentavalent arsenic is reduced probably by GSH or cysteine. Thus, the tissue distribution, retention and toxicity of arsenic following exposure to moderate doses of arsenite and arsenate are similar. At very high doses, more arsenic is retained following exposure to arsenite than to arsenate. The liver is an important initial site of arsenic methylation, but most tissues seem to have methylating capacity. There are major differences in the biotransformation of inorganic arsenic between animal species and population groups. Most experimental animals methylate arsenic more efficiently and excrete less MMA in the urine than do humans. Some mammals (e.g., chimpanzee, marmoset monkey, and guinea pig) have been identified that do not methylate inorganic arsenic at all. Although the rat efficiently methylates arsenic, a major part of the DMA produced is retained in the erythrocytes.

Experimental animal studies found that the binding of arsenic is mainly to high molecular-weight proteins in various tissues; however, arsenic is continuously released from most intracellular binding sites over time following exposure (Vahter et al. 1982). Probably, As(III) is bound to proteins before undergoing subsequent methylation-reduction reactions. Compounds of the type Me_2AsSR, formed following

addition of the second methyl group, are easily oxidized to DMA (Cullen et al. 1989), which is then excreted in the urine. However, the stability might vary, and DMA complexes have been detected in urine (Marafante et al. 1987).

2.1.10.3 Metabolic Transformation

Arsenic metabolism is characterized in many species by two main types of reactions:

(1) reduction of pentavalent to trivalent arsenic, and

(2) oxidative methylation reactions

In these reactions, trivalent forms of arsenic are sequentially methylated to form mono-, di- and trimethylated products using *S*-adenosyl methionine (SAM) as the methyl donor and GSH as an essential co-factor . Methylation of inorganic arsenic facilitates the excretion of inorganic arsenic from the body, as the end-products MMA and DMA are readily excreted in urine. One unusual feature of arsenic metabolism is that there are extreme qualitative and quantitative interspecies differences in methylation to the extent that some species do not appear to methylate arsenic at all (Vahter, 1999).

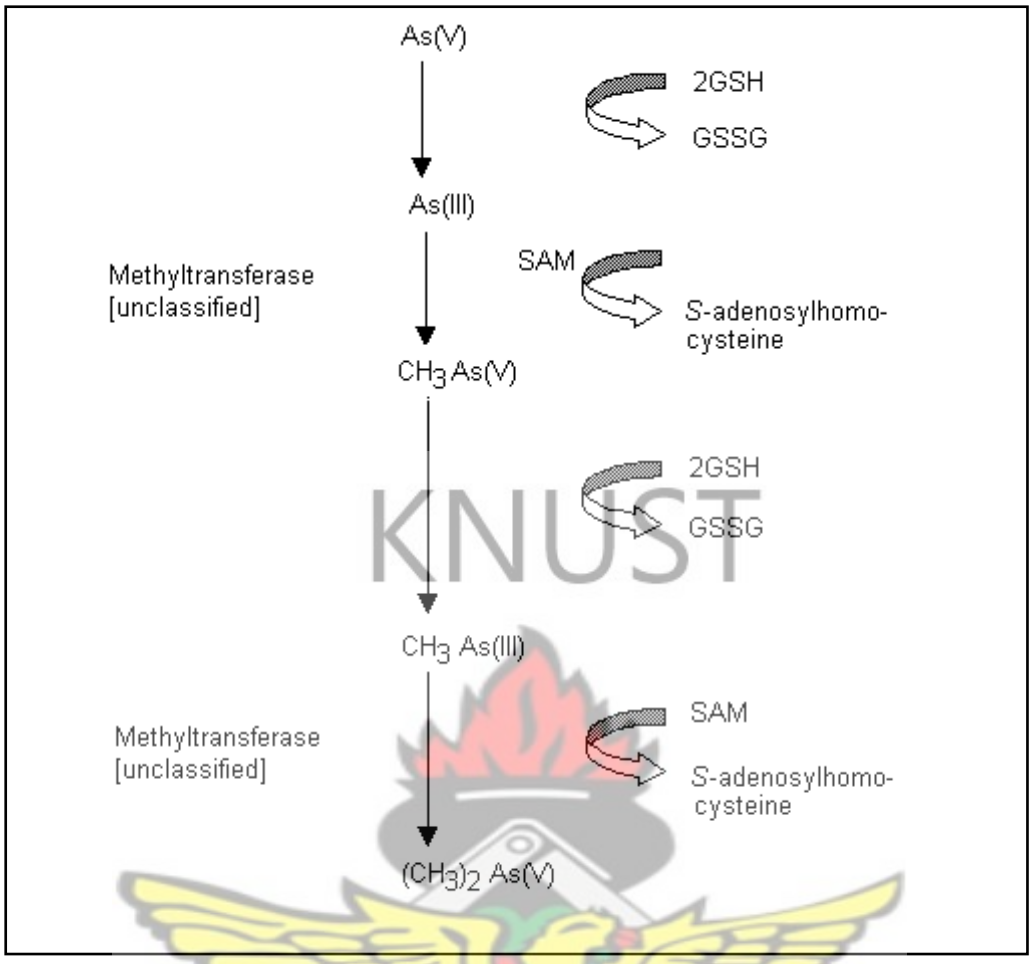


Fig. 2.3 Arsenic methylation in mammals.

Reducing equivalents are supplied by glutathione(GSH) and S-adenosyl methionine (SAM) serves as the methyl donor as in figure 2.3. Reduction of pentavalent to trivalent forms is required for methylation. Trimethylated forms are produced in small amounts if animals or humans are administered DMA

Arsenate reduction is known to occur non-enzymatically under conditions of low oxygen tension (i.e. an anaerobic environment such as exists in the gut) or over time at pH 2 or lower (Vahter et al, 1983). *In vitro* mechanistic studies have demonstrated that the ubiquitous cellular tripeptide GSH is able to reduce arsenate to arsenite in both aqueous systems (Scott et al., 1993) and in intact erythrocytes (Delnomdedieu et

al., 1994). Interestingly, bacteria have the capability to enzymatically reduce inorganic arsenate to arsenite (Rosen, 1995)

Factors such as dose, age, gender and smoking contribute only minimally to the large inter-individual variation in arsenic methylation observed in humans. However, lower methylation efficiency in children has been observed in only one study out of three. Studies in humans suggest the existence of a wide difference in the activity of methyltransferases, and the existence of polymorphism has been hypothesized.

2.1.10.4 Excretion

The major route of excretion of most arsenic compounds is via the urine. Inorganic arsenic is eliminated primarily via the kidney in humans as well as animals. Following exposure to inorganic arsenic, the biological half-time is about 4 days. It is slightly shorter following exposure to As(V) than to As(III) (Pomroy et al. 1980;. In six human subjects who were ingested with radiolabeled ^{74} As-arsenate, 38 % of the dose was excreted in the urine within 48 hrs and 58 % within 5 days (Tam et al. 1979). The results indicate that the data best fit to a three-compartment exponential function, with 66 % excreted with a half-time of 2.1 days, 30 % with a half-time of 9.5 days, and 3.7 % with a half-time of 38 days (Pomroy et al. 1980).

Studies in adult human males voluntarily ingesting a known amount of either trivalent or pentavalent arsenic indicate that 45–75 % of the dose is excreted in the urine within a few days to a week. Relatively few studies in volunteers have included measurement of arsenic in both faeces and urine. However, Pomroy et al. (1980) reported that 6.1 % ± 2.8 % of a single oral dose of arsenic acid (As(V)) was excreted in the faeces over a period of 7 days, compared to 62.3 % ± 4.0 % of the dose excreted in urine. The trivalent form is more extensively methylated, leading to similar long-term excretion.

The methylated metabolites MMA and DMA are excreted in the urine faster than the inorganic arsenic. In humans, about 78% of MMA and 75% of DMA were excreted in the urine within 4 days of ingestion of the dose (Buchet et al. 1981). Similar results were reported for mice in which the half-time of MMA and DMA was about 1 hr (Hughes et al, 1998). The 24-hr whole-body retention was about 2 % of the dose.

In people occupationally, experimentally, or environmentally exposed to inorganic arsenic, the urinary content of metabolites of inorganic arsenic generally consists of 10 – 30 % inorganic arsenic, 10 – 20 % MMA, and 55 – 75 % DMA. Some groups of people who excrete only a few percent of MMA have been identified. That response, together with marked individual variations, can indicate a genetic polymorphism in the arsenic methyltransferases. Experimental studies indicate that the methylation of arsenic might also be influenced by the arsenic species absorbed, by acute high-level exposures, as well as by nutritional factors and diseases.

Arsenic is excreted by routes other than just urine and faeces, but in general these routes of excretion are quantitatively minor. Owing to the ability of arsenic to accumulate in keratin-containing tissues, skin, hair and nails could also be considered potential excretory routes for arsenic, although they would in general be quantitatively minor. Studies in IPCS, 1981, indicated that arsenic is excreted in sweat to some degree. The average concentration of arsenic in sweat induced in a hot and humid environment is 1.5 µg/L, and the hourly loss was 2 µg (Vellar 1969). With an average arsenic concentration in the skin of 0.18 mg/kg, Molin et al, 1976, estimated that the daily loss of arsenic through desquamation was 0.1 - 0.2 µg in males with no known exposure to arsenic.

Other studies including IPCS, 1981 have indicated that arsenic can be excreted in human milk, although the levels are low (Concha et al., 1998). For example, in the Bombay area (India) Dang et al. (1983) reported arsenic levels ranging from 0.2 to 1.1 ng/g in breast milk of nursing mothers 1–3 months postpartum. Concha et al. (1998) found that the average concentration of arsenic in breast milk was quite low (3.1 µg/litre) even when urinary arsenic excretion was as high as 230–300 µg/litre from 3 weeks to 5 months postpartum in a study of Andean women in Argentina consuming drinking-water high in arsenic (~200 µg/litre). Significantly, low-arsenic excretion in breast milk of nursing mothers led to a decrease in urinary arsenic concentration of their infants during the nursing period.

Animal studies have shown retention of arsenic in the skin, hair, squamous epithelium of the upper gastrointestinal tract, epididymis, thyroid, skeleton, and lens of the eye. Arsenite is the main form interacting with tissue constituents, except the skeleton. In human subjects, the hair and nails have the highest concentrations of arsenic (0.02-1 mg/kg of dry weight), and the skin and lungs have fairly high concentrations (0.01-1 mg/kg of dry weight). Data extrapolated from animal studies permit the development and validation of a suitable PB-PK model for inorganic arsenic for humans.

Levels of arsenic or its metabolites in blood, hair, nails and urine are used as biomarkers of arsenic exposure. Blood arsenic is a useful biomarker only in the case of acute arsenic poisoning or stable chronic high-level exposure. Arsenic is rapidly cleared from blood, and speciation of its chemical forms in blood is difficult. Arsenic in hair and nails can be indicators of past arsenic exposure, provided care is taken to prevent external arsenic contamination of the samples. Arsenic in hair may also be used to estimate relative length of time since an acute exposure. Speciated metabolites in urine expressed either as inorganic arsenic or as the sum of metabolites (inorganic

arsenic + MMA + DMA) provide the best quantitative estimate of recently absorbed dose of arsenic. However, consumption of certain seafood, mainly seaweed and some bivalves, may confound estimation of inorganic arsenic exposure because of metabolism of arsenosugars to DMA in the body or the presence of DMA in the seafood. Such food should be avoided for 2 to 3 days before urine sampling for monitoring of exposure to inorganic arsenic.

2.1.11 Mechanism of Toxicity

Arsenic affects mitochondrial enzymes and impairs tissue respiration. This appears to be related to the cellular toxicity of arsenic. Arsenic reacts primarily with enzymes containing two sulfhydral groups. Some sulfhydryl-containing proteins and enzymes are functionally altered when exposed to arsenic. When arsenic accumulates in the mitochondria, the respiration is mediated by the NAD-linked substrates which results in a reaction between the arsenite ion and the dihydrolipoic acid cofactor and this is needed for the oxidation of the substrate. It is further shown that arsenic inhibits succinic dehydrogenase activity resulting in stimulation of ATPase activity by uncoupling oxidative phosphorylation. Studies have shown that the addition of glutathione and British anti-Lewisite (BAL) can reverse some of these arsenic changes. In addition, inhibition of mitochondrial respiration is shown to increase the production of hydrogen peroxide, which in turn, may cause the production of reactive oxidative species (ROS). ROS result in the induction of major stress protein families. Arsenical-induced oxidative stress and ROS may be critical factors in mediating DNA damage and initiation of carcinogenesis.

2.1.11.1 Oxidative Stress

There are evidence to support the concept that arsenite induces oxidative stress in mammalian cells and that the induced oxidative damage can result in genotoxicity. For instance, adding superoxide dismutase to cultured medium reduces the frequency of arsenite-induced SCE in human lymphocytes which indirectly supports this concept (Nordenson et al, 1991). Also, vitamin E can protect human fibroblasts from arsenic toxicity (Lee et al, 1994). Arsenite can also increase the concentrations of a number of proteins that can protect against oxidative stress e.g., metallothionein (Albores et al. 1992) and heme oxygenase (Keyse et al 1989). Although these studies and other similar ones suggest that arsenic induces oxidative stress and results in genotoxicity, more direct evidence clearly needs to be proven. Recent studies by Hei et al. (1998) suggest that reactive oxygen species are involved in the formation of deletion mutations of human chromosome 11 in a human-hamster hybrid cell following arsenic treatment. This is supported, in part, by the data of Gurr et al. (1998), who showed that arsenic induced micronuclei in cells in vitro are at concentrations above 10 µ*M*. The induction of micronuclei was reduced by treatment with NO synthase inhibitors and superoxide dismutase, as well as calcium chelators and uric acid. Another explanation for arsenic's possible role in the induction of genotoxicity is that arsenic could affect the repair of endogenously produced oxidative DNA damage.

2.1.12 Effects of Arsenic

The effects of arsenic are divided into to one on humans and the other on the environment.

2.1.12.1 Health Effects

Soluble inorganic arsenic is acutely toxic, and ingestion of large doses leads to gastrointestinal symptoms, disturbances of cardiovascular and nervous system functions, and eventually death. In survivors, bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy and encephalopathy may be observed.

Long-term exposure to arsenic in drinking-water is causally related to increased risks of cancer in the skin, lungs, bladder and kidney, as well as other skin changes such as hyperkeratosis and pigmentation changes. These effects have been demonstrated in many studies using different study designs. Exposure–response relationships and high risks have been observed for each of these end-points. The effects have been most thoroughly studied in Taiwan but there is considerable evidence from studies on populations in other countries as well. Increased risks of lung and bladder cancer and of arsenic-associated skin lesions have been reported to be associated with ingestion of drinking-water at concentrations ≤50 µg arsenic/litre.

Occupational exposure to arsenic, primarily by inhalation, is causally associated with lung cancer. Exposure–response relationships and high risks have been observed. Increased risks have been observed at cumulative exposure levels ≥ 0.75 (mg/m^3)/ year (e.g. 15 years of exposure to a workroom air concentration of 50 µg/m^3). Tobacco smoking has been investigated in two of the three main smelter cohorts and was not found to be the cause of the increased lung cancer risk attributed to arsenic; however, it was found to be interactive with arsenic in increasing the lung cancer risk.

Even with some negative findings, the overall weight of evidence indicates that arsenic can cause clastogenic damage in different cell types with different end-points in exposed individuals and in cancer patients. For point mutations, the results are largely negative.

Chronic arsenic exposure in Taiwan has been shown to cause blackfoot disease (BFD), a severe form of peripheral vascular disease (PVD) which leads to gangrenous changes. This disease has not been documented in other parts of the world, and the findings in Taiwan may depend upon other contributing factors. However, there is good evidence from studies in several countries that arsenic exposure causes other forms of PVD.

Conclusions on the causality of the relationship between arsenic exposure and other health effects are less clear-cut. The evidence is strongest for hypertension and cardiovascular disease, suggestive for diabetes and reproductive effects and weak for cerebrovascular disease, long-term neurological effects, and cancer at sites other than lung, bladder, kidney and skin.

Levels of arsenic in food are fairly low, as it is not added due to its toxicity. But levels of arsenic in fish and seafood may be high, because fish absorb arsenic from the water they live in. Luckily this is mainly the fairly harmless organic form of arsenic, but fish that contain significant amounts of inorganic arsenic may be a danger to human health.

Arsenic exposure may be higher for people that work with arsenic, for people that live in houses that contain conserved wood of any kind and for those who live on farmlands where arsenic-containing pesticides have been applied in the past.

The occurrence of tumors in high numbers after long-term ingestion of arsenic in relatively young patients, or at anatomic sites where cancer is an extremely rare occurrence (e.g., liver angiosarcoma), has increase the likelihood that many of the documented cancers were induced by arsenic (Popper et al. 1978). The observations also assist in identifying major cancer end points. The most common types of malignancy described in the reports are skin cancer, lung cancer, angiosarcoma of the liver (probably noted because of its rarity), prostate cancer, and bladder cancer. Reports of other cancers also appear: leukemia; other hematopoietic cancers; and cancers of the breast, colon, stomach, parotid gland, nasopharynx, larynx, buccal cavity, kidney, and others. Additional case reports describe internal cancers after the appearance of Bowen's disease, a type of superficial intraepidermal carcinoma that has been linked with arsenic exposure (Hugo et al, 1967).

Exposure to inorganic arsenic can cause various health effects, such as irritation of the stomach and intestines, decreased production of red and white blood cells, skin changes and lung irritation. It is suggested that the uptake of significant amounts of inorganic arsenic can intensify the chances of cancer development, especially the chances of development of skin cancer, lung cancer, liver cancer and lymphatic cancer.

A very high exposure to inorganic arsenic can cause infertility and miscarriages with women, and it can cause skin disturbances, declined resistance to infections, heart disruptions and brain damage with both men and women. Finally, inorganic arsenic can damage DNA. A lethal dose of arsenic oxide is generally regarded as 100 mg. Organic arsenics can cause neither cancer, nor DNA damage. But exposure to high doses may cause certain effects to human health, such as nerve injury and stomachaches. Liver is a major target organ in arsenic toxicity and carcinogenesi (Wu

et al, 2008).Chronic arsenic exposure may lead to irreversible damage to several vital organs; moreover arsenic is established as carcinogen (IPCS, 2001; Chen et al, 2004).

2.1.12.2 Environmental Effects

The arsenic cycle has broadened as a consequence of human interference and due to this, large amounts of arsenic end up in the environment and in living organisms. Arsenic cannot be destroyed once it has entered the environment, so the amounts that we add can spread and cause health effects to humans and animals on many locations on earth.

Aquatic and terrestrial biotas show a wide range of sensitivities to different arsenic species. Their sensitivity is modified by biological and abiotic factors. In general, inorganic arsenicals are more toxic than organoarsenicals and arsenite is more toxic than arsenate. The mode of toxicity and mechanism of uptake of arsenate by organisms differ considerably. This may explain why there are interspecies differences in organism response to arsenate and arsenite. The primary mechanism of arsenite toxicity is considered to result from its binding to protein sulfhydryl groups. Arsenate is known to affect oxidative phosphorylation by competition with phosphate. In environments where phosphate concentrations are high, arsenate toxicity to biota is generally reduced. As arsenate is a phosphate analogue, organisms living in elevated arsenate environments must acquire the nutrient phosphorous yet avoid arsenic toxicity.

Plants absorb arsenic fairly easily, so there may be high-ranking concentrations present in food. The concentrations of the dangerous inorganic arsenics that are currently present in surface waters enhance the chances of alteration of genetic materials of fish. This is mainly caused by accumulation of arsenic in the bodies of

plant-eating freshwater organisms. Birds eat the fish that already contain eminent amounts of arsenic and will die as a result of arsenic poisoning as the fish is decomposed in their bodies.

Arsenic compounds cause acute and chronic effects in individuals, populations and communities at concentrations ranging from a few micrograms to milligrams per litre, depending on species, time of exposure and end-points measured. These effects include lethality, inhibition of growth, photosynthesis and reproduction, and behavioural effects. Arsenic-contaminated environments are characterized by limited species abundance and diversity. If levels of arsenate are high enough, only species which exhibit resistance may be present.

2.1.13 Essentiality and Therapeutic Uses

2.1.13.1 Essentiality of Arsenic

There is a general agreement about the criteria needed to identify a substance as an essential nutrient: that is the substance must be present in all organisms for which it is essential, the reduction of exposure below a certain limit results consistently and reproducibly in a reduction of physiologically important functions.

It is universally accepted that arsenic is present in living matter. Arsenic has not been tested for essentiality in humans nor has it been found to be required for any essential biochemical processes. Data from four species indicate that semisynthetic diets with arsenic concentrations in the range of 35 to 50 ng/g or less in combination with dietary or reproductive stress result in functional impairments (Anke, 1991). Such concentrations might occur naturally in some experimental diets and are similar to those found in most human foods except seafood. The mechanisms and sequence of events leading to functional impairments are not known.

Studies show that arsenic supplementation of low-arsenic semisynthetic diets prevents the occurrence of abnormal reproductive performance in goats and minipigs (350 ng/g) Uthus, 1992) and reduced growth in chicks, and rats (500 to 4,500 ng/g). Although the studies have had no independent confirmation under identical experimental conditions, replications by the original investigators have been consistent with goats and minipigs fed semisynthetic diets, as well as with rats and chicks subjected to additional dietary stress (Nielsen, 1980). Toxic effects of the supplementation have not been studied.

