PHYSICAL AND CHEMICAL CHARACTERISTICS OF WATER AND SOIL IN BURULI ULCER ENDEMIC AND NON-ENDEMIC AREAS IN THE WESTERN, CENTRAL AND NORTHERN REGIONS OF GHANA

BY

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A Thesis Submitted to the Department of Chemistry, College of Science, Kwame Nkrumah University of Science and Technology in Partial Fulfillment of the Requirements for the degree of MASTER OF PHILOSOPHY in Analytical Chemistry

July, 2012

DECLARATION

I hereby declare that this submission is my own work towards the MPhil and that, to the best of my knowledge; it contains no material previously published by any person. No material which has been accepted for the award of any other degree of this university, except where due acknowledgement has been made in the text.

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DEDICATION

I dedicate this work to the Atosona family.

ACKNOWLEDGMENT

I am most grateful to the Almighty God for his strength and protection throughout my entire study. I am also indebted to my supervisor, Dr. R. B. Voegborlo for his invaluable assistance and directions throughout this study. My special thanks goes to Petra Tschakert, Edith Parker, Joseph Oppong, Richard Amankwah, Frank Nyame, Heidi Hausermann, David Ferring, David Azanu, Julianne Hagarty, Lindsay Kromel, Saviour Mantey, Rose Sandow, Yakubu Iddrisu Goro, Charles Abbey, and Emmanuel Effah for their contributions in the field and laboratory. My appreciation also goes to the entire Atosona family for their support throughout my studies and then to all individuals who helped in diverse ways in making this work a success.

ABSTRACT

To determine the relationship between water quality, soil chemistry and Buruli ulcer incidence, water and soil samples were collected from five communities in Ghana within the dry season in 2010 and the wet season in 2011: four communities in the southern part of Ghana (three Buruli-endemic communities: Pokukrom, Betenase, and Ayanfuri, and one control: Kedadwen) and one non-endemic community (Nangruma) in the north.

Water samples were analyzed for the following parameters, pH, Conductivity, dissolved oxygen, turbidity, alkalinity, total hardness, some selected anions (SO_4^{2-}, Cl^-, $PO4^{3-}$ and NO^{2-}) and some trace metals (As, Cd, Fe, Cu, Zn, Pb and Se). Soil samples were also analyzed for pH, Conductivity and trace metals. The UV-Visible Spectrophotometer was used to determine the selected anions, Atomic Absorption Spectrophotometer was used to determine the trace metals and titrimetry was employed to measure alkalinity, hardness and chloride content.

Results from analysis of pH and other related physicochemical parameters revealed that, mean pH values for all the water bodies in the dry season was (5.78) and (5.68) for the wet season water samples. Non-endemic community (Nangruma) recorded basic pH (7.12). Mean arsenic concentrations for all the water bodies in the dry season stood at (0.04 mg/L) and (10.18 mg/L) for the wet season. Mean cadmium concentrations for all the water bodies in the wet season was (0.05mg/L), dry season samples did not contain detectable levels of cadmium. The following were recorded for copper, zinc, selenium, lead (Cu dry season (0.06 mg/L), Cu wet season (0.19 mg/L), Zn dry season (0.21 mg/L), Zn wet season (0.40 mg/L), Se dry season (0.01mg/L), Se wet season (0.01mg/L), Pb wet season (0.16mg/L) dry season water samples did not contain detectable levels of lead. Mean phosphate levels for all the water bodies in the dry season stood at (13.32 mg/L) and (8.56 mg/L) for the wet season. Iron and arsenic were the highest trace metals recorded in the analyzed soil samples (5642.5 mg/kg and 66.55 mg/kg) respectively.

From the results, all the water bodies in the dry and wet seasons recorded slightly acidic pH (pH<7). In all locations, gold-mining pits and pools of stagnant water bodies have a significantly different chemical signature than rivers and naturally occurring swamps; trace metals, thought to aid in the growth of *M. ulcerans*, are present in much higher concentrations in mining pits and stagnant water bodies than in other water body. Phosphate may also be a control. When the levels of physicochemical parameters during the study were compared seasonally, nearly in all cases, concentrations of trace metals levels in samples collected in the wet season were considerably higher compared to the dry season samples.

Despite this, few differences in chemical compositions between the endemic and non- endemic communities does exist, implying that other variables such as human behavior may also in a way control the onset of Buruli ulcer.

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LIST OF ABBREVIATIONS

BU……..……Buruli ulcer

MU……..…...*mycobacterium ulcerans*

WHO……..World Health Organization

PCR……...……...*Polymerase Chain Reaction*

MAC……*Mycobacteria. avium* Complex

WHA……...……World Health Assembly

USD……..……United States Dollar

TB……...……..Tuberculosis

GBUI……..……....Global Buruli Ulcer Initiative

UK……..……United Kingdom

PK……..…….……..Pokukrom

BT……...…Betenase

AF……...Ayanfuri

KD……..…Kedadween

NG……....Nangruma

GPS……..……Global Positioning Systems

KNUST……...……Kwame Nkrumah University of Science and Technology

SPSS……...…….Social Statistical Package Science

ND……..……..Not Detectable

CHAPTER ONE

1.0 INTRODUCTION

Neglected, emerging and re-emerging infectious diseases are an increasing health concern for many parts of the world. Between 1972 and 1999, 35 new disease-causing agents were identified, with many more diseases re-emerging after years of dormancy (WHO, 2003). Buruli ulcer (BU) is one of such emerging disease; it is caused by the environmental mycobacterium called *M. Ulcerans.* Occurring in at least thirty-three countries worldwide, it is the third most common Mycobacterial disease after tuberculosis and leprosy (Merritt *et al.*, 2005). While BU is typically non-fatal, it can result in severe deformities and medical complications if not promptly and properly treated. Cases of BU have been reported in over 30 tropical and subtropical countries, typically in poor rural communities, and most frequently in West Africa (WHO 2007). More than 1,000 cases of BU were reported in Ghana alone in 2010 (WHO 2011). The first case of BU reported in Ghana was in 1972 in the Ga-district (Bayley, 1971). A national case search in 1998 indicated a national prevalence of 20.7/100,000 and a prevalence of 87.7/100,000 for the former Ga-district (now the Ga-West and Ga-South municipalities), the fifth most endemic in the country, yet with the highest burden in terms of healed and active lesions (Amofah *et al*., 2002).

The disease remains poorly understood to scientists, as its mode of transmission is unknown. Treatment is well-understood clinically, although in practice, it can be expensive, long, and often poorly managed (WHO 2012). Many scientists recently have turned their attention to determining the mode of transmission for BU in an effort to prevent this disease as it is the World Health Organization's top research priority (WHO, 2012).

Mycobacterium ulcerans is an environmental pathogen that is commonly associated with water and soil (Ross *et al*., 1997; Hayman, 1991). One worldwide characteristic of the disease is its association with bodies of water (Mueld, 1992, Oluwasanmi *et al.*, 1976).Despite the high morbidity of buruli ulcer, it has been neglected completely. It is therefore not surprising when WHO described it as a neglected tropical disease.

Research on BU suggests that is associated with areas subjected to environmental modification such as logging, irrigation, agriculture, mining, or dam construction (Hayman 1991; Veitch *et al.,* 1997; Merritt *et al*., 2005; Wagner *et al.,* 2008; Merritt *et a,*. 2010). Other researchers have found that direct contact with aquatic environments is a major risk factor. Soil also plays a significant role in the mode of transmission of various diseases since it is a big reservoir for bacteria, viruses and many more biological organisms and many other chemicals. The conditions that aid in the habitation of soil by these organisms may be due to the variations in certain physical and chemical properties of the soil. Soil is a significant reservoir of mycobacterium. Environmental growth of mycobacteria may be enhanced in low pH soils (Iivanainen *et al.*, 1999).

Merritt *et al.* (2005) proposed that "poor water quality influences biological communities, leading to increased growth and proliferation of *M. ulcerans* in aquatic habitats." While some research, e.g., Fyfe *et al*., (2010), has demonstrated a link between *M. ulcerans* and strictly terrestrial animals, the majority of *M. ulcerans* research to date implicates aquatic environments in the potential mode(s) of transmission for BU. Duker *et al*., (2004) suggested a connection between arsenic enrichment in soil and water and incidence of BU. Taken together, these studies

suggest that some difference in environmental conditions, human interaction with the environment, or both must exist between endemic and non-endemic communities to explain the difference in BU morbidity. Williamson *et al*., (2008) conducted a microbiological assay of aquatic environmental samples in both Buruli-endemic and non-Buruli-endemic areas of Ghana, finding that *M. ulcerans* is present equally often in samples from endemic and non-endemic communities, where endemicity was based on passive surveillance. Williamson *et al*., (2012) found that the number of *M. ulcerans*-positive samples correlated with prevalence of BU on a community scale.

A study of mycobacteria in brook waters, conducted by Iivanainen *et al*., (1993), found that culturable counts of slow-growing mycobacteria were most negatively correlated to pH. Likewise, counts were most positively correlated to chemical oxygen demand and metals concentrations. Consequently, one might expect that *M. ulcerans*, a slow-growing mycobacterium, would be likely to thrive in similar environments to those bacteria studied by Iivanainen *et al*., (1993), if indeed *M. ulcerans* can exist outside a host. Using this assumption, *M. ulcerans* should be most prevalent in water with low pH, and that *M. ulcerans* may be seen most commonly in waters with high metals concentrations, particularly iron and heavy metals. This assumption is supported by many studies of BU incidence relative to land use, as well as known chemical trends associated with these land uses (e.g., increased nitrogen in agricultural areas). Areas with high nitrogen and phosphorus concentrations will likely be a preferred environment for the growth of *M. ulcerans*, as environmental nutrient enrichment has been linked to the emergence of other direct-transmission and vector-borne bacterial diseases (Johnson *et al*., 2010). The positive correlation of mycobacterial population with metals concentrations suggests

that BU incidence may be higher near mining sites, as heavy metals are commonly associated with tailing waste from mining activity (Walker *et al*., 2001).

This study seeks to answer the following questions: (1) is there a difference in water quality between endemic and non-endemic communities? (2) is there any difference in water quality between types of water bodies within these communities? and (3) if there are differences, do they relate to the postulated environment for *M. ulcerans* (high metals, low pH, high nutrient concentrations)? This study focuses on mining regions and particularly on "galamsey" (gather-and-sell), or artisanal small-scale, gold mining areas. These areas are characterized by large amounts of localized disturbance, most notably pools of water associated with active ore-washing stations. While *M. ulcerans* is not measured in this study and the incidence of BU is instead used, detection of preferred environments for *M. ulcerans* growth and persistence may be useful in the quest for the Buruli ulcer vector(s). Analysis of localized soil and water chemistry will be done.

To my knowledge, this research is the first study that has examined BU disease prevalence related to water quality, soil chemistry, land use and seasonal changes. The results of this study should provide invaluable information pertaining to the quality of water and soil properties of BU endemic and non- endemic areas of disease.

1.1 OBJECTIVES OF RESEARCH

1.1.1 Primary objective

To determine whether BU disease prevalence is related to water quality and soil chemistry.

1.1.2 Specific objectives

1. To determine the physical and chemical properties of water in BU endemic and non-endemic areas during the dry and wet seasons.
2. To determine the physical and chemical properties of soil in BU endemic and non-endemic areas.
3. To compare some characteristics of water and soil in buruli ulcer endemic areas (Pokukrom, Betenase and Ayanfuri) to that of buruli ulcer free areas "Overseas" (Nangurma) and Kedadwen.
4. To determine any seasonal differences between the characteristics of water in both BU endemic and non-endemic areas.

CHAPTER TWO

2.0 LITERATUSSRE REVIEW

2.1 BURULI ULCER

(Mycobacterium ulcerans infection)

Buruli ulcer (BU) is an emerging disease caused by flesh-eating bacteria. This disease of the skin is caused by mycobacterium ulcerans, a bacteria related to those causing tuberculosis and leprosy. BU due to *M. ulcerans* is a great public threat. In certain geographical regions the incidence of BU is increasing. Children are disproportionately affected. Rates in some villages in Africa are greater than 15 % (Marston *et al.,* 1995). New data has implicated an aquatic insect in the transmission of the disease and this may be the basis for beginning a control programme (Marsollier *et al.,* 2002). BU is painless and slow to develop. A typical Buruli lesion is an extensive, deeply undermined skin ulcer that heals by scarring. There are other presentations including nodules, plaques, oedematous swelling of a whole limb or the abdominal wall and osteomyelitis (Buntine *et al.*, 2002). Death due to BU is rare but permanent deformities are common. The diagnosis of BU is likely if large numbers of acid-fast bacilli are present in smears or histological sections obtained from a suspicious lesion. The presence of *M. ulcerans* can be rapidly confirmed by a specific and sensitive PCR (Ross *et al.*, 1997; Russell *et al.*, 2002).

2.1.1 The Causative Organism and Toxin Secretion

There are well over 100 species of Mycobacteria but most common species include: *M. avium* Complex (MAC), *M. kansasii*, *M. ulcerans, etc*. (Gangadharam *et al*., 1998). Mycobacterium is a genus of gram – positive, aerobic bacteria. Most species are free-ling in soil and water, but the major habitat for some is the diseased tissue of

warm-blooded hosts. Mycobacteria were one of the first types of bacteria recognized to cause disease (tuberculosis and leprosy). The name *Mycobacterium*, which means fungus-bacterium, was introduced in 1896. The name does not imply that *Mycobacterium* are fungi; rather it describes the way that the tubercle bacillus grows on the surface of liquid media as mold-like pellicles (Gangadharam *et al*., 1998).

The *Mycobacterium* genus belongs to the family Mycobacteriaceae and consists of many species, some of which are pathogenic to humans (Pfyffer *et al*., 2007). They have cell walls with very low permeability, contributing to their resistance to therapeutic agents. The mycobacterial cell wall is highly complex and has a lipid content that approximates 60% of the structure (Brennan *et al*., 1995). The cell wall characteristics allow the mycobacterial species to survive in different environments (e.g., in biofilms in water habitats or particulate matter in soils and water) and resist disinfection procedures. *M. ulcerans* belongs to a group of mycobacteria that are potentially pathogenic for humans and animals. These are sometimes called "opportunistic mycobacteria" Most species belonging to this group are found widespread in the environment and may become pathogenic under special circumstances (Brosch *et al*., 2007). *Mycobacterium ulcerans* is a slow growing mycobacterium. Another important factor is oxygen concentration. Reduced oxygen concentration enhances the growth of *Mycobacterium ulcerans*, suggesting a preference of this organism for microaerophilic environments. (Cole *et al*., 2001).*Mycobacterium ulcerans* is unique among mycobacteria in that much of the pathology appears to be mediated by production of toxic macrolides, the mycolactones that are required for virulence. (clancey *et al*., 1962). These soluble toxins have immunosuppressive and cytotoxic properties *in-vitro* and can be isolated from the culture filtrate of the mycobacterium. When injected into healthy guinea

pigs, histopathological changes compatible to Buruli ulcer lesions, were induced. (Smith *et al*., 1970). Mycolactones induce cell death by apoptosis, which may explain the absence of an inflammatory immune response despite the extensive tissue damage. (Oluwasanmi *et al*., 1976). In contrast to the wild type *M. ulcerans,* mycolactone negative mutants fail to colonize the salivary glands of water insects, suggesting that these molecules may play a role in the ability of *M. ulcerans* to colonize reservoir species (clancey *et al*., 1962, Bayley *et al*., 1971). Until now no cell receptor has been found to explain the cascade of effects induced by mycolactones (Debacker *et al*., 2004).

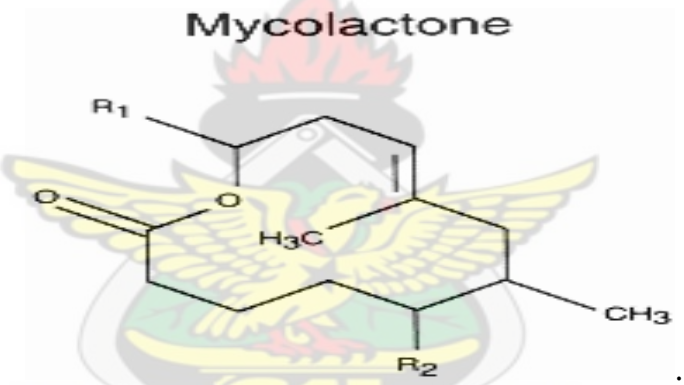


Fig. 1 Mycolactone core structure: 12-membered ring to which two polyketide-derived side chains (R1 and R2) are attached. (Source: George *et al.*, *Science* 1999).

2.1.2 Symptoms

M. ulcerans causes distinctive, often severe, skin lesions. It is thought that the primary mode of infection with this species is through cuts from vegetation (e.g., grass) which allow the organisms to enter the skin. Lesions develop as small, palpable, painless, subcutaneous swellings approximately 4 to 10 weeks after infection. The growing nodule which is firm and attached to the skin remains

superficial and extends laterally involving fat and fascia around muscle bundles or the muscles themselves. The skin overlying the lesion loses pigmentation, becomes filled with fluid and necrotic and often ulcerates. The ulceration typically has undermined edges and enlarges over many months (Feldman, 1974). The infection is mostly on the limbs, most often on exposed areas but not on the hands or feet. In children all areas may be involved, including the face or abdomen. A more severe form of infection produces diffuse swelling of a limb, which, unlike the papule or nodule, can be painful and accompanied by fever. Infection may frequently follow physical trauma, often minor trauma such as a small scratch.

2.1.3 Epidemiology

Infection is acquired from the environment and person-to-person spread has not been described. BU endemic areas are usually near tropical marshes, rivers or lakes but transmission also occurs in temperate southern Australia (Horsburgh *et al.*, 1997). BU is typically unevenly distributed within an endemic country. New areas of micro-endemicity may appear unpredictably (Johnson *et al.,* 1996). Unlike TB and leprosy, BU is contracted by exposure to a contaminated environment rather than from infected people. Most patients with BU are children below the age of 15. A study from Amansie West in Ghana reported the median age as 12 years, with 49% of cases aged 10 - 14 years. Only 20 % were over 50 years old (Amofah *et al.*, 1993). Recent reports from Benin suggest that there is also an increased attack rate of BU in the elderly, resulting in an age-specific incidence (F. Portaels, personal communication). The precise mode of transmission has not been established, but recent work has suggested that aquatic insects and biofilms attached to aquatic plants harbour *M. ulcerans* (Marsollier *et al.,* 2002). Transmission of *M. ulcerans* from

infected insects to laboratory mice has been demonstrated, but whether this is how humans become infected remains unknown. Transmission by aerosol or through direct contact with contaminated soil has also been proposed. It is possible that more than one mode of transmission exists. It has been suggested that environmental changes such as logging, mining and nutrient enrichment of waterways is contributing to the spread of BU.

2.1.4 History of Buruli Ulcer

Buruli ulcer, which has also been referred to as Bairnsdale, Searles, and Kumusi ulcer, depending on the geographic region where it was historically reported (Radford, 1974; Horsburgh and Meyers, 1997), is the most common name of *M. ulcerans* infection. A team lead by Professor Peter MacCallum in Australia, in 1948 provided a detailed description of the disease among six patients from the Bairnsdale area near Melbourne. This team was the first scientists to isolate the causative organism, *Mycobacterium ulcerans*. The disease is still referred to as the Bairnsdale ulcer in southern Australia.

