

**SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA**

**ASSOCIATION BETWEEN ARSENIC EXPOSURE AND BURULI
ULCER (BU) IN THE GOLD-MINING COMMUNITIES OF
AMANSIE WEST DISTRICT**

BY

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**THIS DISSERTATION IS SUBMITTED TO THE UNIVERSITY
OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE AWARD OF MASTER OF PUBLIC
HEALTH DEGREE**

JULY, 2016

DECLARATION

I hereby declare that this research dissertation is an original work by me under supervision in the Department of Biological, Environmental and Occupational Health, towards obtaining a Masters of Public Health (MPH) Degree, and that it contains no material previously published by another person or accepted for the award of any other degree, except where due acknowledgement has been made in the text.

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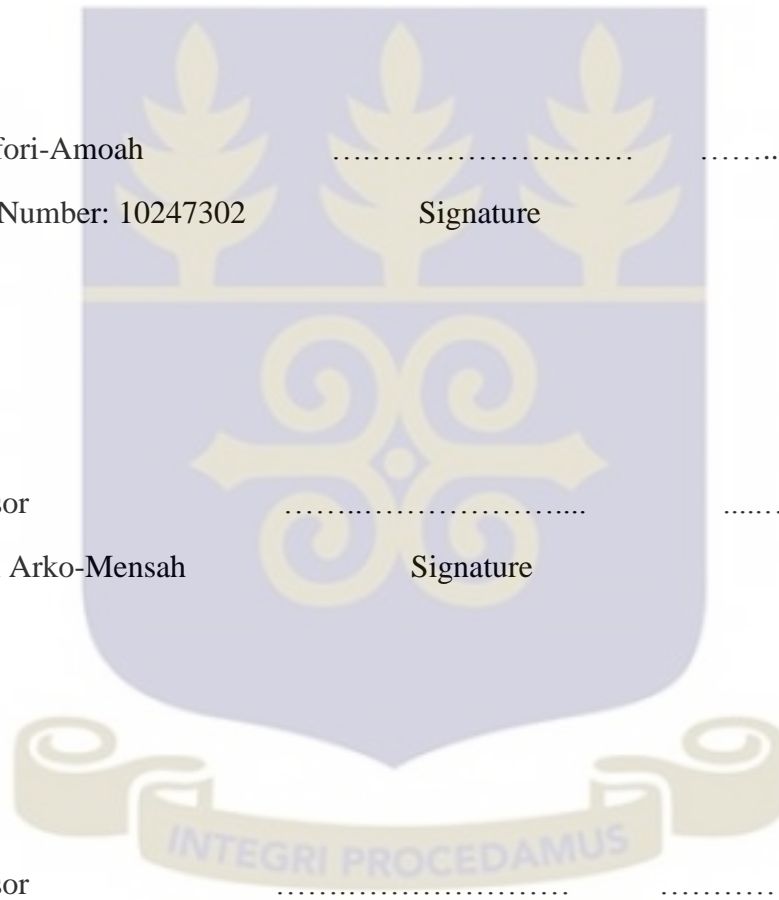
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DEDICATION

This work is dedicated to the Almighty God, for His mercy, grace and providence throughout the entire work. I also dedicate this work to my Aunt, Diana Acquah and her husband, for their understanding, loving kindness and support throughout my stay with them during the course of this programme.



ACKNOWLEDGEMENTS

I give glory and thanks to the Almighty God for the strength and enablement to go through the MPH programme successfully.

I wish to express my sincere gratitude to my ablest supervisors, Prof. Julius Fobil and Dr. John Arko-Mensah, for their unending guidance and unflinching support throughout the work.

This study was supported by the West Africa-Michigan Collaborative Health Alliance for Reshaping Training, Education and Research in Global Environmental and Occupational Health (WEST AFRICA-MICHIGAN CHARTER II).

My profound appreciation, also, goes to Mr. Prince Owusu of the Ecological Laboratory Unit, University of Ghana, and all the staff at the St. Martin's Hospital, Agroyesum, as well as Misters Samuel Opoku Asiedu and Jones Lamptey of Kumasi Centre for Collaborative Research into Tropical Medicine (KCCR); for their immense support throughout the research work.

My final thanks go to all my MPH course-mates and colleagues, especially Ms. Maria Mensa-Wonkyi, Mr. Bright Gambilla Sandow and Ms. Eunice Matilda Mends.

ABSTRACT

INTRODUCTION: Buruli ulcer (BU), as the name suggests is an ulcerative skin disease caused by *Mycobacterium ulcerans*. Due to the high endemicity of BU in communities of the Amansie West District where artisanal mining is common, previous studies have focused on a possible relationship between levels of arsenic in environmental media to BU incidence. Thus far, a correlation has been found between arsenic concentrations in environmental media and growth of *M. ulcerans*, suggesting a higher BU prevalence in arsenic-rich gold-mining environment.

OBJECTIVE: The aim of this study was to investigate whether there is an association between arsenic levels in biological samples (i.e. blood and/or urine) and the prevalence of BU, and to determine whether or not differences exist across different BU subject groups (vis-à-vis: old-, new- and non- BU cases) in the gold-mining communities of the Amansie West District.

METHODOLOGY: Individuals, male or female, who have been medically diagnosed as having BU, and their non-BU relatives or other healthy individuals living in the Amansie West District were recruited into the study (upon informed consent). Blood and urine samples were collected and analyzed for arsenic levels using an Atomic Absorption Spectro-Photometer (AAS).

RESULTS: Arsenic was undetected in all (i.e. blood and urine) samples; below the detection level of the instrument used ($\leq 0.01 \mu\text{g/L}$).

CONCLUSION: Arsenic was undetectable in the blood and urine of neither BU patients nor healthy individuals from the Amansie West District, suggesting that arsenic levels alone may not account for the aetiology of *M. ulcerans* infection.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
As	Arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
BU	Buruli ulcer
DMA	Dimethylarsinic acid
EcoLab	Ecological Laboratory Unit of University of Ghana
EPA	Environmental Protection Agency
GC	Gas Chromatography
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IL	Interleukin
LOD	Level of Detection
MCL	Minimal Concentration Level
MMA	Monomethylarsonic acid
mg/L	milligram-per-litre
MU	<i>Mycobacterium ulcerans</i>
NAA	Neutron Activation Analysis
ND	Arsenic concentration below detection limit
ppb	parts-per-billion
Th-cells	T-lymphocyte helper cells

TNF	Tumour necrosis factor
$\mu\text{g/L}$	microgram-per-litre
WHO	World Health Organization



CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Arsenic exposure is reported to be one of the major global health problems, affecting over 300 million people worldwide. It is known that, at concentrations $> 50 \mu\text{g/L}$, inorganic arsenic is associated with elevated risks of several cancers (e.g., bladder, kidney, liver, lung, skin, prostate, etc), cardiovascular diseases and high blood pressure (Hagarty *et al.*, 2015; Gyasi *et al.*, 2012a).