2.1.13.2 Therapeutic Uses

Arsenic over the years has been observed to have therapeutic properties for certain disorders. The introduction of inorganic arsenic as a therapeutic agent in the modern medical era is generally attributed to Thomas Fowler, a British physician whose treatise "Medical Reports of the Effects of Arsenic in the Cure of Agues, Remitting Fevers, and Periodic Headaches" was published in 1786.

Inorganic arsenic was also recommended in the treatment of pernicious anemia, chorea, leukemia, and Hodgkin's disease (Osler 1894) by Sir William Osler. Prescribed doses commonly delivered approximately 5-10 mg of inorganic arsenite orally per day (Langehan 1921). The chronic use of inorganic arsenic in this manner was sometimes associated with the development of cutaneous hyperpigmentation or, less commonly, peripheral neuropathy and other multisystemic signs of chronic arsenic poisoning (Osler, 1894).

Inorganic arsenic continued to be used as a therapeutic agent through the mid-twentieth century, by which time its recognized uses were confined predominantly to leukemia, psoriasis, and chronic bronchial asthma (Goodman and Gilman

1955).Although inorganic arsenic might still occasionally be encountered in non-Western traditional medicines or folk remedies (Kew et al. 1993; Espinoza et al. 1995), its availability in medications listed in official Western formularies ended in the 1970s.

2.1.14 Analysis of Arsenic Compounds

In 1775 arsenic compounds were discovered by Scheele to react under reducing conditions to produce a volatile gas, arsine (AsH_3) (Partington 1962) and this provided a tool to counteract the use of arsenic oxide, As_2O_3, for homicide which had reached epidemic proportions during the Middle Ages. The discovery led to the development of the Marsh test for arsenic, in which arsine is volatilized out of the reaction mixture and is detected, for example, by decomposition to an arsenic mirror. Then again, in the Gutzeit modification, (Vogel, 1954) is based on the arsine is generated from arsenic compounds by the addition of zinc granules to concentrated sulphuric acid. The arsine can be detected by means of a strip of filter paper moistened with silver nitrate or mercuric chloride. The arsine reacts with silver nitrate to give a grey spot, and with mercuric chloride to give a yellow to reddish-brown spot. Both procedures can be made semi quantitative. These were the first applications of an analytical method now referred to as "hydride generation" that is widely applicable to the analysis of elements that form volatile hydrides when treated with an appropriate reducing agent, usually sodium borohydride (Vallee 1973).

In older studies, zinc and hydrochloric acid were used as reducing agents. The Gutzeit method was used in a recently published Japanese study (Tsuda et al. 1995) in which well water was found to contain arsenic in the parts-per-million range—the measurements were made in 1959. A variation of this method, Natelson's method

(Natelson 1961), was used in the early study of arsenic in the well water of Taiwan (Tseng et al. 1968). That method uses colorimetric detection and is said to detect arsenic at 40 µg/L. Arsine produced from arsenate and arsenite is sometimes reacted with silver diethyldithiocarbamate solution to produce a red solution of undetermined chemical nature that can be measured colorimetrically (Irgolic 1994). The colorimetric method is easy to use, is inexpensive in terms of equipment and operator costs, and is commonly used by the water-supply industry, though there are limitations with regard to sensitivity and arsenic speciation. Total-arsenic determination commonly involves oxidation of the sample, by using digestion or ashing, with a mixture of chemicals, including HNO_3-H_2SO_4-H_2O_2 or HNO_3-H_2SO_4-$HClO_4$ for wet digestion and MgO-$MgNO_3$ for dry ashing (Irgolic et al. 1995). The arsenic is then determined by using one of a number of methods ranging from hydride generation (colorimetric or spectroscopic detection or neutron activation) to spectrophotometry e.g., graphite-furnace atomic absorption (GFAA) or inductively coupled plasma-atomic emission spectrometry (ICP-AES)).

2.1.14.1 Hydride Generation Atomic Absorption Spectroscopy

Hydride generation technique may be conveniently characterized by three steps:

1. generation of the volatile analyte;
2. its collection (if necessary) and transfer to the atomizer;
3. decomposition to the gaseous metal atoms with measurement of the AA response.

Several reactions have been utilized for the production of the hydrides; all rely on the formation of atomic hydrogen as a reductant. The classical metal–acid reaction employing Zn–HCl is limited to the generation of AsH_3, SbH_3, and SeH_2:

$$Zn + 2HCl \longrightarrow ZnCl_2 + 2H \xrightarrow{E^m} EH_m + H_2 \text{ (excess)}$$

Equation 2.4

where E is the analyte element and may or may not equal m. This system requires that these analytes be present in their lower oxidation states prior to reaction. Reduction to the hydride with sodium tetrahydroborate as shown in equation 2.5 is considerably more efficient:

$$NaBH_4 + 3H_2O + HCl \longrightarrow H_3BO_3 + NaCl + 8H \xrightarrow{E^{m+}} EH_m + H_2(\text{excess})$$

Equation 2.5

And this reaction can be used to generate the hydrides of antimony, arsenic, bismuth, germanium, lead, selenium, tellurium, and tin.

The hydride that is formed is flushed from the generating chamber with argon, helium, or nitrogen. There are essentially three methods used to generate hydrides with $NaBH_4$:

1. continuous systems where sample and reagent are pumped and mixed in a continuous fashion and then passed to some type of gas–liquid separation device;
2. batch systems, wherein a pellet of NaBH4 or an aliquot of the reductant solution is added to the sample from which the volatile products are purged with a flow of transfer gas;
3. Flow Injection (FI) systems in which discrete volumes of sample are merged with flowing streams of acid and/or reductant.

The hydride technique is an absolute procedure in that the measured response is directly proportional to the absolute mass of the analyte element and not to its

concentration in the solution. In practice, an aliquot of acid is usually added to the batch vessel followed by an accurately dispensed volume of sample.

The transient hydride plume produced by either the batch or FI approach may be directly transferred to the atomization cell because reduction reactions are sufficiently rapid. Alternatively, the hydride is frequently collected in a cold trap (usually a cryogenic U-tube filled with a suitable adsorbent that is subsequently warmed to desorb the analyte) connected to the generator.

2.1.14.1a Atomization

Conventional acetylene-based flame systems have found little use as atomization cells for hydrides. The relatively cool argon (entrained air)–hydrogen or simple air–hydrogen flame is advantageous as it exhibits low background absorption at lower wavelengths (15% at 193.7 nm). However, the excess hydrogen generated along with the hydrides often perturbs the flame, changing its composition and absorption characteristics, and the low kinetic temperature makes it more susceptible to interferences. Currently, the most popular atomization source for hydride generation AAS is the heated quartz T-tube (typically 10mm diameter_100–150mm length). Both argon–hydrogen and air–acetylene flames have been utilized to heat open-ended silica tubes to which the hydrides are delivered in a stream of carrier gas from the generator via the central arm of the T. The quartz tube can also be heated electrically (700–1001C) with the advantage of longer analyte residence time in the optical path and the possibility of obtaining the optimum atomization temperature for each element. Often, the tube is sealed with removable quartz windows at either end and fitted with nipples at the extreme ends as exits for the gas flow. These features result in improved sensitivity over the flame heated cells. Deterioration and aging of the

interior surface of the quartz tube invariably occurs, leading to a decrease in sensitivity.

2.1.14.1b Atomization Mechanisms

Atomization of the hydrides is currently believed to proceed via interaction with free hydrogen radicals; oxygen also plays an active role. In argon/hydrogen diffusion flames and quartz tube atomizers, a cloud of hydrogen radicals is formed by reactions between hydrogen and oxygen as observed in the reaction equations:

$H^{\circ} + O_2 \rightarrow OH^{\circ} + O^{\circ}$ Equation 2.6

$O^{\circ} + H_2 \rightarrow OH^{\circ} + H^{\circ}$ Equation 2.7

$OH^{\circ} + H_2 \rightarrow H_2O + H^{\circ}$ Equation 2.8

The concentration of hydrogen radicals is several orders of magnitude higher than that of hydroxyl radicals. In the quartz tube, this occurs either in a flame burning at the end of an oxygen delivery capillary (for a flame-in-tube device) or at the beginning of the hot zone for an externally heated tube (above 6001C). Only a small portion of the volume of the atomizer is filled by the cloud, as determined by the gas dynamics, geometry of the tube andits temperature profile. The hydride is atomized within the radical cloud in accord with sequential collisions:

$EH_x + H^{\circ} \rightarrow EH^{\circ}_{x-1} + H_2$ Equation 2.9

$EH^{\circ} + H^{\circ} \rightarrow E + H_2$ Equation 2.10

The number of hydrogen radicals is primarily determined by the oxygen supply to the atomizer and, if insufficient, thermal decomposition of the hydride may occur (if the temperature is high) and lead to the formation of dimeric (and tetrameric for arsenic) species or, in the absence of hydrogen, to oxides, with consequent loss of sensitivity.

Also used for atomization sources are the flame-intube devices, in which the excess hydrogen generated during the reduction step is used to carry the hydrides to a T-shaped quartz tube. A small amount of oxygen or air is added to support the combustion of a small flame. Although more complicated than the simple quartz tube, this system does not exhibit any significant analytical advantages over the latter but has been found useful for mechanistic studies relating to atomization processes.

2.1.14.1c Detection

Detection systems employed with hydride generation approaches are conventional AA spectrometers, usually fitted with intense electrodeless discharge or hollow cathode lamp sources. Quartz tube cells are of suitable dimensions to be compatible with the optical systems of all modern spectrometers. Background correction is usually achieved in double-beam optics using deuterium sources, and Zeeman-effect background correction can be implemented when the graphite furnace is used as the atomization cell.

2.1.14.1d Interferences

Chemical interferences may occur in the liquid phase during formation and release of the hydride or in the gas phase during its transport to the atomizer or within the atomizer. The extent and severity of these interference effects vary widely and are dependent on the instrumentation and hydride generation system used. A ‘physical’

interference, often referred to as a kinetic interference, may arise as a result of a difference in the release rate of the hydride from solution due to a volume effect or perhaps to sample foaming. These interferences are only encountered in direct systems where the measurement is performed online; they do not occur when the hydride is collected.

Spectral interferences are essentially absent in hydride generation AAS because the analyte is completely separated from the sample matrix. Minor fluctuations that may occur in the baseline during sample introduction are easily compensated for with conventional background correction systems.

2.1.14.2 Quantitative Analysis by Atomic Absorption

The atomic absorption process consists of the following steps: light at a specific wavelength of initial intensity I_o passes through the absorbing layer containing ground-state analyte atoms. The initial light intensity decreases by an amount determined by the atom concentration in the absorbing layer and the reduced intensity I is measured. The absorbance A, i.e., the logarithm of the ratio between the initial light intensity and the reduced intensity, is proportional to the concentration of free analyte atoms in the absorbing layer according to the Beer–Lambert law:

$$A = \log I_0/I = abc$$

where a is the absorption coefficient, a constant characteristic for the absorbing species at a specific wavelength, b is the length of the absorbing layer, and c is the concentration of the absorbing species.

Several related terms are used to define the amount of light absorption: the transmittance

$$T = I/I_0,$$

Also expressed in percentage terms,

$$T\ \% = 100 * I/I_0$$

The percent absorption

$$A\% = 100 * T\%,$$

which is the complement of percent transmission.

For analytical purposes the absorbance A is mostly used, which is proportional to the concentration of free gaseous atoms of the analyte under definite experimental conditions. The formation of free gaseous atoms from the analyte species present in the sample solution involves several processes, e.g., desolvation, volatilization, dissociation of the chemical compounds.

The efficiency of these processes for the given experimental conditions is included in the calibration equation. The calibration relationship is established by measuring the absorbances of standard solutions containing known amounts of analyte and plotting the absorbance data against concentration.

Hydride generation techniques significantly improve atomic absorption spectrometry (AAS) concentration detection limits while offering several advantages:

1. separation of the analyte from the matrix is achieved which invariably leads to improved accuracy of determination;
2. preconcentration is easily implemented;
3. simple chemical speciation may be discerned in many cases;
4. the procedures are amenable to automation.

Disadvantages with the approach that are frequently cited include interferences from concomitant elements (notably transition metals), pH effects, oxidation state influences (which may be advantageously used for speciation) and gas-phase atomization interferences (mutual effect from other hydrides).

2.2 Buruli Ulcer

Buruli ulcer (BU) is a disease of the skin and subcutaneous tissues caused by *Mycobacterium ulcerans*, a slow-growing environmental bacterium that grows optimally at temperatures from 30° C - 33°C. The causative organism *Mycobacterium ulcerans*, although different, belongs to the same family of organisms that cause leprosy and tuberculosis.

The restricted growth temperature is thought to limit infection to the cooler parts of the body; most ulcers occur on the extremities; lesions on the lower extremities are almost twice as common as those on the upper (Marston et al. 1995; WHO, 2000). The disease affects all age groups, but children under the age of 15 years are predominantly affected. There are no sex differences in the distribution, but BU commonly affects rural communities with limited access to health care.

Buruli ulcer presents itself in several ways; clinically it often starts as a painless, raised skin lesion or papule or with a hard sub-cutaneous nodule. Infection often leads to extensive destruction of skin and soft tissue with the formation of large ulcers usually on the legs or arms. Infection extends from the skin into the subcutaneous tissue and often invades underlying muscle tissue. In other cases it presents as an extensive area of edema or swelling. Tissue underlying these areas of edema is necrotic and the edematous region usually breaks down to form a large ulcer. The disease also can present as a firm, painless plaque of a well-demarcated lesion of

irregular edges with a reddened or discolored appearance. Ulceration can be extensive and disfiguring, often affecting 50% or more of a limb

The disease is of public health importance because the exact mode of its transmission is unclear, and its treatment with antimicrobials has not been very successful especially when the disease gets to the ulcerative stage (WHO, 2001; Amofah et al, 2002). It progresses slowly which is usually painless and patients are systemically well (Duker et al, 2004). These factors contribute to lesions which are often extensive because many patients do not seek medical authorities until there is considerable tissue destruction (Thanjaraj et al. 1999; WHO, 2000) accompanying complications, fever and pain due to the secondary infection of the lesions (National Institutes of Health, 1996). Furthermore, the absence of effective treatment worsens the prognosis due to complications that leads to impairment of body functions with long-term socio-economic impact (Walsh et al, 1999; Gyasi et al 2012a). In spite of all these data, plausible clue leading to a full pathogenesis of this tropical disease is yet to be fully explored.

The study by Gyasi et al., 2012a in the Amansie West District, Ghana where in a cross sectional cohort study, arsenic levels were monitored over a period of 18 months and compared based on endemicity and also a work published in 2004, Duker and a group of workers established that arsenic in the environment could be implicated in BU infection using spatial dependency in the same district.

Increased human activities (eg. mining) and urban development remove riparian forests and also change water quality through both point and non-point source pollution; these factors may in turn be tied to ecological changes and Buruli ulcer incidence. Duker *et al.* (2004) found significant spatial relationships among villages

in Buruli ulcer affected areas and arsenic-enriched surface waters and adjacent farmlands. The authors suggested that increased Buruli ulcer risk was related to immunosuppression resulting from the consumption of arsenic-enriched drinking water and food crops. This hypothesis has not been clinically tested. Additionally, many water bodies associated with increased sedimentation and eutrophication have characteristically low dissolved oxygen concentrations that are documented to enhance the growth of *M. ulcerans*

Arsenic in water and soil has been proposed to be a possible contributory factor in *Mycobacterium ulcerans* infection in Buruli Ulcer (BU) endemic areas Gyasi et al, 2012a. In another study by Gyasi et al, 2012b, 0.8 - 4.8 mg/L of arsenic was introduced to into ICR mice which were synonymous to arsenic detected from streams and soils in Buruli Ulcer endemic communities of the Amansie West District of the Ashanti Region of Ghana, via their drinking water. Mice with arsenic exposure of 4.0 - 4.8 mg/L developed inflammation, erythema and open ulcers on skin (with scab formation).

The high levels of arsenic in tissue (possibly from accumulation) cause inflammation, erythema and open ulcers on the skin, and have the potential to cause liver and spleen damage, and red blood cells microcytosis in ICR mice. Its immunosuppressive potential could enhance vulnerability to *Mycobacterium ulcerans* infections in Buruli Ulcer endemic areas.

2.3 Mining

Arsenic is a widely dispersed element in the Earth's crust and exists at an average concentration of approximately 5 mg/kg. Arsenic primarily exists as arsenopyrite and as a constituent in several other sulfide minerals. Significant natural contamination of surface waters and soil can arise when arsenic-rich geothermal fluids come into contact with surface waters. When humans are implicated in causing or exacerbating arsenic pollution, the cause can almost always be traced to mining or mining-related activities. Arsenic minerals exist in the environment principally as sulfides, oxides, and phosphates. Historical arsenic contamination exists in Cornwall, UK; an example of a recent arsenic pollution event is that of Ron Phibun town in southern Thailand, where arsenic-related human health effects have been reported. US; and consumption of contaminated foodstuffs (China) and exposure to wood preserving arsenicals (Europe and North America).

Arsenic and gold are often related in hard rock gold mines, where gold often occurs as tiny blobs within the arsenopyrite. During gold mining the gold is removed, and arsenic is left behind in earth extracted during mining which is called mine tailings. Mine tailings near goldmines may contain high levels of arsenic and this is washed down the river and out to sea. Because gold- and arsenic-bearing minerals coexist, there is a hazard of mobilizing arsenic during gold mining activities. The Ashanti region of central Ghana currently faces this as a real risk.

The mineral deposit in the district is composed of pyrite, arsenopyrite, minor chalcopyrite, sphalerite, galena, native gold and secondary hematite which are associated with arsenic and sulphur (Robb *et al*, 1999).

The mining processes release airborne particles and large quantities of arsenic pollutants into the atmosphere and water bodies in the environment. These may however have adverse effect on vegetation, animals and finally on human being living in the area

CHAPTER THREE

3 Materials and Experimental Methods

This chapter deals with the materials and equipment used for the analyses, the samples and sampling procedures, the procedures for determining the physicochemical parameters, the arsenic concentrations in maize, fish and human urine samples collected from some towns in the Amansie West District.

3.1 Materials Used For the Analysis

The materials used in the analysis are, the chemical reagents, the glass wares and equipment, maize, fish and human urine samples

3.1.1 Reagents

All reagents were of analytical grade (BDH chemical limited, Poole, England) except otherwise stated. Distilled water was used for the preparation of all solutions.

- 98 % Arsenic trioxide (As_2O_3),
- 37 % Hydrochloric acid (HCl), 11.18 M
- 37 % Hydrochloric acid, 6 M
- 63 % Nitric acid (HNO_3) 15.6 M
- 60 % Perchloric acid ($HClO_4$), 2 M
- 96 % Potassium iodide, 2 % ($^{w}/_{v}$) (analar Phillips reagent, England)
- 99 % Sodium borohydride ($NaBH_4$), 0.6 % ($^{w}/_{v}$)
- 96 % Sodium hydroxide (NaOH) 0.5 % ($^{w}/_{v}$)
- 98 % Sulphuric acid (H_2SO_4), 13.39 M
- Orion thermoscientific standard buffer solutions of pH 4.01, 7 and 10.01

3.1.2 Glassware and Equipments

The main glasswares used for the chemical analyses are:

- beaker (50 ml, 100 ml)
- conical flasks (50-250 ml)
- digestion and filter flasks
- measuring cylinder (50 ml)
- pipette (5 ml, 10 ml)
- volumetric flask (50 ml, 250 ml)

The major equipments used in the analyses are:

- Orion 5 star thermo scientific meter (measures pH, conductivity and temperature), USA
- Varian AA240FS (Fast Sequential AAS) Spectrophotometer, Australian
- Analytical balance
- Hot plate (burner) and oven

3.1.2.1 Cleaning of Glassware

Glasswares were first were extremely dirty were soaked in detergent solution and 10% HNO_3 overnight depending on the degree of dirt accumulated on the glassware, washed under running tap water and rinsed with 1 L distilled water and were then dried.

3.2 Sampling Site, Sample Collection and Preparation

The area within which the sampling was done, how the samples were collected and how they were treated before the analyses were done are described here

3.2.1 Sampling Site

The Amansie West district is located in the south-western part of the Ashanti Region. It shares common boundary on its Western part with the Atwima District. On its northern part, can be found the Bosomtwe District, while a regional boundary separates it from Western Region on its southern part as shown in Figure 1.1.