Many cases occurred in Buruli County (now called Nakasongola District) in Uganda, giving rise to the most widely used name for the disease – Buruli ulcer, this was in 1960's. The disease has emerged rapidly in several parts of the world, since 1980, particularly in West Africa. Throughout the 20th century, cases were consistently and increasingly reported from around the world, and during the past decade there has been both a rise in prevalence rates and an expanding geographic distribution, independent of increased surveillance (Horsburgh *et al*., 1997; WHO, 2000; Amofah *et al*. 2002). Although the global extent of this disease is unknown, the worldwide

burden is probably grossly underestimated, due to lack of reporting, difficult access to healthcare for infected individuals, and localized outbreaks in rural areas of developing nations (WHO, 2000). This prompted action by WHO in 1998. Considering the increasing geographical spread, severe consequences and limited knowledge of *mycobacterium ulcerans* infection disease, the World Health Assembly (WHA) in 2004 adopted a resolution1 to improve the surveillance and control of BU and accelerate research to develop better tools for its control.

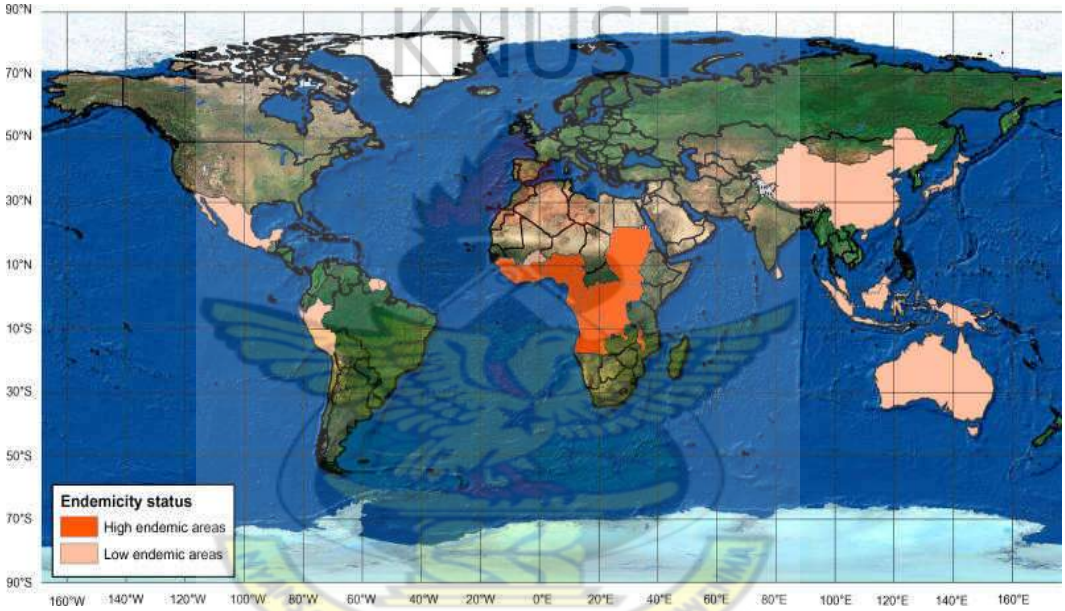


Fig. 2 Countries reporting Buruli ulcer (Johnson *et al.*, 2005).

2.1.5 Global Distribution of Buruli Ulcer

Incidence of Buruli ulcer has been reported in 33 countries in Africa, the Americas, Asia and the Western Pacific, mainly in tropical and subtropical regions but the disease is not well-known and is therefore under-reported. In the countries of reported cases, Buruli ulcer occurs in some specific places, often in wet areas. Of the estimated 7,000 cases of Buruli ulcer reported annually (Walsh, 2008) more than

4,000 cases occur in Sub- Sahara Africa, with the largest number reported from the West African countries. In Côte d'Ivoire, approximately 24 000 cases have been recorded between 1978 and 2006. In Benin, nearly 7000 cases have been recorded between 1989 and 2006; in Ghana more than 11 000 cases have been recorded since 1993 (WHO, 2008). In Australia, more cases of BU are being reported recently – 25 in 2004, 47 in 2005 and 72 in 2006. Most of the recent cases have come from the State of Victoria and the town of Point Lonsdale. Increasing number of cases is being reported from Cameroon, Congo, Gabon, Sudan, Togo and Uganda. After 30 years of no official report, an assessment carried out in south-eastern Nigeria in November 2006 confirmed some BU cases. Some patients have been reported from China, but the extent of the disease is not known. Recent reports suggest, for the first time, that Brazil may be endemic in the areas bordering French Guyana. These numbers may only be an indication of the presence of the disease but do not reveal the magnitude of the problem. The greatest risk factors for acquiring Buruli ulcer include residing in an endemic area, close proximity to specific bodies of water, and age less than 15 years (WHO, 2008, Walsh, 2008).

The infection occurs in well-defined areas throughout the world, mostly tropical areas - in several areas in Australia, in Uganda, in several countries in West Africa, in Central and South America, in Southeast Asia and New Guinea. It is steadily rising as a serious disease, especially in West Africa and underdeveloped countries, where it is the third leading cause of mycobacterial infection in healthy people, after tuberculosis and leprosy. In East Africa, thousands of cases occur annually and in these areas the disease has displaced leprosy to become the second most important mycobacterial disease of man (after tuberculosis).The disease is more likely to occur

where there have been environmental changes such as the development of water storages, sand mining and irrigation. Buruli ulcer is currently endemic in the Benin, Côte d'Ivoire, Ghana, Guinea, Liberia, Nigeria, Sierra Leone and Togo. (Amofah *et al*., 1993). In Ghana, 1999 data indicated that the prevalence rate of the disease in the Ga West District was 87.7 per 100,000, higher than the estimated national prevalence rate at 20.7 per 100,000 generally, and 150.8 per 100,000 in the most disease-endemic districts.

Table 2.1 Increased BU incidence in some West African countries

Country	Year of first case	1988-1997	1998-1999
Benin	-	2300	4000
Côte d'Ivoire	1978	10000	15000
Ghana	1971	2000	6000
Togo	1995	40	-

Source: Grosset *et al*., 2000, Meyer *et al*., 1996, Amofah *et al*., 2002 and Aujoulat *et al*., 2003.

2.1.6 Prevalence of Buruli Ulcer in Ghana

Incidence of Buruli ulcer has increased over the last several years. For instance, in Ghana, the number of new cases reported has been 685 in 2003, 1021 in 2004, 1097 in 2005, and 1010 in 2006. The first probable case of Buruli ulcer in Ghana was reported in the Greater Accra Region in 1971; the presence of additional cases along the tributaries of the Densu River in the area was considered likely (Bayley, 1971). In 1989, van der Werf et al. described 96 cases in the Asante Akim North District of Ashanti Region (van der Werf *et al*., 1989). This report was followed by the description of a major endemic focus in Amansie West District in the same region

(Amofah *et al*., 1993). Since then, isolated cases have been found in scattered communities in many parts of the country, generating much political and media concern and interest. In 1993, a passive surveillance system for reporting Buruli ulcer was initiated in Ghana. By the end of 1998, approximately 1,200 cases had been reported from four regions. Gross underreporting was suspected, however, as the media continued to report cases in remote rural communities. Because most cases were known to be in relatively deprived, inaccessible areas, the routine reporting system was judged inadequate to provide a true picture of the extent of disease and the geographic distribution of cases for design of a national control program. True incidence data, however is difficult to determine due to poor surveillance measures and case confirmation.

2.1.7 Overall Burden of the Disease

BU is an important disease because the incidence is increasing, it is expensive to treat, and it is most common in regions that lack advanced medical facilities. An example is seen in a recent study that has estimated the cost per case in Ghana at 780 USD (Asiedu *et al*., 1998). BU has become a major burden for poor agricultural communities in West Africa. In Australia, one of the very few Organization for Economic Co-operation and Development countries where transmission of *M. ulcerans* occurs, the cost per case has been conservatively estimated at 12,000 USD (Drummond *et al*., 1999).A recent report from Ghana has estimated a national prevalence of 20.7/100 000 in 1999 (Amofah, 2002). In one highly endemic region in Ghana an annual incidence of 280/100 000 was reported - higher than TB in the same region. The disease rates in Uganda have been estimated at 2 – 5 % of the

population and in Côte d'Ivoire and Ghana; the rates in some villages have been estimated at 16 % and 22 % respectively (Amofah *et al.,* 1993; Marston *et al.,* 1995). Disabilities resulting from BU disease are severe and it has been estimated that 25% of cases are left with some disability in Côte d'Ivoire (Marston *et al.,* 1995) and 58 % in a recent study in Ghana. The global burden of BU has not been established but concern about the emergence of BU prompted WHO to create a specific programme for BU in 1998 (GBUI: Global Buruli Ulcer Initiative). In terms of number of cases, Buruli ulcer is probably the third most common mycobacterial disease in immune competent humans after tuberculosis and leprosy.

However, due to the lack of precise data, the burden of the disease at global and national levels is not entirely known. Of particular note are the numerous endemic areas for Buruli ulcer (at least 32 countries) caused by *M. ulcerans*. Although considered the third most common mycobacterial infection of humans after TB and leprosy, the actual burden of this disease is unknown. Potential transmission by an insect vector raises specific issues related to public health and the potential to manage the disease. Some areas of Benin and Cote d'Ivoire, at present, the number of cases may exceed those of tuberculosis and leprosy. In Cote d'Ivoire, over 5000 cases have been recorded since 1995. In some communities in this country, up to 16 % of the population has been found to be affected by buruli ulcer. I communities in Ghana 22 % of the people had the disease. A survey done in one of the endemic districts (population of 106560) in Ghana estimated the prevalence at 3.19 per 1000. The disease most commonly affects impoverished inhabitants in remote rural areas with limited access to health care. It often occurs in close proximity to slow-flowing or stagnant bodies of water. All age groups, particularly children under 15 years of

age, are affected. No racial or socioeconomic group is exempt. As of today, the modes of transmission are not entirely known. The organism probably enters the body through small breaks in the skin from contaminated soil, water, or vegetation. Recent evidence suggests that in some cases insects may be involved in the transmission of the disease. There is also anecdo evidence to support person-to-person transmission. Estimating the amount of infection and disease associated with environmental mycobacteria is both difficult and inherently inaccurate.

2.2 RESERVOIR(S) AND MODE(S) OF TRANSMISSION

The disease often occurs in close proximity to water bodies, but no specific activities that bring people into contact with water have been identified (i.e. fetching of water, fishing, washing, bathing, etc). The mode of transmission of Buruli ulcer is not entirely known. Recent evidence suggests that insects may be involved in the transmission of the infection (Portaels *et al*., 1999). These insects are aquatic bugs belonging to the genus Naucoris (family Naucoridae) and Diplonychus (family Belostomatidae). Trauma is probably the most frequent means by which *M. ulcerans* is introduced into the skin from surface contamination (Stienstra *et al*., 2001). The initial trauma can be as slight as a hypodermic needle puncture or as severe as gunshot or exploding land mine wounds (Meyers *et al*., 1974). Other studies have suggested aerosol spread but these are not proven (Veitch *et al*., 1997). Epidemiological evidence has not clearly supported person-to-person transmission. However, Muelder & Nourou found that 10 out of 28 patients had relatives whom had also had the disease, and cautioned against the dismissal of person-to-person transmissions (Muelder *et al*., 1990). Given the number of patients who shed large numbers of bacilli from their wounds and live in very close contact with relatives,

more cases should have been observed. The cases reported by Muelder & Nourou could perhaps have been exposed to a common source of infection.

After considering the various suspected agents, (Portaels *et al.,* 2001*)* proposed the hypothesis that human beings as well as domestic and wild animals could be contaminated or infected by biting insects such as water bug (Portaels *et al*., 2001). Aquatic bugs are cosmopolite insects found throughout temperate and tropical regions especially rich in freshwater. They represent about 10% of all species of Hemiptera associated with water and belong to two series of the suborder Heteroptera: the Nepomorpha, which include four super families whose members spend most of their time under water, and the Naucoridae, which include a single family, the Naucoridae, whose members are commonly termed creeping water bugs. Whether found in temperate countries like France or tropical ones like Ivory Coast, aquatic bugs exhibit the same way of life, preying, according to their size, on mollusks, snails, young fish, and the adults and larvae of other insects that they capture with their raptorial front legs and bite with their rostrum. These insects can inflict painful bites on humans as well. In the Ivory Coast, where Buruli ulcer is endemic, the water bugs are present in swamps and rivers, where human activities such as farming, fishing, and bathing take place. Present findings (Marsollier *et al*., 2002) describing the experimental transmission of *M. ulcerans* from water bugs to mice are in good agreement with the possibility of this mode of transmission to humans by bites.

Also in strong support of this hypothesis was the localization of *M. ulcerans* within the salivary glands of Naucoridae (Marsollier *et al*., 2002). Local physiological conditions of this niche appear to fit the survival and the replication needs of *M.*

ulcerans but not those of other mycobacteria. Surprisingly, infiltration of the salivary glands of Naucoridae by *M. ulcerans* does not seem to be accompanied by any tissue damage similar to the ulcerative skin lesions developed by bitten individuals and mediated by the cytotoxic activity of the mycolactone(George *et al*., 2000) and other toxins produced by *M. Ulcerans* (Dobos *et al*., 2001). The inactivation of the latter toxins could be the result of salivary enzymatic activities, which remain to be determined. Mycobacterium ulcerans was first cultivated and characterized from the environment in 2008 (Portaels *et al*., 2008).

2.3 ENDEMIC REGIONS AND ASSOCIATION WITH WATER

In many areas, *M. ulcerans* infection has only occurred after significant environmental disturbance. In a paper published in 1948 describing the disease, the first patients from the Bairnsdale District in Victoria presented in 1939. (MacCallum *et* al., 1948), there had been terrible floods in the district, when all road and rail links had been cut and there had been considerable destruction of property. In Uganda, Barker examined cases of *M. ulcerans* infection (Buruli ulcer disease) occurring in the Busoga District on the east side of the Victoria Nile, north of Lake Victoria (Barker, 1971). Although cases were known in the other parts of the country, cases were unknown in the district before 1965. Barker postulated that the outbreak was related to the unprecedented flooding of the lakes of Uganda between 1962 to 1964 as a result of heavy rainfall. In Nigeria, cases have occurred among Caucasians living on the campus of University of Ibadan only after 1965 (Oluwasanmi *et al*., 1976) when a small stream flowing through the campus was dammed to make artificial lake. The first case reported in Côte d'Ivoire was a French boy of seven years who lived with his parents beside Lake Kossou, (Perraudin *et al*., 1980) an

artificial lake in the center of the country. In Liberia, cases have been reported in the north of the country (Ziefer *et al*., 1981) following the introduction of swamp rice to replace upland rice. This introduction has been associated with construction of dams on the May or river and extended wetlands. In Papua New Guinea, the infection occurs mainly in relation to the Sepik and Kumusi rivers; in the later areas, the disease is known as the "Kumusi ulcer" (Radford *et al*., 1974). The disease occurred after flooding and devastation, which followed the eruption of Mount Lamington in 1951. Reid described how older people living in the villages blamed the volcano for the disease (Radford *et al*., 1974).

2.4 DISTRIBUTION OF MYCOBACTERIA

Mycobacteria are found in soil and water and often (but not always) the inoculation event can be traced to a specific exposure. In many of the infections due to these organisms transmission occurs via minor trauma to the skin. For *Mycobacterium ulcerans*, water appears to be the major source. While mycobacterial skin disease is believed to be worldwide, certain infections have limited geographic occurrences. BU has been reported in many tropical and some temperate countries, and it is endemic in parts of sub Saharan Africa. There are also established foci in the Americas, Asia, Australia and Papua New Guinea (Asiedu *et al.*, 2000).

2.5 ENVIRONMENTAL MYCOBACTERIA

Environmental mycobacteria can be found in diverse environments and most appear to exhibit a saprophytic lifestyle. However, some have the ability to infect animals, birds and humans, and have evolved mechanisms by which they can invade and grow within host cells. Because the number of organisms shed back into the

environment from infected animals can be relatively small, and heavy and widespread colonization of some environments occurs, it remains rather unclear what role animal/human infection plays in the lifecycle of many of these organisms. Because these organisms are widespread in the environment, and there is little evidence that person-to-person transmission is common, there is an implicit assumption that environmental mycobacterial infections derive from water, food, the environment or contact with animals. There is evidence to support this assumption in many cases, although the source of infection in most remains unclear. A variety of mycobacterial species causing human disease have been linked to contaminated water. However, some of these links can result from diagnostic uncertainty associated with differentiating contamination of patients or their specimens from human disease caused by environmental mycobacteria.

2.6 OCCURRENCE OF MYCOBACTERIA IN WATER

Environmental mycobacterial species have been repeatedly isolated from natural and municipal waters. They occur in surface water, notably ponds, streams, and estuaries. Mycobacterial characteristics such as surface hydrophobicity and charge, as well as certain physiochemical factors like salinity, temperature, humidity and wind currents can influence the distribution of mycobacteria in water systems (Falkinham, 2001).

The waterborne mycobacteria are members of a large and very significant family of human pathogens. *Mycobacterium* is the single genus in the family Mycobacteriaceae, order Actinomycetales. From the standpoint of human health, the most significant of environmental mycobacteria are the *Mycobacterium avium* complex (MAC) and *M. ulcerans* which is of interest in this study. *M. ulcerans* is the causative agent of Buruli ulcer, a debilitating disease characterized by large necrotic

skin ulcers that is currently widespread throughout West and Central Africa. It is extremely difficult to isolate *M. ulcerans* from the environment, although the pathogen may occupy niche environments such as the salivary glands of particular aquatic insects (Masollier *et al.*, 2002). *M. ulcerans* is known to cause disease in humans; it produces large necrotic skin lesions caused by massive necrosis of subcutaneous fat. Histopathology shows a marked absence of a host inflammatory immune response and massive numbers of bacilli are found extra cellularly. This unusual pathology has been linked to the presence of a macrolide toxin produced by *M. ulcerans* called mycolactone (George *et al.*, 1999).

2.7 RISK FACTORS

Most infections are thought to occur by local inoculation as a result of accidental or unapparent trauma. The main risk factor for BU is contact with an endemic region. The period of exposure can be very short, but most affected people are residents of these areas. There is circumstantial evidence that wearing clothing (trousers) may be protective (Marston *et al.*, 1995). Exposure to contaminated solutions or devices is another risk factor.

In severely immune compromised patients, it is likely that the skin lesions have occurred as a result of haematogenous dissemination rather than direct inoculation. Immunodeficiency, abnormal defensive barriers as a result of skin injury and exposure to certain sources (i.e. soil, water and contaminated solutions or devices) are risk factors associated with infection.

2.8 ENVIRONMENTAL FACTORS

The disease buruli ulcer often occurs in places near water bodies, for example slow-flowing rivers, ponds, swamps, lakes. The germ that causes the disease lives in a wet environment but the exact place is not known. It is suspected that some water insects may transmit the disease.

2.9 ENVIRONMENTS OF BU OUTBREAKS

2.9.1 Agricultural environments as Pathways of MU Infection

Farming activities in close proximity to a river have also been considered as a risk factor in MU infections (Marston *et al*., 1995). For example, a study by (Barker *et al*., 1973), which relates to farming (i.e., crop irrigation), drinking water and frequency of MU infection showed that the disease (BU) was found in 6% of families using boreholes, 25% of families using seasonal swamps and 53% of families using permanent swamps at the edge of a section of the Nile in Uganda. The construction of dams for agricultural purposes is also related to the extension of wetlands, which enhance MU infections (Ziefer *et al*., 1981). The Benin incidence of BU, especially around Zangnanado, could be related to recent construction of canals for irrigation purposes for rice cultivation.