Arsenic is among the top 20 most abundant trace elements on the earth's crust. Its association with some non-weathering resistant mineral deposits (e.g. sulphide minerals) has contributed to its release in large amounts into the environment. Arsenic is widely present in natural waters and soluble over a wide range of pH conditions. Arsenic is used in hardening of alloys and in production of pigments, semiconductors, rodenticides, fungicides etc. Because of its usefulness and exploitation, contamination of this heavy metal is widespread across a range of environmental media (Gyasi *et al.*, 2014; Mazumder, 2000).

Arsenic occurs naturally in groundwater from dissolution of arsenic-bearing aquifers, with concentrations typically ranging from $<1-1000 \mu\text{g/L}$. Several cases of arsenic poisoning have resulted from intake of contaminated drinking water. Severe health effects due to arsenic-contaminated drinking water have been reported mainly in populations of low socio-economic status and poor nutrition. This is because the disposal/removal of highly reactive methylated arsenic species from the body

becomes very poor, due to the deficient nutritional and immune condition of the malnourished individual (Hagarty *et al.*, 2015; Mazumder, 2000).

Elevated levels of arsenic have been associated with several adverse health outcomes, such as different forms of cancers, vascular diseases, dermatological ailments, diabetes, respiratory diseases, cognitive decline, adverse pregnancy outcomes and infant mortality (Quansah *et al.*, 2015). Again, arsenic is known to interact with and inhibit several enzymes in the body leading to several multi-systemic non-cancer effects, which could predispose one to a defective immune system. For example, immunosuppression due to arsenic has been found to affect antigen processing of splenic macrophages, with consequent defective mechanism of T-helper cells. Down-regulation of the immune system is known to be a risk factor for the development of buruli ulcer (BU), an ulcerative skin disease caused by *Mycobacterium ulcerans* (MU) (Gyasi *et al.*, 2014; Gyasi *et al.*, 2012b; Addo *et al.*, 2005; Mazumder, 2000).

In an analytical chemistry study conducted in water samples (collected from different sources) for trace metal concentrations, higher levels of most trace metals were found in samples from artisanal mining, also known as galamsey pits and BU hot spots, as compared to other water bodies (Atosona, 2012). For instance, concentrations of arsenic, cadmium, lead and other metals were found to be highest in galamsey pits and BU hot spots. Higher levels of arsenic in the environment might be contributing to increased BU incidence in these galamsey communities by supporting the growth of *M. ulcerans*. On the other hand, arsenic in drinking water could have a general immuno-suppressive effect, making the population more susceptible to buruli ulcer (Hagarty *et al.*, 2015; Atosona, 2012; Gyasi *et al.*, 2012a).

Buruli ulcer (BU) is a human ulcerative skin disease, which usually begins as a painless nodule or papule in the skin, and may progress to massive skin ulceration, which often results in grossly deforming sequelae. Infection leads to extensive destruction of skin and soft tissue with the formation of large ulcers usually on the legs or arms. If untreated, infected patients often suffer long-term functional disability such as restriction of joint movement as well as obvious cosmetic problem, with inflammation extending to deep fascia and contractual deformities (Berkowitz *et al.*, 2015; Walsh *et al.*, 2009).

BU is rated as the third most common mycobacterial infection worldwide, after tuberculosis and leprosy by the World Health Organization (WHO, 2012). The main form of treatment is wide excisional surgery, including amputation of limbs, which requires prolonged hospitalization; which presents a high risk of physical deformities and places a huge burden on hospital resources and budgets. In a WHO report in 2012, Ghana was noted to be the second most endemic country for buruli ulcer after Cote d'Ivoire globally. There have been more than 2000 reported cases in the last ten years, with outbreaks occurring in at least 90 districts in Ghana. Buruli ulcer was first brought to public attention in Ghana in 1993, when severe cases were reported from the Amansie West district of the Ashanti Region (Bonyah *et al.*, 2013; Owusu-Sekyere, 2013; Asare, 2010; Walsh *et al.*, 2009).

Moreover, results from a statistical modelling study using spatial neighbour method revealed a spatial dependency of BU prevalence on proximity to drainage channels and farmlands containing > 15 mg/L arsenic, and thus purported that BU has a positive association with arsenic exposure (Duker *et al.*, 2006; Duker *et al.*, 2004). In this case, the role of arsenic may be of an immuno-depressant; increasing the

susceptibility of opportunistic pathogens like MU to cause BU-disease. It is, however, important to note that in spite of the plethora of studies carried out by numerous researchers globally on BU infections, including the World Health Organization, the pathogenesis and aetiology of this skin disease still remains quite unclear.

1.2 Problem Statement

Duker *et al.* in 2006 enumerated the possible influence of environmental contamination (i.e. arsenic poisoning) on *M. ulcerans* infection. Elevated arsenic levels has been suggested to adversely affect the human immune system, implicated as a vital environmental risk factor for development of *M. ulcerans* infections, particularly BU (Bonyah *et al.*, 2013; Gyasi *et al.*, 2012a). Also, subjects exposed chronically to arsenicals are said to be prone to viral and bacterial infections, as well as other arsenic-induced lesions (Gyasi *et al.*, 2012b; Addo *et al.*, 2005).

Proximity analysis carried out to determine spatial relationships between BU-affected areas and arsenic-enriched farmlands and drainage channels in the Amansie West District, showed that settlements along arsenic-enriched drainages and farmlands had higher BU prevalence than settlements farther away from arsenic-enriched sites (Duker *et al.*, 2004). However, is there any evidence of association between arsenic exposure or contamination and BU in affected patients? In addition, what are the levels or concentrations of arsenic and other heavy metals in BU- and non-BU individuals living in exposed environment? For instance, in a BU-endemic highly mining-dominated environment, are there significant differences in the blood and/or urine concentrations of arsenic in affected and non-affected individuals?

Subsequently, a very important public health research question of interest worth investigating is, “is there any evidence of association between arsenic exposure (in terms of blood and urine arsenic concentrations) and BU?” To provide an answer to this question, there was the need to undertake an analytical study of arsenic levels in BU patients and healthy subjects living in arsenic-exposed mining communities of the Amansie West District in the Ashanti Region of Ghana.

1.3 Justification

The aetiology of *M. ulcerans* infection still remains poorly understood among members of the scientific community, as the mode of transmission is still unclear. Thus, scientists have recently turned their attention to determining the pathogenesis of BU in an effort to prevent this disease, as it is considered by the WHO as a neglected and re-emerging disease which needs immediate research attention.

While BU is typically non-fatal, it can result in severe deformities and medical complications, if not promptly and properly treated. In Ghana, BU still remains one of the top priority health problems and has become a typical disease with devastating deformities among children (as about 70% of those affected are children less than 15 years of age). The consequence for these children is prolonged morbidity (which often leads to serious disruption of school or even discontinuation of schooling), and further complications such as contracture deformities and/or amputations (which might leave them with permanent disabilities). This is a cause for worry and concern, as these children would later grow into adulthood and become a serious burden to the society. More so, the disease could have serious physical and cosmetic problems with further

repercussions such as stigmatization, social isolation, diminished marriage prospects for girls and women, and sometimes even divorce for affected individuals.