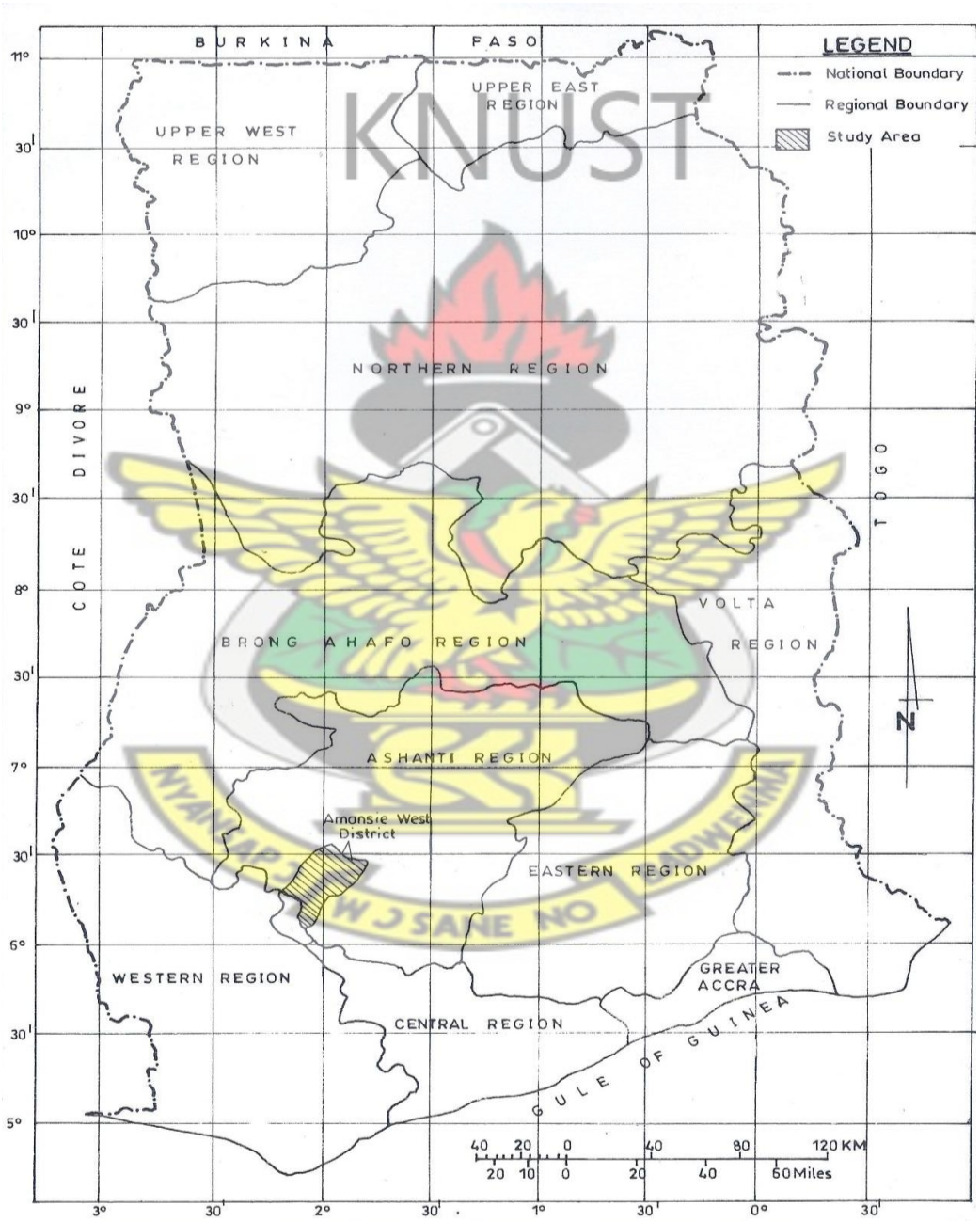


Fig. 3.1 Map of Ghana showing Amansie West District in the Ashanti Region

The Amansie West District lies between latitudes 6°N and 6°45'N and longitudes 1°30'W and 2°15'W. It covers an area of about 1364 km^2. The district capital, Manso Nkwanta, is about 40 km south of Kumasi. The topography of the district is generally undulating with an elevation of 210 m above sea level. The most prominent feature is the range of hills, which stretches across the north-western part of the district, especially around Manso-Nkwanta and Abore. These hills have an elevation between 560 m and 630 m. The district is drained in the north by the Offin and Oda rivers and their tributaries such as Jeni, Pumpin and Emuna. Vegetation in the district is composed mainly of secondary forests, thicket, forb regrowth (i.e., soft-stemmed leafy herbs, mostly the weeds, which appear on farms and have to be cut regularly) and swamp vegetation. Vegetation thrives in ferric fluvisols, which are the major soil types in the district. These soils have been developed through yearly rainfall ranging from 125 to 200 cm with temperatures of 22 to 30 °C. The landscape of the district varies from gentle to broken (Duker *et al*, 2004).

The district is underlain by lower proterozoic birimian and, to a lesser extent, Tarkwaian rocks. Throughout Ghana, Birimian rocks of West Africa are mainly volcanic greenstones with intervening sedimentary rocks and granitoid intrusions, in places containing deposits composed of pyrite, arsenopyrite, minor chalcopyrite, sphalerite, galena, native gold and secondary hematite (Robb et al, 1999)

The district has about 310 settlements (though not all these settlements are mapped) with a population of 108,726 in the year 2000. There are approximately equal percentages of males and females (49 % and 51 %, respectively), of whom 70 % are farmers and 22 % are engaged in legal and 'galamsey' (or illegal) mining (Duker 2005).

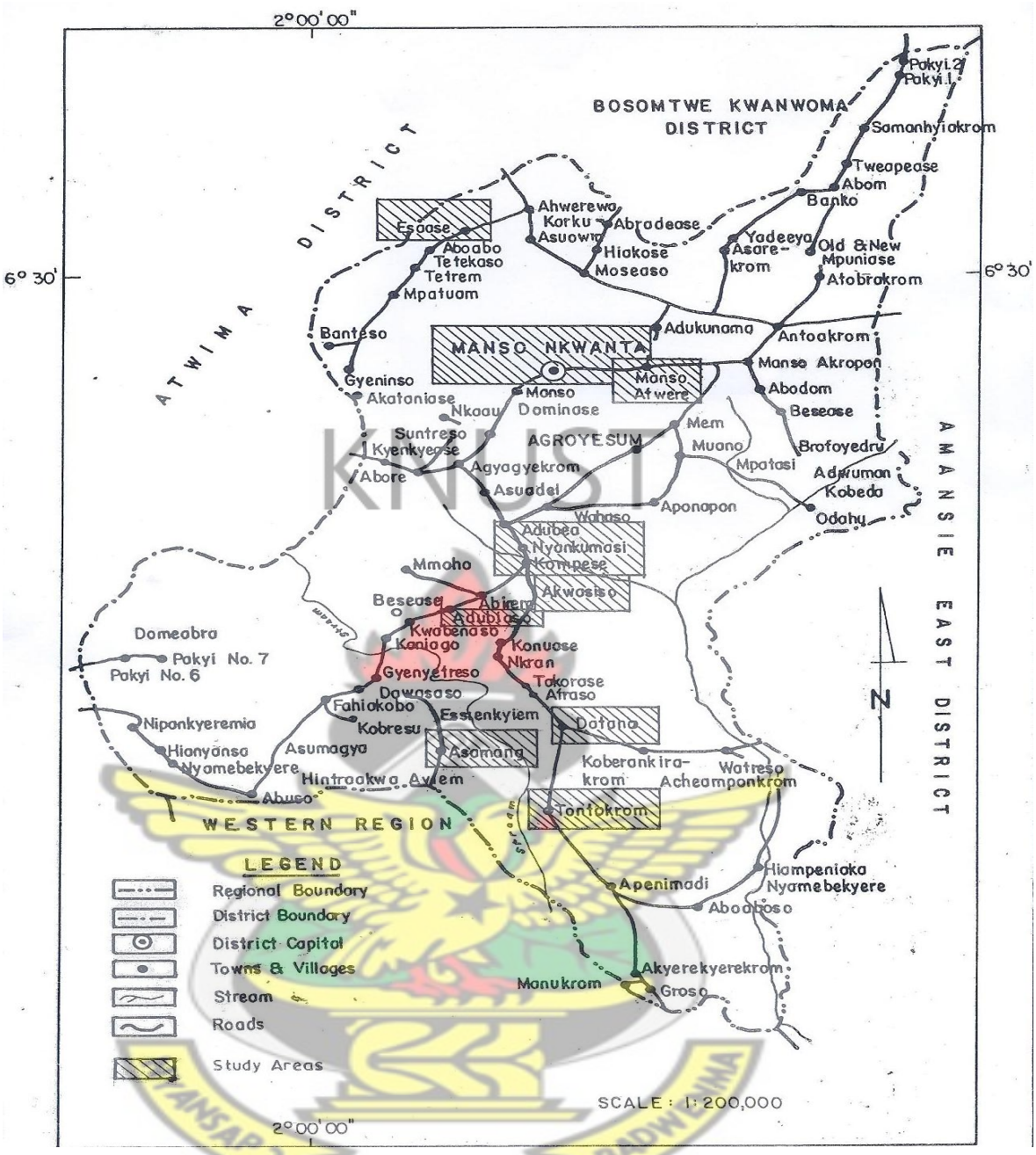


Fig. 3.2 Map of Amansie West District showing some of the study areas

The study area is from the north through the central to the southern part of the Amansie West District as depicted in the figure above.

3.2 .2 Sampling

Sampling procedures for the maize, fish, and urine samples are described below.

3.2.2.1 Sampling of Maize

At the time of the sampling, maize from the farms had all been harvested so maize were sampled from households that had stored their maize grown within the Amansie West District in barns.

About 50 to 100 g of maize samples were taken from five to six different household within towns located in the district. Families confirmed that the maize were from farms within the district.

These samples were put into separate transparent polythene bags and labeled. The samples were labeled B, T, DT, DD, NN, NK, YK, AS, TK, AK, KN, AT, TT, MP and ES which represent the following towns Bonsaso, Tontokrom, Datano, Dadiase, Nkensere Nkwanta, Nyankumasi, Yawkrow, Asamang, Takorasi, Akwasiso, Koninase, Atwere, Tetrem, Mpatuom and Esaase respectively from where the samples were taken. All the maize samples were then put in a dried resealable polyethene bags and brought to the laboratory. The samples were then stored in a cool dried locker.

3.2.2.2 Sampling of Fish

The fish were sampled from the River Offin and streams on daily basis. Those from River Offin were picked upstream and downstream. The fish collected upstream around Adobewura were labeled U1 to U15 and the ones collected downstream at Ntonbroso were labeled D1 to D15.

In towns where some maize and urine were sampled, there are streams that mining activities were going on which made parts of the water stagnant. The fish sampled

from these streams were labeled ST1 to ST13. The fish were then kept under ice in an ice chest, transported to the laboratory then stored in a freezer for further analysis

3.2.2.3 Sampling of Human Urine

The urine samples were collected in the morning on different days from twenty three (23) males and fourteen (14) females of various occupations based on questionnaires (socio-demographic questions) shown in Appendix S answered by subject in the community, i.e. traders, miners, pupils, farmers and teachers and the source of their food stuffs whether is within the community or outside the community.

The samples were collected into polyethylene bottles and duly labeled. pH and conductivity measurement were carried out on the samples. The samples were then brought to the laboratory and store in a fridge for further analyses

3.2.3 Sample Preparation

The maize samples from the various household and barns were mixed and divided into two parts for each town and then labeled as B1, B2, T1, T2, DT1, DT2, DD1, DD2, NN1, NN2, NK1, NK2, YK1, YK2, AS1, AS2, TK1, TK2, MP1, MP2, KN1, KN2, AT1, AT2, TT1, TT2, AK1, AK2, ES1 and ES2 representing the various towns. They were then milled into a finely powdered form using a blender and stored in well labeled resealable polythene bags.

The fish were allowed to thaw; the edible parts of the raw fish were removed, chopped and divided into two parts. One part was blended in distilled water to form 1% (w/v) fish suspension and the other part was dried in an oven at a temperature of about 60 °C for 2 hours. They were milled into powdered form, packaged nicely in polythene bags and labeled as above from the sampling site for further analysis.

3.3 Preparation of reagents and Experimental Procedure for the Determination of Physicochemical Parameters and the Arsenic levels in the Samples

In this section, how the reagents used in the analyses were prepared and the experimental methods used in the various determinations are outlined.

3.3.1 Reagents Preparation

- Stock Standard Arsenic Solution: 0.264 g of As_2O_3 was dissolved in minimum volume of 20 % ($^{w}/_{v}$) NaOH, neutralized with HNO_3 and was diluted to 1 liter to give 200 µg/mL As for the stock arsenic standard solution.

Solutions for Recovery: 5 µg/mL , 10 µg/mL, 20 µg/mL and 30 µg/mL of Arsenic solutions were prepared by diluting 2.5, 12.5, 25 and 37.5 mL respectively of the 200 µg/mL Arsenic stock solution and made to volume in100 mL volumetric flasks.

- 0.6 % Sodium borohydride solution was prepared by weighing 3.000 g of $NaBH_4$, dissolved into 500 mL volumetric flask and made to volume.
- 0.5 % Sodium hydroxide was prepared by dissolving a weighed 5 g of sodium hydroxide in a beaker. It was then transferred into a 500 mL volumetric flask and made to the mark with distilled water. 20 % NaOH was also prepared in the same way but the weighed sodium hydroxide was 20 g.
- The 6 M hydrochloric acid was prepared by diluting 268.4 mL of the stock (11.18 M) hydrochloric acid in a 500 mL volumetric flask

3.3.2 pH Determinations on the Sampling Field

Before the pH determinations, the pH probe was calibrated and it was done as follows:

First, three distinct Orion Thermo scientific standard buffer solutions (with pHs of 4.01, 7.00 and 10.01) which were already prepared were used for the calibration. The

buffer solution of pH 4.01 made of deionized water, potassium hydrogen phthalate and amaranth dye, buffer solution of pH 7 is made of deionized water, Na_2HPO_4, KH_2PO_4, Na_2CrO_4, and $K_2Cr_2O_7$ and buffer solution pH 10.01 is also made of deionized water, $NaHCO_3$, Na_2CO_3, methyl paraben.

The pH probe was immersed into the lowest pH solution (4.01) and the pH reading was allowed to stabilize. The control knob was adjusted until the expected pH (4.01) was read. The probe was rinsed in distilled water, immersed in the other solutions (of pH 7 and 10.01) and the knob was adjusted to read the expected values. The pH meter was calibrated by immersing the pH electrode, in turn in 25 mL of the buffer solutions, and then regulating the knob of the pH meter until the expected reading was obtained. The electrode of the pH meter was washed thoroughly with distilled water before it was immersed in another buffer solution. The calibration was done at a temperature of 25 °C.

The pH of the urine samples were determined of which after every determination, the electrode was wash thoroughly with distilled water. 1 % HCl was added to the samples, the plastic bottles were labeled, kept under ice in an ice chest and transported to the laboratory and kept in a fridge.

3.3.3 Determination of Conductivity of the Urine on the Field

The conductivity probe was calibrated before any determinations were made. The calibration was done by immersing the electrode which had been washed with distilled water into 25 ml standard 0.01 N KCl solution. The conductivity meter was adjusted to read 1413 µS/m for the KCl solution and the value saved on the meter.

The electrode was then removed, washed with distilled water and then immersed again into the KCl solution to confirm the conductivity (APHA, 1998).

The conductivity readings were recorded by immersing the probe into the urine samples each and rinsing the probe with distilled water after every reading.

3.4 Determination of Physicochemical Parameters

The pH, moisture and ash contents of the maize and fish samples, conductivity of urine samples were determined as follows:

3.4.1 pH Determinations

3.4.1a Maize

A weighed amount of 5 g of each milled maize sample was soften by soaking in 100 mL of distilled water in a 250 mL pyrex beaker and swirled for about 15 minutes to get a homogeneous mixture. The mixture was then filtered into a small sterilized plastic container and the pH of the filtrate determined.

The pH for the maize solutions was determined by immersing the pH electrode in the filtrates of the maize samples. The pH value indicated on the pH meter was recorded when the reading was stable in each case. The electrodes were washed thoroughly with distilled water after every determination to avoid cross contamination. For each sample, the pH determinations were made in triplicate and the mean taken to represent the pH of the sample.

3.4.1b Fish

The fish suspensions were filtered and the pH was determined by immersing the pH meter into the filtrate and recording the value. The pH was measured with a glass electrode (Orion 5 star Thermo Scientific meter) after standardizing the pH meter with pH 4.01, 7.00 and 11.01 buffers.

3.4.2 Determination of Ash Content of Maize

An aluminium dish was heated in an oven at 250 °C for one hour and cooled. The dish was weighed accurately and about 5 g of the milled maize sample added and was spread out evenly on it, then reweighed. The sample was dried and charred by heating at 250 for about 5 hours. The dish containing the ash was allowed to cool and re-weighed. The dish was reheated for 30 minutes, cooled and reweighed. This was done till successive weighings were constant. All maize samples were treated as above for the ash content determination.

The ash content was determined as follows:

$$Ash\ Content(\%) = {Weight\ of\ Ash}/{Weight\ of\ maize} \times 100$$

3.4.3 Determination of the Moisture Content of Fish

An aluminium container with a loose fitting lid was dried in an oven at 100°C and the weight measured accurately. 5 g of the edible part of the fish sample (chopped) was spread evenly over the bottom of the container; the lid put on and then weighed again. The container was placed in an air oven at a temperature of 101 °C for 5 hours. The samples were then allowed to cool in at ambient temperatures and then reweighed.

The drying process was repeated at the same temperature and at time intervals of 1 hour until a constant weight was achieved.

$$Moisture\ Content\ (\%) = \frac{Weight\ of\ wet\ fish\ - Weight\ of\ dried\ fish}{Weight\ of\ wet\ fis} \times 100$$

3.5 Determination of Arsenic Concentration in Maize, Fish and Urine Samples

Total Arsenic concentrations were determined in maize, fish and urine samples from some selected towns in the Amansie West District. The samples were first digested and filtered before the concentrations were determined. The analytical method was atomic absorption spectroscopy for the determination of the total arsenic as outlined below.

3.5.1 Digestion of Maize, Fish and Urine Samples

The digestion procedure used was adapted from Toxicological Profile for Arsenic, Analytical methods, - ATSDR..

3.5.1a Fish and Maize

The fish were allowed to thaw and dried in the oven until all the moisture was removed and then the dried fish were then ground using blender into fine powdered form. The maize (kernel of corn) was removed from the cob and ground into finely powdered form using a blender. Then 5 g of the powdered form was weighed and transferred into separate digestion flask.

For the digestion, 20 mL of HNO_3 and H_2SO_4 in the ratio of 3:1 was added to 5 g of the powdered samples. The mixture was heated to about 100 °C and there was evolution of brown fumes. The solution was allowed to cool then 20 mL of $HClO_4$ is added. The heating was continued until the solution was reduced to about 5 mL. The solution was allowed to cool again, filtered through a Whatman filter paper and 1 mL of HCl was added. The sample solution was transferred into a 250 volumetric flask and made up to volume with distilled water. The samples solution were stored in bottles with plastic cork and analyzed. The digestion process was repeated for all the fish samples. The sample reagent blank was also prepared in the same way

3.5.1bUrine

The digestion of the urine samples were taken through the following procedures

To each 25 mL of the urine samples measured into separate digestion flask, 40 mL of HNO_3 and $HClO_3$ were added. The sample solution was heated to about 120 ºC then the heating temperature was raised to about 180 ºC until solution was reduced to volume between 5 and 10 ml. The solution was cooled, transferred into a 250 volumetric flask and diluted to volume with 0.1 M HNO_3. The sample solutions were stored in bottles with plastic cork for further analysis. The sample reagent blank was also prepared in the same way

3.5.2 Determination of Levels of Arsenic Using Hydride Generation Atomic Absorption Spectroscopy

The atomic absorption spectroscopy is based on the property of atoms of elements emitting or absorbing at a specific electromagnetic radiation under certain physical conditions. The instrument was equipped with argon to drive the hydride system.

3.5.2a Instrumentation

The instrument was set up according to manufacturer's specification. The HCl (6 M) and $NaBH_4$ (0.6%) generate the hydride.

Table 3.1 Instrumental Parameters for the Atomic Absorption in the Varian AA240FS (Fast Sequential AAS) Spectrophotometer for Arsenic

PARAMETER	VARIAN AA 240FS
Lamp Current	10 mA (Arsenic hollow cathode)
Wavelength	193.7 nm
Slit	0.5 nm
HCl	6 M at 1 mL/min flow rate
NaOH	0.5%
Detection limit	0.001 mg/L
$NaBH_4$	0.6% at 1.5 mL/min
Fuel	Acetylene
Support/Oxidant	Air

Before any measurement was made, potassium iodide was added to all digested samples to convert any pentavalent arsenic into trivalent

1. Three different capillaries were inserted into the digested solution, the HCl (6 M) and $NaBH_4$ (0.6%) simultaneously.
2. The hydride generation system sucked up (aspirated) the digested sample, sodium borohydride and HCl and mixed them up.
3. A volatile hydride of the analyte (arsenic) was created from the reaction.

4. The gas-liquid separator separated the gas (hydride) and any liquid present.
5. The liquid drained down and collected with a container (waste) but the hydride flowed to the optical cell.
6. The hydride form of the metalloid was decomposed and created atoms of the element of interest.
7. The monochromator removed scattered lines of other wavelengths and by so doing, only a narrow spectral line or band reached the photomultiplier tube (PMT).
8. The PMT as the detector determined the intensity of photons of the analytical line and the results (concentration in mg/L) was displayed.

Procedures 2 to 8 are in-built processes.

3.6 Recovery

The standards were taken through the above procedure, followed by the blanks (prepared using only the reagents) and then the samples. All determinations were made in triplicate for reproducibility. During the analyses, distilled water was run in between each determination to avoid cross contamination and also, after every five determinations, the standards were run to check the reliability of the instrument

CHAPTER FOUR

4 Results and Discussions

Results obtained for the chemical analyses of the distilled water used in the analyses, the blanks, the maize, fish and urine samples are presented and discussed in this section.

4.1 Results obtained for the distilled water and blanks used for the Analysis

The pH of the distilled water used for the analysis recorded 6.76 and this may be due to the dissolution of atmospheric carbon dioxide (CO_2) into the water that has made it slightly acidic. The conductivity of the distilled water was recorded as 4.5 μS/cm as a result of the water may not be fully deionized.