2.9.2 Rivers and Streams as Pathways of MU Infection

Aujoulat and his team, indicate that, in Côte d'Ivoire, increased incidence of BU was very much related to areas around dammed rivers (Aujoulat *et al*., 1996). The first report of MU infection in Côte d'Ivoire was a 7-year old boy living with his parents near an artificial lake (Lake Kossou) in the centre of the country (Peraudin *et al*., 1980). In Nigeria, BU incidence among Caucasians on the campus of Ibadan

University (Oluwasani *et al*., 1976) was associated with a small stream near the university, which was dammed to make an artificial lake. Similarly in Liberia, there were reports of BU cases after a dam construction following the introduction of swamp rice to replace upland rice (Ziefer *et al*., 1981). In Ghana BU is clustered along the Densu River (Mensah-Quainoo, 1998). An impoundment on the southern part of the river (Weija Dam) stores water for the western part of the capital city, Accra. BU occurred in settlements both upstream and downstream of the impoundment. However, the upstream part and along the impoundment where wetlands have been created as a result, BU incidences were higher than in the downstream part south of the impoundment where settlements were on higher elevations.

2.9.3 Swamps and Related Environments

Many of the MU infections occurred after flooding. Bainsdale, Australia, experienced its worst floods on record in 1935 and the first recorded case of BU in 1939 (MacCallum *et al*., 1948). (Barker, 1971) also postulated that the outbreak of BU incidences north of Lake Victoria in the Busoga district in Uganda was related to unprecedented flooding from 1962 to 1964, which occurred as a result of heavy rains. Several references have been made to renewed outbreaks of BU after flood events (Meyer *et al*., 1996; Barker, 1974; Portaels, 1989, 1995; Radford, 1974; Ravisse, 1977; Ravissse *et al*., 1975; Burchard *et al*., 1986). Outbreaks of BU on Philip Island were seemingly related to a road construction, which resulted in the formation of marshlands at the headwaters of an estuary (Johnson *et al*., 1995). Also on Philip Island, a golf course irrigated with recycled sewage and nearby swamp was associated with an outbreak of BU between 1993 and 1995 (Ross *et al*., 1997;

Stinear *et al*., 2000; Veitch *et al*., 1997). In this particular outbreak it was hypothesized that MU was transmitted via aerosols since it had been demonstrated that cells of MU could be aerosolized from suspensions of tap water (Hayman, 1991). Another evidence suggesting that water was not the only source of MU (but rather aerosols) was the occurrence of an outbreak in Kinyari (Uganda) refugee camp, located adjacent to swampy regions near the Nile River. The re-location of the refugees from the site drastically reduced MU infection (Bradley, 1971). MU has also been associated with slowly flowing or stagnant waters (Portaels, 1995; Meyers, 1994). Other places where BU outbreaks occurred in marshy environments include French Guiana (Pradinaud *et al*., 1974), Cameroun (Ravisse, 1977; Ravisse *et al*., 1975).

2.10 HEAVY METALS

Heavy metals are a natural constituent of the Earth's crust. Living organisms require trace amounts of some of these heavy metals which are considered as essential metals, mainly cobalt, copper, iron, manganese, and zinc. Excessive levels of these essential metals however can be detrimental to living organisms (Venchikov, 1998). Human activities have drastically altered the biochemical and geochemical cycles and balance of some of these heavy metals. They therefore tend to be stable, persistent and contaminate the environment since they cannot be destroyed (Bugenyi *et al.*, 1989). They are introduced into aquatic systems as a result of the weathering of soils and rocks, from volcanic eruptions, and from a variety of human activities involving mining processes, or use of metals and/or substances that contain metal pollutants. The most common heavy metal pollutants are arsenic, cadmium, chromium, copper, nickel, lead and mercury. Excessive levels of the metals in the

aquatic ecosystem (environment) can affect aquatic life and pose a lot of health risk to people drinking from such polluted water or consuming foods from the polluted water.

Heavy metal poisoning could result, for instance, from drinking contaminated water (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain (Wolf, 1982, Markowitz, 2003). Heavy metals are dangerous because they tend to bioaccumulate (i.e. their concentration increase in a biological organism over time, compared to their concentration in the environment). The metals accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted (El-Rayis *et al*., 1986). They can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater (Pelig *et al*., 1991).

2.11 HOW METALS GET INTO THE ENVIRONMENT

2.11.1 The Environment

Heavy metals are introduced into aquatic systems as a result of the weathering of soils and rocks, from volcanic eruptions, and from a variety of human activities involving the mining processes, or use of metals and/or substances that contain metal pollutants (Förstner *et al*., 1981). The most common heavy metal pollutants are arsenic, cadmium, chromium, copper, nickel, lead and mercury. There are different types of sources of pollutants: point sources (localized pollution), where pollutants come from single, identifiable sources. The second type of pollutant sources are non-point sources, where pollutants come from dispersed (and often difficult to identify) sources (Jernelöv, 1975). There are only a few examples of localized metal pollution,

like the natural weathering of ore bodies and the little metal particles coming from coal-burning power plants via smokestacks in air, water and soils around the factory (Förstner *et al*., 1981).The most common metal pollution in freshwater comes from mining companies. They usually use an acid mine drainage system to release heavy metals from ores, because metals are very soluble in an acid solution. After the drainage process, they disperse the acid solution in the groundwater, containing high levels of metals. When these metals contaminants reach waters, they either, settle to the bottom, becoming part of the lake sediments or stay in the water as metal ions. Depending on water movement (currents) above the lake bottom, or human activities (mining or shipping), the metals and their attached contaminants may be either picked up and moved elsewhere (resuspended) or may remain permanently on the lake bottom, eventually being buried beneath other particles settling to the bottom (Fahmy *et al*., 1981, Akoto *et al*., 1990).

2.11.2 The Soil

Amongst the range of contaminants that may be found in soils, potentially toxic elements or heavy metals are of particular interest for a number of reasons. Firstly, they show a tendency, under normal circumstances, to accumulate in soils and have a long persistence time because of the interactions with particular soil components. Secondly, they are ubiquitous in soils and arise from both natural and anthropogenic sources, with pathways including inheritance from the parent rocks, application of wastes, as well as local and long-range atmospheric and fluvial deposition of emissions from industry and mining (El-Rayis, *et al*., 1986, Saad *et al*., 1985).

2.12 WATER POLLUTION

There are two main sources of water pollution; point sources and non-point sources. Point sources include factories, wastewater treatment facilities, septic systems, and other sources that are clearly discharging pollutants into water sources. Non-point sources are more difficult to identify, because they cannot be traced back to a particular location. Non-point sources include runoff including from farms, fertilizer, chemicals and animal wastes from farms, fields, construction sites and mines. Landfills can also be a non-point source of pollution, if substances leach from the landfill into water supplies.

2.13 GOLD MINING

Mining operations have negative effects on surface and underground water in a number of ways:

1. The mining process exposes heavy metals and sulphur compounds that were previously locked away in the earth. Rainwater leaches these compounds out of the exposed earth and heavy metal pollution that continues long after the mining operations have ceased.
2. Similarly, the action of rainwater on piles of mining waste (tailings) transfers pollution to freshwater supplies.
3. Huge pools of mining waste "tailings" are often stored behind containment dams. Most tailings impoundments leak at some point in the mines life. In the event like this, water pollution is guaranteed.

Mining companies in developing countries sometimes dump mining waste directly into rivers or other bodies of water as a method of disposal. Water supplies in the

study area are from rivers, streams and rainfall and are characterized by their natural geographical distribution and accessibility, and unsustainable water use.

2.13.1 Mining and Water Pollution

Mining affects fresh water through heavy use of water in processing ore and through water pollution from discharged mine effluent and seepage from tailings and waste rock impoundments. Increasingly, human activities such as mining threaten the water sources on which we all depend. There is growing awareness of the environmental legacy of mining activities that have been undertaken with little concern for the environment. Mining by its nature consumes, diverts and can seriously pollute water resources.

2.13.2 Mining and the Environment

The adverse environmental impact of mining activities on the environment is well documented (Heath *et al*., 1993; Veiga *et al*., 1997; Warhurst 1999; Warhusrt, 1994). Particular attention has been directed towards the impacts of large scale and small-scale gold mining activities on environmental contamination. While the land degradation caused by the gold mining is pronounced, chemical contamination from the gold extraction process imposes a double burden on the environment, with harmful health implications for mining communities and people residing in close proximity to such activities (Yelpaala, 2004). In Ghana several studies in mining towns have revealed that environmental problems such as land degradation, pollution and others are associated with mining activities.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 LOCATION AND GEOLOGY OF STUDY AREAS

The study, which comprises of water and soil chemistry, was conducted in five selected communities namely: Pokukrom, Betenase, Ayanfuri, Kedadwen and Nangruma. (Fig 3.1)

The five study areas are made up three endemic areas: Pokukrom, Betenase, and Ayanfuri, and two non-endemic areas: Kedadwen and Nangruma. These areas were selected based on data from Ghana's National BU Control Programme (2008).

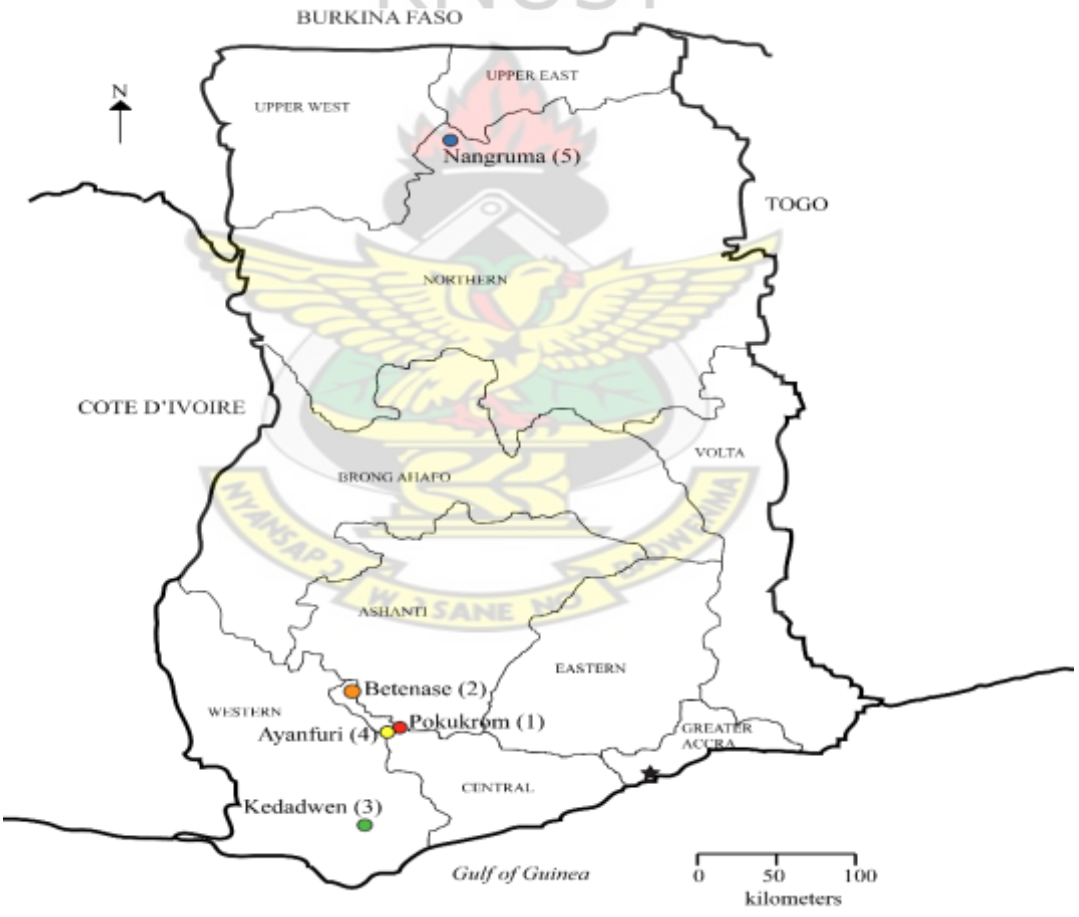


Fig. 3.1 Map of study areas in dots.

Pokukrom, Betenase and Ayanfuri are located in the Upper Denkyira East District in the Central Region (Fig 3.1). The District lies within latitudes 5°. 30' and 6°. 02' north of the equator and longitudes 1° W and 2° W of the Greenwich Meridian. About twenty nine (29) of the population in the district have active Buruli Ulcer cases. Twelve (12) of the active cases are from Pokukrom, eight (8) in Ayanfuri and nine (9) in Betenase. The fouth community, Kedadwen located in the Western Region is free of Buruli Ulcer cases and was thus kept as control (Fig 3.1).

The Upper Denkyira East Municipality covers a total land area of 1700 square kilometers, which is about 17% of total land area of the Central Region. The area falls under a forest-dissected plateau, rising to about 250m above sea level. There are pockets of steep sided hills alternating with flat-bottomed valleys. The major river in the area is the River Offin. A number of streams which are tributaries of either the rivers Offin or Pra flow through the municipality. Prominent among them are the Subin Ninta, Aponapon and Tuatian in the south and Dia, Afiefi and Subin in the north Wikipedia Free Encyclopedia. (2007).

The fifth study area Nangruma is located in the Northern Region of Ghana (Fig 3.1). The district is located roughly within longitudes 0°35'W and 1°45'W and Latitude 9°55'N and 10°35'N. It has a total land area of 5,013 km². The community was also kept as control since Buruli Ulcer cases have not been reported there. The District has a generally undulating terrain characterised by gentle slopes from north-east to south-west. There are however, a few isolated visible outcrops and uplands of not more than 10% slope. Isolated hills, which break the monotony of the landscape, can be found around Karimenga, Shelinvoya and the outskirts of Wulugu. The Geological formation in the West Mamprusi District is underlain mainly by the

Middle Lower Voltaian, which comprises of sandstone, arkose, mudstone and shale. The western part of the district is underlain by the lower Voltaian formation consisting of sandstones and grit. The northern tip is underlain by the Birimian rock formations. Birimian rocks are metamorphosed lavas, which ply Units, schists, tufts and greywacke. Regarding the middle Voltaian, the depth and the degree of weathering depends on the lithology. The district is drained by the White Volta and its tributaries the sissili and the Kulpawn Rivers. Flooding by the White Volta is an annual problem caused mainly by numerous small rivers which flow into it especially below pwalugu Wikipedia Free Encyclopedia. (2007).

3.2 APPARATUS

- Pyrex 50 ml graduated stoppered test tube (26 x 200mm).
- Aluminium top hot plate, HP 1- 2, 457 x 305 x 150 mm (Clifton, UK).
- Aluminium Heating Blocks, 95 x 75 x 50 mm.
- 250ml beakers (Pyrex).
- 50ml and 100ml volumetric flask.
- 50ml Pipette
- 50ml measuring cylinder
- Watch glass
- 2mm sieve
- Whatman No. 40 filter paper
- Agate mortar and pestle
- Wash bottle
- Funnel

- Unicam 929 Atomic Absorption Spectrophotometer
- Conductivity meter
- pH meter
- Wagtech 5000 Photometer

3.3 TREATMENT OF SAMPLING BOTTLES AND GLASSWARE

All glass wares and bottles were soaked in detergents solution overnight after which they were rinsed with distilled water and soaked in 10% HNO_3 solution overnight. They were then rinsed again with distilled water and dried in an oven at 70 degrees celcius.

3.4 REAGENTS

All reagents used were of analytical reagent grade (BDH Chemicals Ltd, Poole, England) unless otherwise stated. Double distilled water was used for the preparation of all solutions.

- **Potassium dichromate solution (1M)** was prepared by dissolving 49.024 g of dry $K_2Cr_2O_7$ in 800 ml of distilled water and diluted to 1000 ml.
- **Concentrated. Sulphuric acid**, analytical reagent grade (BDH Chemicals Ltd, Poole, England) 0.10M H_2SO_4. 8 ml of the concentrated H_2S0_4 was diluted into a 500 mL volumetric flask so that the acid concentration was approximately 0.10M.
- **Ferrous ammonium sulphate solution (0.2M)** was prepared by dissolving 78.39g ferrous ammonium sulphate in 50ml conc. H_2SO_4 and diluted to 1000 ml with distilled water in a $1dm^3$ volumetric flask.
- **Ferroin Indicator solution** (phenanthroline monohydrate- ferrous sulphate)

$[C_{12}H_8N_2]_3FeSO_4$ was prepared by dissolving 1.485 g of 1,10 phenanthroline monohydrate ($C_{12}H_8N_2.H_2O$) in 100 ml of 0.025M ferrous sulphate (0.695 g of ferrous sulphate, $FeSO_4.7H_2O$ in 100 ml of distilled water).

- **EDTA solution**, 0.01M was prepared by dissolving 3.725g of EDTA disodium salt in deionised water. The solution was transferred quantitatively into a 1000 ml volumetric flask and made up to the mark with deionised water. It was then stored in a polyethylene bottle.
- **Standard Calcium Solution,** 1.0g of dried calcium carbonate was weighed accurately and transferred to a 500ml conical flask. 21ml of 1M hydrochloric acid solution was added slowly. The content of the flask was swirled until all the carbonate was dissolved. 200ml of water was added, boiled to expel the carbon dioxide and cooled. It was dried for 4 hrs at about 105 degrees Celsius before use. Few drops of methyl red indicator solution was added and adjusted to an intermediate orange colour with 1M hydrochloric acid solution. It was transferred quantitatively, to a 1000 ml volumetric flask and made up to the mark.

  1ml of this solution= 1mg calcium carbonate.
- **Sodium Hydroxide 2M Solution,** 8g of NaOH was dissolved in 100 mL of freshly distilled water. The solution was stored in a polyethylene bottle.
- **Buffer Solution pH 10,** 67.5g of ammonium chloride [NH_4CI] was dissolved in 570 ml of ammonia solution and 5.0 g of the sodium magnesium salt of EDTA added and diluted to 1000 ml with deionised water. The solution was stored in a polyethylene bottle.
- **Eriochrome Black T (EBT), 0.5 % solution,** Dissolve 0.5g in 100 ml of ethanol-water (80+20 v/v).

- **Standard Silver Nitrate ($AgN0_3$) solution 0.1M,** 5g of $AgN0_3$ was dried for about 2 hours at 100 degrees Celsius and allowed to cool. An accurate weight of 4.25g of solid $AgN0_3$ was weighed and dissolved in 250ml of distilled water in a volumetric flask.
- **Sodium Carbonate (Na_2CO_3),** Na_2CO_3 was dried in an oven at 250 °C and allowed to cool in a dessicator before used. 0.1050 g of Na_2CO_3 was weighed and transferred to a clean 250mL conical flask. About 50 mL of CO_2free distilled water was added to dissolve the solid. 5 drops of methyl orange indicator was added to the flask.

3.5 SAMPLING AND SAMPLE PREPARATION

3.5.1 Soil Samples

Soil samples were collected from the five communities namely: Pokukrom (PK), Betenase(BT), Ayanfuri (AF), Kedadween (KD) and Nangruma (NG). Coordinates using GPS were obtained for the sites. Samples from Nangruma ("Overseas") and Kedadwen were kept as control samples since the areas are free of buruli ulcer cases.