Research on BU suggests that, there is a positive correlation between mycobacterial population density and metal concentrations, indicating that BU incidence may be higher near mining sites; as heavy metals are commonly associated with tailing waste from mining activity (Owusu-Sekyere, 2013).

This study intended to draw a parallel between arsenic concentrations in BU and non-BU individuals in endemic communities in the Amansie West District; one of the highest BU-endemic districts in Ghana, and has experienced exploitation of arsenic-bearing mineral deposits and historic atmospheric emissions of As_2O_3 from the roasting of sulphide ores (from mine operations and 'galamsey' activities).

1.4 RESEARCH QUESTIONS

- i) Are there differences in the concentrations of arsenic in the blood/urine of groups of BU cases, and uninfected controls?
- ii) What is the association between arsenic exposure and BU across these subject groups (if differences exist)?

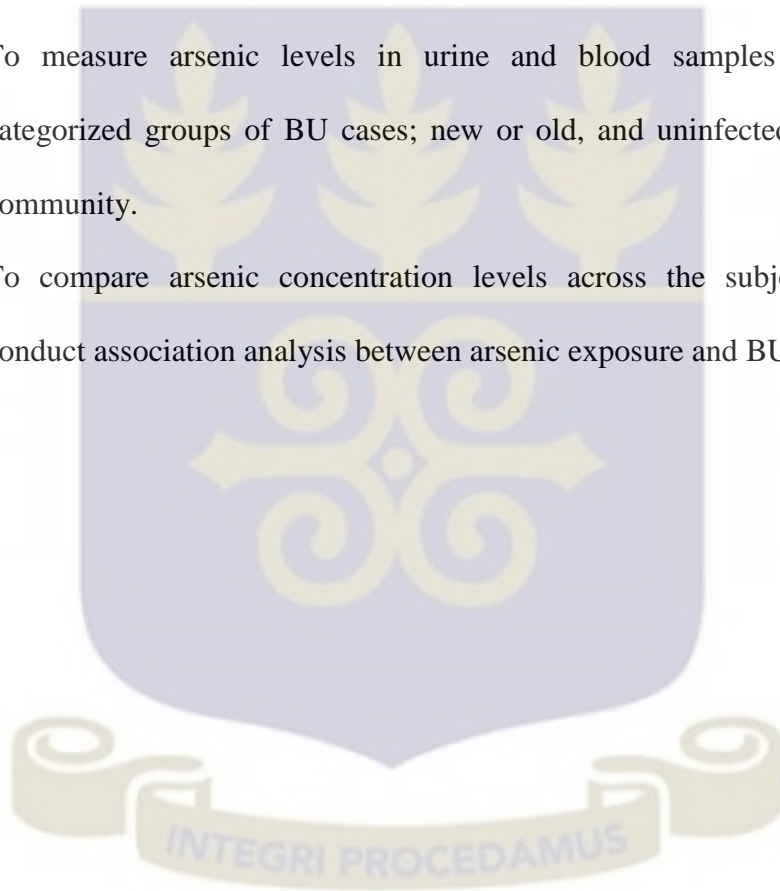
1.5 RESEARCH OBJECTIVES

1.5.1 General Objective

To compare arsenic levels in urine and blood of BU cases and non-BU controls in the endemic gold-mining communities of the Amansie West District, in order to establish the relationship between arsenic exposure and risk of Buruli Ulcer.

1.5.2 Specific Objectives

- i) To measure arsenic levels in urine and blood samples collected from categorized groups of BU cases; new or old, and uninfected subjects in the community.
- ii) To compare arsenic concentration levels across the subject groups, and conduct association analysis between arsenic exposure and BU (if possible).



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BURULI ULCER (BU) DISEASE

2.1.1 The Causative Organism

BU is a skin disease caused by *Mycobacterium ulcerans* (MU) and is rated as the third most common mycobacterial infection after tuberculosis and leprosy. MU is a slow-growing organism, which grows optimally under micro-aerophilic conditions at 25 °C and 28 °C than at higher temperatures. The bacteria secrete a necrotizing and immunosuppressive toxin called “mycolactone”. The main effect of this toxin is to produce fat necrosis, which may extend beyond the site of infection. The toxin is known to suppress the production of interleukin-2 (IL-2) and tumour necrosis factor (TNF); thereby down-regulating T-helper-1 (Th1) (i.e. the pro-inflammatory immune response). Development of MU infection therefore has much to do with the state of the individual's immune response (Duker *et al.*, 2006).

However, it is reported that the MU toxin alone could not induce systemic immunosuppression; host susceptibility factors (which could be genetic or environmental) also play a vital role. For instance, studies indicate that those developing BU may already have immune deviation resulting in down-regulation of Th1 and increased Th2 (humoral immune response) or have inherent defect in their immune system that leads to failure to develop any strong response to mycobacterial antigens (En *et al.*, 2008; Duker *et al.*, 2006).

2.1.2 Aetiology/Pathogenesis

The BU disease is reported to often affect mostly impoverished inhabitants in remote and rural areas, with children being the most vulnerable (Bonyah *et al.*, 2013). For

example in Ghana, about 70% of BU patients and cases recorded from the endemic areas in the Ashanti Region were found to be younger than 15 years (Walsh *et al.*, 2009).

It has been speculated that BU is acquired when MU enters the body through a skin rupture, or through a non-ruptured but unusually unhealthy or thin skin (Gyasi *et al.*, 2014). Studies conducted suggest that arsenic in the environment may play a contributory role in MU infection as well as the spatial distribution of BU (Duker *et al.*, 2004). Bioaccumulation of arsenic in the fatty tissues of the skin, due to its high lipid solubility, is said to provide a favourable environment for MU in the skin, as arsenic is known to facilitate growth of microorganisms. Hence, it has been hypothesized that arsenic might induce MU adhesion to human tissues as well as influence the ability of MU to establish BU (Duker *et al.*, 2006).

More so, areas where BU was noted to be a serious health threat, the concentrations of arsenic in surface and ground water were found also to be higher than average. For example, the Amansie West District, which accounts for most of the BU cases in Ghana, happens to have high levels of arsenic in water bodies with concentrations frequently exceeding the World Health Organization level of MCL of 10 µg/L, possibly released through the intensive gold mining activities there (Duker *et al.*, 2004). Although BU hotspots are reported to be found at areas where artisanal mining is concentrated, the aetiology of *M. ulcerans* is still not clearly defined.

2.1.3 Symptoms/Clinical presentations of BU

Buruli ulcer is primarily a disease of subcutaneous fat and is often characterized by deep and necrotizing skin lesions, mostly on the arms and legs (Figure 1). The disease

usually begins as a firm, painless nodule, less often as diffuse, sometimes painful oedema; which without appropriate therapy causes massive skin ulceration, resulting in grossly deforming sequelae. It can be characterized as a non-ulcerative and secondary ulcerative disease. According to the clinical case definition of the WHO, the pre-ulcerative stage includes papules, nodules, plaques and oedematous form (Figure 1a). When the skin ulcerates, it shows an extensive zone of necrotic subcutaneous fat (necrotizing panniculitis), which extends laterally, undermining the dermal edges of the ulcer (as showed in Figure 1b). Later, a granulomatous healing response occurs and fibrosis, scarring, as well as calcification and contractures with permanent disabilities may result. Sometimes, the bone is affected causing gross deformities (Asare, 2010; Duker *et al.*, 2006).