The analysis of arsenic of the blank for the analyses of maize and fish recorded in triplicate 0.001 mg/L, 0.000 mg/L and 0.000 mg/L and the average was 0.000 mg/L. The blank prepared for the urine analysis was recorded in triplicate 0.001 mg/L, 0.000 mg/L and 0.001 mg/L and the mean concentration is 0.001 mg/L which was subtracted from all readings of the urine arsenic concentrations

These values were factored into the results presented in this study.

4.2 Maize

The pH, conductivity, ash content and arsenic concentrations of the maize samples are presented in the table on the next page with their standard deviation.

Table 4.1 The pH, Conductivity, Ash Content and Arsenic Levels in Samples of Maize from Some parts of Amansie West District.

Samples	pH	Conductivity (µS/cm)	Ash Content (%)	Mean Arsenic Concentrations (mg/L)	Arsenic Concentrations (mg/kg)
B1	6.49	3030.61	20.81	0.005±0.002	0.25
B2	6.73	2960.93	19.73	0.004±0.003	0.20
T1	6.19	2708.52	21.31	0.007±0.002	0.35
T2	6.03	2424.01	19.70	0.007±0.005	0.35
DT1	6.54	3254.70	17.45	0.005±0.003	0.25
DT2	6.65	3310.57	19.21	0.004±0.002	0.20
DD1	6.5	3192.49	18.94	0.005±0.001	0.25
DD2	5.98	1837.82	20.03	0.007±0.004	0.35
NN1	5.88	1672.49	17.76	0.006±0.001	0.30
NN2	5.59	1508.43	19.82	0.008±0.002	0.40
NK1	5.71	1729.37	17.87	0.008±0.005	0.40
NK2	5.89	1830.45	16.91	0.006±0.003	0.30
YK1	6.57	3267.68	17.23	0.004±0.001	0.20
YK2	6.49	3094.41	18.89	0.005±0.007	0.25
AS1	6.02	2231.27	19.78	0.004±0.002	0.20
AS2	5.92	1747.93	20.73	0.006±0.001	0.30
TK1	6.9	3371.41	17.06	0.003±0.000	0.15
TK2	5.7	1645.87	19.90	0.005±0.003	0.25
AK1	6.17	2692.43	18.52	0.007±0.002	0.35
AK2	6.59	3153.59	19.90	0.005±0.001	0.25
KN1	5.6	1618.72	18.52	0.008±0.001	0.40
KN2	5.41	1590.38	19.65	0.009±0.000	0.45
AT1	6.7	3275.06	16.94	0.004±0.001	0.20
AT2	6.02	2239.69	18.45	0.006±0.003	0.30
TT1	6.45	3038.74	19.63	0.007±0.002	0.35
TT2	6.35	2910.19	18.97	0.006±0.001	0.30
MP1	5.43	1632.26	17.01	0.004±0.001	0.30
MP2	6.02	2278.58	16.90	0.006±0.002	0.30
ES1	6.53	3269.81	18.31	0.005±0.001	0.35
ES2	6.56	3191.57	15.87	0.008±0.003	0.40

The study showed that the pH of maize sample solutions was found to be slightly acidic ranging from 5.41 to 6.90 with an average at 6.18. DD2, NN1, NN2, NK1, NK2, AS2, TK2, MP1, KN1, KN2, had their pH less than 6. From the results in Table

4.1, it can be observed that the pH has a little effect on the concentration of arsenic in maize though from literature, a decrease in pH gives a higher arsenic concentration (Ferguson et al, 1972). For instance, in ES_1 the pH was 6.53 and the arsenic concentration was 0.35 mg/kg and as the pH increased a little inYK1 to 6.57 and the arsenic concentration decreased to 0.02 mg/kg. The graph in Appendix D showed a poor correlation between pH and arsenic concentration of which was 0.423. The pH may be due to the varied composition of the soil, the hydrogen ion content of the soil from which the maize plant absorbed it's nutrients from and the arsenic content of the soil since the mining activities do not operate within the same perimeter of every farm. The mobility and solubility of metals (arsenic) increase with decreasing pH. At low pH, hydrogen ions compete with metals for exchange sites on particles and this is reflected in the results obtained. Mean arsenic concentration for the soils sampled in the various communities of Amansie West was found to be 1.464 mg/kg by (Gyasi et al, 2012a) and this value is high enough for the maize plant to absorb in to various tissues of the plant including the kernels.

The study also revealed the conductivity of ions in the maize sample solutions ranged from 1508.43 to 3371.41 µS/cm with the average being 2523.13 µS/cm. Conductivity depends on the presence of ions, their total concentration, mobility, valence and relative concentrations and on the temperature of the measurement (APHA *et al*., 1998). High conductivity corresponds to high concentration of ions in solution.

From the scattered diagram in Appendix E, a significant correlation was not found between the conductivity and the arsenic concentration in maize ($r^2 = 0.276$). This is to be expected since conductivity does not only depend on arsenic, it may be high as observed in the Table 4.1 above but that does not mean it is necessarily arsenic rather it could be due to the presence of other ions.

From Appendix F the maize labeled KN2 which was from Koninase recorded the highest concentration of Arsenic of 0.045 mg/kg at a corresponding pH of 5.41 and TK1 (Takorasi) recorded the lowest concentration of Arsenic of 0.015 mg/kg at pH of 6.90. These variations may be due to the maize from Koninase farms being around the perimeter of the mining activities and that from Takorasi may be far from the mining operation centers or the soil pH may have a higher effect on the absorption of arsenic into the maize plant and subsequently to the maize kernels of Koninase and a lower effect from the maize from Takorasi

4.3 Fish, *Clarias agboyensis*

The results of the physicochemical analysis and the arsenic concentration of the fish sampled from the river Offin and streams in the Amansie West District are presented and discussed below.

4.3.1 Fish from Upstream

Table 4.2 Physicochemical Properties and Arsenic Concentration Levels in the *Clarias agboyensi* from upstream of River Offin around Adobewura.

Labeled Samples	pH	Moisture Content (%)	Ash Content (%)	Mean Arsenic Concentration (mg/L)	Arsenic Concentration (mg/kg)
U1	6.59	63.16	15.64	0.042±0.003	2.10
U2	5.34	64.87	13.14	0.065±0.001	3.25
U3	6.81	55.98	16.27	0.039±0.004	1.95
U4	5.98	61.8	10.82	0.054±0.002	2.52
U5	6.06	58.47	9.98	0.051±0.002	2.51
U6	5.49	60.79	13.43	0.063±0.001	3.15
U7	6.87	57.55	12.22	0.038±0.001	1.90
U8	6.23	63.83	14.73	0.059±0.004	2.95
U9	6.47	66.75	13.38	0.043±0.002	2.15
U10	6.12	59.03	12.89	0.053±0.001	2.65
U11	6.19	49.2	12.87	0.055±0.002	2.75
U12	6.61	59.58	10.35	0.042±0.003	2.10
U13	6.45	60.65	12.79	0.049±0.005	2.45
U14	6.84	54.89	15.25	0.038±0.002	1.90
U15	5.47	65.2	11.02	0.061±0.003	3.05

The study revealed the pH measurement for the fish *Clarias agboyensis* sampled from upstream U1 to U15 around Adobewura ranged from 5.34 to 6.87 with the mean value being 6.23. The high acidity recorded 5.34 (Table 4.2) led to an increase in the release of arsenic in the fish solution and this has been observed in the concentration of arsenic in the fish U2 of 3.25 mg/kg as shown in Appendix I. The highest pH recorded 6.87 of the fish U7 recorded the lowest arsenic concentration of 1.90 mg/kg. Also

1.90 mg/kg of arsenic concentration was recorded for the fish U14 which had the pH of 6.84.

There was an extremely good correlation (r^2 = 0.873) between arsenic concentration and pH which was depicted in Appendix G. As the pH of the fish solution decreased, the arsenic concentration increased although there were slight variations in the pH of the fish which resulted in the same arsenic levels of some of the fish for instance, U7 and U14 had 1.90 mg/kg. This variation may be due to the difference in hydrogen ion activity of the water, the age difference of the fish, and movement of the fish from one point of the river to another and also other substances that were introduced into the water may affect the arsenic intake of the fish.

The average moisture and ash contents were respectively 60.12 % and 12.98 % and these two did not have any association with the arsenic concentration levels in the fish but the material component did not have any reflection in the results obtained.

4.3.2 Fish from the downstream

Table.4.3 Physicochemical properties and Arsenic Levels in the fish *Clarias agboyensi* from downstream of River Offin

Labeled Samples	pH	Moisture Content (%)	Ash Content (%)	Mean Arsenic Concentration (mg/L)	Arsenic Concentration (mg/ kg)
D1	6.64	56.23	12.78	0.042±0.001	2.10
D2	5.21	59.34	15.35	0.079±0.004	3.95
D3	5.96	61.05	11.23	0.063±0.001	3.20
D4	6.49	58.45	9.79	0.054±0.002	2.70
D5	5.02	65.72	14.38	0.096±0.005	4.80
D6	6.91	62.19	11.56	0.039±0.001	1.95
D7	5.47	59.45	14.43	0.065±0.001	3.25
D8	6.24	63.85	13.83	0.052±0.002	2.65
D9	6.53	58.37	15.31	0.043±0.003	2.15
D10	5.07	62.78	14.9	0.081±0.002	4.05
D11	6.89	57.63	12.01	0.039±0.002	1.95
D12	6.02	65.17	9.89	0.057±0.001	2.85
D13	5.51	64.69	15.98	0.072±0.002	3.60
D14	5.89	56.84	11.76	0.055±0.003	2.75
D15	5.21	61.99	14.19	0.063±0.001	3.15

The pH recorded for the fish *Clarias agboyensi* solution D1 to D15 from downstream (Ntonbroso) ranged from 5.02 to 6.91. From Table 4.3, there is a relationship between the recorded arsenic concentration and the pH of the fish solution, as the pH increased the arsenic concentration decreased. The highest arsenic concentration recorded was 4.80 mg/kg and this was at a pH of 5.02 and the lowest arsenic concentration recorded was 1.95 mg/kg and this was at a pH of 6.91 for the fish D6. There was another arsenic concentration of 1.95 mg/kg recorded which was at a pH of 6.89. There was a very good correlation coefficient of 0.851 between the pH and the arsenic concentration of the fish found in the graph of Appendix I. From the bar chart in

Appendix J, D5 recorded the highest arsenic level of 4.80 mg/kg likewise D6 and D11 each recorded the lowest arsenic level of 1.95 mg.kg.

The moisture content of the D1 to D15 ranged from 56.23 % to 65.72 % and averaged 60.91 %. These values may not represent the actual moisture content because there may be other substances like the oil which may have evaporate with the water content. The ash content also varied from 9.79 % to 15.98 % and also averaged 13.16%. These two parameter moisture content and ash content did not have any effect on the arsenic concentration since there was a very poor correlation between these parameters and arsenic concentration.

4.3.3 Fish from the Streams and Ponds

Table 4.4 Physicochemical Properties and Arsenic Levels of the fish *Clarias Agboyensis* from Some Streams.

Labeled Samples	pH	Moisture Content (%)	Ash Content (%)	Mean Arsenic Concentration (mg/L)	Arsenic Concentration (mg/kg)
ST1	7.15	62.49	10.68	0.034±0.002	1.70
ST2	6.65	60.05	15.92	0.041±0.001	2.05
ST3	5.83	56.16	11.32	0.057±0.005	2.85
ST4	6.28	49.43	14.56	0.045±0.003	2.25
ST5	6.89	55.78	13.54	0.038±0.001	1.90
ST6	5.11	57.67	12.43	0.067±0.002	3.35
ST7	6.58	65.98	14.61	0.054±0.001	2.70
ST8	6.25	58.46	11.52	0.049±0.002	2.45
ST9	6.34	59.10	13.83	0.043±0.004	2.15
ST10	5.86	56.55	15.31	0.058±0.002	2.90
ST11	6.69	58.77	13.34	0.038±0.001	1.90
ST12	5.03	63.39	11.50	0.079±0.002	3.95
ST13	5.45	64.98	15.34	0.059±0.001	2.95

From Appendix G, there was a good correlation of 0.873 for pH with Arsenic concentration as depicted. And from Appendix H, ST6 recorded 3.35 mg/kg at pH of 5.11, ST11 recorded 3.15 mg/kg at pH of 5.21, ST14 recorded 2.95 mg/kg at pH of 5.45 and ST9 also recorded 2.15 at pH of 6.34 which shows that as the pH increased arsenic concentration also decreased.

At the highest pH of the 7.15, ST1 recorded the lowest arsenic concentration of 1.7 mg/kg and the lowest pH of 5.03, ST12 recorded the highest arsenic concentration of 3.95 mg/kg. Although there were some irregularities for example fish ST5, the arsenic concentration was 1.90 mg/kg and this was recorded at pH of 6.89 and for ST11 the arsenic concentration was 1.90 at a pH of 6.69 There was a significant relation between pH and arsenic concentration.

The moisture content of the fish was averagely recorded as 59.67 % and the moisture content had no relation with the pH and the arsenic concentration of the fish solutions. The ash content recorded 13.46 % as the mean value for the fish and the ash content had lower values as a result of the high moisture content and other substances like the oil in the fish volatilizes when the fish was exposed to high temperatures. The mean arsenic concentration recorded was 2.58 mg/kg but ranged from 1.7 mg/kg for to 3.95 mg/kg according to the table above.

From the Tables 4.2, 4.3 and 4.4, the pH recorded for the fish ranged from 5.34 to 6.87, 5.02 to 6.91 and 5.03 to 7.15 respectively. The pH of the fish solution from downstream had the lowest pH value (being slightly acidic) and the streams and ponds had the highest pH value (slightly alkaline) and these variations may be due to the source of waste from the mining activities that go into the river which might have accumulated making the fish sampled downstream having lower pH. The downstream

fish were highly acidic as compared to the upstream ones and these may be due to the upstream being the source and other effluent may be introduced into the river along from other tributaries.

From Appendix M, fish sampled downstream had higher mean arsenic concentration levels and the decreased in the acidity may be due to some biochemical processes for substances undergoing degradation or other chemical being introduced into the water might have taken place as the water flowed downwards, streams fish also were slightly acidic since the water is not able to flow freely as a result of the mining activities.

The differences in the pH of the fish reflected the same pattern of differences in the arsenic concentration of the fish although there were some irregularities which may be due to the age difference of the fish.

From Tables 4.2, 4.3 and 4.4, the arsenic concentration were from 1.7 mg/kg to 3.95 mg/kg, 1.90 mg/kg to 3.25 mg/kg and 1.95 mg/kg 4.80 mg/kg

If the arsenic in fish is compared to the JECFA, 2003 provisional tolerable daily intake levels of 0.002 mg/kg, it means arsenic in the fish is far higher than the JECFA daily intake if all the arsenic is inorganic in the fish but if not then there are additions of the organic ones since the determination was made for As(III). It has been stated before that most of the arsenic in fish is in the less toxic organic forms.

4.4 Human Urine

4.4.1 Physicochemical Analysis Results of Urine

Table.4.5 Field Test Result for the Physicochemical Properties of Human Urine Samples from Subject Living in some parts of the Amansie West District.

Labeled Samples	pH	Conductivity (µS/cm)	Temperature (ºC)	Labeled Samples	pH	Conductivity (µS/cm)	Temperature (ºC)
AS	8.18	25000.54	31.2	BC	6.12	19290.15	35.3
AK	7.75	27040.38	32.5	BT	6.23	30756.58	31.8
AD	8.23	11691.30	30.4	BM	6.98	25198.95	34.6
AG	6.45	21454.29	36.3	BZ	7.98	16737.50	33.1
AH	7.96	25356.64	33.8	BR	7.02	31485.37	35.7
AL	6.31	18574.21	35.4	BF	8.43	25664.43	32.3
AB	6.34	29063.47	31.9	BP	7.28	27825.75	32.9
AT	6.25	15950.53	35.9	DS	7.85	17347.22	33.6
AM	8.12	17186.42	32.1	DK	7.06	12693.52	34.9
AZ	5.73	26325.59	35.4	DG	5.98	26227.74	31.3
AR	6.04	36918.80	36.1	DH	6.05	31026.16	32.7
AF	5.97	29403.72	34.5	DL	5.56	18940.38	33.2
AP	7.35	23295.36	32.9	DB	6.9	20228.70	30.3
BS	8.01	18364.19	36.2	DT	6.53	16751.52	33.6
BK	6.1	27673.85	29.8	DM	6.04	28307.06	32.9
BD	5.99	24129.27	36.9	DR	5.97	23149.81	30.9
BG	6.05	20565.08	32.4	DZ	7.12	19601.25	31.7
BH	8.2	18928.67	33.7	DF	6.95	37425.64	29.5
BL	7.1	31505.36	32.9				

The pH recorded for human urine samples ranged from 5.54 to 8.20 with an average of 6.77 in Table 4.5 which is within the pH range of a normal (background) human being thus 4.6 - 8, average being around 6.0.

The conductivity measured varied from 11691.25 µS/cm to 37425.64 µS/cm with averaged at 23703.46 µS/cm. These values fall within the conductivity of a normal (background) urine which ranged from 1100 µS/cm and 33900 µS/cm which averaged 21500 µS/cm (Maricker, 2010). Conductivity deals with the total ions, mobility,

valency and relative concentrations of ions in solutions and these values reflect these parameters.

The conductivity and the pH(a measure of hydrogen ion activity) of the human urine samples did not interrelate in any to the arsenic concentration of human urine.

The temperatures for the urine samples within which the pH and conductivity measurements were also recorded ranged from 29.5 °C to 36.9 °C and averaged at 33.26 °C.

4.4.2 Arsenic concentration in Urine

The arsenic concentration presented in the table below with the standard deviation

Table.4.6 Arsenic Concentration in Human Urine of Subject living in some part of the district.

Labeled Urine	Age (yrs)	Duration of Stay (yrs)	Gender	Mean Arsenic Concentration (mg/L)
AS	6	6	M	0.004
AK	27	5	M	0.004
AD	22	8	F	0.003
AG	39	27	M	0.006
AH	21	9	F	0.003
AL	23	4	F	0.006
AB	28	16	F	0.007
AT	26	26	M	0.008
AM	19	4	M	0.004
AZ	29	8	M	0.012
AR	32	9	M	0.009
AF	20	13	F	0.01
AP	29	10	M	0.005
BS	27	11	F	0.004
BK	21	6	F	0.006
BD	32	18	F	0.006
BG	18	10	M	0.007
BH	20	7	M	0.003
BL	17	14	M	0.005
BC	18	18	F	0.009
BT	25	22	M	0.008
BM	23	7	F	0.005
BZ	19	12	M	0.004
BR	27	19	M	0.005
BF	23	3	M	0.003
BP	25	8	F	0.005
DS	21	5	M	0.004
DK	25	15	M	0.006
DG	33	6	M	0.012
DH	24	9	F	0.009
DL	23	17	F	0.014

DB	19	10	M	0.005
DT	25	15	F	0.007
DM	19	12	M	0.008
DR	29	20	M	0.011
DZ	34	18	F	0.005
DF	13	13	M	0.006

From Table 4.6, the arsenic concentration ranged from 0.003 mg/L to 0.014 mg/L and the average was recorded as 0.0064 mg/L. The median total urine arsenic of some adults in US is 0.0034 mg/L (Navas-Acien et al, 2009). The human urine labeled DL had the highest arsenic concentration of 0.014 mg/L which was from a twenty three (23) year old female who had stayed in the district for seventeen (17) years. AD, AH, BH and BF labeled urines had their arsenic concentration to be 0.003 mg/L which was the lowest. AZ, AF, DG, DL and DR labeled had their arsenic concentration to be and above the normal level of 0.01 mg/L (ToxGuide for Arsenic, 2007) for an unexposed individual.

From Table 4.6, it has been observed that arsenic concentration increased with the pH of the human urine samples, as the pH of the urine samples increased the arsenic concentration decreased. For instance, in AZ, AF, DG, DL, and DR, their pH were recorded as 5.73, 5.97, 5.98, 5.56 and 5.97 respectively and the arsenic concentration were or above 0.010 mg/L. The arsenic concentration was above the normal arsenic level in urines of subjects that did not take seafood so the higher values may be due to two things;

1) the exposure to arsenic in the environment and

2) the intake of seafood

The scattered diagram of arsenic concentration against conductivity in Appendix N showed that there will not be any correlation observing the way the points are scattered, so one cannot use conductivity to predict the arsenic concentration but rather laboratory experiment and other factors could be investigated. This conductivity values may not be necessarily due to arsenic in the urine rather from other ionic substances in the urine.

The duration of stay of all the subjects did not have any relation with the arsenic concentration.

It has been observed that from Appendix R, the DL sampled from a twenty three (23) years old female who has stayed in the area for seventeen (17) years had the highest arsenic concentration of 0.014 mg/L. Also from the graph, BF sampled from a twenty three (23) year old male who has stayed in the area for three years recorded the lowest arsenic concentration of 0.003 mg/L.

Appendix O shows the arsenic content of males and females from which urine was sampled from and the female urine recorded the highest mean arsenic concentration of 0.007 mg/L and the males recorded 0.006 and this may indicate that the females may be more exposed to arsenic than the males.

From Appendix P, the number of years a person stayed in the community may have influenced the quantity of urinary arsenic. Subjects who have stayed more than twenty years had higher urinary arsenic of 0.00733 mg/L and those who have stayed between eleven (11) to twenty (20) years also had their mean arsenic concentration to be 0.00713 mg/L. The subject who stayed in the area for less than ten (10) years had their mean urinary arsenic concentration to be 0.00574 mg/L implying here that the number of years a person is exposed to arsenic has some kind of influence on the

excreted arsenic. When arsenic is ingested, it is not all that is excreted but rather it is absorbed into the human system, metabolized and excreted later.