A total of fifty eight (58) surface soil samples (0 – 15 cm) were collected randomly from cultivated fields (cassava, plantain farms, etc), cocoa farms. Small scale mining otherwise known as "Galamsey" sites, logged areas and matured forest.

Each soil sample was collected in a plastic bag and sealed. All samples were transported to the Chemistry laboratory at Kwame Nkrumah University of Science and Technology (KNUST).

Samples were air dried for three days and sieved through a 2 mm sieve and were analyzed for pH, conductivity and trace metals.

3.5.2 Water Samples

Water samples were taken from the five communities namely: Pokukrom (PK), Betenase (BT), Ayanfuri (AF), Kedadween (KD) and Nangruma (NG). Coordinates using GPS were obtained for the sites. Samples from Nangruma ("Overseas") and Kedadwen were kept as control samples since the areas are free of buruli ulcer cases. A total of ninety-four (94) water samples were withdrawn from wells, boreholes, rivers, galamsey mining pits, swamps, and potential "Buruli ulcer hot spots," where applicable, in the selected community. "Buruli ulcer hot spots" are specific areas in the study communities which were identified by community members, those places were named as such by community members as areas that they felt posed a risk for contracting Buruli ulcer; these are areas which consist mainly of pools of stagnant water. Samples from rivers and streams were sampled at points where inhabitants constantly cross, these were also points where children constantly come in contact with as they play and swim most often. The analyzed samples were collected within the period of January 2010 in the dry season and July 2011 in the wet/rainy season. Each water sample was collected in three bottles:

- 500mL unpreserved sample for analysis of pH, major cations, major anions, and sulfate,
- 500mL preserved with H_2SO_4 for analysis of ammonia, nitrate, nitrite, and phosphate, and
- 100mL preserved with HNO_3 for analysis of trace metals.

Sample bottles were each rinsed at least three times with the water to be sampled. Sample bottles were filled directly from the water body when possible. Concentrated H_2SO_4 and HNO_3 were added, where necessary, to samples immediately upon collection to preserve samples. All samples were transported to the Chemistry

laboratory at Kwame Nkrumah University of Science and Technology (KNUST). Samples were refrigerated upon arrival at the laboratory. Samples were analyzed for major ions, nutrients and trace metals.

3.6 DETERMINATION OF SOIL pH

Soil suspension for soil pH determination was prepared by weighing 20g of soil into a beaker and adding 100ml of distilled water. Using the ORION 5 STAR thermoscientificmeter pH meter, the electrode of the pH meter was rinsed with distilled water and lowered into the sample. The pH meter was allowed to stabilize and the pH value of the sample was read.

3.7 DETERMINATION OF SOIL CONDUCTIVITY

Soil suspension for conductivity determination was prepared by weighing 20g of soil into a beaker and adding 100ml of distilled water. Using the ORION 5 STAR thermoscientificmeter conductivity meter, the electrode was rinsed with distilled water and lowered into the sample. The conductivity meter was allowed to stabilize and the conductivity value of the sample was read.

3.8 DIGESTION PROCEDURE FOR DETERMINATION OF METALS IN SOIL

Exactly 0.5 g of a pulverized sample was weighed into a 250ml beaker and 20ml conc. HNO_3 was added. It was then heated until all the brown fumes had ceased (about 30 minutes). It was then allowed to cool and after which it was filtered with Whatman No. 40 filter paper after a little dilution with distilled water into a 100ml volumetric flask. The filter paper was washed several times with small amount of

distilled water into the filtrate after which the solution was made to the mark with distilled water. A blank solution was prepared using the same procedure without the sample.

3.9 ANALYSIS OF SOIL SAMPLES

The concentration of the metals (i.e. As, Cd, Cu, Fe, Pb, Se, Zn) were determined with the Unicam 929 Atomic Absorption Spectrophotometer.

3.10 DETERMINATION OF WATER pH

3.10.1 Procedure

Calibration of the pH electrode

The electrode was placed in the buffer solution of pH 4.00. It was ensured that the value for pH reads 4.00. The reading was allowed to stabilize. The electrode was rinsed with distilled water and again placed in pH 10 solution. It was also ensured that the pH meter reads 10.00, and allowed the reading to stabilize. The water sample was placed in a 50ml beaker. The pH of the water sample was then measured with the pH meter until the pH stabilized and the value was recorded. The pH electrode was rinsed with deionised water between each measurement. The electrode was stored in its storage solution when not in use.

3.11 CONDUCTIVITY

3.11.1 Calibration of the conductivity cell

The potassium chloride solution (KCl) with concentration of 0.01M known as the reference solution, which at 25^{o}c has a conductivity of 1413µs/cm was used to

standardize the conductivity meter. The conductivity cell was rinsed with at least three portions of 0.01M KCI solution.

3.11.2 Procedure for the measurement of conductivity

The electrode of the conductivity cell was rinsed with at least three portions of the sample; it was then lowered into the sample. The conductivity in µs/cm units of the sample was then measured.

3.12 ALKALINITY

3.12.2 Procedure for the measurement of alkalinity

This was determined by measuring exactly50 ml of water into a clean 250 mL conical flask. 2 drops of phenolphthalein indicator was then added and the resulting mixture titrated against standard 0.10M H_2SO_4 solution until the pink colour disappeared. The burette reading was recorded and three drops of methyl orange indicator was added to the solution and titrated against the standard 0.10M H_2SO_4 solution to the first permanent pink colour at pH 4.5.

3.12.3 Calculations

$$\text{Alkalinity (mg/L)} = \frac{\text{V X M X 1000}}{\text{Vol of sample}}$$

Where V = volume of acid used

M = molarity of H_2SO_4

3.13 TOTAL HARDNESS

3.13.1 Procedure for determination of total hardness

EDTA Titrimetric method was used to determine the total hardness in the water samples. Determination of total hardness was carried out by measuring 50mL of the water sample into a 250mL conical flask. About 4mL of ammonium chloride in concentrated ammonia as the buffer solution and 6 drops of Erichrome black T indicator solution were added prior to titration. The content in the conical flask was titrated against 0.01M EDTA to the endpoint indicated by a distinct colour change from violet to blue colouration. Titration was repeated for consistent titre values from which an average titre was calculated (APHA, 1992).

$$\text{Total Hardness in mg/L } CaCO_3 = \frac{\text{Vol of EDTA X M X 1000}}{\text{Vol of sample}}$$

Where M = Molarity of EDTA used.

3.14 CHLORIDE ION CONCENTRATION

3.14.1 Procedure for determination of chloride concentration

The Argentometric method was used to determine the chloride concentration in the sample. 50 mL of water sample was pipetted into a 250 mL conical flask. 1 mL of 0.25 M potassium chromate (K_2CrO_4) was added to the conical flask. Water sample was titrated against the standard $AgNO_3$ solution slowly while swirling gently until the colour changed from yellow to brick-red. Blank (distilled water) was titrated using the same procedure. Volume of $AgNO_3$ for the blank was subtracted from the average volume of sample. This volume was used to determine the concentration of chloride ion in the water sample.

The value was calculated using the formula

Cl^- (mg/L) = $(A - 0.2) \times 35.450 / \text{Vol of sample}$

Where A = titre value

M = molarity of $AgNO_3$

3.15 DIGESTION PROCEDURE FOR DETERMINATION OF METALS IN WATER

Exactly 15 ml of conc. HNO_3 was added to 50ml of the sample. The mixture was evaporated to a small volume (about 15ml), cooled and diluted to the mark with distilled water. A blank solution was prepared using the same procedure without the sample.

3.16 ANALYSIS OF WATER SAMPLES

3.16.1 Measurement of metals

The concentrations of the metals (i.e. As, Cd, Cu, Fe, Pb, Se, Zn) and the metalloids (As and Se) were determined with the unicam 929 Atomic Absorption Spectrophotometer.

3.16.2 Measurement of Sulphate, Phosphate, Nitrate, Ammonia, and Fluoride

Concentrations of nitrate, nitrite, phosphate, ammonium, fluoride, manganese, sulphate, sulphide were determined using the Wagtech 5000 Photometer.

The Photometer method was used to determine the concentration of SO_4^{2-}, PO_4^{3-}, NO^{3-}, NH_3 and F^- in the water sample. Analytical water test tablets (photometer grade) reagents for specific test were used for the preparation of all sample solutions. The samples were allowed to stand for the colour to develop. The tablets were added to the samples to form complexes with the analyte which impart colour to the

samples. The required wavelength for the specific test was selected by moving the slide control. Distilled water was used to zero the instrument. The ON button was pressed and kept depressed until the display reads 100 (100% T). The sample was immediately submitted to the instrument to read the % transmittance. The displayed reading was taken to a calibration chart from which the concentration of the analyte was determined from the read % transmittance.

3.17 STATISTICAL ANALYSIS

The data obtained in this study were subjected to statistical analysis using both Microsoft Excel (2007 Edition) and Statistical Package for Social Science (SPSS) software. The relationships between the water bodies, communities and physicochemical parameters concentrations in water samples were evaluated by ANOVA. The relationship between the dry and wet seasons was evaluated by student t-test. All errors were calculated at 95% confidence level.

CHAPTER FOUR

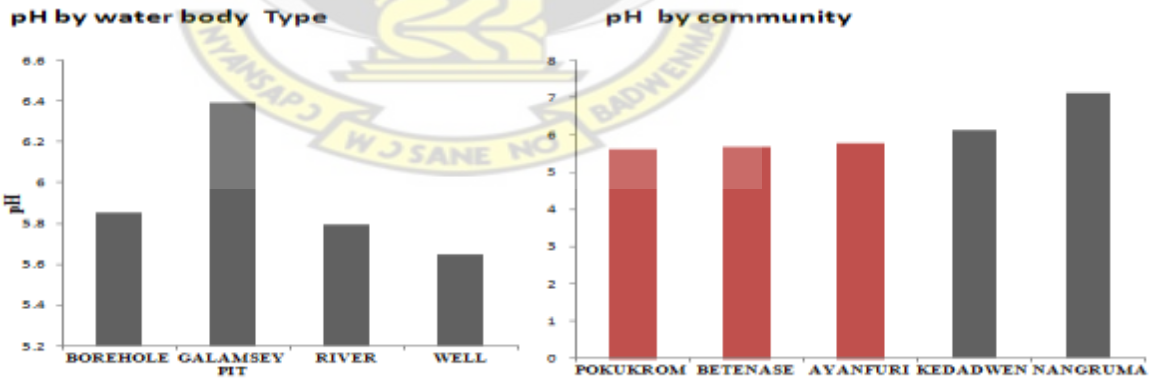
4.0 RESULTS AND DISCUSSION

The results of pH, trace metals and nutrients analyzed for the water and soil samples in the dry and wet seasons are organized and discussed based on the water body types, soil source types and the communities. Red in the graphs indicates endemic communities and blue indicates non-endemic communities**.**

4.1 WATER

4 1.1 pH

In the dry season, results from analysis of pH and other related physicochemical parameters for the dry season samples revealed that mean values of pH for all water bodies in the entire study during the dry season varied within the range from 5.6 to 6.6. The highest pH of 6.6 was recorded for galamsey pits samples; while the lowest pH of 5.6 was recorded for well, samples. From the mean pH values, all the water bodies tend to be slightly acidic (pH < 7) (Figure 4.1a).

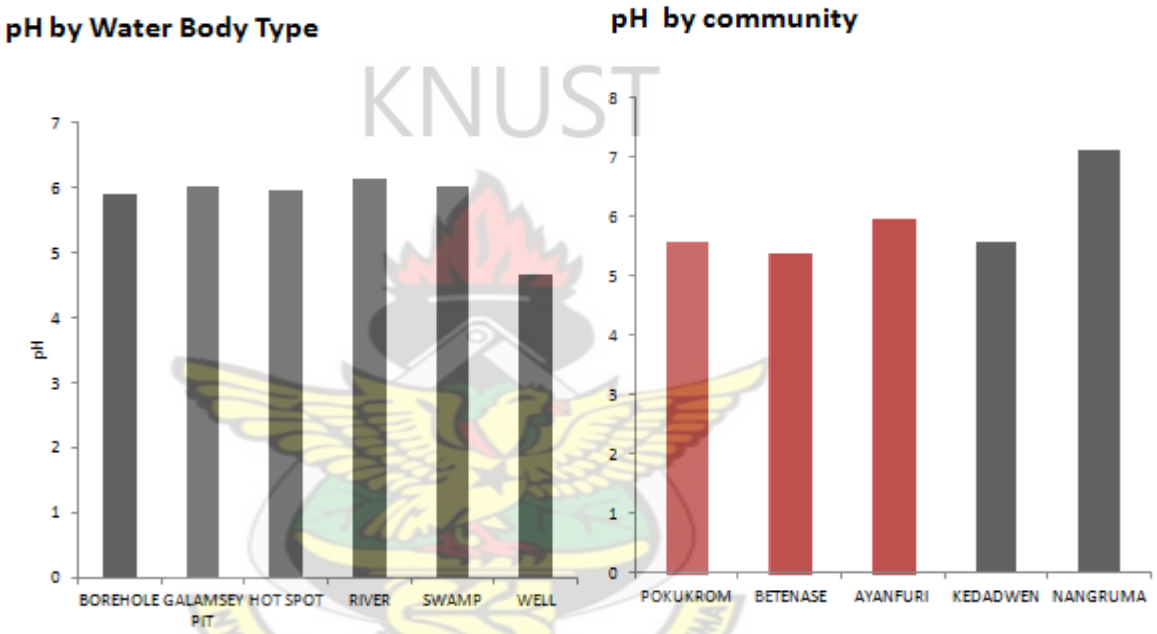


(4.1a) **(4.1b)**

Fig.4.1 Plots of pH of water during the dry season, a) water body type, and b) community Red and black indicates endemic and non-endemic communities respectively.

Wells and rivers were observed to be the water bodies with high acidity, but well water samples are more acidic (Figure 4.1a). There were no significant differences for pH between the water bodies during the dry season according to ANOVA at 95% confidence level. All the mean values of pH obtained fell within this range but were slightly below the natural background level of 7.0. Based on figure 4.1a , the pattern of the pH values of all the water body types can be written in descending order as follows: Galamsey pits > boreholes > rivers > wells. The mean pH values for the communities during the dry season varied within the range of 5.6 to 7.12. The highest pH of 7.12 was obtained for non-endemic community (Nangruma); while the lowest pH of 5.60 was obtained for an endemic community (Pokukrom). Endemic communities (Southern communities), Pokukrom, Betenase, and Ayanfuri from the results are all acidic recording 5.60, 5.66 and 5.76 respectively. However samples from Kedadwen, a non-endemic community (Southern community), was also observed to be acidic (6.13). From the mean pH values of water samples from Nangruma, a non-endemic community, located in the north, the water samples were observed to be neutral or slightly basic. There were significant differences between endemic and non-endemic communities for pH during the dry season according to ANOVA at 95% confidence level. These results suggest more suitable conditions for *M. ulcerans* growth in southern Ghana than in northern Ghana, but the similarity in pH values between endemic communities Pokukrom, Betenase, and Ayanfuri and non-endemic community Kedadwen in the south implies that pH may not be a controlling factor for *M. ulcerans* growth in this region. Based on Figure 4.1b, the pattern of the pH values in the water at all the communities can be written in descending order as follows: Nangruma > Kedadwen > Ayanfuri > Betenase > Pokukrom.

The wet season's sample results for pH revealed that mean values of pH for all water bodies studied ranged from 4.66 to 6.14. The highest pH of 6.14 was obtained for river samples; while the lowest pH of 4.66 was obtained for well samples. From the mean pH values, all the water bodies tend to be slightly acidic (pH< 7) (Figure 4.1c). There were no significant differences in pH between the water bodies during the wet season according to ANOVA at 95% confidence level.



(4.1c) **(4.1d)**

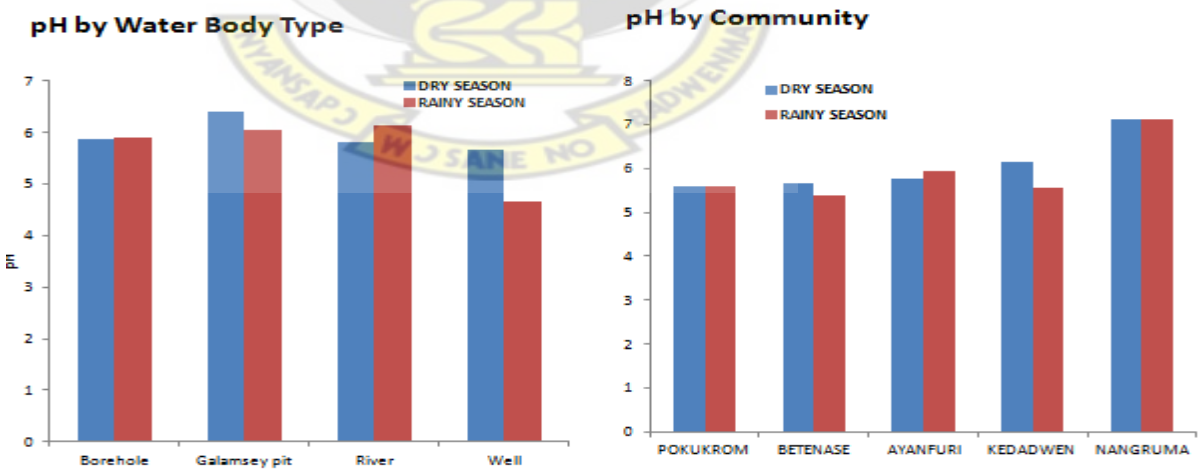
Fig. 4.1 Plots of pH during the wet season, c) water body type, and d) community. Red and black indicates endemic and non-endemic communities respectively.

Based on figure 4.1c, the pattern of pH values in the water samples can be written in descending order as follows: River>swamp=galamsey pits>BU hot spot>borehole>well (Figure 4.1c). The water samples had acceptable pH values in the range of 4.6 to 6.1. When pH was organized by community, Nangruma, a non-

endemic community had the highest pH of 7.1; while Betenase, an endemic community had the lowest pH of 5.38. Based on figure 4.1d the pattern of mean pH values in the water from of all the study communities can be written in descending order as follows: Nangruma>Ayanfuri>Pokukrom>Kedadwen>Betenase (figure 4.1d). Endemic communities, Pokukrom, Betenase, and Ayanfuri recorded acidic pH values of 5.5, 5.38 and 5.9 respectively. Kedadwen a non-endemic community was observed to also have acidic pH value (5.5) (Figure 4.1d). There were no significant differences in pH between endemic and non-endemic communities during the wet season according to ANOVA. From the mean pH values water samples from Nangruma in Northern Ghana was observed to have high pH 7.1 (slightly basic) values compared to the rest of the four study areas in southern Ghana. The above results suggest more suitable conditions for *M. ulcerans* growth in southern Ghana than in northern Ghana, but the similarities in observed pH values between endemic and non-endemic communities in the south may imply that pH is not a controlling factor for *M. ulcerans* growth in this region.

Seasons seem to have an effect on the number of MU infection as reportedling, some authors (Revill *et al*., 1972; Meyers *et al*., 1996).A series of epidemiological studies show the existence of seasonal variation in the appearance of Buruli ulcer cases. It seems that the number of cases increase during dry periods or after inundations (Darie *et al*., 1993, Portaels *et al*., 1989). These conditions are probably favourable for the development of *M. ulcerans*, because of the concentration of possible vectors in areas that are frequently visited by humans. The quality of water is never constant; it is constantly changing in response to daily, seasonal and climatic rhythms (Marian *et al.,* 2010).