Figure 1: Picture of Buruli ulcer at (a) the pre-ulcerative stage: nodule, and (b) secondary ulcerative stage.

Because of the local immunosuppressive properties of mycolactone, or perhaps as a result of other unknown mechanisms, the disease progresses without pain and fever (En *et al.*, 2008); which may partly explain the reason those affected most often do not seek prompt treatment.

2.1.4 Treatment and Control of BU

Currently, excisional surgery with or without skin grafting remains the recommended therapy for BU. There have been anecdotal accounts of successful antibiotic therapy of early lesions. Studies have established chemotherapeutic combination of rifampicin and streptomycin as the most effective way of killing the *M. ulcerans*, and more recent work shows that the organism can be killed in human tissue by taking the stated treatment combination for at least eight weeks (Walsh *et al.*, 2009).

The use of protected sources of water for domestic purposes reduces exposure to MU contaminated sources, and consequently may reduce BU infections. In addition, since most endemic areas are rural populations (who are poor and consider hospital treatment expensive), and as even the mode of transmission of the disease is unclear, alternative interim precautionary measures through education and limiting contact with environmental sources of MU (of the rural population) are seen as a way to reduce infections and consequent economic burden imposed by the BU disease (Ahorlu *et al.*, 2013; Duker *et al.*, 2006).

2.1.5 Prevalence of BU in the Amansie West District

The Amansie West District is where the first case of BU was reported in Ghana in 1971 (Duker *et al.*, 2004), and is currently the most BU endemic district in Ghana. Between 1991 and 2001, more than 2000 cases of BU were said to have been reported from the district (Duker *et al.*, 2004). Prevalence of BU in the district is 151 cases per 100,000 inhabitants, which is way above the national rate of 22.7 cases per 100,000 inhabitants. The most endemic towns include Tontokrom, Mpatuam, Manso Mem, MansoAtwere, Edubia, Watreso, Abore, Keniago, Essuowin, Ahwerewa and Datano

(Bonyah *et al.*, 2013). The treatment for Buruli ulcer disease in the district is provided at the St Martin's Catholic Hospital at Agroyesum which has specialized BU-disease facilities following WHO treatment guidelines (Owusu-Sekyere, 2013).

2.2 ARSENIC TOXICITY

2.2.1 The Arsenic Element and Its Exposure

Arsenic (As) is one of the naturally occurring elements that can be found in the earth's crust. Arsenic is mainly referred to as a metal though it is chemically classified as a metalloid, having both metallic and non-metallic properties. It is found in the environment as compounds mostly combined with other elements such as oxygen, chlorine, sulphur, carbon and hydrogen. Arsenic is very common in most geological environments: igneous, metamorphic and sedimentary rocks, and occurs as a chalcophile, oxyanionic or metalloid element often associated with sulphide ores. Examples of As-compound ores include arsenopyrite, orpiment and realgar.

One can be exposed to arsenic through various pathways including inhalation, accidental soil ingestion, as well as through food and drinking water. The primary routes of arsenic entry into the body are ingestion and inhalation; although dermal absorption also occurs, but to a lesser extent. Exposure through contaminated water used for drinking, food preparation and irrigation of food crops poses the greatest threat to public health (WHO, 2010). WHO guidelines suggest a permissible limit of 10 micrograms of arsenic in a litre of water (i.e. 10 µg/L).

The relative toxicity of an arsenical depends primarily on its form, valence state, solubility, physical state and purity, and rates of absorption and elimination (ATSDR, 2007). Inorganic arsenic is known to be generally more toxic than organic arsenic. Also, forms of arsenic that are more rapidly absorbed are said to be more toxic, while those most rapidly eliminated tend to be less toxic (ATSDR, 2007). The toxicity of arsenic compounds in humans is predominantly a function of the rate of removal from the body. In general, arsine is considered to be the most toxic form, followed by the arsenates (arsenic-III), the arsenates (arsenic-V) and organic arsenic compounds.

2.2.2 Sources of Arsenic Contamination

Sources of arsenic contamination include both natural and anthropogenic sources. Naturally, arsenic occurs in over 200 minerals. The desorption and dissolution of these minerals results in a high level of arsenic in ground water, especially in lakes, rivers, deltas and alluvial planes. Also, arsenic found in soil sediments finds its way in water sources through wind-blown dust, water run-off and leaching. Volcanic eruptions are another source of arsenic contamination (Armah *et al.*, 2011).

Anthropogenic sources of arsenic mobilization and contamination include weathering of mine waste rock and gold tailings, burning of fossil fuels, agricultural practices involving the use of fungicides, herbicides and insecticides that contain arsenic. Arsenic is associated with ores containing metals, such as copper and lead; and thus may enter the environment during the mining and smelting of these ores. Small amounts of arsenic also may be released into the atmosphere from coal-fired power plants and incinerators because coal deposits and coal-waste products often contain some arsenic (Armah *et al.*, 2010).

2.2.3 Arsenic Contamination in Ghana

Contamination of groundwater by arsenic in different parts of the globe is a consequence of natural and/or anthropogenic sources, resulting in adverse effect to human health and ecosystem; and Ghana is no exception. In Ghana, industrial activities like mining largely account for high levels of arsenic in drinking water sources (Akabzaa *et al.*, 2014).

A study conducted in Akyem Abuakwa to assess the impact of artisanal gold mining on water sources found elevated levels of arsenic in drinking water sources (Kortatsi *et al.*, 2008). Also, a study conducted by Asante *et al.* in 2007 to elucidate the contamination status of multi-trace element levels in sources of drinking water and human urine in Tarkwa found arsenic concentrations above the WHO guidelines. Again, the average concentration levels of several metals in mine-impacted streams of Tarkwa and Prestea were also found to be above the maximum allowable limits in drinking water stipulated by WHO and EPA-Ghana. These metals included arsenic, mercury, copper, manganese, among others (Kusimi & Kusimi, 2012).

2.2.4 Health Effects of Arsenic Contamination

Although the arsenic element has several industrial and pharmaceutical uses, it is also a protoplasmic poison that presents severe health disorders. Chronic exposure to arsenic causes arsenicosis (WHO, 2010) and other serious disease outcomes such as cancer of the bladder, liver and lungs (ATSDR, 2007), adverse pregnancy complications (Quansah *et al.*, 2015), as well as neurological and cognitive developmental disorders (Bellinger, 2013). Arsenicosis is a disabling disease with typical features including skin lesions, pigmentation of the skin and patches on the

palm of the hand and soles of the feet, in addition to other skin changes such as hyperkeratosis, upon excessive repeated exposure to arsenic (i.e. Figure 2). Arsenicosis is known to affect more than 140 million people in at least 70 countries, including Afghanistan, Argentina, Bangladesh, Cambodia, Chile, China, India, Mexico, Mongolia, Myanmar, Nepal, Pakistan, Taiwan, Vietnam, Sub-Saharan Africa and the United States (WHO, 2010).