Appendix Q also reveals that as a person grows in age he easily excretes arsenic, subjects who were above thirty (30) years had their mean urinary arsenic concentration of 0.0076 mg/L to be higher than subjects between twenty one (21) and thirty (30) years had their mean to be 0.0071 mg/L, eleven (11) to twenty (20) years had 0.0061 mg/Land subjects less than ten years had 0.0042 mg/L.

From Navas-Acien et al, (2009), the total arsenic concentration recorded 0.0034 mg/L and the results of this study were above the Navas-Acien et al normal value. Bioaccumulation may also occur over a period of time and this may become a threat which could lead to effects that may be dangerous to human health. These amounts are also released as waste which end up in the environment adding to water bodies and at the same time serving as a threat to the environment.

The results obtained for arsenic levels in maize and fish were within ranges that are normal to human systems compared with Swiss tolerance limits for food or fodder crops (0.2 mg/kg and 4 mg/kg, respectively) (Gulz, 2002).

The WHO limit set for arsenic in cereals (maize) and fish is 10mg/kg g (WHO, 2003) and for this study, the results were far below this value so these values. Dietary arsenic represents the major source of arsenic exposure for most of the general population. The actual total arsenic concentrations in the fish and maize from various points in the district vary widely depending on growing conditions (type of soil, water, geochemical activity) and processing techniques for these food items.

Buruli ulcer has been found to be endemic around arsenic rich environments (Duker *et al*, 2005) though there are no literatures proving how arsenic plays a role in the

epidemiology of the disease. Chemical analysis were done to check the levels of arsenic and the rate of bioaccumulation of arsenic in the body system is slow, arsenic is easily excreted from the body within few days of exposure to it but upon continual exposure, the arsenic tend to accumulate and may play a role in the Buruli ulcer infection. The results of this study may not be that harmful to cause any significant effect such as the Buruli ulcer but continual exposure may lead to long term effects.

The Buruli ulcer is a disease that occurs on subject whose limbs are exposed to water bodies which are stagnant either standing in the water working especially miners or children swimming in the water and the disease seem to be on the decline as a result of good hygienic practices and diet that the people undertake according to Ministry of Health report (2011).

4.5 Recovery

Table.4.7. Recovery of Arsenic concentration of the HGAAS

Concentration of standard arsenic solution (mg/L)	**Concentration recovered (mg/L)**	**Percent recovery**
5	4.856	97.12
10	9.793	97.93
20	19.071	95.36
30	28.986	96.62

Average recovery = 96.76 %

The average percent recovery for the triplicate determination of arsenic standard solutions after every five determinations recorded 96 % and the range of recovery is 95.36 to 97. 93. This proved that the instrument was reliable in the determination of arsenic in the maize, fish and urine samples.

CHAPTER FIVE

5. Summary, Conclusion and Recommendations

5.1 Summary of results

The average total arsenic levels in maize ranged from 0.015 mg/kg to 0.045 mg/kg dry weight and the pHs of the maize sample solutions ranged from 5.41 to 6.90 which falls within the acidic region. The conductivity of ions in the maize sample solutions varied from 1508.43 to 3371.41 µS/cm with the average being 2523.66 µS/cm.

The edible part of the fish *Clarias agboyensis* found upstream recorded the mean arsenic concentration to be 2.584 mg/kg which ranged from 1.90 mg/kg to 3.25 mg/kg. The pH measurement recorded for the fish from upstream ranged from 5.34 to 6.87 with the mean value being 6.23. The ash content recorded 12.98 % as the mean value for the fish and the moisture content of the fish were averagely 60.12 % from upstream.

The fish downstream recorded the arsenic concentration which ranged from 1.95 mg/kg to 4.80 mg/kg. The pH recorded for the fish varied from 5.02 to 6.91 with the average being 5.93. The average moisture and ash contents were respectively 60.90 % and 13.16 %.

The mean total arsenic concentration recorded for fish from streams and ranged from 1.7 mg/kg to 3.95 mg/kg. The pH of the fish ranged from 5.03 to 7.15. The moisture content of the fish ranged from 55.78 % to 65.98 % and averaged 59.67 %. The ash content also varied from 10.68 % to 15.92 % and also averaged 13.46 %.

The mean arsenic concentration of fish upstream, downstream and ponds and rivers recorded downstream fish had the highest arsenic concentration of 3.00 mg/kg, followed by streams and ponds of 2.55 mg/kg and upstream recording 2.48 mg/kg

The total arsenic concentration of human urine measured ranged from 0.003 mg/L to 0.014 mg/L. The temperature of the urine samples recorded ranged from 29.5 °C to 36.9 °C and averaged at 33.26 °C. The conductivity measured varied from 11691.30 µS/cm to 37425.64 µS/cm with average at 23705.10 µS/cm. The pH recorded for human urine samples ranged from 5.54 to 8.20 with an average of 6.77.

From Gyasi et al, 2012 they found out that when mice were exposed to high levels of arsenic from 4.0 - 4.8 mg/L the mice developed inflammation, erythema and open ulcers on skin (with scab formation) and from this study the results 0.015 mg/kg to 0.045 and 1.90 mg/kg to 4.80 mg/kg are very low in the maize and fish respectively.

5.2 Conclusion

The study has presented a general idea about the concentration distribution of arsenic in maize, fish and human urine from some parts of the Amansie West District and their contamination levels in the district.

Comparing, the level of arsenic in the raw maize and fish is higher than the arsenic excreted through urine. The pH of human urine samples ranged from 5.54 to 8.20, the part within the acidic region is a matter of concern. Comparing pH with the arsenic released, the lower the pH of a sample the higher the arsenic released in the sampled materials although there were some discrepancies.

The mean arsenic concentration of fish sampled downstream was higher than that for fish sampled upstream and streams and ponds.

Arsenic concentration in human subject urines samples was found to be higher for people who have stayed longer in the district than for people who have stayed for less period of time. Subjects who have advanced in age also had higher arsenic

concentration than those subjects who are young. Also it was observed that female subjects although they were less in number had their urinary arsenic concentration to be higher than those of their male counterparts

The arsenic concentration levels found in this study may not be able to cause Buruli ulcer but when they accumulate to high levels in a more conducive acidic environment, it may help *Mycobacterium ulcerans* to cause the Buruli ulcer infection.

5.3 Recommendations

1. Future work should investigate arsenic levels for food substances coming from outside the Amansie West District.
2. Effluent from mines should be treated before discharge into water bodies.
3. Farming within the mining area should be prohibited.
4. Farming of maize should be grown on mounds (aerated soil) to reduce the absorption of arsenic into the plant and finally into the maize kernel.
5. Findings of this research should be made available to the Environmental Protection Agency, Food and Drugs Board, Community water and sanitation.

REFERENCES

Abdelghani, A. A, Reimers, R. S., Anderson, A. C., Englande, A. J., Lo, C. P., Shariatpanahi, M. (1981). Transport and distribution of arsenic in sediments. Heavy metals in the environment. 'Proceedings of the 3rd International Conference". Amsterdam, September 1981, Geneva, WHO, pp 665–668.

Agency for Toxic Substances and Disease Registry (ATSDR), (2005). CERCLA Priority List of Hazardous Substances. GA, US Department of Health and Human Services. Available at http://www.atsdr.cdc.gov [accessed on 19th Dec 2011.]

Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological profile for arsenic. Available at http://www.atsdr.cdc.gov/toxprofiles/tp2.html. [Accessed on 19th Dec, 2011]

Ahmann, D., Krumholz, L. R., Hemond, H.F., Lovley, D.R., Morel, F.M.M. (1997). Microbial mobilization of arsenic from sediments in the Aberjona watershed. *Environmental Science Technology* 31:2923-2930.

Ahmann, D., Roberts A. L., Krumholz, L. R., Morel, F. M. (1994). Microbe grows by reducing arsenic. *Nature* 371:750.

Akeredolu, F. A., Barrie, L. A., Olson, M. P., Oikawa, K. K., Pacyna, J. M., Keeler, G. J. (1994). The flux of anthropogenic trace metals into the Arctic from the mid-latitudes in 1979/80. *Atmospheric Environment*, 28(8): 1557–1572.

Albores, A., Koropatnick, J., Cherian, M. G., Zelazowski, A. J. (1992). Arsenic induces and enhances rat hepatic metallothionein production in vivo. *Chemical-Biological Interaction* 85:127-140.

Al Rmalli SW, Haris PI, Harrington CF, Ayub M. (2005). A Survey of Arsenic in Foodstuffs on Sale in the United Kingdom and Imported From Bangladesh. *Science of Total Environment*, pg 37, 23–30.

Amasa, S. K. (1975). Arsenic pollution at Obuasi goldmine, town, and surrounding countryside. *Environmental Health Perspective*, pgs 12, 131–135.

American Public Health Association (APHA), American Water Works Association, Water pollution control Federation (1998). *Standard Methods for the Examination of Water and Waste Water*. Washington D.C., 18th Edition, Pp 76-89.

Amofah. G., Bonsu, F., Tetteh, C., Okrah, J.,Asamoa, K., Asiedu, K., J. Addy, (2002). Buruli Ulcer in Ghana: Result of National Case Search, *Emergency Infectious Diseases.*, 8(2), 167 – 170.

Amonoo-Neizer H. E., Amekor, M. K. E. (April 1993). Determination of Total Arsenic in Environmental Samples from Kumasi and Obuasi, Ghana. *Environmental Health Perspectives,* Vol 101, Number 1.

Amundsen, P., Staldvik, F. J., Lukin, A. A., Kashulin, N. A., Popova, O. A. Reshetnikov, Y.S (1997). Heavy metal contamination in freshwater fish from the border region between Norway and Russia. *Science of the Total Environment*, 201, 371- 378.

Andreae, M. O. (1983). Biotransformation of arsenic in the marine environment. In Lederer. W. H., Fensterheim, R. J. (eds). *Arsenic: industrial, biochemical, environmental perspectives*. New York, Van Nostrand Reinhold, pp 378–392.

Anke, M. (1991). The essentiality of ultra trace elements for reproduction and pre-and postnatal development. in Chandra, R.K. (ed) "Trace Elements in Nutrition of Children—II", Pp. 119-144. New York: Raven.

Asante, K. A., Agusa, T., Subramanian, A. O., Ansa-Asare, D., Biney C. A., Tanabe S. (2007).Contamination status of arsenic and other trace elements in drinking water and residents from Tarkwa, a historic mining township in Ghana. *Chemosphere*, 66, 1513–1522.

Asiedu, K., Etuaful, S. (2000). Economic and social impact. In *BURULI ULCER: Mycobacterium ulcerans infection* Edited by: Asiedu, K., Scherpbier, R., Raviglione, M. World Health Organisation, Global Buruli Ulcer Initiative, Geneva: 57-60.

Baig, J. A., Kazi, T. G., Arain, M. B., Afridi, H. I., Kandhro, G. A., Sarfraz, R. A., Jamali, M. K., Shah, A.Q. (2009). Evaluation of arsenic and other physico-chemical parameters of surface and ground water of Jamshoro, Pakistan. *Journal of Material Hazard*. Mater. 166, 662– 669.

Beeler, T. (1990). Oxidation of sulfhydryl groups and inhibition of the (Ca21 1 Mg21)-ATPase by arsenazo III. *Biochim. Biophys. Acta* 1027:264–267.

Beyer, W. N., Cromartie E. J. (1987). A survey of Pb, Cu, Zn, Cd, Cr, As, and Se in earthworms and soil from diverse sites. *Environmental Monitoring Assesements*, 8(1): 27–36.

Bhumbla, D. K., Keefer, R. F. (1994). Arsenic mobilization and bioavailability in soils. In: Nriagu J. O. (ed), *Arsenic in the environment": Part I: Cycling and characterization.* New York, John Wiley & Sons, pp 51–82.

Braman, R. S., Foreback, C. C. (1973). Methylated forms of arsenic in the environment. *Science*, 182: 1247–1249.

Brockbank, C. I., Batley, G. E., Low, G. C. (1988). Photochemical decomposition of arsenic species in natural waters. *Environmental Technology Letters*, 9(12): 1361–1366.

Buchet, J. P., Lauwerys, R. Roels, H. (1981). Comparison of the urinary excretion of arsenic metabolites after a single dose of sodium arsenite, monomethylarsonate or dimethylarsinate in man. *International Archives of Occupational Environmental Health* 48:71-79.

Callahan, M. A., Slimak, M. W., Gabel, N. W., May, I. P., Fowler, C. F., Freed, J. R., Jennings, P., Durfee, R. L., Whitmore, F. C., Maestri, B., Mabey, W. R., Holt, B. R., Gould, C. (1979). Water-related environmental fate of 129 priority pollutants. Vol I. Introduction and technical background, metals and inorganics, pesticides and PCBs. EPA-440/4-79-029a. Washington, DC, U.S. Environmental Protection Agency, Office of Water Planning and Standards.

Camerman, N. Trotter, J. (1964). Stereochemistry of arsenic Part XI: "Cacodyl disulphide" dimethylarsino dimethyldithioarsinate. *Journal of Chemical Society*. (London) 1964:219-227

Chen, Y., Ahsan, H. (2004). Cancer burden from arsenic in drinking water in Bangladesh. *American Journal of Public Health.* 94: 741-744.

Coles, D. G., Ragaini, R. C., Ondov, J. M., Fisher, G.L., Silberman, D., Prentice, B. A. (1979). Chemical studies of stack fly ash from a coal-fired power plant. *Environmental Science Technoogyl*, 13(4): 455 459

Concha, G., Vogler, G., Lezeano, D., Nermell, B., Vahter, M. (1998). Exposure to inorganic arsenic metabolites during early human development. *Toxicological Science.* 44:185-190.

Cooper. A. J. L., (1983). Biochemistry of Sulphur Containing Amino Acids, *Ann. Rev Biochem.* 52: 187-222

Cullen, W.R., McBride, B. C., Reglinski, J. (1984). The reaction of methylarsenicals with thiols: Some biological implications. *Journal of Inorganic Biochemistry*. 21:179-194

Cullen, W. R., Reimer, K. J. (1989). Arsenic speciation in the environment. *Chemical Review*. 89:713-764

Dang, H. S., Jaiswal, D. D., Somasundaram, S. (1983). Distribution of arsenic in human tissues and milk. *Science of the Total Environment*. 29:171-175.

Das, H. K., Mitra A. K., Sengupta, P.K., Hossain, A., Islam, F. Rabbani, G. H. (2004). Arsenic concentrations in rice, vegetables, and fish in Bangladesh: A preliminary study. *Environmental International*; 30: 383-87.

De Kimpe, J., Cornelis, R., Mees, L., Van Lierde, S., Vanholder. R. (1993). More than tenfold increase of arsenic in serum and packed cells of chronic hemodialysis patients. *American Journal of Nephrology*. 13:429-434.

Delnomdedieu, M., Basti, M. M., Styblo, M., Otvos, J. D., Thomas, D. J. (1994). Complexation of arsenic species in rabbit erythrocytes. *Chemical Resistance Toxicology* 7:621-627.

Diorio, C., Cai, J., Marmor, J. Shinder, R., DuBow. M. S. (1995). An *Escherichia coli* chromosomal *ars* operon homolog is functional in arsenic detoxification and is conserved in gram-negative bacteria. *Journal of Bacteriology*. 177:2050-2056.

Dixon, H. B. F. (1997). The biochemical action of arsonic acids especially as phosphate analogs. *Advanced Inorganic Chemistry* 44:191-227.

Dodd, M., Grundy, S. L., Reimer, K. J. Cullen W. R. (1992). Methylated antimony(V) compounds: Synthesis, hydride-generation properties, and implications for aquatic speciation. Appl. *Organometallic Chemistry* 6:207211.

Duker, A. A. (2005). *Spatial analysis of factors implicated in Mycobacterium ulcerans infection in Ghana.* PhD Thesis, International Institute for Geo-information Science & Earth Observation, Enschede, The Netherlands

Duker, A. A., Carranza, J. M. E., Hale, M. (2004). Spatial dependency of Buruli ulcer prevalence on arsenic-enriched domains in Amansie West District, Ghana: implications for arsenic mediation in Mycobacterium ulcerans infection. *International Journal of Health Geographics.*; vol 3: pp 19.

Edmonds, J. S., Francesconi, K. A., (1987) Transformations of arsenic in the marine environment. *Experientia*, 43: 553–557.

EPA. (1985). Ambient water quality criteria for arsenic, U.S. Environ. Protection Agency Rep. 440/5-84-033. 66 pp.

Espinoza, E. O., Mann, M. J., Bleasdell, B. (1995). Arsenic and mercury in traditional Chinese herbal balls. *N. Engl. J. Med.* 333:803-804.

Ferguson, J. F., Gavis, J. (1972). A review of the arsenic cycle in natural waters. *Water Research*, 6: 1259–1274.

Food Standards Agency (FSA) (October 2005). ARSENIC IN FISH AND SHELLFISH 82/05

Francesconi, K. A., Edmond, J. S. (1997). Arsenic and marine organisms. *Advanced Inorganic Chemistry*. 44:147-189.

Garelick H, Jones H, Dybowska A, Valsami-Jones E (2008). Arsenic pollution sources. *Rev Environ Contam Toxicol.* 197:17-60.

Ghosh, M. M., Yuan, J. R. (1987). Adsorption of inorganic arsenic and organoarsenicals on hydrous oxides. *Environ. Progress* 6:150-157.

Gorby, M. S. (1994). Arsenic in human medicine. In Nriagu J. O. (ed), *Arsenic in the environment, Part II: Human Health and Ecosystem Effects*. New York: Wiley; pp 1-16.

Gurr, J. R., Liu, F., Lynn, S., Jan, K. Y. (1998). Calcium-dependent nitric oxide production is involved in arsenite-induced micronuclei. *Mutat. Res.* 416:137-148.

Guy, G. R., Cairns, J., Ng, S. B. Tan, Y. H. (1993). Inactivation of a redox-sensitive protein phosphatase during the early events of tumor necrosis factor/interleukin-1 signal transduction. *J. Biol.Chem.* 268:2141–2148.

Gyasi, S. F., Awuah, E., Larbi, J. A, Koffuor, G. A. (2012a). Arsenic in Water and Soil: A Possible Contributory Factor in *Mycobacterium ulcerans* Infection in Buruli Ulcer Endemic Areas. *Asian Journal of Biological Sciences, 5(2): 66-75.*

Gyasi, S. F., Awuah, E., Larbi J. A., Koffour, G. A., Owusu-Afriyie, O. (2012b). Clinical, Hematological and Histopathological Responses to Arsenic Toxicity in ICR Mice Using Arsenic Levels Synonymous to Buruli Ulcer Endemic Communities in the Amansie West District of Ghana. *European Journal of Experimental Biology*, 2 (3):683-689

Gulz, P. A. (2002). *Arsenic Uptake of Common Crop Plants from Contaminated Soils and Interaction with Phosphate* Dissertation submitted to the Swiss Federal Institute of Technology Zurich for the degree of Doctor of Natural Science.

Hasegawa, H. (1997). The behavior of trivalent and pentavalent methyl arsenicals in Lake Biwa. *Applied Organometallic Chemistry* 11:305-311

Haswell, S. J., O'Neill, P., Bancroft, K. C. (1985). Arsenic speciation in soil-pore waters from mineralized and unmineralized areas of south-west England. *Talanta*, 32: 69–72.

Hei, T. K., Liu, S. X., Waldren, C. (1998). Mutagenicity of arsenic in mammalian cells: Role of reactive oxygen species. Proc. Natl. Acad. Sci. USA 95:8103-8107.

Hering, J. G., Chen, P. Y., Wilkie, J. A., Elimelech M. L. (1997). Arsenic removal from drinking water during coagulation. *J. Environ. Eng*. 123:800-808.

Hostynek, J. J., Hinz, R. S., Lorence, C. R., Price, M., Guy, R. H. (1993). Metals and the skin. *Crit. Rev. Toxicol.* 23:171-235.

Hughes, M. F., Kenyon. E. M. (1998). Dose-dependent effects on the disposition of monomethylarsonic acid and dimethylarsinic acid in the mouse after intravenous administration. *J. Toxicol. Environ. Health* 53:95112.

Hugo, N. E., Conway, H. (1967). Bowen's disease: Its malignant potential and relationship to systemic cancer. *Plast. Reconstr. Surg.* 39:190-194.

Huq, S. M. I., Naidu. R. (2003). Arsenic in groundwater of Bangladesh: contamination in the food chain. In Ahmed, M. F. (ed). *Arsenic contamination: Bangladesh perspective*. Dhaka, ITN- Bangladesh, 203-206.

Hutton, M., Symon, C. (1986). The quantities of cadmium, lead, mercury and arsenic entering the U.K. environment from human activities. *Sci Total Environ*, 57: 129–150.

IPCS (International Programme on Chemical Safety), (1981). Arsenic. Geneva, World Health Organization.

IPCS (International Programme on Chemical Safety), (2001). Environmental Health Criteria 224: 2nd ed Arsenic and Arsenic Compounds. World Health Organization,

Irgolic, K. J. (1994). Determination of total arsenic and arsenic compounds in drinking water. Pp. 51-60 in Chappell, W.R., Abernathy, C.O., Cothern C.R., (eds). *Arsenic: Exposure and Health,* Northwood, U.K.: Science and Technology Letters.