All the water bodies studied in the wet and dry seasons showed pH values varying from 4.6 to 6.1, indicating moderately acidic water (pH< 7) (figure 4.1e). *M. ulcerans* is believed to strive in acidic medium (Iivanainen *et al*., 1993). Wells, rivers, BU hot spots and swamps in the dry and wet seasons were observed to be the water bodies with high acidity, but well water samples are more acidic, compared to the other water bodies. Thus, based on these results, pH may be an indicator of the ability of *M. ulcerans* to grow in certain water bodies. The highest pH 6.1 was recorded for the dry season, while the lowest was in the wet season (figure 4.1e). There were no significant differences for pH values in boreholes between the two seasons according to student T- test; however, the differences between galamsey pits, rivers and wells in the two seasons for pH were significant according to student T-test. From the mean pH values obtained, all the water bodies in both seasons fell below the potable range (the WHO recommended pH range for potable water is 6.5-8.5) (Kortatsi, 2002).



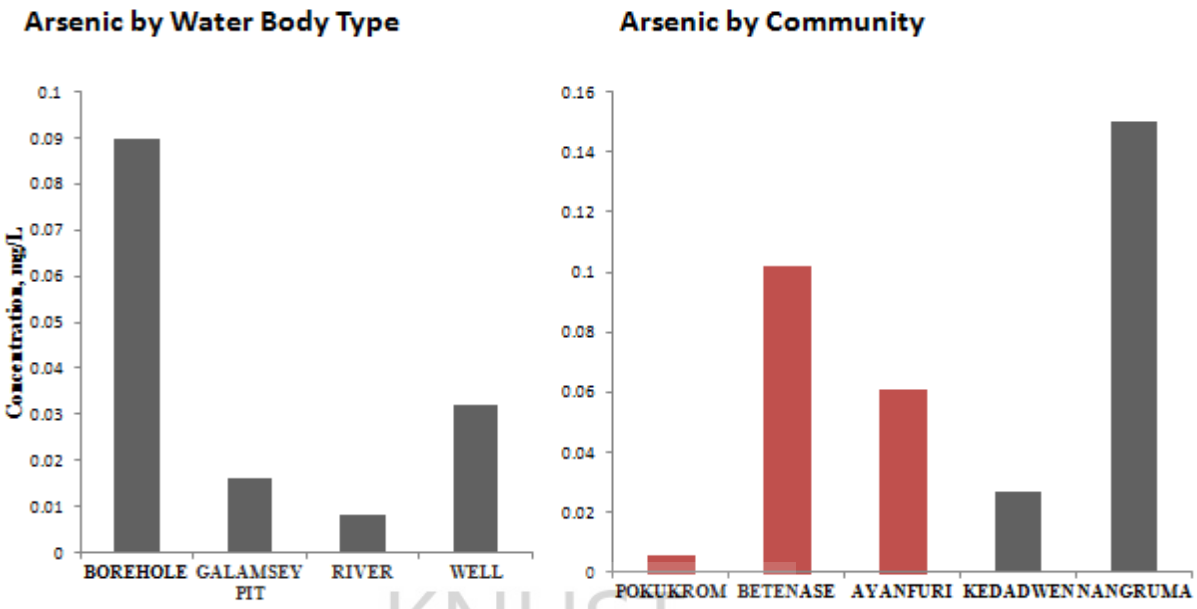
(4.1e) **(4.1f)**

Fig. 4.1 Plots of pH of water for both dry and wet season, e) water body type, and f) community. Blue indicates dry season and red wet season.

The highest mean pH (7.1) was observed for Nangruma samples, whereas pH (5.6) was the minimum in Pokukrom samples. Four of the study areas (Pokukrom, Betenase, Ayanfuri and Kedadwen) showed slightly acidic pH, while Nangruma a non-endemic community showed consistently alkaline pH for both seasons. Nangruma, a non-endemic community consistently recorded the highest pH values during the dry and wet season. Kedadwen, a non-endemic community also showed acidic pH similar to the endemic communities. There was no seasonal difference in pH values for Pokukrom and Nangruma (Figure 4.1f) according to student T-test.

4.1.2 Trace metals

Arsenic results during the dry season revealed that, mean arsenic concentrations for all the water bodies stood at 0.034 mg/L, ranged from 0.008 to 0.08 mg/L. However, mean arsenic concentration was observed to be highest in borehole samples compared to the other water bodies (figure 4.2a). The results also revealed that, well water samples contained the next highest arsenic content after the boreholes during the dry season (0.0322 mg/L). Arsenic content in boreholes and wells (0.08 and 0.0322 mg/L) exceeded the WHO Limit of 0.01 mg/L for consumption. The high levels of arsenic in boreholes and wells might be due to the fact that mining activities has been the cause of both surface and groundwater chemical pollution because of discharged mine effluent and seepage from tailings and waste rock impoundments. There were significant differences in arsenic concentration between the water bodies for the dry season according to ANOVA.



(4.2a) **(4.2b)**

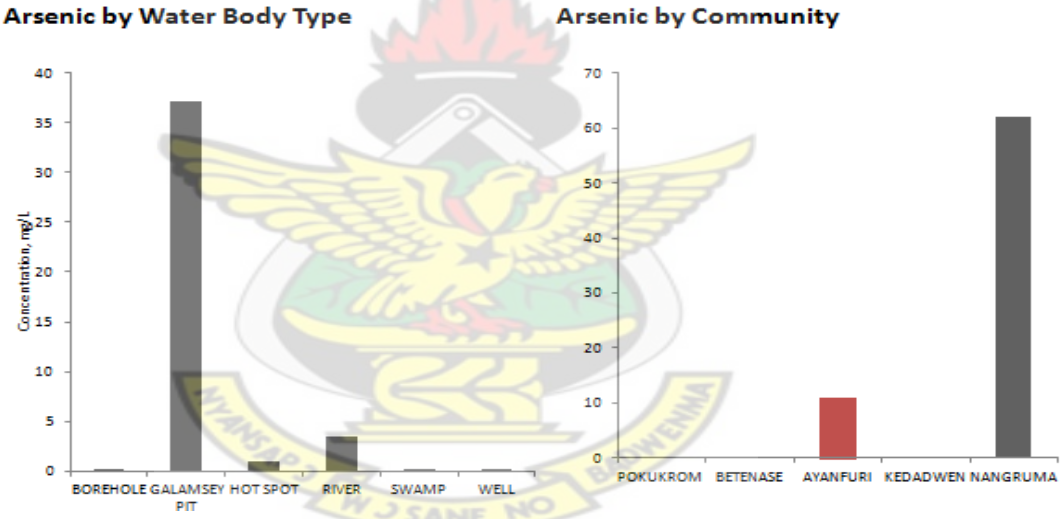
Fig. 4.2 Plots of arsenic for water during the dry season, a) water body type, and b) community. Red indicates an endemic community and black non-endemic community.

Based on Figure 4.2a, the pattern of the arsenic occurrence in the water body types can be written in descending order as follows: Boreholes > wells > galamsey pits > rivers.

When the mean levels of arsenic concentration in the dry season was analyzed based on community, it stood at 0.069 mg/L, range from (0.01 to 0.15mg/L). It was however revealed that arsenic concentration was highest in Nangruma (a non–endemic community) (0.15mg/L), compared to the other study areas (figure 4.2b), and lowest in Pokukrom; an endemic community (0.01mg/L). There were significant differences between endemic and non-endemic communities according to ANOVA. Arsenic content in four out of the five communities exceeded the WHO Limit of 0.01 mg/L. The community with levels lower than the WHO limit is Pokukrom. Endemic communities, Pokukrom, Betenase, and Ayanfuri show some high arsenic

concentrations, but the highest arsenic concentrations are seen in Nangruma (a non-endemic community) (Figure 4.2b). Within Nangruma, arsenic concentrations are highest in borehole samples. Based on figure 4.2b, the pattern of the arsenic occurrence in the water at all the communities can be written in descending order as follows: Nangruma > Betenase > Ayanfuri > Kedadwen >Pokukrom.

Trace metals have been assumed to be beneficial for the growth *M. Ulcerance.* Mining activities is often associated with high trace metals concentration; it is not surprising that results of arsenic concentration in the wet season are seen to be high in galamsey pits samples (Figure 4.2c).



(4.2c) **(4.2d)**

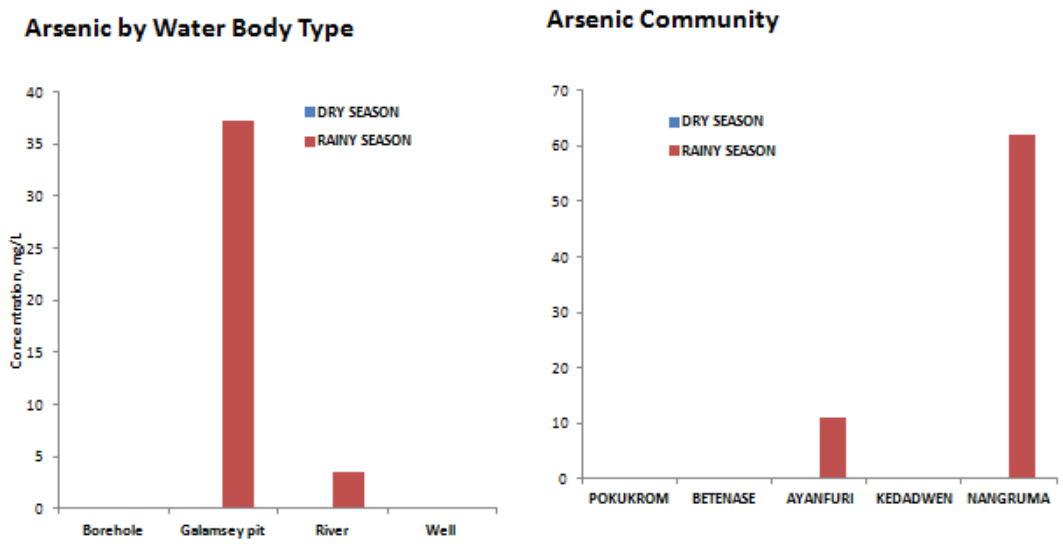
Fig. 4.2 Plots of arsenic for water during the wet season, c) water body type, and d) community. Red indicates an endemic community and black non- endemic community.

Wet season analyses results of arsenic for all the water bodies, revealed that the highest mean concentrations of arsenic (As) (37.16 mg/L), was recorded in galamsey pits; The lowest arsenic (0.01mg/L), concentration was observed in boreholes

(Figure 4.2c). Out of all the water body types only wells had safe amounts of arsenic. The rest of the water bodies had arsenic at trace and unsafe levels. Levels were as high as 3.55 mg/L, 1.02 mg/L and 0.06 mg/L in rivers, BU hot spot and swamp respectively. There were no significant differences in arsenic concentration between the water bodies in the wet season according to ANOVA. The arsenic contents exceeded the WHO Limit of 0.01 mg/L (WHO, 2008). This presents a high risk to consumers. The results revealed that, arsenic concentrations are also high in BU hotspots and rivers compared to other water body types. Order of magnitude of arsenic in water bodies is: Galamsey pits>river>BU hot spot>swamp>well>borehole (Figure 4.2c). Endemic communities namely: Pokukrom, Betenase, and Ayanfuri were observed to have some high arsenic concentrations, but the highest arsenic concentrations are observed in Nangruma (Figure 4.2d). Within Nangruma, arsenic concentrations are highest in galamsey pits. There was significance difference between the endemic and non-endemic communities according to ANOVA. The order of magnitude of arsenic in the communities is: Nangruma >Ayanfuri>Pokukrom>Betenase>Kedadwen (Figure 4.2d).

Seasonal variation of the trace metal accumulation in the water bodies and study communities was observed to exhibit a unique seasonal pattern, in that samples collected in the wet season had considerably higher concentrations of metals than those collected in the dry season. This observation could be attributed to runoff increase during the wet season. Also, almost all the metals studied (As, Zn, Cu, Pb and Cd) were high in galamsey pits when water samples were considered based on water body type. The metals were also high in Nangruma (non-endemic community) throughout the study for wet season. All the metals investigated in the study (arsenic,

copper, cadmium, lead, selenium and zinc), had their highest concentrations recorded in the wet season. The order of magnitude of the metal occurrences in the wet season is: arsenic>zinc>copper>lead>cadmium>selenium. This may be due to runoff effect during the wet season that enters the water bodies. In addition, in the dry season which is warmer, metal levels are likely to be reduced by biochemical processes. In Ghana, temperatures are high in the dry season and this increases the biochemical activities in the water bodies. Since there may not be runoffs into the water bodies, the concentrations of the metals may be reduced. The contrary holds true in the wet season. In the season, very high metal concentrations were consistently found in galamsey pits and rivers. Concentrations of the metals investigated in the study based on communities followed similar trend as that of the water bodies, in that the highest concentrations at the community level are observed in the wet season samples as compared to the dry season samples. Ayanfuri, an endemic community and a non-endemic community (Nangruma) in the study recorded the highest trace metal concentration for both seasons. The highest mean concentrations of arsenic (37.16 mg/L), zinc (0.72 mg/L), copper (0.59 mg/L), lead (0.45 mg/L) and cadmium (0.08 mg/L) were observed during the wet season. The highest selenium concentration (0.02 mg/L) occurred in the dry season (Figure 4.2e).



(4.2e) **(4.2f)**

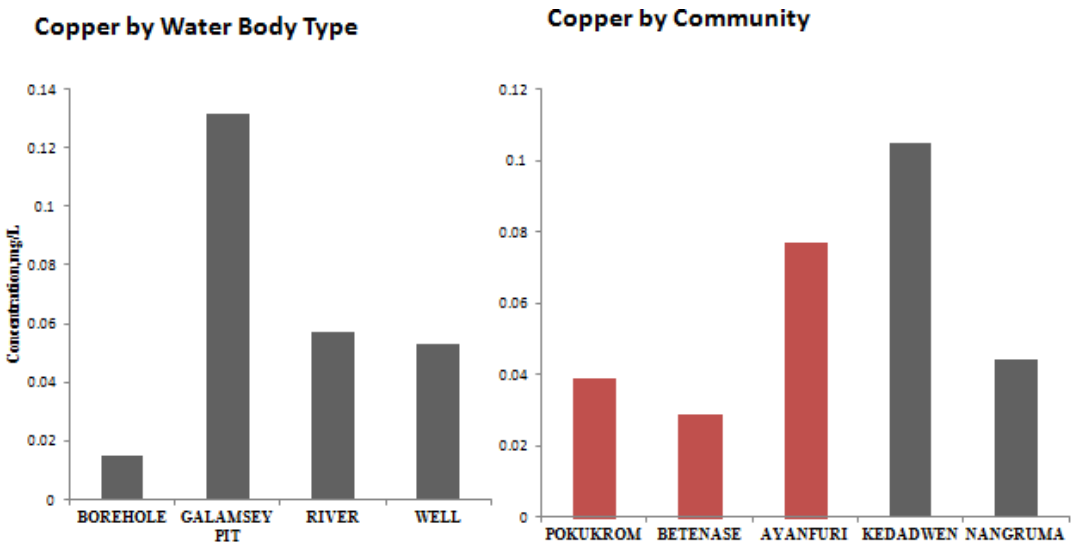
Fig. 4.2 Plots of arsenic in water for both dry and wet season, e) water body type, and f) community. Red indicates wet season.

Arsenic is the highest metal recorded throughout the study (Figure 4.2e and 4.2f). High arsenic values of 61.95 mg/L were recorded during the wet season, but arsenic dropped during the dry season (0.01 mg/L). During the wet season arsenic was highest in galamsey pits and rivers. There were no significant differences between boreholes, galamsey pits, rivers and wells for arsenic during the two seasons according to student T-test. Boreholes, rivers and wells which are a source of drinking water in the study areas, happen to contain high levels of arsenic. This is alarming since several dermatological diseases (Bowen's disease, hyperkeratosis, hyperpigmentation) are related to arsenic ingestion and exposure (Mensah-Quainoo *et al.*, 1998). Bioaccumulation of arsenic in the fatty tissues of the skin (Gorby, 1994) due to its high lipid solubility (Isensee, 1973, Schoolmeester *et al*., 1980) may provide a favourable environment for *M.Ulcerens* in the skin because arsenic is known to help microorganisms grow (Mahieu *et al.*, 1981). This is likely to make inhabitants who consume water from these sources prone to *M.Ulcerans* infection.

Duker and his team (2006) hypothesized, that arsenic induces *M.Ulcerens* adhesion to human tissues, and also added that arsenic influences the ability of MU to establish BU. Trend of arsenic when organized by communities is similar to arsenic by water body type, in that, arsenic is high in the wet season compared to the dry season (Figures 4.2e and 4.2f). There were no significant differences in arsenic concentration in Betenase between the two seasons according to student T- test; however, the differences in arsenic concentration for Pokukrom, Ayanfuri, Kedadwen and Nangruma between the two seasons were significant according to student T-test. The highest arsenic content among the communities is seen in a non-endemic community Nangruma. Ayanfuri, an endemic community also had high levels of arsenic. (Figure4.2f). The high levels of arsenic concentrations in Nangruma (a non-endemic community) located in northern Ghana compared to other communities in southern Ghana, could probably be caused by the different mining techniques employed in these communities. For example, miners in the southern communities, Pokukrom, Betenase, Kedadwen, and Ayanfuri, employ surface mining (open mining) where mining is done by stripping surface vegetation and layers of bedrock in order to reach buried gold ore deposits and recovering it from an open pit in the ground. On the other hand, miners in Nangruma (a non-endemic) community in the north employ underground mining, which involves digging tunnels or shafts into the earth to reach buried ore deposits. Ore, for processing, and waste rock, for disposal, are brought to the surface through the tunnels and shafts and crushed. When the ore is crushed, arsenic can be released in larger amounts in Nangruma, because of the abundance of arsenic-bearing minerals, particularly arsenopyrite, compared to amounts that would have come from runoff from other sources in the case of the southern study areas. Surface waters close to these

galamsey drainage pits or gold mines may get contaminated by arsenic. Arsenic may be washed from galamsey drainage pits to rivers and swamps which might explain the reason for the high levels of arsenic in Nangruma a non- endemic community compared to other study communities in the south. Based on the above, the trace metal could easily be trapped into near-by drainage channels during floods; this may end up in rivers, wells and other surface water bodies. This actually presents a high risk to consumers.

Mean concentrations of copper in the water samples ranged from 0.02 to 0.13 mg/L for boreholes and galamsey pits respectively during the dry season. Copper results from this season in all the water bodies were observed to be highest in galamsey pits followed by rivers (Figure 4.3a). Thus galamsey pits and rivers may be locations for contracting BU, since high trace metal concentrations are postulated to be beneficial for *M. ulcerans* growth (Duker *et al*., 2004). Based on figure 4.3a, the pattern of copper occurrence in water samples from all the water bodies in this season can be written in descending order as follows: Galamsey pits>rivers>wells>boreholes. There were significant differences in copper concentration between the water bodies for the dry season according to ANOVA. When organized by community concentrations were highest in Kedadwen (a non-endemic community) and lowest in Betenase (an endermic community) (Figure 4.3b). Nangruma (a non-endemic community) also shows high copper concentrations.