Moreover, long-term exposure to arsenic in drinking-water is said to be related to increased risks of cancer in the liver (i.e. angiosarcoma), lungs, bladder and kidney, as well as skin and internal cancers. Occupational exposure to arsenic, primarily by inhalation, is also known to be associated with lung cancer. Research conducted on arsenic-exposed workers found a systematic gradient in lung cancer mortality rates, depending upon duration and intensity of exposure. A higher risk of lung cancer was found among workers exposed predominantly to arsenic trioxide in smelters and to pentavalent arsenical pesticides (ATSDR, 2007).



Figure 2: Picture of (a) skin cancer on the palm and (b) keratosis of a patient who ingested arsenic over a prolonged period of time from a contaminated well. (Courtesy of Arsenic Foundation)

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

This is a case-control study; and it involved laboratory testing of arsenic levels in blood and urine samples collected from BU- and non-BU individuals. Here, old cases of Buruli ulcer served as the control group for new BU-cases, while non-BU subjects served as community-matched controls for both old and new BU-cases.

This was to help assess also whether there would be any significant differences in levels of arsenic in old- and new- BU cases possibly (due to variations in arsenic bio-accumulation and/or elimination over time).

3.2 Study Setting

The study was conducted in the Amansie West District of the Ashanti Region, where the disease was first given public recognition and national documentation in Ghana. All recruitment including screening and interactions such as information gathering and sample/specimen collection were conducted in the district. The St. Martin's Hospital at Agroyesum (in the district), which has specialized Buruli ulcer disease facilities, was used for sample/specimen collection.

Metal concentration analysis was done in a level-2 biosafety laboratory at the Ecological Laboratory Unit (EcoLab), University of Ghana, Legon-Accra.

3.3 Study Area

Geographical Location

The Amansie West District is located in the south-western part of Ashanti Region of Ghana. The District shares boundaries with the Amansie East District in the west,

Atwima Mponua District in the east, Atwima Nwabiagya District in the north and Amansie Central in the south. The district falls within latitudes $6^{\circ} 35'$ and $6^{\circ} 51'$ North and longitudes $1^{\circ} 40'$ and $2^{\circ} 05'$ West. The district covers an area of about 1,364 km² and forms about 5.4 percent of the total land area of the Ashanti Region, with Manso Nkwanta as its district capital (Owusu-Sekyere, 2013; Duker *et al.*, 2006).

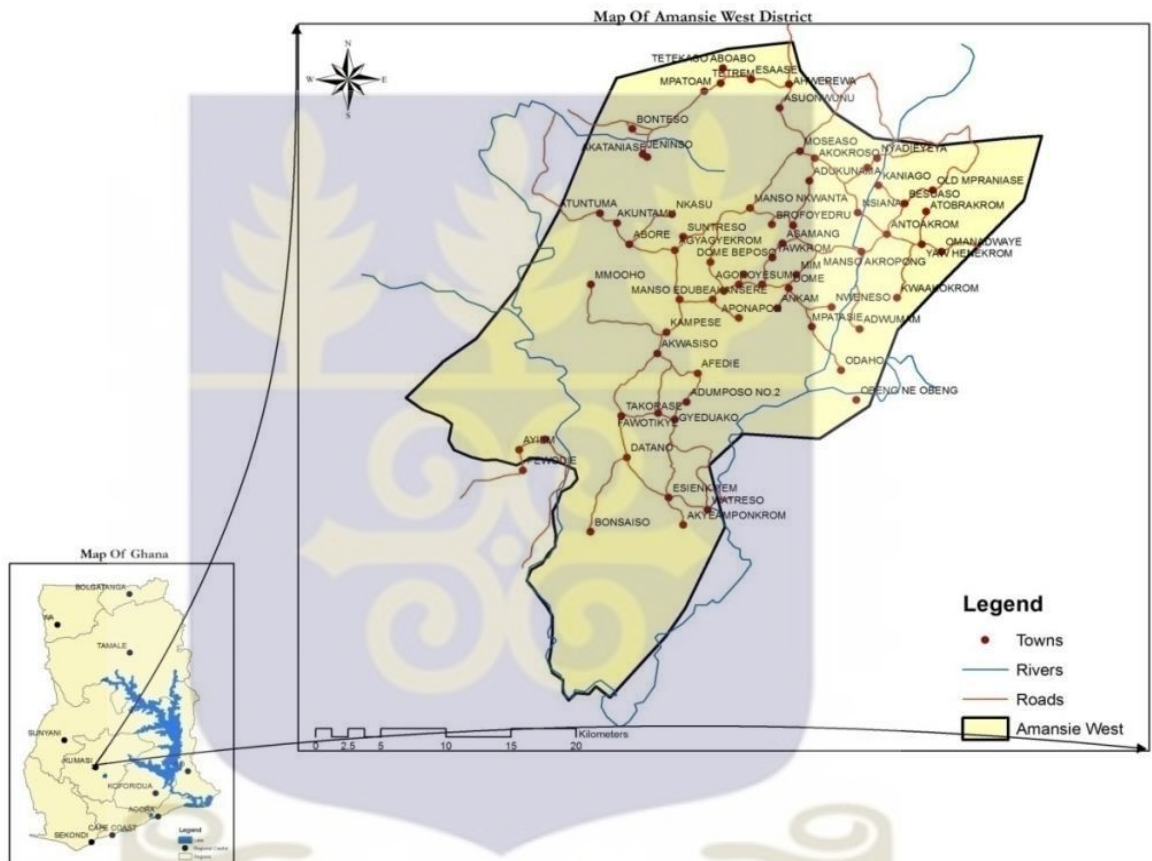


Figure 3: Map of Amansie West District (Owusu-Sekyere, 2013).

The district is populated by various segmented identity subgroups of Asantes and settler migrants from other parts of Ghana. These groups rely mainly on subsistence farming and artisanal small scale mining, commonly called ‘galamsey’ for sustenance (Owusu-Sekyere, 2013). The district has about 310 settlements with a population in 2000 of 108,726. 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 *et al.*, 2004).

Geology and soil

The district is underlain by Lower Proterozoic Birimian and Tarkwaian geological formations. Economically, the Birimian and Tarkwaian rocks are regarded as the most important formations due to their mineral potentials. Birimian rocks of West Africa are mainly volcanic greenstones with intervening sedimentary rocks and granitoid intrusions; in some places containing deposits composed of pyrite, arsenopyrite, minor chalcopyrite, sphalerite, galena, native gold and secondary hematite (Duker *et al.*, 2004, 2006).

These geological formations are the reasons for the existence of high mineral deposits in the district. Consequently, many gold-mining activities are predominant in the district. Soils are deep, open and acidic in many places due to heavy leaching of arsenic and other heavy metals from the gold-mining sites and “galamsey” activities.

3.4 Study population

The study population included participants, male or female, Buruli ulcer patients who had been medically diagnosed with laboratory confirmation for BU, and their non-BU relatives or other healthy individuals living in the Amansie West District.