Irgolic, K. J., Greschonig, H., Howard, A. G. (1995). Arsenic. Pp 168 to 184 in Encyclopedia of Analytical Science, A. Townshend, ed. London: Academic.

Jacobson, S. E., Mares, F., Zambri, P. M. (1979). Biphase and triphase catalysis. Arsenated polystyrene catalysts for the epoxidation of olefins by aqueous hydrogen peroxide. *J. Am. Chem. Soc.* 101:6946-6950.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2003. Summary and conclusions of the sixty first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), pp. 18-22. Available on http://www.who.int/pcs/jecfa/Summary61.pdf (accessed on1st April 2012)

Kew, J., Morris, C., Aihie, A., Fysh, R., Jones, S., Brooks, D. (1993). Arsenic and mercury intoxication due to Indian ethnic remedies. *Br. Med. J.* 306:506-507.

Keyse, S. M., Tyrrell, R. M. (1989). Heme oxygenase is the major 32-Kda stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. *Proc. Natl. Acad. Sci*. USA 86:99-103.

Knox, S., Langston, W. J., Whitfield, M., Turner, D. R., Liddicoat, M. I. (1984). Statistical analysis of estuarine profiles. II. Application to arsenic in the Tamar Estuary (S.W.England). *Estuar Coast Mar Sci,* 18: 623–638.

Landry, J., Lambert, H., Zhou, M., Lavoie, J. N., Hickey, E., Weber, L. A., Anderson, C. W. (1992). Human HSP27 is phosphorylated at serines 78 and 82 by heat shock and mitogen-activated kinases that recognize the same amino acid motif as S6 kinase II. *Journal of. Biological Chemistry* 267:794–803.

Langehan, H. A. (1921). A Century of the United States Pharmacopoeia: 1820-1920. Liquor Potassii Arsenitis. Bull. Univ. Wisc. Ser. No. 1153,Gen. Ser. No. 936.

Latimer, W. M., Hildebrand J. H. (1951). Reference Book of Inorganic Chemistry, 3rd Ed. New York: Macmillan.

Lee, T. C., Ho, I. C. (1994). Differential cytotoxic effects of arsenic on human and animal cells. *Environ. Health Perspect.* 102(Suppl 3):101-105

Lovley, D. R., Phillips, E. J. P., Lonergan, D. J. (1991). Enzymatic versus nonenzymatic mechanisms for Fe(III) reduction in aquatic sediments. *J. Environ. Sci. Technol.* 25:1062-1067.

Luo, W., Lu, Y., Wang. G., Shi Y., Wang, T., Giesy, J. P. (2008). Distribution and availability of arsenic in soils from the industrialized urban area of Beijing, China *Chemosphere* 72(5):797-802

Lumsdon, D. G., Fraser, R. A., Russel, J. D., Livesey, N. T. (1984). New infrared band assignments for the arsenate ion adsorbed on synthetic goethite (a-FeOOH) *J. Soil Sci.* 35:381-386.

Mahieu, P., Buchet, J. P., Roels, H. A., Lauwerys, R. (1981). The metabolism of arsenic in humans acutely intoxicated by As_2O_3. Its significance for duration of BAL therapy. *Clinical Toxicology*, 18(9):1067-1075.

Manning, B. A., Goldberg, S. (1997). Adsorption and stability of arsenic(III) at the clay mineral-water interface. *Environ. Sci. Technol*. 31:2005-2011.

Maricker, F (Aug 2010). Electrical Conductivity and Total Dissolved Solids in Urine Urol Res 38 (4) 233-5. Epub 2009 Nov 17

Marston, B. J., Diallo, M. O., Horsburgh, C. R., Diomande J. I., Saki, M. Z., Kanga, J., Patrice, G., Lipman, H. B., Ostroff S. M., Good, R. C. (1995). Emergence of Buruli ulcer disease in the Daloa region of Cote D'ivoire. *Am. J. Trop. Med. Hyg.* 52: 219-224.

Mattschullat, J. (2000). Arsenic in the geosphere- A review. *Science, Total Environment.* 249, 297-312

Melliker, J. R., Nriagu, J. O., (2008). Arsenic International Encyclopedia of Public Health 233-238

Ministry of Health Report (2011). *Buruli ulcer in Amansie west District of Ashanti region,* volume 17, pg 99-104.

Mok, W. M., Wai, C. M. (1994). Mobilization of arsenic in contaminated river waters. In: Nriagu JO (ed). *Arsenic in the environment: Part I: Cycling and characterization.* New York, John Wiley & Sons, pp 99–117.

Molin, L., Wester, P. O. (1976). The estimated daily loss of trace elements from normal skin by desquamation. *Scand. J. Clin. Lab. Invest*. 36:679682.

Nakamura, M., Matsuzono, Y., Tanaka, S., Hashimoto, Y. (1990). Chemical form of arsenic compounds and distribution of their concentrations in the atmosphere. *Appl Organomet Chem,* 4: 223–230

Natelson, S. (1961). Part II. Methodology. In Thomas, C. C., *Microtechniques of Clinical Chemistry,* 2nd Ed. Pp. 113-116 Springfield, Ill.:.

National Institutes of Health, (1996). Guide for care and use of laboratory animals. Publication no. 83-23. Office of Science and Health Reports, Department of Health and Human Services.Bethesda, MD

Navas-Acien, A., Umans, J. G., Howard, B. V., Francesconi, K. A., Crainiceanu, C. M.(2009). Urine Arsenic Concentrations and species Excretion Patterns in American Indian Communities Over a 10-year Period: The Strong Heart Study. *Environmental Health Perspective* 117(9):1428-1433

Nielsen, F.H. (1980). Evidence of the essentiality of arsenic, nickel, and vanadium and their possible nutritional significance. in Draper, H.H. (ed), *Advances in Nutritional Research*, Vol. 3, pp. 157-172. New York: Plenum.

Nordenson, I., Beckman, L. (1991). Is the genotoxic effects of arsenic mediated by oxygen free radicals? *Hum. Hered*. 41:71-73.

NRC (National Research Council). (1977). Medical and Biological effects of environmental pollutants – Arsenic. National Academy of Sciences, Washington, DC.

Nriagu, J. O. (ed.) (May 1994). Arsenic in the Environment, Part 2, Human Health and Ecosystem Effects Advances in Environmental Science and Technology (Volume 27)

Nriagu, J. O., Azcue, J.M. (1990). Food contamination with arsenic in the environment. Pp. 121-143 in Nriagu, J. O., Simmons, M. S. (eds), *Food Contamination from Environmental Sources.* New York: John Wiley & Sons

O'Neil, M. J., ed. (2001). The Merck Index, 13th Ed., Whitehouse Station, NJ, Merck & Co., pp. 135–138

Osler, W. (1894). 'Principles and Practice of Medicine'. New York: Appleton.

Parris, G. E., Brinckman, F. E. (1976). Reactions which relate to environmental mobility of arsenic and antimony. II. Oxidation of trimethylarsine and trimethylstibine. *Environ Sci Technol*, 10(12): 1128–1134.

Partington, J. R. (1962). VI. Chemistry in Scandinavia. II. Scheele. Pp. 205234 in A History of Chemistry, Vol. 3. London: Macmillan.

Pomroy, C., Charbonneau, S. M., McCullough, R.S., Tam, G.K.H. (1980). Human retention studies with ^{74}As. *Toxicol. Appl. Pharmacol*. 53:550-556.

Popper, H., Thomas, L. B., Telles, N. C., Falk, H., Selikoff I. J. (1978). Development of hepatic angiosarcoma in man induced by vinyl chloride, thorotrast, and arsenic. *Am. J. Pathol*. 92:349-369.

Quaghebeur, M., Rate, A., Rengel, Z., Hinz, C. (2005). Heavy metals in the environment. Desorption kinetics of arsenate from Kaolinite as influenced by pH. *J. Environ. Qual*. 34, 479-486

Robb, L. J., Yao, Y., Armstrong, R. A., Murphy, P. J. (1999). Gold in the Birimian granites of Ghana: a metamorphic origin. In Rotterdam S, Balkema A. A. (eds), *Mineral Deposits:Processes to Processing*. 1033-1036

Sagan, L. S., Zingaro, R. A., Irgolic K. J. (1972). Alkoxy-, alkylthio-, and (organyseleno)dialkylarsines. *J. Organomet. Chem*. 39:301-311.

Sanders, J. G. (1980). Arsenic cycling in marine systems. *Mar Environ Res*, 3: 257–266.

Sathawara, N. G., Parikh. D. J., Agarwal, Y. K. (2004). Essential heavy metals in environmental samples from western India. *Bul. Environ. Contam. Toxicol.* 73: 756

Schoolmeester, W. L., White, D. L. (1980). Solubility of arsenic in fats: Arsenic poisoning. *South Med J* 73(2):198-208.

Scott, M. J., Morgan, J. J. (1995). Reactions of oxide surface. Oxidation of As(III) by synthetic birnessite. *Environ. Sci. Technol.* 29:1898-1905.

Scott, N., Hatlelid, K. M., MacKenzie, N. E.,Carter, D. E. (1993). Reactions of arsenic(III) and arsenic(V) species with glutathione. *Chem. Res. Toxicol.* 6:102-106

Scudlark, J. R., Church, T. M. (1988). The atmospheric deposition of arsenic and association with acid precipitation. *Atmos Environ,* 22(5): 937–943.

Smedley, P. L. (1996). Arsenic in rural groundwater in Ghana. *J. Afr. Earth Sci*., 22(4), 459–470.

Sun, X., Doner, H. E. (1996). An investigation of arsenate and arsenate bonding structures on goethite by FTIR. *Soil Sci*. 161:865-872.

Tamaki, S., Frankenberger, W. T. (1992). Environmental biochemistry of arsenic. *Rev Environ Contam Toxicol,* 124: 79–110.

Tao, S. H., Bolger, P. M. (1998). 'Dietary Intakes of Arsenic in the United States'. Paper presented at the Third International Conference on Arsenic Exposure and Health Effects, July 12-15, San Diego, Calif.

Thangaraj, H. S., Evans, M. R. W., Wansbrough-Jones, M. H. (1999). *Mycobacterium ulcerans*: Buruli ulcer. *Trans. Royal Soc. Trop. Med. and Hyg.* 93: 337-340.

The MAK-Collection (2005). Part II: BAT Value Documentations, Vol. 4. DFG, Deutsche Forschungsgemeinschaft Copyright © 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim ISBN: 3-527-27049-3

ToxGuide for Arsenic (October 2007). available at http://www.atsdr.cdc.gov/toxguides/toxguide-2.pdf [accessed 20th Mar, 2012]

Tseng, W. P., Chu, H. M., How, S. W., Fong, J. M., Lin, C. S., Yeh, S. (1968). Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. *Journal of National Cancer Institute.* 40:453-463.

Tsuda, T., Babazono, A., Yamamoto, E., Kurumatani, N., Mino, Y., Ogawa, T. Kishi, T., Aoyama, H. (1995). Ingested arsenic and internal cancer: A historical cohort study followed for 33 years. *American Journal of Epidemiology*. 141:198-209.

Turpeinen, R., Pantsar Kallio, M., Haggblom, M., Kairesalo, T., (1999). Influence of microbes on the mobilization, toxicity and biomethylation of arsenic in soil. *Science of the Total Environment*, 15(236): 173–180.

US EPA (US Environmental Protection Agency) (1982). An exposure and risk assessment for arsenic. EPA 440/4-85-005. Washington, DC, US Environmental Protection Agency

Uthus, E. O. (1992). Evidence for arsenic essentiality. *Environ. Geochem. Health* 14:55-58.

Vahter, M. (1994). Species differences in the metabolism of arsenic compounds. *Appl. Organomet.* Chem. 8:175-182.

Vahter, M. (1999). Methylation of inorganic arsenic in different mammalian species and population groups *Sci Progr*, 82: 69–88.

Vahter, M., Envall, J. (1983). In vivo reduction of arsenate in mice and rabbits. *Environ. Res.* 32:14-24.

Vahter, M., Marafante, E., Lindgren, A., Dencker, L. (1982). Tissue distribution and subcellular binding of arsenic in marmoset monkeys after injection of ^{74}As-arsenite. *Arch Toxicol*, 51: 65–77.

Vahter, M., Norin, H. (1980). Metabolism of ^{74}As-labeled trivalent and pentavalent inorganic arsenic in mice. *Environ. Res.* 21:446-457.

Vallee, B. L. (1973). 'Arsenic. Air Quality Monographs', No. 73-18. Washington, D.C.: American Petroleum Institute.

Vellar, O. D. (1969). 'Nutrient Losses Through Sweating'. Thesis. Oslo University, Oslo, Norway.

Vogel, A. E. (1954). 'Special tests for small amounts of arsenic'. In: Vogel AE (ed) A textbook of macro and semi-micro qualitative inorganic analysis, 4th ed. London, Longmans, pp 242–247.

Wakao, N., Koyatsu, H., Komai, Y., Shimokawara, H., Sakurai, Y., Shiota, H. (1988). Microbial oxidation of arsenite and occurrence of arsenite-oxidizing bacteria in acid mine water from a sulphur-pyrite mine. *Geomicrobiol J*, 6: 11–24.

Walsh, D. S., Meyers, W. M., Kreig, R. E., Walsh, G. P. (1999). Transmission *of Mycobacterium ulcerans* to the nine banded armadillo. *Am J Trop Med Hyg.* 61(5), 694–697.

Walsh, L. M., Keeney, D. R. (1975). Behavior and phytotoxicity of inorganic arsenicals in soils. *ACS Symp Ser*, 7: 35–52.

Walsh, P. R., Duce, R. A., Fasching, J. L. (1979). Considerations of the enrichment, sources, and flux of arsenic in the troposphere. *J Geophys Res*, 84(4C): 1719–1726.

Waychunas, G. A., Rea, B. A., Fuller, C. C., Davis, J. A. (1993). Surface chemistry of ferrihydrite: Part 1. EXAFS studies of the geometry of coprecipitated and adsorbed arsenate. *Geochim. Cosmochim. Acta* 57:22512269.

Welch, A. H., Lico, M. S., Hughes, J. L. (1988). Arsenic in groundwater of the western United States. Ground Water, 26(3): 333–347.

Wewerka, E. M., Bertino, J. P. L., Wagner, P., Williams, J. M., Wanek, P. L., Wangen, L. E. (1978). 'Trace element characterisation of coal wastes', second

annual progress report. DOE LA-7360-PR; EPA-600/7-78-028a, Washington DC.

WHO (2000). 'BuruliI Ulcer: Mycobacterium ulcerans infection'. Edited by: Asiedu K, Scerpbier R, Raviglione M. WHO/CDS/CPE/GBUI/1.WHO, Geneva.

WHO (2001). 'Arsenic and Arsenic Compounds' (Environmental Health Criteria 224), 2nd Ed., Geneva, International Programme on Chemical Safety , Geneva.

Woolson, E. A. (1983). In: Fowler BA ed. Biological and environmental effects of arsenic. Amsterdam, *Elsevier Science*, pp 51–139

Wu, J., Liu, J., Michael Waalkes, M. P., Cheng, M. L., Li, L., Li, C. X., Yang, Q. (2008). High Dietary Fat Exacerbates Arsenic-Induced Liver Fibrosis in Mice *Experimental Biology and Medicine* 233:377-384. Editorial 1994 *Clinical Chemistry*.

Wuana, R. A., Okieimen, F. E. (2011). HeavyMetals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *International Scholarly Research Network.* 10.5402/2011/402647

Yamanaka, K., Ohtsubo, K., Hasegawa, A., Hayashi, H., Ohji, H., Kanisawa, M., Okada, S. (1996). Exposure to dimethylarsinic acid, a main metabolite of inorganic arsenics, strongly promotes tumorigenesis initiated by 4-nitroquinoline 1-oxide in the lungs of mice. *Carcinogenesis* 17:767-770.

Yang, L., Peterson, P.J., Williams, W.P., Wang, W., Hou, S., Tan, J., (2002). The relationship between exposure to arsenic concentrations in drinking water and the development of skin lesions in farmers from inner Mongolia, *China*, *Ingenta Connect*, 24(4), 293–303.

Yost, L.J., Schoof, R.A., Aucoin, R. (1998). Intake of inorganic arsenic in the North American diet. *Hum. Ecol. Risk Assess.* 4:137-152.

Zhang, X., Cornelis, R., de Kimpe, J., Mees, L., Lameire, N. (1998). Study of arsenic-protein binding in serum of patients on continuous ambulatory peritoneal dialysis. *Clin. Chem*. 44:141-147.

Zingaro, R. A., Thomson J. K. (1973). Thio and seleno sugar esters of dialkylarsinous acids. *Carbohydr. Res*. 29:147-152.

APPENDICES

Appendix A

Calculation of Moisture Content of fish

Moisture content (%) is determined as

$$Moisture\ Content = \frac{Weight\ of\ wet\ fish\ - Weight\ of\ dried\ fish}{Weight\ of\ wet\ fish} \times 100$$

For U5, weight of dried fish = 2.211 g

Weight of wet fish = 5 g

$$Moisture\ content\ = \frac{5\ g - 2.211\ g}{5\ g}\ \times\ 100$$

$$Moisture\ content\ = \frac{2.789\ g}{5\ g}\ \times\ 100$$

$$Moisture\ content\ = 55.78\ \%$$

Appendix B

Calculation of Ash content of maize and fish

$$Ash\ content\ = \frac{Weight\ of\ ash}{Weight\ of\ maize} \times 100$$

For DT, weight of ash = 0.960 g

Weight of maize = 5 g

$$Ash\ content\ = {}^{0.961\ g}/_{5\ g} \times 100$$

$$= 19.21\ \%$$

Appendix C

Calculation of Arsenic concentration of maize into mg/kg

Maize from B1 concentration in mg/L = 0.005

Volume of sampled (digested) solution = 250 ml

Mass of sampled maize = 5g

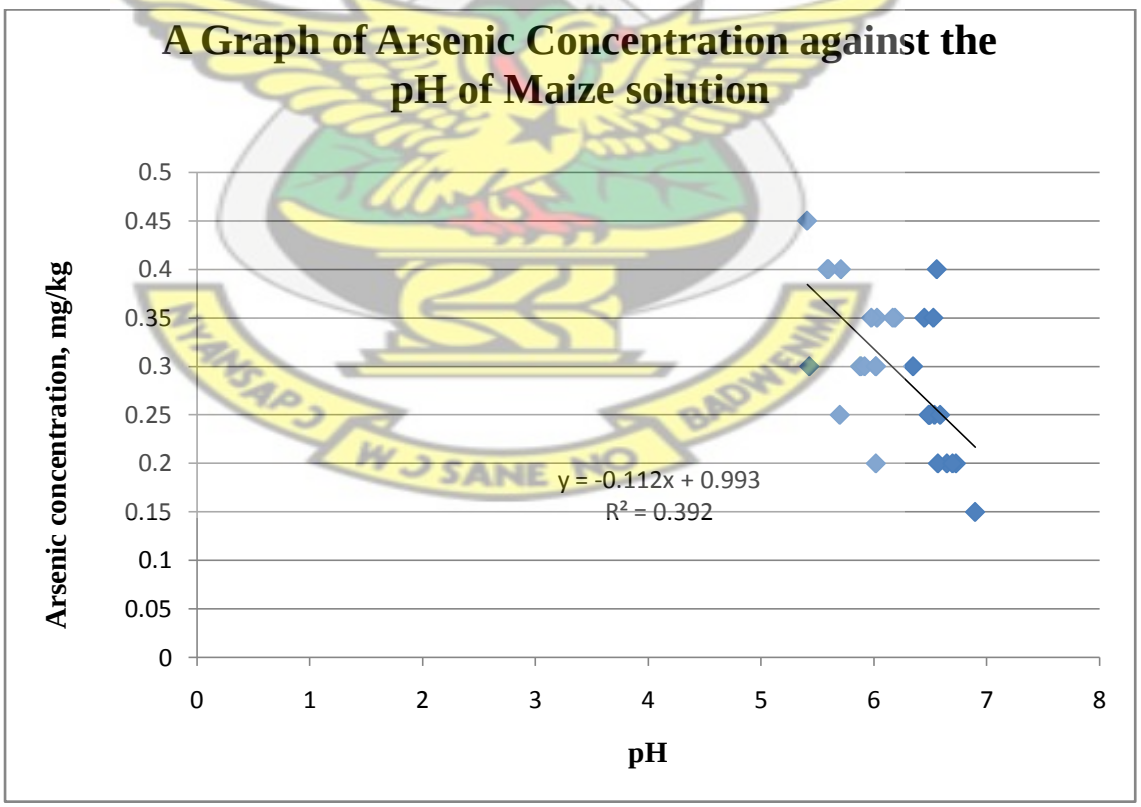
$$Concentration\ in\ {}^{mg}/_{kg} = \frac{\frac{0.005mg}{L}}{5\ g} \times 250\ mL$$

$$Concentration\ in\ {}^{mg}/_{kg} = 0.25\frac{\mu g}{g}$$

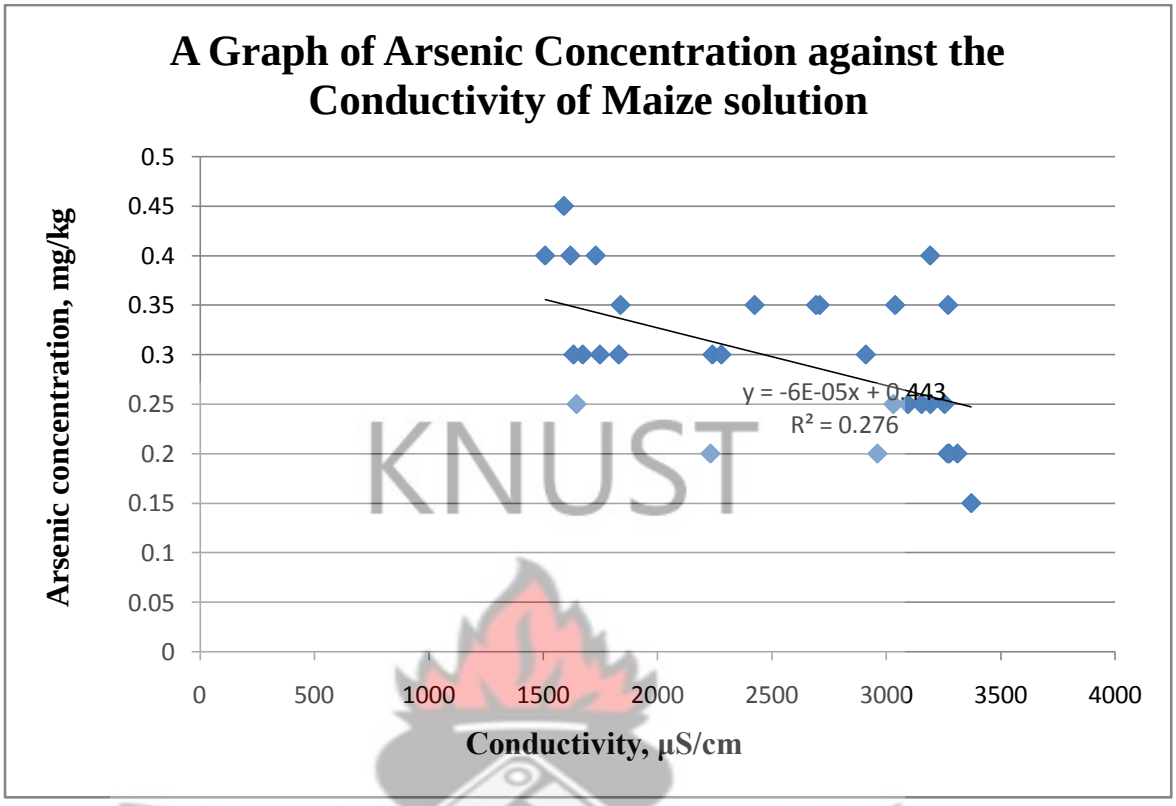
$$= 0.25mg/kg$$

This same procedure was used in the calculation for the arsenic concentration of the maize and fish into mg/kg