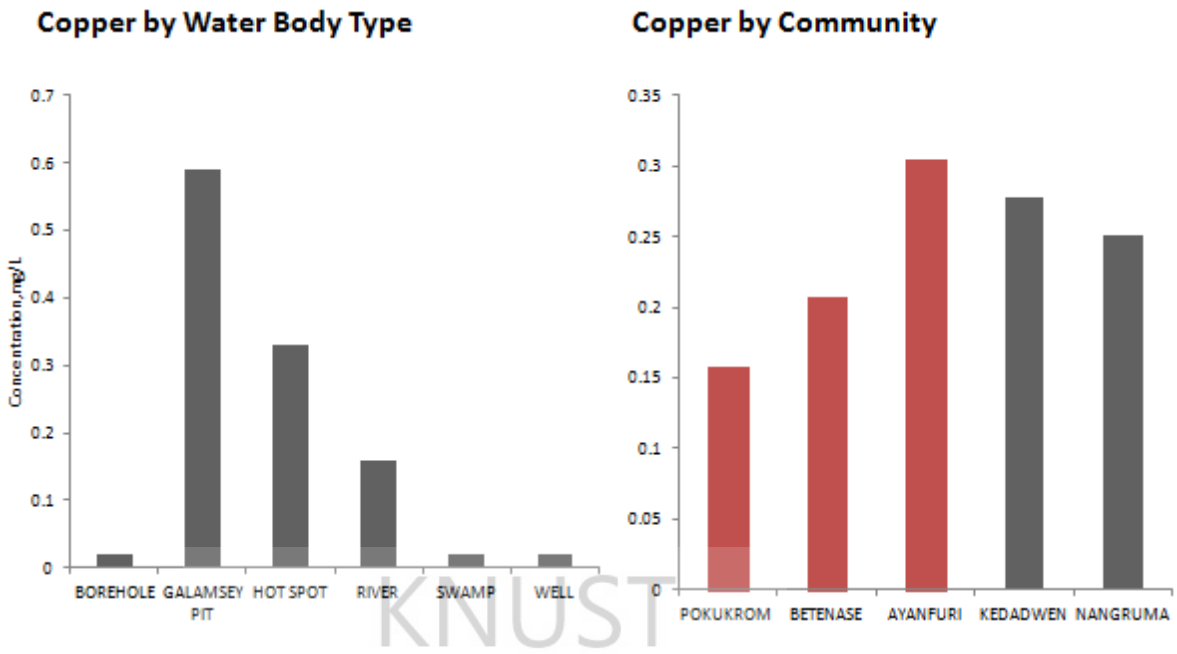


(4.3a) **(4.3b)**

Fig. 4.3 Plots of copper in water during the dry season, a) water body type, and b) community. Red indicates an endemic community and black non-endemic community.

Based on figure 4.3b, the pattern of copper occurrence in the water samples from all the communities in the dry season can be written in descending order as follows: Kedadwen > Ayanfuri > Nangruma> Pokukrom > Betenase. There were significant differences in copper concentration between the endemic and non-endemic communities for the dry season according to ANOVA.

The wet season copper concentrations followed similar trends to copper concentration in the dry season, in that galamsey pits recorded highest concentrations compared to the other water bodies (Figure 4.3c). There were no significant differences in copper concentration between the water bodies for the wet season according to ANOVA. All the water bodies contained trace and allowable amounts of copper, since recorded values were below their maximum allowable values.

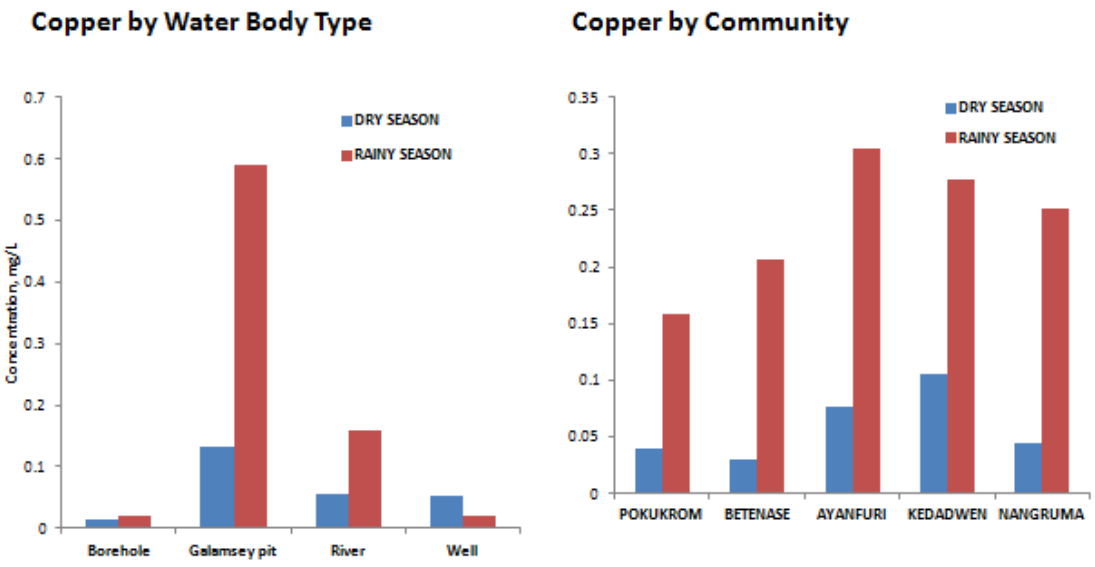


(4.3c) **(4.3d)**

Fig. 4.3 Plots of copper in water during the wet season, c) water body type, and d) community. Red indicates an endemic community and black non-endemic community.

When organized based on community (Figure 4.3d), high concentrations are seen in Ayanfuri which is an endemic community, but concentrations are low in the other endemic communities (Betenase and Pokukrom). Nangruma, a non-endemic community also showed high copper concentrations. There were no significant differences in copper concentration between the endemic and non-endemic communities according to ANOVA.

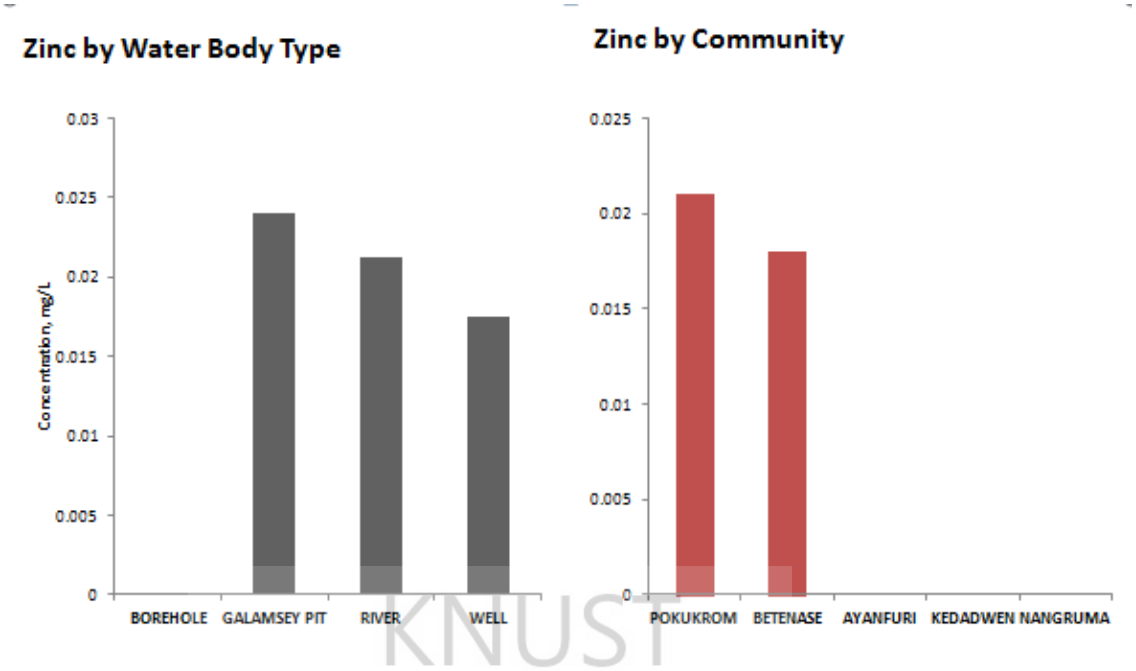
Mean concentrations of copper for both seasons range from 0.02 to 0.59 mg/L. Copper concentrations are highest in the wet season compared to the dry season (Figure 4.3e and 4.3f).The difference is significant according to students T-test.



(4.3e) **(4.3f)**

Fig. 4.3 Plots of copper in water for both dry and wet seasons, e) water body type, and f). Blue indicates dry season and red wet season.

Dry season results for zinc concentration were observed to be high in galamsey pits water samples followed by river samples (Figure 4.4a). From the results, the trend of zinc occurrence in the dry season is similar to that of copper. All the water bodies and sites contained trace and allowable amounts of zinc. Zinc concentration in all the water bodies and sites were below the limit of 3.00 mg/L set by WHO (2000). There were significant differences in zinc concentration between the water bodies in the dry season according to ANOVA. Zinc, when organized by community (Figure 4.4b), concentrations are lowest in Kedadwen (a non-endemic community) and highest in Pokukrom (an endemic community). There were significant differences in copper concentration between endemic and non-endemic communities for the dry season according to ANOVA.

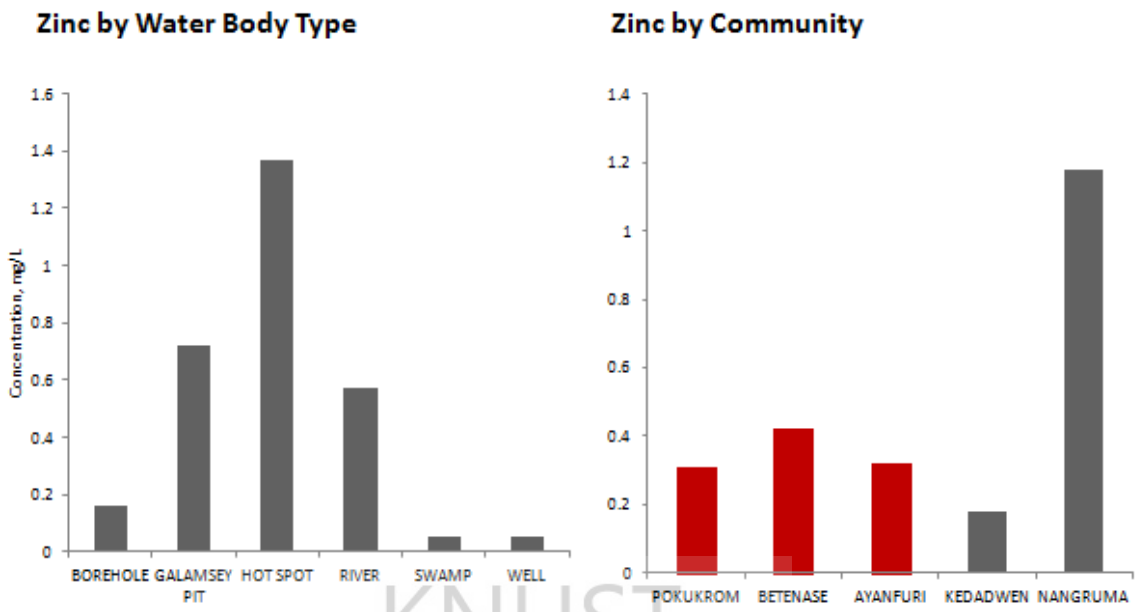


(4.4a) **(4.4b)**

Fig. 4.4Plots of zinc in water during the dry season a) water body type, and b) community. Red indicates an endemic community and black non-endemic.

Wet season occurrence of zinc is similar to that of the dry season. Concentrations are highest in galamsey pits and BU hot spots (Figure 4.4c). Here concentrations are elevated in Betenase and Nangruma relative to other communities (Figure 4.4d). There were no significant differences according to ANOVA between endemic and non-endemic communities.

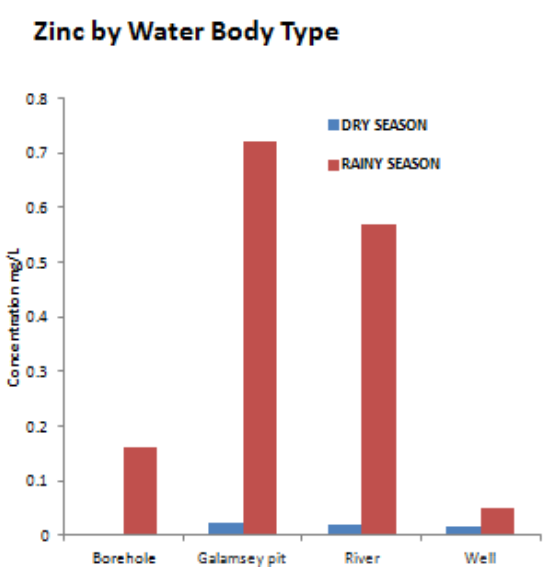
Zinc concentration values for all the water bodies for both the wet and dry seasons ranged from ND to 0.72 mg/L. Higher values were recorded in the wet season (galamsey pits) while lower values were recorded in the dry season (borehole) (Figure 4.4e), There were significant differences in zinc concentration for boreholes, galamsey pits, rivers and wells between the two seasons according to student T- test at 95% confidence level.

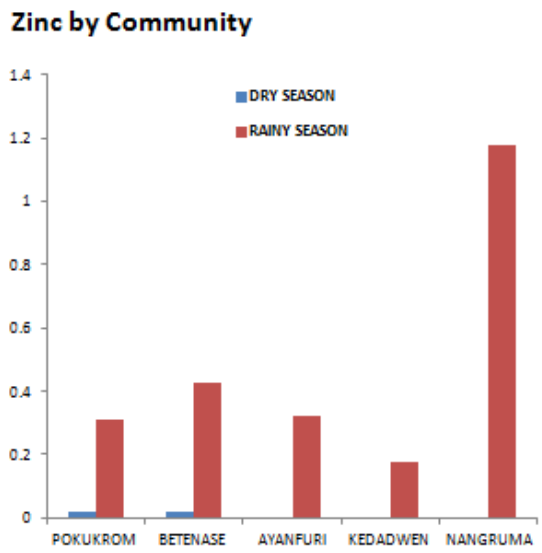


(4.4c) **(4.4d)**

Fig. 4.4 Plots of zinc in water during the wet season, c) water body type, and d) community. Red indicates an endemic community and black non-endemic.

Zinc concentrations when organized by the dry and wet seasons for the communities ranged from ND to 1.18 mg/L. It was highest in the wet season and lowest in the dry season (Figure 4.4f). The differences in zinc concentration for Pokukrom, Betenase, Ayanfuri, Kedadwen and Nangruma between the two seasons were significant according to student T-test.

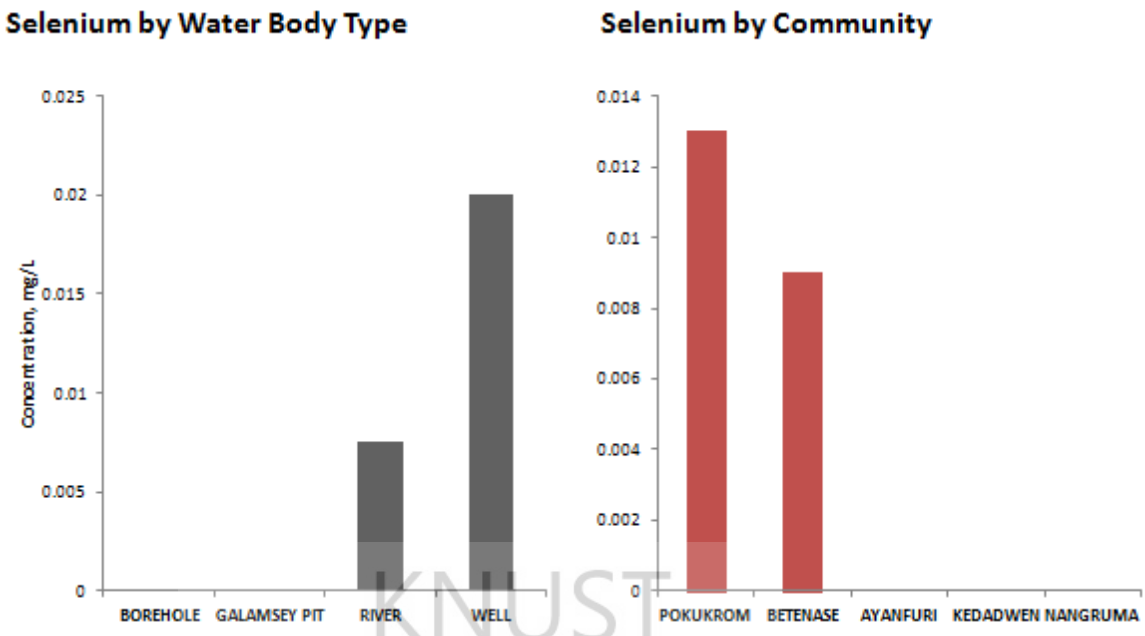




(4.4e) **(4.4f)**

Fig. 4.4Plots of zinc in water for both dry and wet seasons, e) water body type, and f) community. Blue indicates dry season and red wet season.

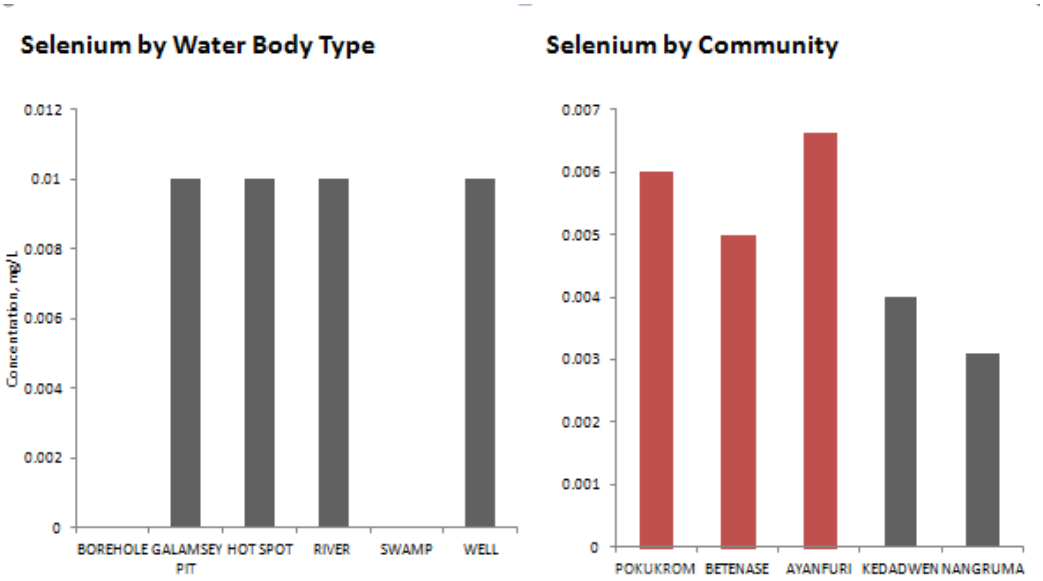
During the dry season, results of selenium concentrations were highest for well water samples followed by river samples, compared to the other water body samples. Boreholes and galamsey pits water samples did not contain detectable levels of selenium (Figure 4.5a). The differences were significant according to ANOVA. Water samples from Pokukrom, an endemic community had the highest concentrations of selenium (Figure 4.5b). Betenase (an endemic community), also had high selenium concentration. The difference for selenium concentration between the communities was significant according to ANOVA at 95% confidence level.