3.5 Inclusion and Exclusion criteria

All participants of this study should have lived within the Amansie West District for a period of at least six months. The justification for the inclusion of these subject populations is that arsenic exposure is predominant in gold-mining communities in Ghana. Individuals living in arsenic-rich mining communities are likely to be

exposed; hence the importance of ascertaining levels of arsenic (i.e. presumably 6 or more months of arsenic exposure). BU-cases should have been confirmed of having BU disease medically at the St. Martin's Hospital (reference BU centre), and/or by PCR. Only individuals who agreed to participate and met the eligibility criteria were enrolled into the study.

Individuals with any of the following were, however, excluded: (i) BU patients who had not stayed within the Amansie West District for at least six months; (ii) people who didn't give their consent for the taking of their urine or blood samples; (iii) individuals who were in severe illness or have anaemia.

3.6 Ethical Consideration and Community entry

Ethical clearance and approval were sought for from the Ghana Health Service Ethical Committee before the start of the research. The Amansie West District Health Directorate and the administration of St. Martin's Hospital were then contacted and permission given. The community unit committees for the various communities within the district were also consulted. The research work was conducted hand-in-hand with District disease control officers, community health staffs, as well as community volunteers experienced with similar research in the communities.

3.7 Sampling/recruitment technique

Reported BU cases were retrieved from medical records of the St. Martin's Hospital at Agroyesum and the District Health Directorate. Cases were followed up in the communities and those identified recruited for the study. Hence, sampling was

purposive (i.e. individuals encountered were recruited and as such used for the research).

Informed individuals with reported BU cases were selected (upon informed consent) and categorized in relation to relevant criteria (such as gender, age, ethnicity, locality, occupation, etc.), to allow for maximum variation. Snowball sampling (i.e. using participants and community volunteers to identify additional cases) was also used in order to increase respondents' confidence and consequently reduce response bias.

3.8 Informed consent

Informed consent was sought and obtained from all study participants. At the initial meeting, an oral script introducing the study was read for those who could read and write, and by a translator for those who could not read and write. If the subject was interested in participating in the study, the written consent form was read by the participant and/or by a translator and any questions raised by the subject was answered. When the individual agreed to participate, blood and urine samples were then collected.

All prospective subjects had the information sheet read and/or explained to them and made to sign the informed consent form, and for those below age 18 their parents/guardians had to sign an assent form on their behalf before recruitment into the study. A translator and local volunteer were engaged to assist in communication and recruitment of participants.

3.9 Collection of Biological Specimens

Blood samples were obtained directly from subjects for the purpose of this research. A total volume of about 3–5ml was collected by a phlebotomist using standard laboratory procedures into tubes containing silicon-gel and clot activator (Channel MED, China), and were left to clot at room temperature. Clotted samples were centrifuged at 3000 rpm for 5 min. Sera were then collected into sterile Eppendorf tubes, frozen, stored on ice (at about 4–8°C) and transported to Ecological Laboratory Unit, University of Ghana, for arsenic analysis.

Urine samples were obtained directly from subjects for the purpose of this research study. Participants were provided with sterile metal-free plastic containers and instructed to urinate into them. Urine samples were then transferred into labelled 50 mL sterile, metal-free plastic urine containers. About 15 mL of the urine was then drawn into plastic tubes with an in-built plunger (Sarstedt S-monovette, Germany), stored on ice (at about 4–8°C) and transported to EcoLab for laboratory analysis.

3.10 Detection of Arsenic Levels in Biological Samples

The measurements of arsenic levels in the urine and blood samples were performed using a PerkinElmer PinAAcle™ 900T Atomic Absorption Spectro-Photometer (AAS) (Shelton, CT, USA). This instrument is equipped with the intuitive WinLab AT Furnace Software for optimal performance. The heavy metal detection equipment was coupled to a FIAS 400 flow injection analysis system that incorporates two peristaltic pumps, a 5-port flow injection valve and a regulated gas supply.

Using a FIAS-AAS system, a sample loop on the flow injection valve was filled with the acidified sample, blank, or standard. The valve was then automatically switched to the inject position and the sample was mixed with a pumped stream of reductant, sodium borohydride for hydrides or stannous chloride. At the point of reaction with the reductant, arsenic was produced, along with hydrogen from the sodium borohydride, resulting in a two-phase mixture. A flow of argon was added to this mixture and then carried through a gas/liquid separator. This allowed the gaseous phase which contained the analyte vapour to enter the quartz cell on the AAS for analysis, while the remaining liquids were pumped to a waste container. Values detected for arsenic were recorded accordingly.

Arsenic was measured as Total Arsenic in all the biological samples. The level of detection (LOD) for the equipment used is 0.01 µg/L.

3.11 Statistical Design and Analysis

Each participant was given a code for the purpose of this research. With the help of Stata statistical software, all study participants were categorized into three groups (i.e. old-, new- and non- BU cases), and their distributions and proportions determined.

CHAPTER FOUR

4.0 RESULTS

4.1 Distribution of Study Participants

A total of forty-six (46) individuals were recruited for the study. They comprised twenty-four (24) males representing about 52% and twenty-two (22) females, which is about 48% of the total. There were thirty-three (33) identified BU- (both old and new cases), and thirteen (13) being non-BU control individuals (Table 1).

Table 1: The Distribution of BU-cases and non-BU controls in the Amansie West District of Ghana.

Groups	Sex	
	Males	Females
Old-BU	7	10
New-BU	13	3
Non-BU	4	9

4.2 Arsenic concentration Levels

Arsenic (As) concentrations in both blood and urine samples of study participants (i.e. both BU- and non-BU individuals) in the Amansie West District were below the detection level of the instrument used (i.e. $0.01\mu\text{g/L}$), as showed in Table 2.

Table 2: Mean Arsenic Concentrations ($\mu\text{g/L}$) in Blood and Urine of BU-cases and non-BU controls of the Amansie West District of Ghana.

Groups	Arsenic concentration in blood and urine Mean ($\mu\text{g/L}$)	
	Blood Arsenic	Urine Arsenic
Old-BU	ND	ND
New-BU	ND	ND
Non-BU	ND	ND

CHAPTER FIVE

5.0 DISCUSSIONS

This study was conducted in a typical BU-endemic mining district in Ghana (i.e. the Amansie West District), to measure and compare the levels of arsenic in urine and blood of BU cases and non-BU controls.

Studies conducted in artisanal mining communities of the Amansie West District, known to be endemic for BU focused on establishing a statistical and spatial correlation between these arsenic-enriched areas and MU infections (Gyasi *et al.*, 2014; Bonyah *et al.*, 2013; Gyasi *et al.*, 2012a; Duker *et al.*, 2006). These earlier research works monitored and measured arsenic concentrations in the environment; water and soil samples, and suggested that arsenic in the environment could play a catalytic role in the development of BU disease. In confirming the involvement of arsenic exposure to MU infections, more studies were conducted using murine animal models; where experimental mice were given water containing arsenic at concentrations synonymous to those recorded in the BU-endemic communities of the Amansie West District. Results of those studies also revealed that arsenic in drinking water, at levels ranging between 0.8 to 4.8 mg/L, made the mice susceptible to Buruli ulcer disease (Gyasi *et al.*, 2012b; Addo *et al.*, 2005).