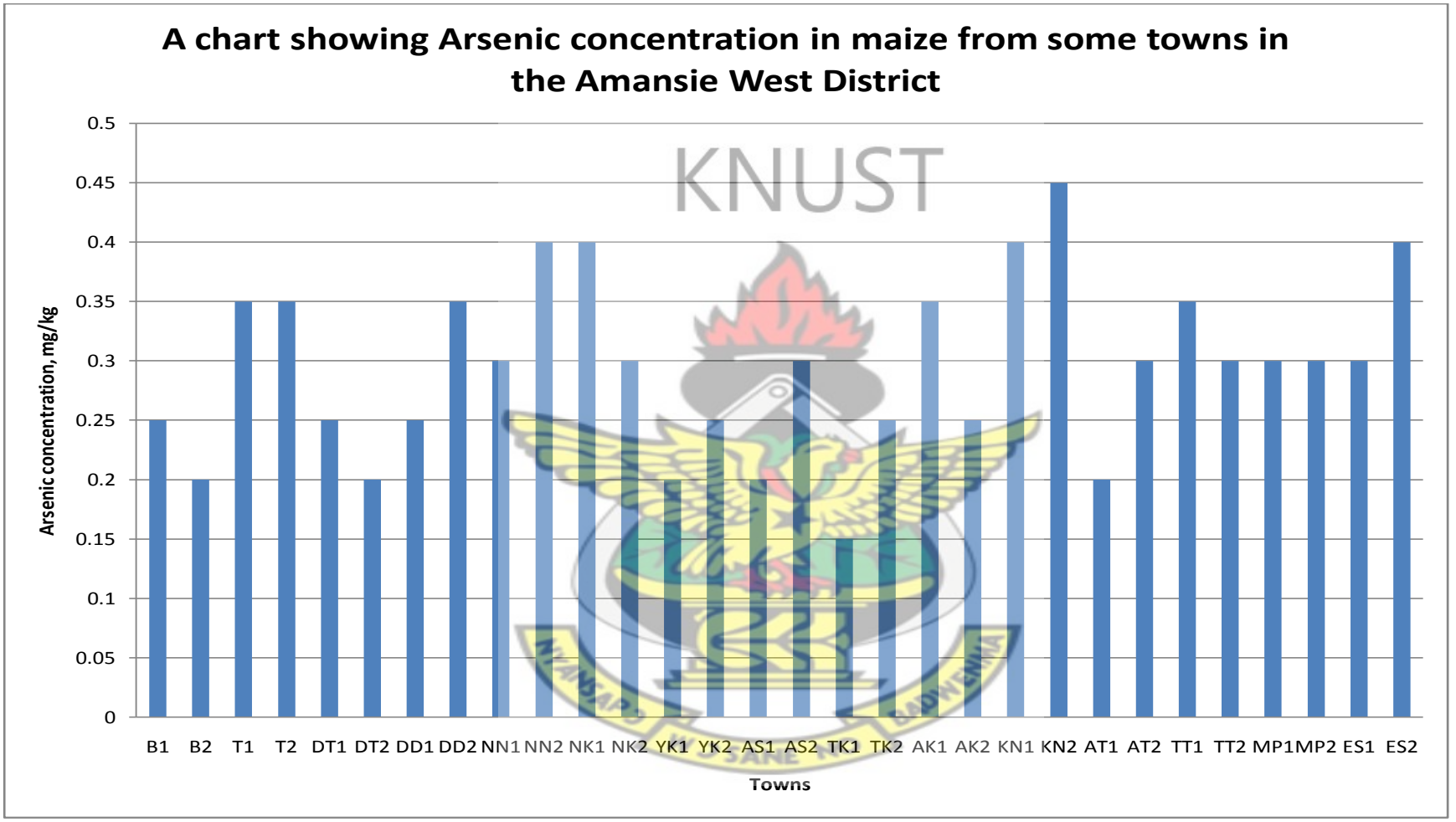
Appendix D



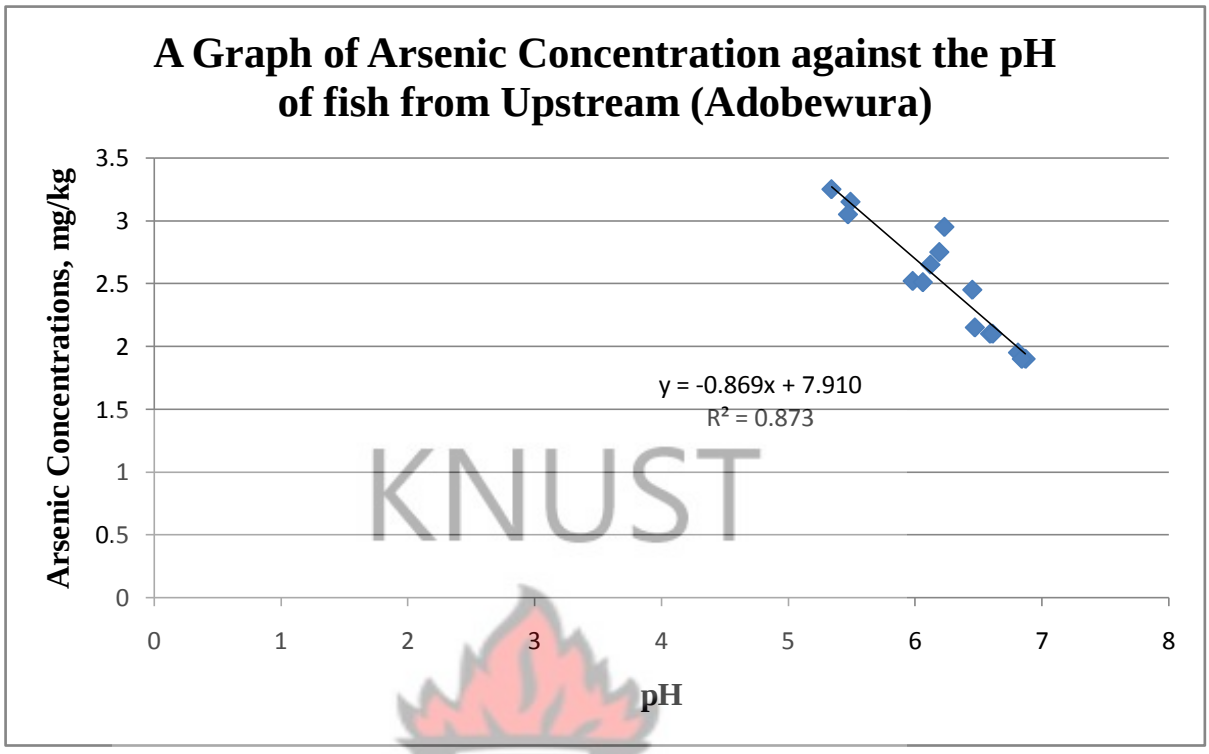
Appendix E



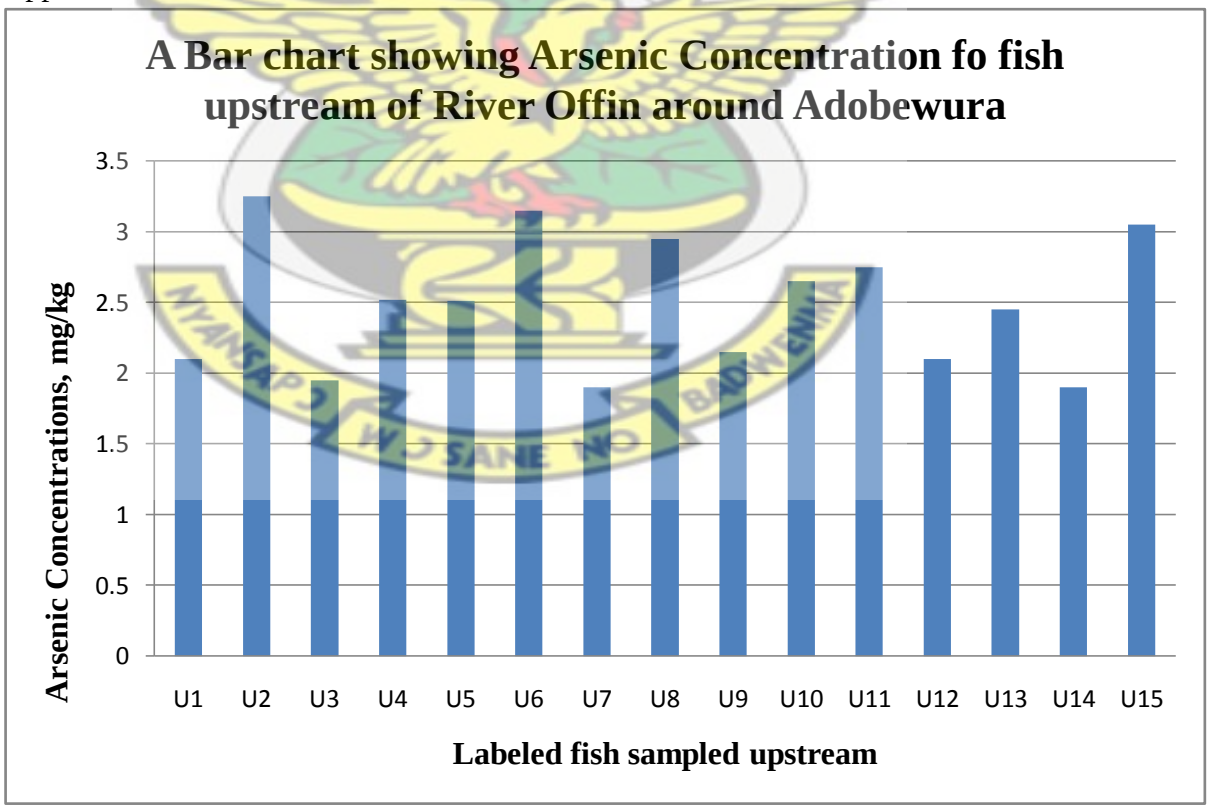
Appendix F



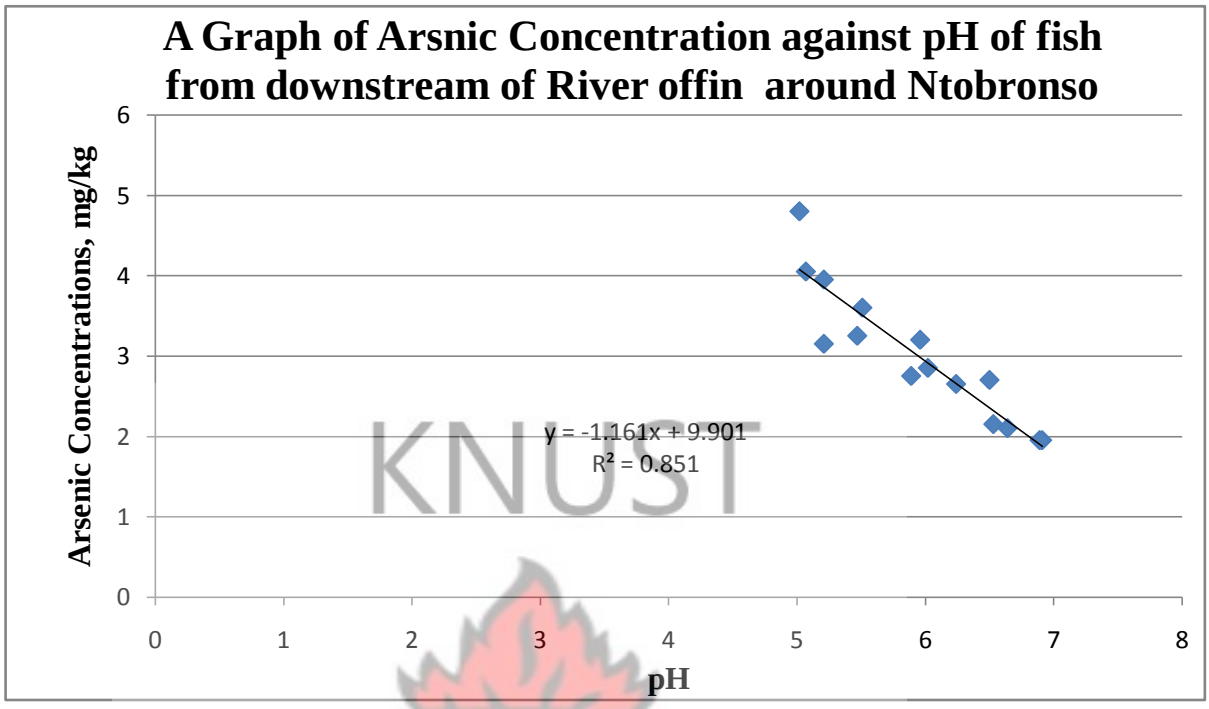
Appendix G



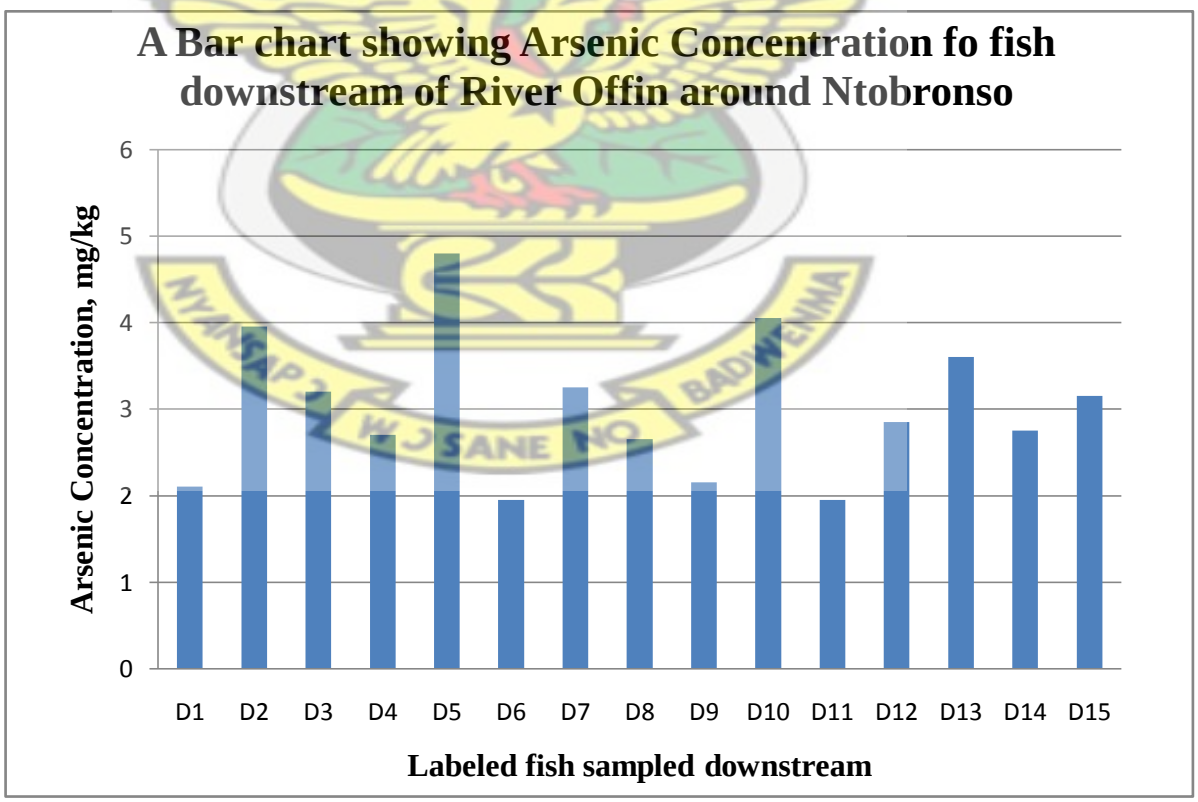
Appendix H



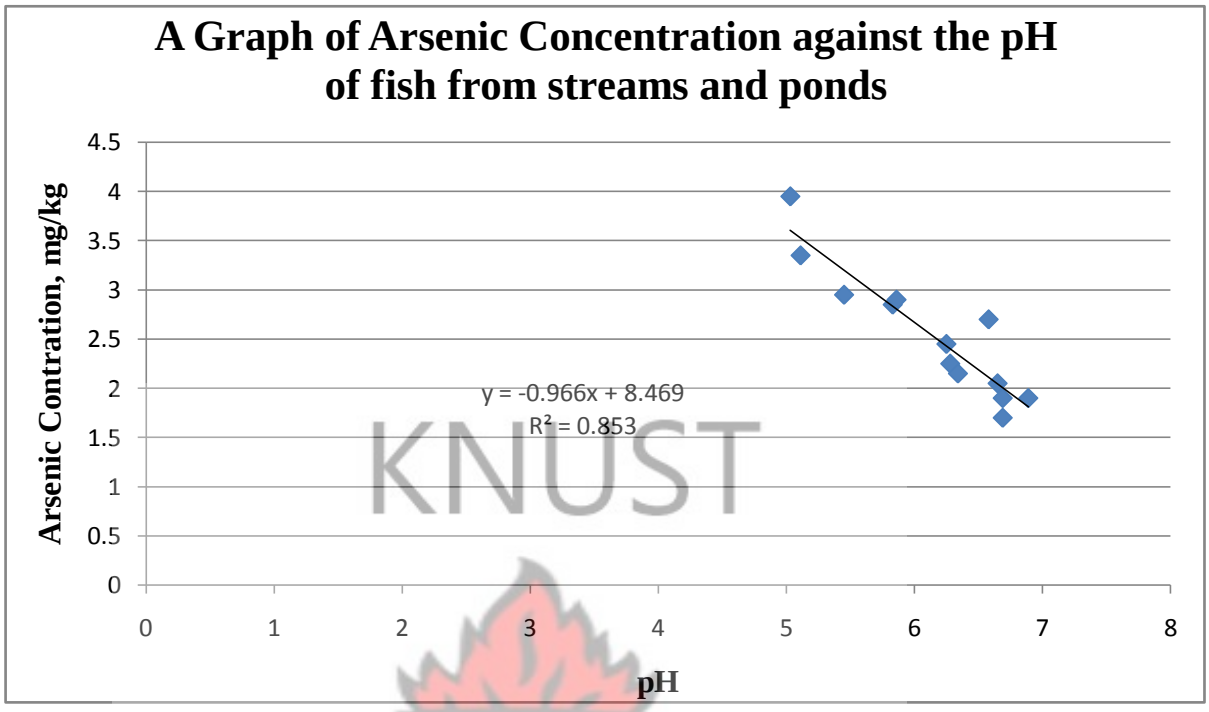
Appendix I



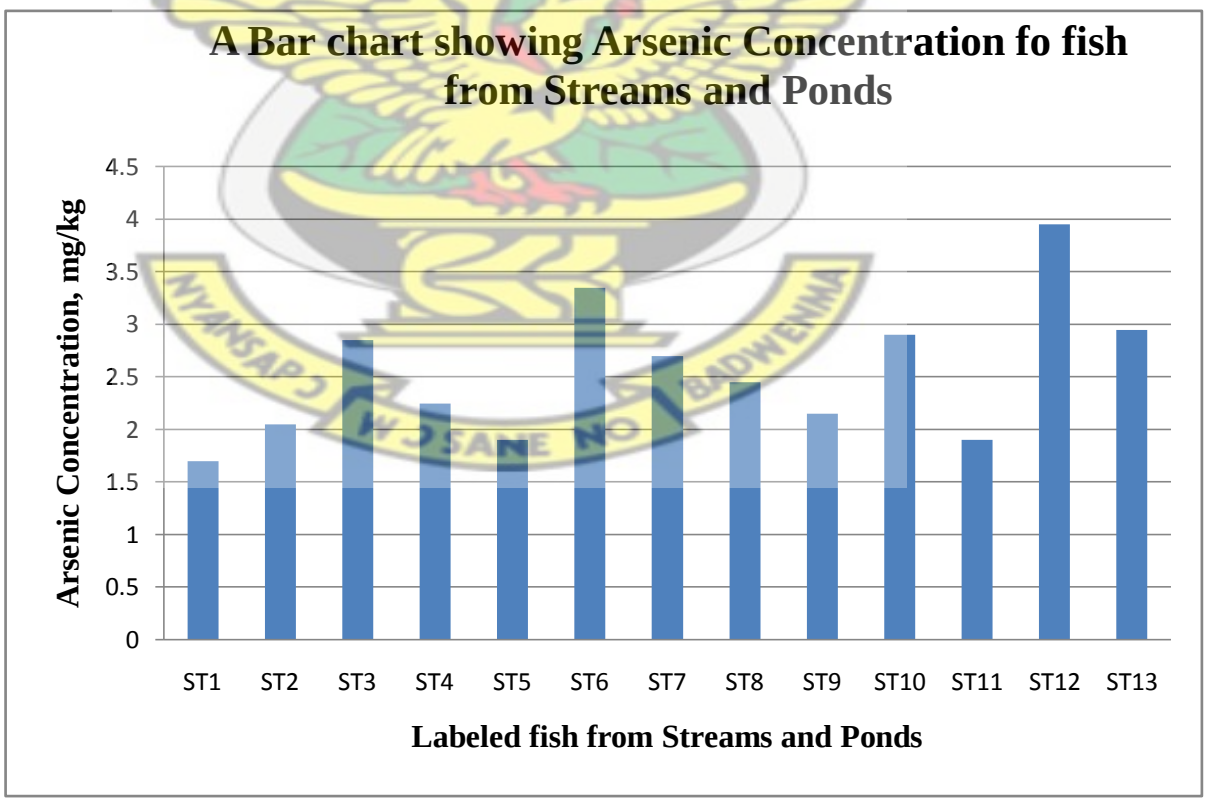
Appendix J



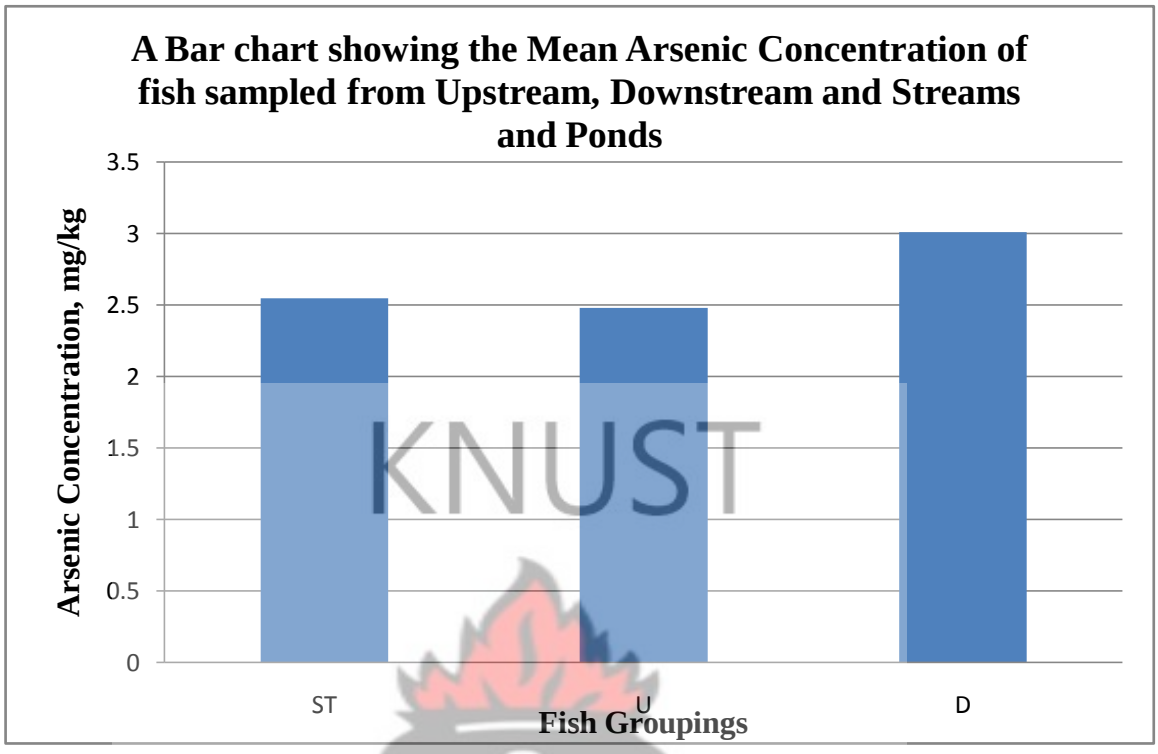
Appendix K



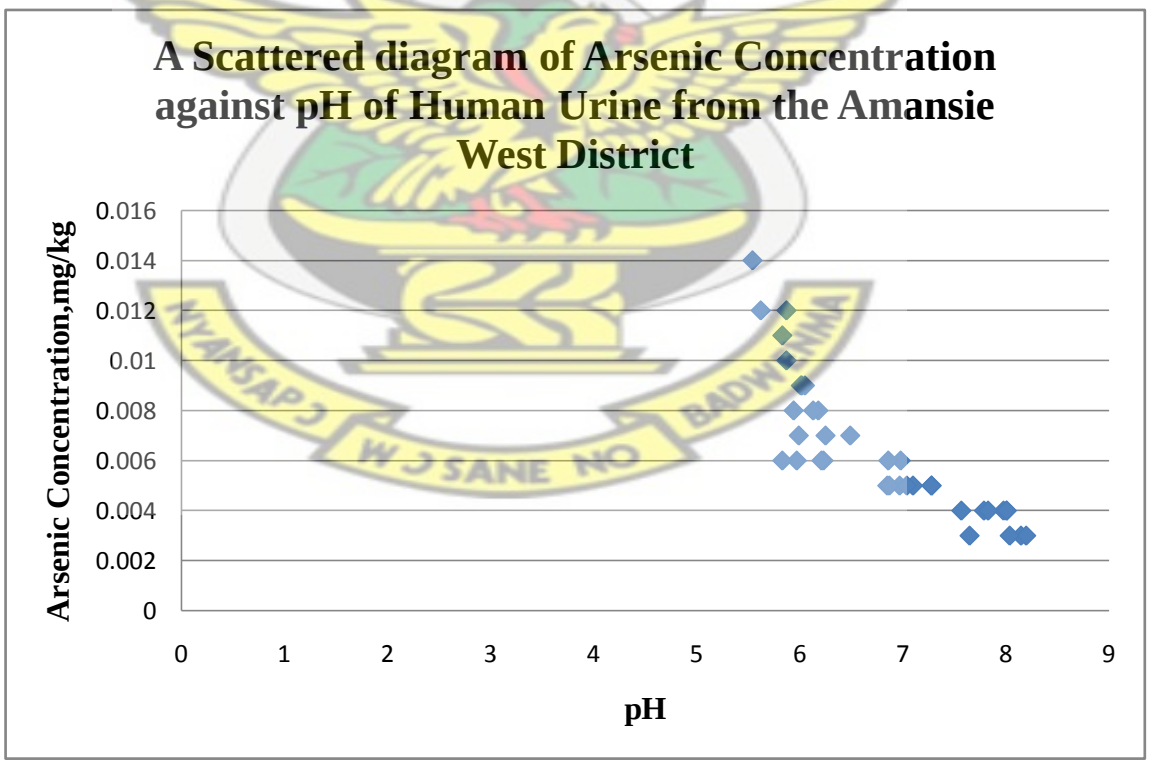
Appendix L