(4.5a) **(4.5b)**

Fig. 4.5 Plots of selenium in water during the dry season, a) water body type, and b) community. Red indicates an endemic community and black non-endemic community.

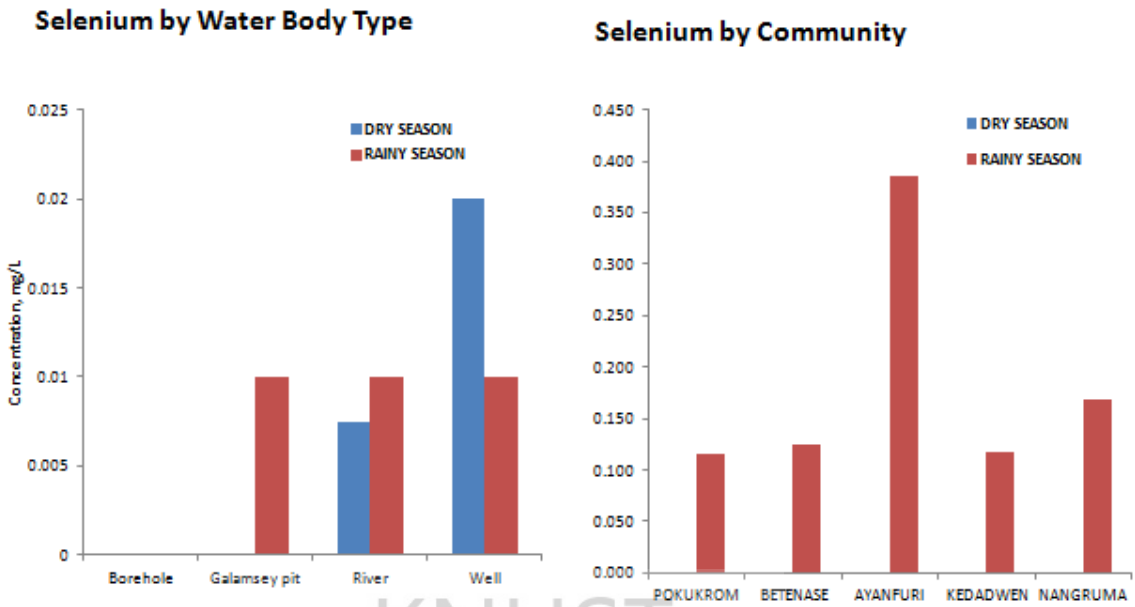
Selenium in the wet season samples recorded high concentrations in galamsey pits. Rivers, wells and BU hot spots also recorded high levels of selenium, but boreholes and swamps had no detectable levels of selenium (Figure 4.5c). There were no significant differences for selenium between the water bodies in both seasons, according to ANOVA. On the other hand, Ayanfuri recorded the highest concentrations of selenium (Figure 4.5d). Endemic communities Pokukrom, Betenase, and Kedadwen also recorded high selenium concentration; Nangruma (a non- endemic) community recorded the least. However there were no significant differences, between the communities according to A



(4.5c) **(4.5d)**

Fig. 4.5 Plots of selenium in water during the wet season, c) water body type, and d) community. Red indicates an endemic community.

Mean concentrations of selenium range from ND to 0.02 mg/L for all the water bodies in both seasons. Maximum values were recorded in the dry season for well samples; while minimum values were recorded in the wet season for borehole water samples (Figure 4.5e). When organized by community, the wet season had the highest mean selenium concentration (Figure 4.5f). Seasonal differences were observed between water bodies and between communities according to student T-test.



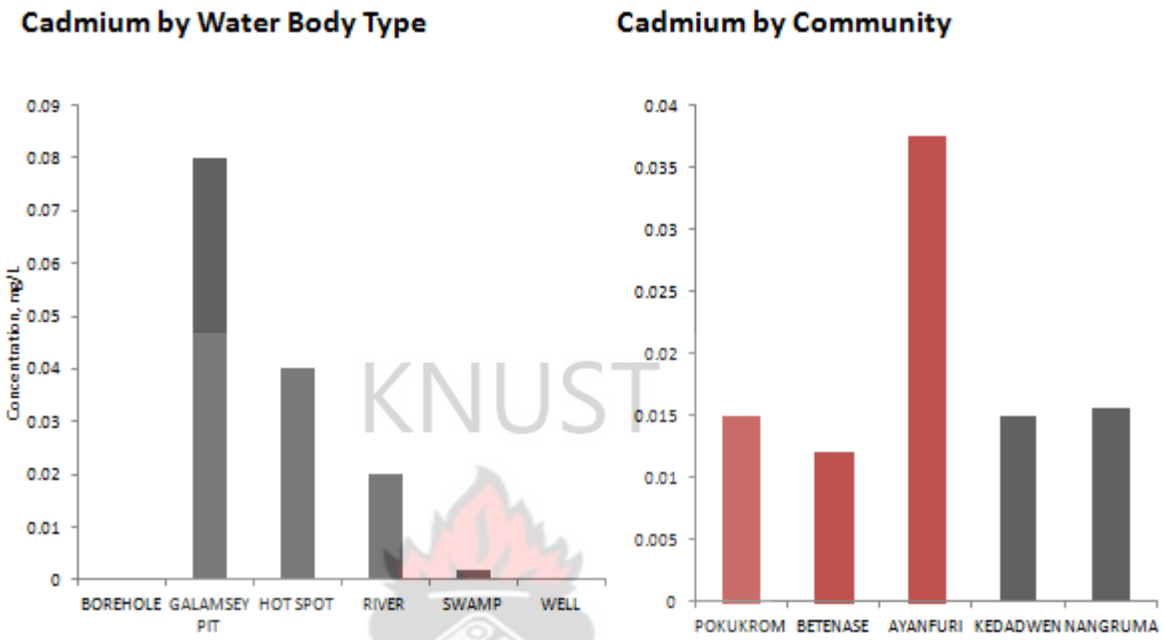
(4.5e) **(4.5f)**

Fig. 4.5 Plots of selenium in water for both dry and wet season, e) water body type, and f) community. Blue indicates dry season and red wet season.

In the case of cadmium, it is one of the most toxic elements with reported carcinogenic effects in humans (Goering *et al*., 1994). Dry season samples did not contain detectable levels of cadmium. Concentrations of cadmium in the wet season varied within the range of 0.00 to 0.08 mg/L. Concentrations were high in galamsey pits followed by BU hot spots (figure 4.6a). Thus these water bodies may pose higher risks for BU infection. Trend of cadmium concentration in the water bodies is in the order: Galamsey pit>BU hot spot > river = swamp > borehole = well, but the differences were not significant according to ANOVA.

Wet season cadmium results for wells and boreholes samples did not contain detectable levels. When cadmium was organized by community, the highest cadmium of 0.038mg/L was obtained for Ayanfuri samples; while the lowest cadmium of 0.012 mg/L was obtained for Betenase samples. Pokukrom, Kedadwen and Nangruma also showed high cadmium concentrations (Figure 4.6b). Trend was

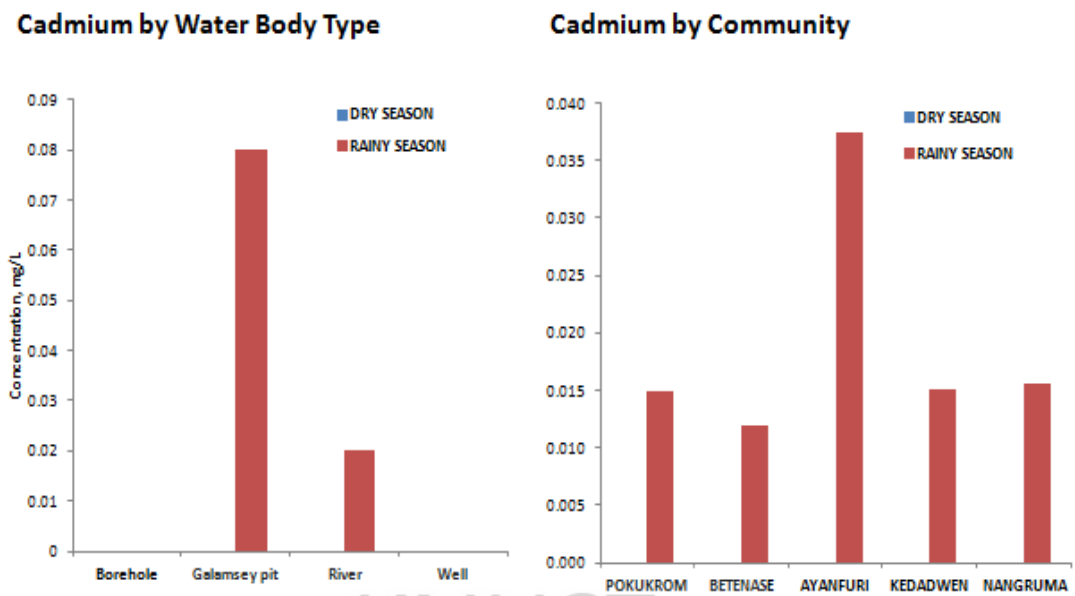
Ayanfuri > Nangruma > Kedadwen > Pokukrom > Betenase, but differences were not significant according to ANOVA.



(4.6a) **(4.6b)**

Fig. 4.6 Plots of cadmium in water during the wet season, a) water body type, and b) community. Red indicates an endemic community and non-endemic community.

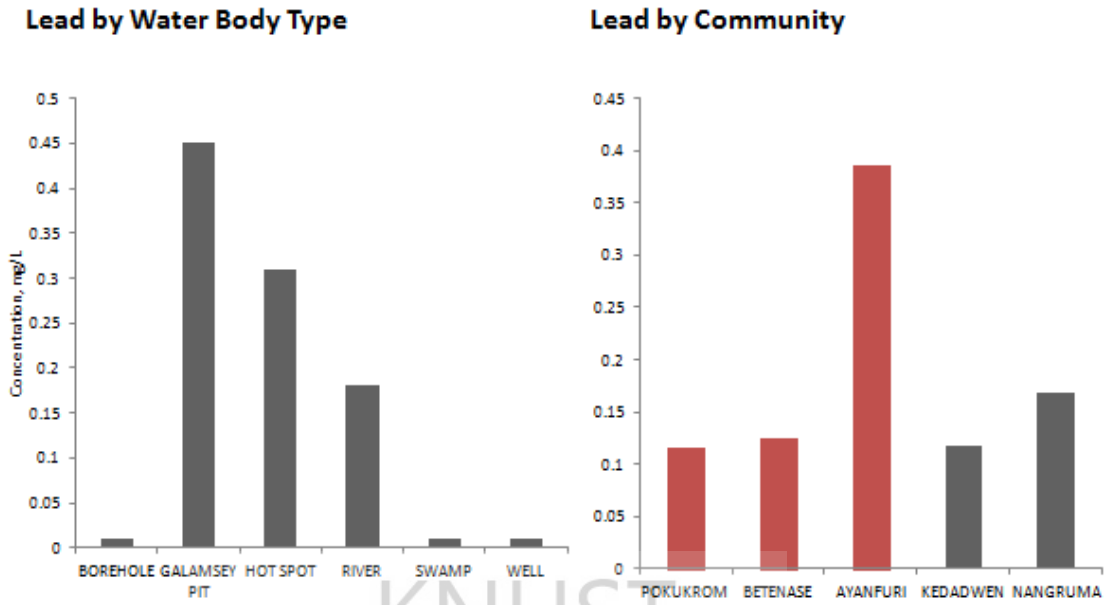
Cadmium mean values ranged from ND to 0.08 mg/L. Maximum values were recorded in the wet season (galamsey pits) (Figure 4.6c). The dry season had no detectable levels of cadmium. Boreholes and wells in the wet season also had no detectable levels of cadmium. Ayanfuri recorded the highest cadmium concentration in the wet season compared to the dry season. (Figure 4.6d). There were no detectable levels of cadmium in samples for the dry season from the study communities. The differences in cadmium concentration for both seasons were significant according to student T-test.



(4.6c) **(4.6d)**

Fig. 4.6 Plots of cadmium in water for both dry and wet season, c) water body type, and d) community, in the dry and wet seasons. Red indicates wet season.

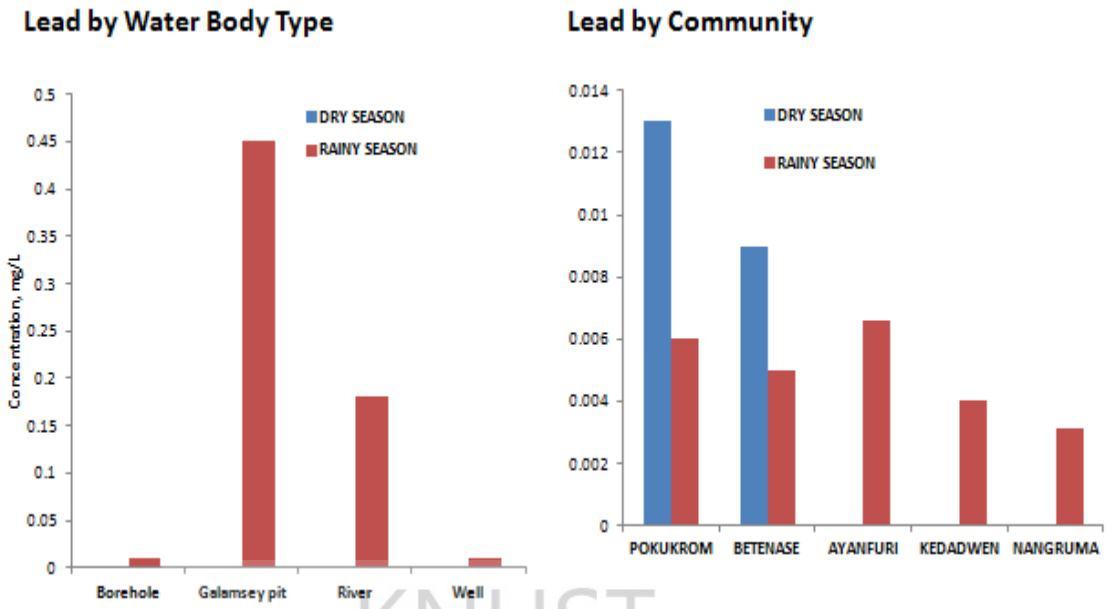
Dry season samples did not contain detectable levels of lead. Galamsey pits in the wet season recorded the highest values of lead followed by BU hot spots compared to the other water bodies in this season but the differences were not significant (Figure 4.7a). Lead concentrations were notably higher in Betenase followed by Nangruma compared to the other communities (Figure 4.7b), but the differences were not significant. Though lead, as a trace metal, may contribute to the favorable environment for *M. ulcerans* growth, there were no significant differences for lead concentrations between endemic and non-endemic communities according to ANOVA. Mean concentrations of lead for both seasons ranged from ND to 0.45 mg/L, for the water bodies.



(4.7a) **(4.7b)**

Fig. 4.7 Plots of lead in water during the wet season, a) water body type, and b) community. Red indicates an endemic community and black non-endemic community.

The wet season has consistently higher concentrations compared to the dry season, with a significant difference between them according to student T-test (figure 4.7c).For the communities (Figure 4.7d), the difference between them is also significant according to student T-test. High concentrations (0.013 mg/L) are seen in the dry season and particularly occurred at Pokukrom.

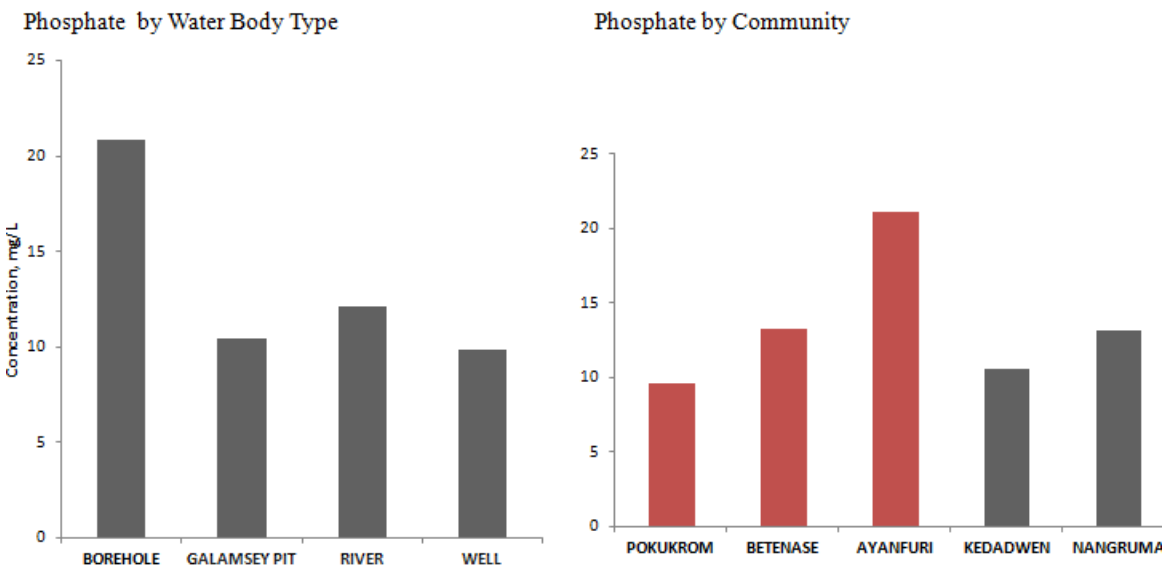


(4.7c) **(4.7d)**

Fig. 4.7 Plots of lead in water for both dry and wet season, c) water body type, and d) community. Blue indicates dry season and red wet season.

4.1.3 Nutrients

Phosphate levels in the dry season were observed to be highest in borehole water samples, followed by river samples. Phosphate concentrations in rivers and galamsey pits were also observed to be high (Figure 4.8a).The mean values for phosphate contents of the water samples from the communities varied within the range of 9.56 to 21.08 mg/L. Pokukrom samples showed the least phosphate concentrations among the five communities and Ayanfuri samples the highest (Figure 4.8b).

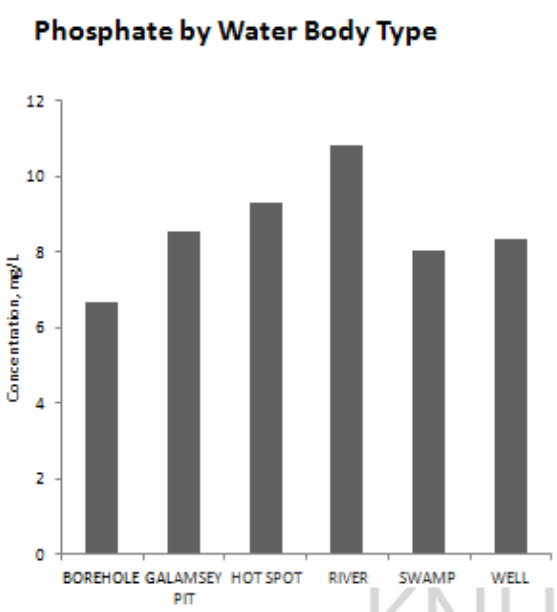


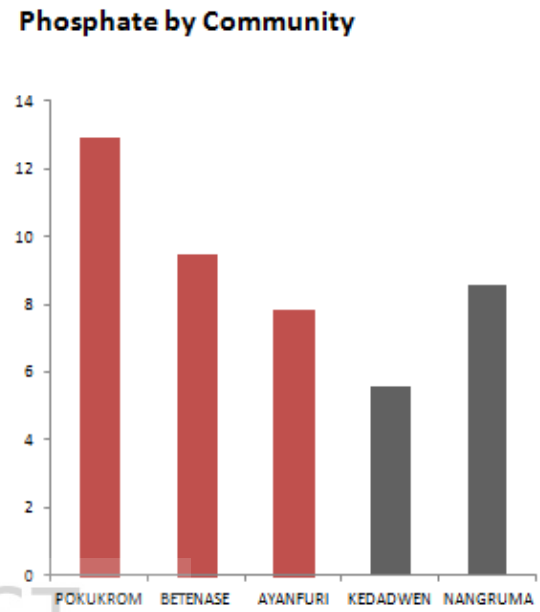
(4.8a) **(4.8b)**

Fig. 4.8 Plots of phosphate in water during the dry season, a) water body type, and b) community. Red indicates an endemic community and black non-endemic community.