The studies above suggested some form of relationship between arsenic exposure and BU disease. However, a keen research question one could ponder over, which necessitated this particular research work is, “is there any evidence of association between arsenic levels in biological matrices such as blood and/or urine and the incidence of BU? And if so, “is there any significant difference in levels/concentrations of arsenic in BU- and non-BU individuals living in these

arsenic-rich communities”? To unravel and provide answers to these questions, a case-control study was carried out in the BU-endemic mining-dominated communities of the Amansie West District to assess whether there were significant differences in blood and/or urine concentrations of arsenic in BU and non-BU individuals.

Results from this study showed no detectable levels of arsenic, below (0.01 μ g/L; detection limit of PinAAcleTM 900T AAS for arsenic) in either BU- or non-BU individuals living in the Amansie West District. This implies that the levels of arsenic concentrations in the biological samples of both BU- and non-BU cases were either very low for the analytical instrument used to detect, or there was no arsenic in the blood and urine samples of study participants.

Earlier works done on environmental media (e.g. water and soil) in the Amansie West District suggested contamination of the district with high arsenic levels, i.e. 0.8 – 1.2 mg/L for water samples, and 1.46 – 1.82 mg/kg for soil samples (Bonyah *et al.*, 2013; Gyasi *et al.*, 2012; Duker *et al.*, 2004). As there are high levels of arsenic in soil and water samples in the Amansie West District, one would generally expect that there should be reasonable concentrations in the individuals living in these arsenic-polluted environments. However, this study conducted on BU- and non-BU individuals in the same Amansie West District showed undetectable levels of arsenic. This suggests that there might be no direct correlation between arsenic concentrations in environmental media (e.g. water and soil samples) and biological matrices (e.g. blood and urine samples).

Studies carried out by other researchers have shown that, arsenic occurs naturally in the Amansie West district, and is widely distributed in large quantities in the soil,

associated with non-weathering-resistant mineral deposits such as sulphide minerals (Gyasi *et al.*, 2014; Gyasi *et al.*, 2012a). This contributes to its release in large amounts into the environment; hence the high levels of arsenic concentrations in environmental media, such as soil and water samples.

It is well documented that once ingested, soluble forms of arsenic are readily absorbed from the gastrointestinal tract. Absorption rate is estimated to range from about 40 to 100% for humans. Arsenate (i.e. As-V form) is said to be better absorbed than arsenite (i.e. As-III form), because arsenate is less reactive with membranes of the gastrointestinal tract (ATSDR, 2007). Arsenic in drinking water is mostly in the arsenate form, and hence complete absorption of arsenic from water may occur. Once absorbed, arsenic is transported by the blood to different organs and cells in the body, where it is metabolized (by the liver). Inorganic As-V and As-III have different mechanisms of action. Arsenate (As-V) is known to behave very much like phosphate and can substitute for phosphate in normal cell reactions, interacting with normal cell functions; whilst arsenite (As-III) has a high affinity for thiol (-SH) groups in proteins, causing inactivation of a variety of enzymes. Metabolism of arsenic in humans involves two processes. After entering a cell, arsenate is reduced to arsenite, and the arsenite is then methylated to form monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). These methylated forms are readily excreted, and may account for the undetectable levels in the biological matrices (ATSDR, 2007; Roy & Saha, 2002; Vahter & Concha, 2001).

The form of arsenic significantly affects the rate at which arsenic is excreted from the body. After ingestion, inorganic arsenic that is not immediately excreted or absorbed by tissues is progressively detoxified through the bio-methylation process in the liver.

The half-life of inorganic arsenic in humans is about 10 hours. About 70% of exposed arsenic in the human body is excreted mainly in the urine; most of a single, low-level dose is excreted within a few days after ingestion. Also, humans are said to rapidly excrete most blood arsenic, with 50 to 90% cleared in two to four days (ATSDR, 2011; Navas-Acien & Guallar, 2008; Hall *et al.*, 2006).

Again, levels of all arsenic forms in blood are reported to decrease within a few hours (Hall *et al.*, 2006), and therefore the blood is usually used as a monitor of recent arsenic exposure. Consequently, the determination of arsenic concentrations in blood samples is not required as frequently as urine. However, the comparison of both matrices is important, especially if blood samples are analyzed when monitoring for other analytes (ATSDR, 2011; Hall *et al.*, 2006). Subsequently, the determination of inorganic arsenic and its metabolites in urine is often used as a convenient and valid estimate of a possible arsenic exposure (Navas-Acien & Guallar, 2008). But as explained earlier, the As-element (after recent exposure) is rapidly methylated *in vivo* and then excreted (as MMA and DMA) in urine within 4–5 days. Thus, arsenic concentrations in biological specimens (i.e. blood and urine samples) decrease within a few days of exposure, irrespective of the toxicity levels in the surrounding immediate environmental media (such as water and soil samples). Hence, this might account for the very low or below-detection levels of arsenic concentrations recorded in this research work.

This is consistent with earlier thoughts that arsenic alone in the environment or human body cannot explain the endemicity of BU. Other host factors, either genetic, immunological or physical, could play a significant role.

Besides, a variety of analytical methods can be used to determine concentrations of trace elements such as arsenic in environmental and biological samples. These include fluorometry, neutron activation analysis (NAA), atomic absorption spectroscopy (AAS), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), gas chromatography (GC), spectrophotometry, x-ray fluorescence analysis, etc (ATSDR, 2015; Berg, 2012). The specific analytical method and equipment used would affect the values and detection levels of arsenic concentrations received and/or recorded.

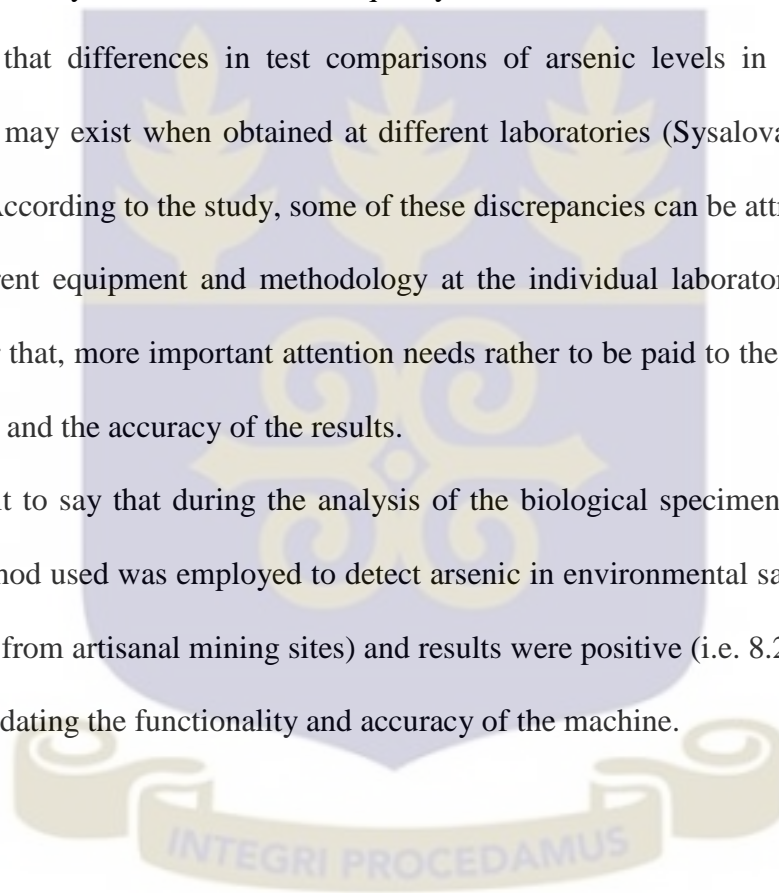
AAS techniques are commonly used for the determination of arsenic in environmental samples. Water samples, including freshwater, river water, sea water, and surface waters, as well as industrial wastes, sediments, mud and soil samples are mostly analyzed by AAS techniques to detect arsenic levels (ATSDR, 2015; Niedzielski & Siepak, 2003; Niedzielski *et al.*, 2002). However, AAS techniques do not have sufficiently high sensitivity and low detection limits, to be very effective in detecting the presence of arsenic in biological samples. ICP-AES or ICP-MS methods (with hydride vapour generation) are most common techniques used to determine total arsenic levels in biological samples, due to their lower detection limits than absorbance detection methods (ATSDR, 2015; Rajakovic *et al.*, 2013; Szkoda *et al.*, 2006). These techniques are specially suited for the analysis of low-concentration samples, and have very high sensitivity. ICP techniques offer multi-element capabilities, but instrumentation is however costly and background interference may also be a problem.