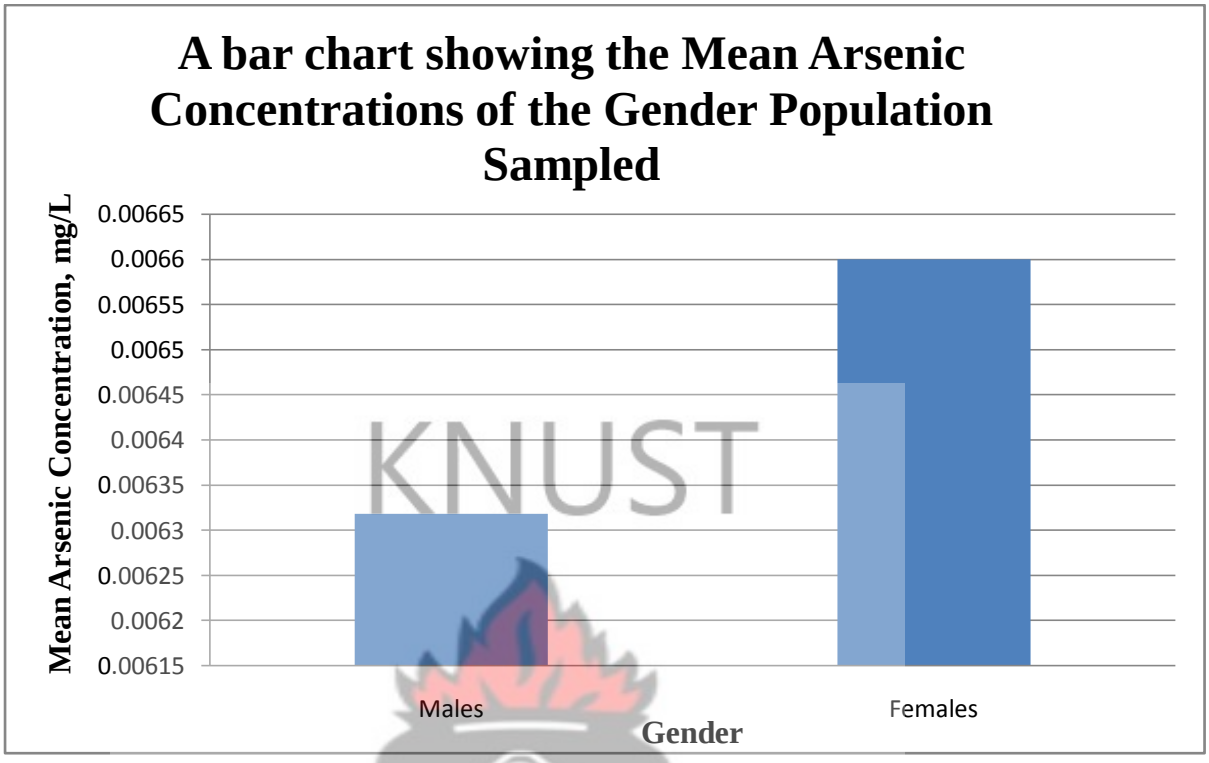
Appendix M



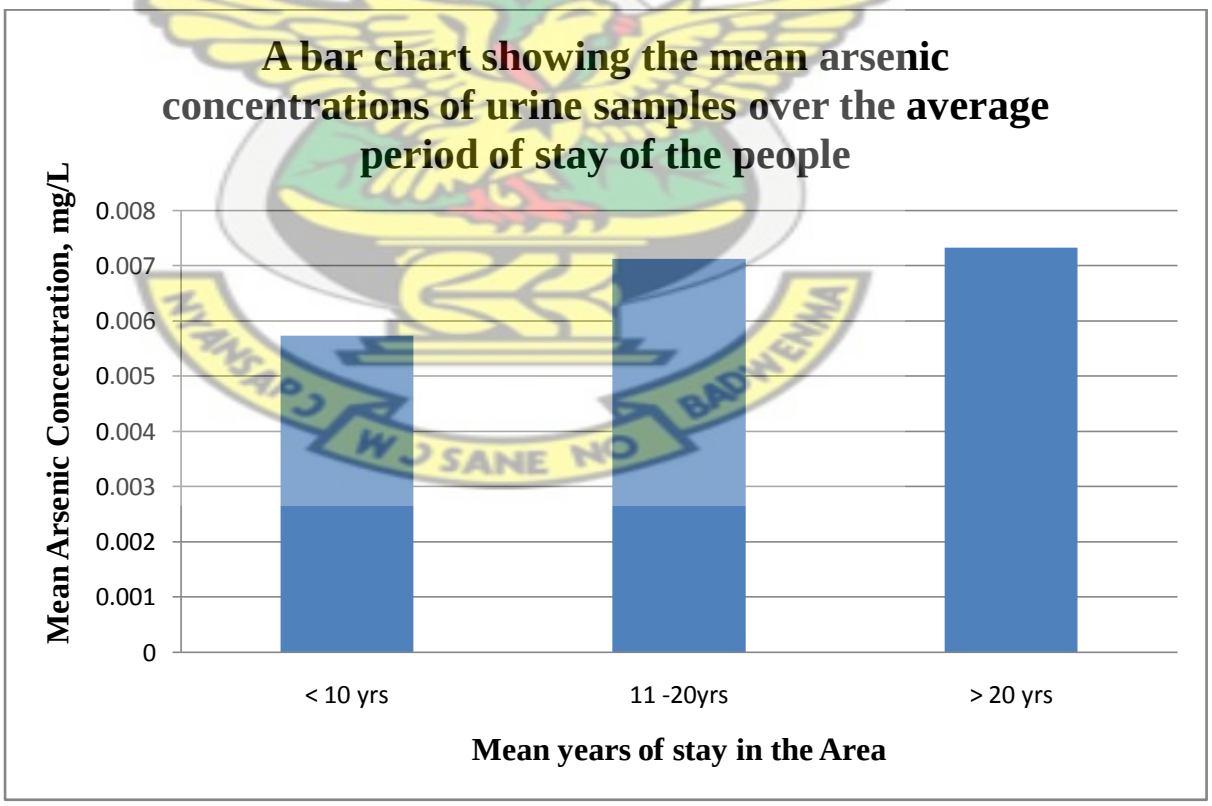
Appendix N



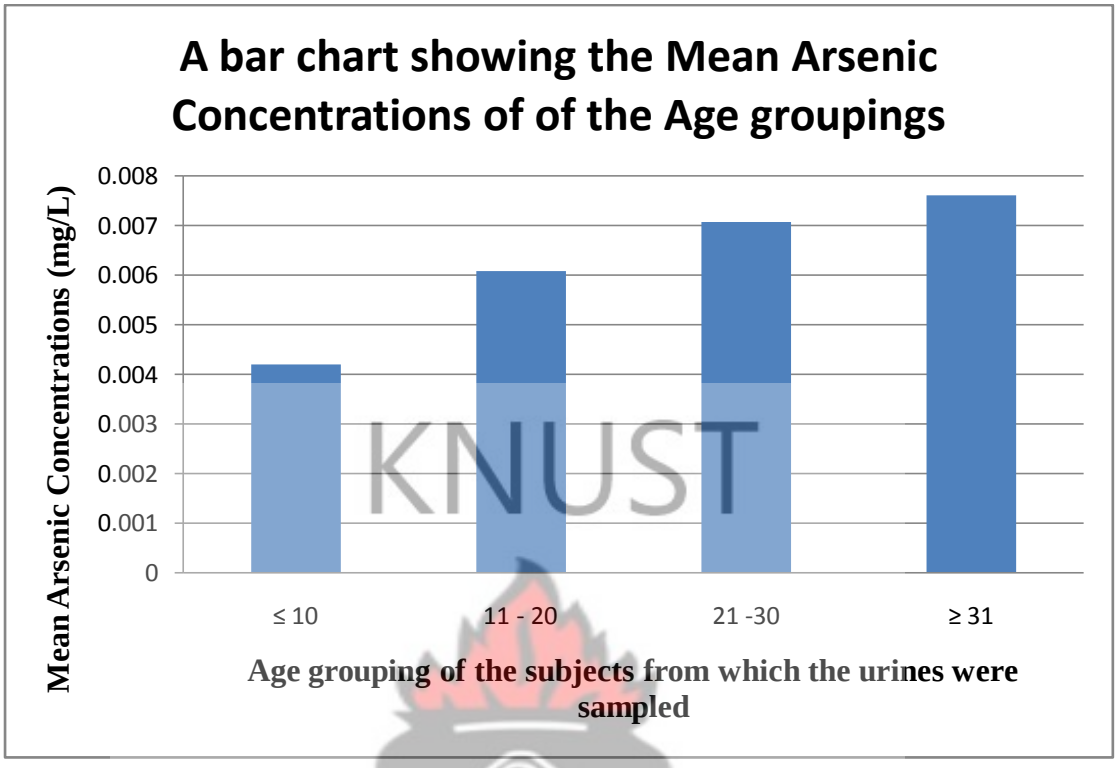
Appendix O



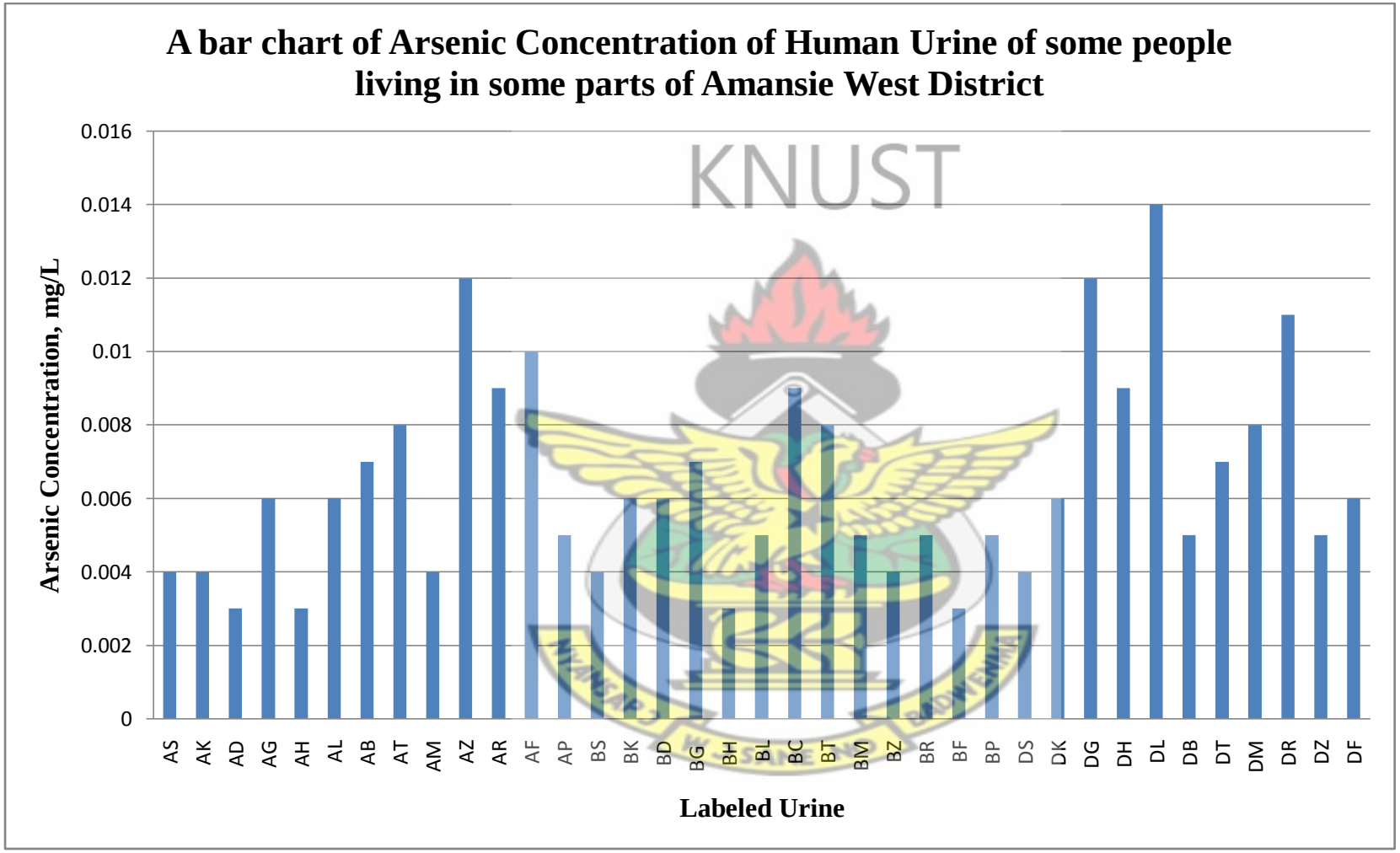
Appendix P



Appendix Q



Appendix R



Appendix S

QUESTIONNAIRE

Male [ ] or Female [ ]

Age range……

How long have you been staying in your community……

Occupation:

Educationist [ ]

Farmer [ ]

Trader [ ]

Mining [ ]

Pupil [ ]

Source of food stuffs:

From your community [ ]

From outside your community [ ]