The analytical equipment used for arsenic concentration in this research work was the PerkinElmer PinAAcle™ 900T Atomic Absorption Spectro-Photometer, which uses the AAS method. This may account for the inability to detect arsenic levels within the

blood and urine samples of the participants, as the concentrations might have been lower than its detection limit (i.e. $\leq 0.01 \mu\text{g/L}$). It pre-supposes that if either ICP-atomic emission spectroscopy (ICP-AES) or ICP-mass spectrometry (ICP-MS) had been used, we might have recorded some arsenic concentrations in the biological samples, as these techniques have lower detection limits (i.e. $< 0.01 \mu\text{g/L}$).

Again, a study done to ensure the quality of arsenic determinations in body fluids showed that differences in test comparisons of arsenic levels in blood and urine samples may exist when obtained at different laboratories (Sysalova & Spevackova, 2003). According to the study, some of these discrepancies can be attributed to the use of different equipment and methodology at the individual laboratories; but stressed however that, more important attention needs rather to be paid to the validation of the methods and the accuracy of the results.

Suffice it to say that during the analysis of the biological specimens, the equipment and method used was employed to detect arsenic in environmental samples (i.e. water samples from artisanal mining sites) and results were positive (i.e. $8.23 - 16.12 \mu\text{g/L}$), thus validating the functionality and accuracy of the machine.



CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Arsenic was undetectable in the blood and urine of neither BU patients nor healthy individuals from the Amansie West District. Therefore, no direct correlation could be established between arsenic concentrations in biological matrices and BU disease. This study suggests that elevated arsenic levels alone may not account for the endemicity of BU disease.

Also, it can be purported that high levels of arsenic in environmental media in a given geographical area may not necessarily reflect in levels in biological matrices from individuals living in that area.

However, it needs to be mentioned that the sample size was rather small, and that therefore results might not be completely representative.

6.2 Recommendations

- i) It is recommended that the present study be repeated, but the metallic analysis determined using ICP-MS or ICP-AES equipment (which has lower detection limit and higher sensitivity).
- ii) Further studies should also be conducted to assess and compare arsenic concentration levels in environmental samples from the Amansie West District and As-levels in biological matrices (of BU- and non-BU individuals) in the same area. This will allow for stronger association analysis to be conducted between arsenic exposure and BU disease.

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APPENDICES

INFORMED CONSENT FORM

Project Title: Association between Arsenic Exposure and Buruli Ulcer (BU) in the Gold-mining Communities of Amansie West District.

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General information about the study

This is a research study being undertaken by the University Of Ghana School Of Public Health, in order to obtain stronger evidence of the association between arsenic exposure and Buruli ulcer (BU) infections. Research on BU suggests that, there is a positive correlation of *Mycobacterium ulcerans* (i.e. BU disease-causing organism) population with arsenic concentrations, indicating higher BU prevalence in arsenic-rich gold-mining environment. The purpose of this study is therefore to investigate the relationship between arsenic exposure and BU, and determine whether or not differences exist across BU-subject groups in the gold-mining communities of the BU-endemic Amansie West District. Findings will be useful in comparing and conducting association analyses between BU infections and arsenic exposure in the environment. This will then provide more knowledge and evidence on arsenic exposure as an environmental factor affecting BU infections.

Procedures

Individuals, male or female, who have been medically diagnosed with BU, and having lived or stayed in the Amansie West District for a period of at least six years, will be included in this study. If you are eligible and agree to participate, you will be required to provide blood and urine samples for arsenic analysis. We will as well ask you questions about your background, time/period of living with the disease, and functional health. The process is expected to last about 45 minutes.

Possible Risks and Discomforts

The study may involve some risks. We anticipate some discomfort and fear of supplying one's blood or biological samples to unknown researchers. In addition, we will have to prick your finger to collect the blood samples. If you feel uncomfortable providing your samples or being pricked, you are free to decline or opt out.

Possible Benefits

There is no direct benefit to the participants of this study. However, the samples you will provide will help in conducting analysis on the evidence of association between arsenic exposure and Buruli ulcer infections. This knowledge will help us in ascertaining environmental arsenic exposure as aetiological factor for BU infections. Overall, the study will not only deepen evidence of the association between arsenic exposure and BU infections, but ultimately go a long way in helping to provide the necessary preventive measures and control mechanisms or interventions.

Voluntary Participation and Right to Refuse

Your participation in this study is absolutely voluntary. Additionally, you are at liberty to withdraw from the study or stop the procedure at any time. However, we will encourage you to participate, as your complete involvement is very important in helping us to evaluate the relationship between arsenic exposure and Buruli ulcer.

Confidentiality

We would like to assure you that whatever information you provide will be handled with strict confidentiality, will be used purely for research purposes, and will never be used against you. Data analysis will be done at the aggregate level to ensure anonymity. Your name or personally identifying information will not be published in any report. Some staff of the research team may sometimes review the research records, but no unauthorized individual(s) will be able to access your information.

Compensation

There is no compensation for participating in this study.

Contact for Additional Information

If you have questions later, you may contact:

Jones Ofori-Amoah, Department of Biological, Environmental and Occupational Health, School of Public Health, College of Health Sciences, University of Ghana, Legon-Accra. Telephone numbers: 0242042060; 0501382444
Email address: emperordjarv@gmail.com

Your rights as a Participant

If you have any questions about your rights as a research participant, you can contact the Administrator of the GHS Ethical Review Committee at the following address:

Hannah Frimpong,
GHS-Ethical Review Committee,
Research and Development Division,
Ghana Health Service,
P. O. Box MB 190,
Accra.
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