Phosphate levels in the water samples were observed to be higher in both the endemic and non-endemic communities. The phosphate concentration of all the samples analyzed exceeded the WHO Limit of 2 mg/L for potable water. It is remarkable that phosphate exceeded the levels stipulated by WHO for water sources utilized for various purposes.

The wet season river samples recorded the highest phosphate levels. Boreholes recorded the lowest phosphate values (Figure 4.8c).



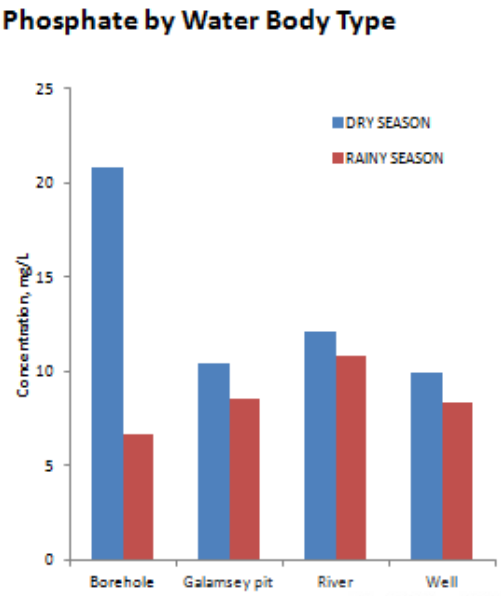


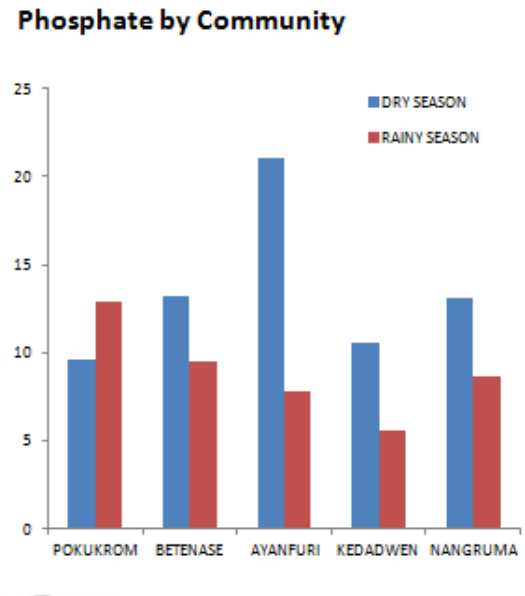
(4.8c) **(4.8d)**

Fig. 4.8 Plots of phosphate in water during the wet season, c) water body type, and d) community. Red indicates an endemic community and black non-endemic community.

High concentrations of phosphate were observed to occur in all the endemic communities (Figure 4.8d). Nangruma (non-endemic) community also recorded high phosphate concentration. This may be notable, as phosphate could contribute to the growth of *M. ulcerans* in the environment. Kedadwen recorded the lowest values.

Phosphate levels in the water bodies for the dry and wet seasons ranged from 6.67 to 20.84 mg/L. Higher values were recorded in the dry season, while lower values were recorded in the wet season (Figure 4.8e), but the differences were not significant according to student T-test. Borehole samples recorded the highest phosphate content in the dry season (20.84 mg/L), and the reverse was observed in the wet season, in that, borehole samples recorded the lowest phosphate concentration in the wet season (6.67 mg/L).





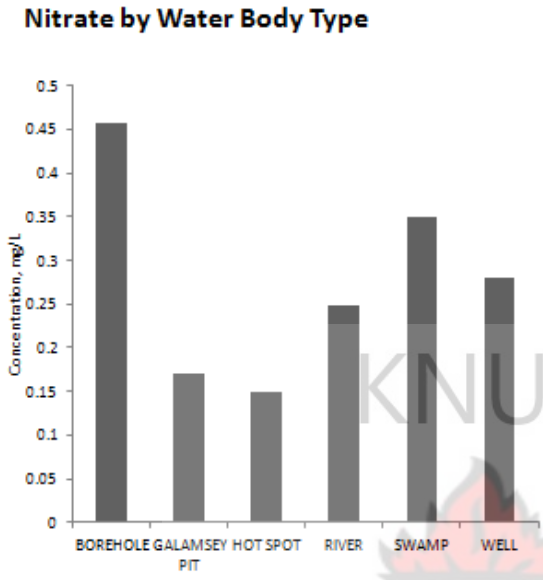
(4.8e) **(4.8f)**

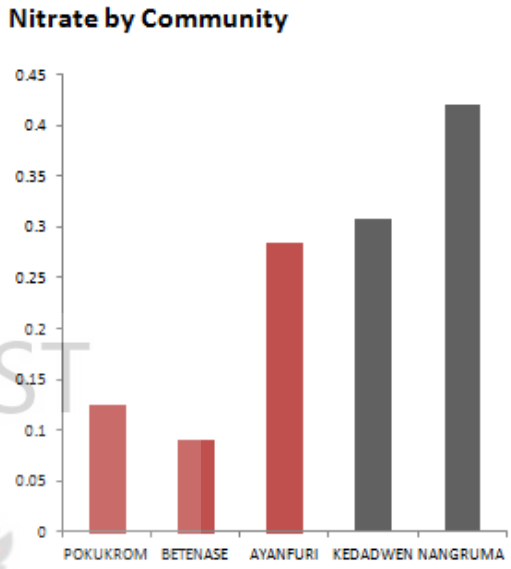
Fig. 4.8 Plots of phosphate in water for the dry and wet seasons, e) water body type, and f) community. Blue indicates dry season and red wet season.

When phosphate results was organized by community, phosphate was high for four communities out of the five communities in the dry season compared to the wet season (Figure 4.8f).

The magnitude of nitrate in the water bodies recorded in the wet season is in the order: Borehole (0.46 mg/L) >swamp (0.35 mg/L) > wells (0.28mg/L) > rivers (0.25 mg/L) > galamsey pits (0.17mg/L). From the results of the study mean concentrations of nitrate was observed to be highest in boreholes compared to the other water body types (Figure 4.9a).

From the results, it was also revealed that BU hot spots and swamps contained high nitrate levels (Figure 4.9a). Nangruma, a non-endemic community recorded the highest nitrate concentrations among the communities (Figure 4.9b).





(4.9a) **(4.9b)**

Fig. 4.9 Plots of nitrate in water during the wet season, a) water body type, and b) community. Red indicates an endemic community and black non-endemic community.

4.2 SOIL

In this study, soil samples were collected from five communities namely: Pokukrom (PK), Betenase (BT), Ayanfuri (AF), Kedadween (KD) and Nangruma (NG). Samples from Nangruma ("Overseas") and Kedadwen were control samples since the areas are free of BU cases. A total of fifty eight (58) surface soil samples (0 – 15 cm) were collected from agricultural cultivated fields (cassava, plantain farms, etc), cocoa farms, small scale mining sites otherwise known as "Galamsey" sites, logged areas and matured forest. The samples were collected within the period of June and

July 2011 which is the wet season. The samples were analyzed for some heavy metals namely arsenic, zinc, copper, cadmium, lead, iron and selenium.

4.2.1 Trace metals-soil

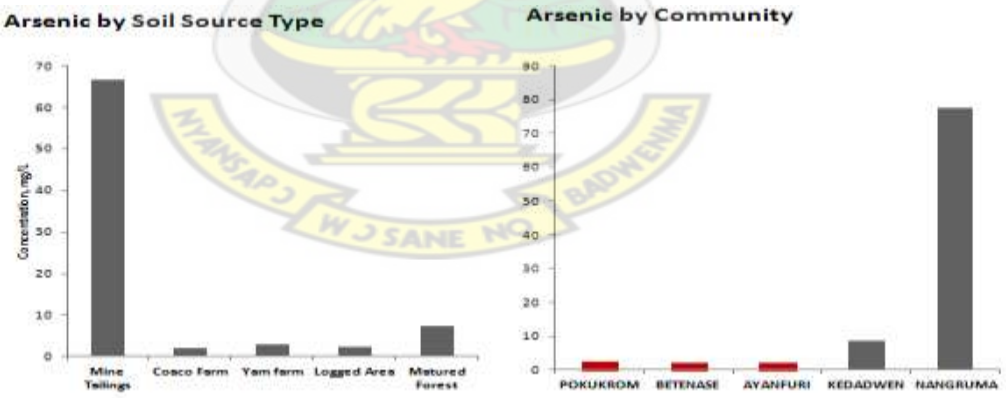
Heavy metals have been associated with *M. Ulcerans* infection. Mining process exposes heavy metals that were previously locked away in the earth. Heavy metal pollution is caused when such metals as arsenic, cobalt, copper, cadmium, lead, and zinc contained in excavated rock are exposed and come in contact with water. Soil also plays a significant role in the mode of transmission of various diseases since it is a big reservoir for bacteria, viruses and many more biological organisms and many other chemicals. These conditions that aid in the habitation of soil by these organisms may be due to the variations in certain physical and chemical properties of the soil. In the study, the average concentrations of the metals in the soils from different sources were in decreasing order: 5642.50 mg/kg for iron in mine tailings, 66.55 mg/kg for arsenic in mine tailings, 20.29 mg/kg for copper in logged area, 8.27 mg/kg for zinc in mine tailings, 3.15mg/kg for selenium in mine tailings, 2.95 mg/kg for lead in mine tailings, 1.75mg/kg for cadmium in mine tailings.

Similarly, when the average concentrations of the metals in the soil were organized based on community a decreasing order was obtained as 7377.00 mg/kg for iron at Kedadwen, 77.33 mg/g for arsenic at Nangruma, 24.00 mg/kg for copper at Nangruma, 11.66 mg/g for zinc at Nangruma, 3.15 mg/kg for selenium at Betenase, 2.45 mg/kg for lead at Pokukrom, 2.24 mg/g for cadmium at Pokukrom.

In this study arsenic and zinc were the most concentrated of the metals when organized by soil type and by communities. The two metals were both high in the different soil sources and community.

Overall results of soil samples showed much variation in the distributions of arsenic, zinc, copper, cadmium, lead, iron and selenium. Of all the soil types investigated for heavy metals concentrations, mine tailings and to a lesser extent matured forest and logged areas showed the highest metal distribution throughout the study. This could be because the major source of metals in the catchment is from mining activities. Because of elevated trace metal concentration, mine tailings, matured forest and logged areas may be risky locations for contracting BU.

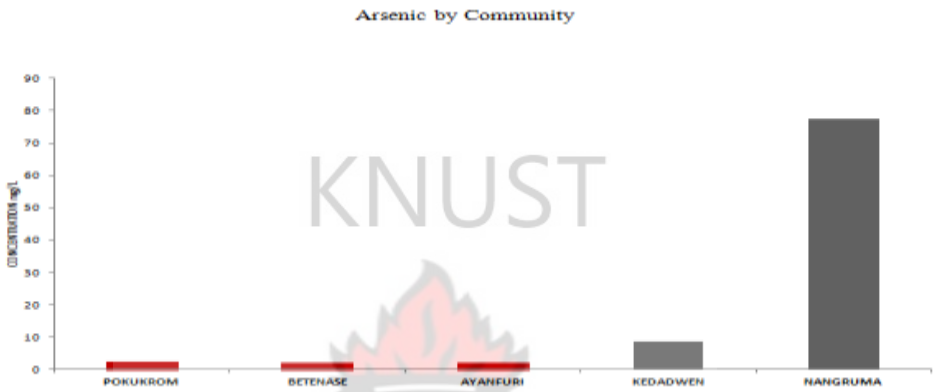
The mean arsenic values ranged from a minimum of 2.13 mg/kg in cocoa farm, to a maximum of 66.55mg/kg in mine tailings. Arsenic concentrations were found to be highest in mine tailings (Figure 4.2a). Arsenic contents in matured forest and yam farm are also high relative to other soil types, but the difference between the soil types was not significant according to ANOVA. The magnitude of arsenic contents in soil types were the order: Mine tailings > matured forest> yam farm> logged area> Cocoa farm.



(4.1a) **(4.1b)**

Fig. 4.1 Plots of arsenic in soil during the wet season, a) source of soil, and b) community. Red indicates an endemic community and black non-endemic community.

The two non-endemic communities (Nangruma and Kedadwen) in the study tend to record highest levels of arsenic content compared to endemic communities (Figure 4.2b). Differences between the communities for arsenic concentration was not significant according to ANOVA. Endemic communities, Pokukrom, Betenase, and Ayanfuri show some high arsenic concentrations, but not as high as seen in Nangruma a non-endemic community (Figure 4.2b).



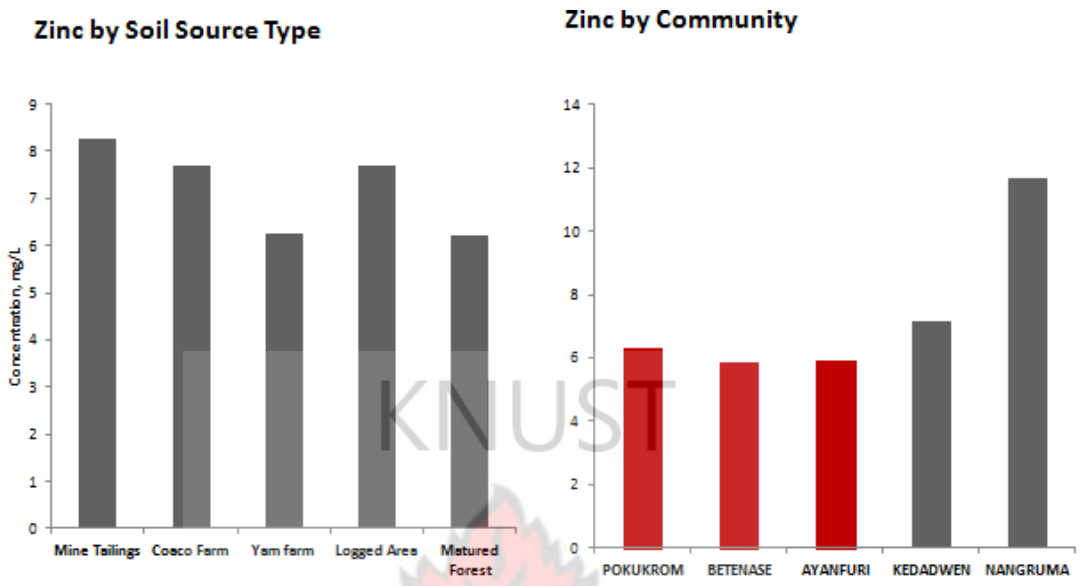
(4.1b)

Fig. 4.1 Plots of arsenic in soil during the wet season. Red indicates an endemic community and black non-endemic community.

Within Nangruma, arsenic concentrations are highest in mine tailings. This trend is likely caused by the differences in mining practices between the endemic and non-endemic communities. The magnitude of arsenic contents when organized by community is in the order Nangruma > Kedadwen> Pokukrom> Betenase > Ayanfuri. The highest arsenic concentration is recorded in the two non-endemic communities, Nangruma and Kedadwen.

The mean level of zinc in the different sources of soil ranged from 6.22mg/kg for matured forest samples to 8.27mg/kg for mine tailings samples. Zinc concentration show similar trends to arsenic, in that mine tailings and logged areas have consistant

higher concentration than other soil types (Figure 4.2a) with significant differences according to ANOVA.



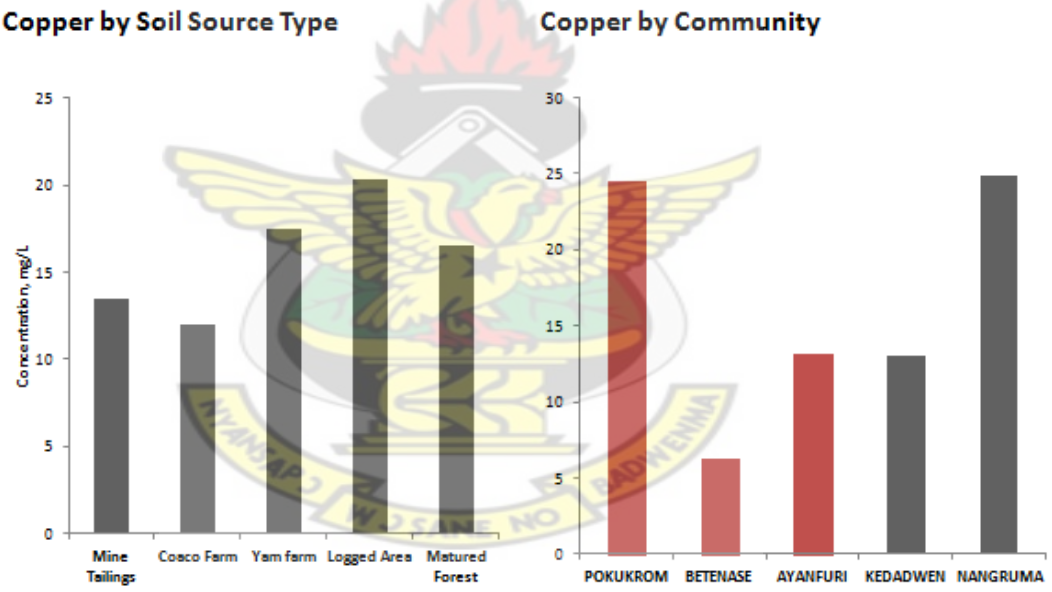
(4.2a) **(4.2b)**

Fig. 4.2 Plots of zinc in soil during the wet season, a) source of soil, and b) community. Red indicates an endemic community and black non-endemic community.

The mean level of zinc in the communities ranged from 5.85 mg/kg for Betenase samples to 11.66 mg/kg for Nangruma samples. Trends for zinc are similar to that of arsenic when organized by community. High concentration for both metals is recorded in Nangruma and Kedadwen (mean; 11.66 and 7.17mg/kg) respectively (Figure 4.2b) which are non-endemic communities. Concentrations are low in endemic communities. The difference in zinc concentrations between the communities is significant according to ANOVA. The lowest zinc concentration was obtained for yam farm soil samples; while the highest zinc was obtained for mine

tailings. When organized by community, Nangruma showed the highest concentration; while Betenase and Ayanfuri showered the lowest zinc contents.

Copper in the analyzed soil had mean levels of 6.25 and 24.85 mg/kg for Betenase and Nangruma, and higher concentrations are seen in Nangruma compared to the other communities but the difference was not significant according to ANOVA. Copper shows similar trends in concentration to zinc, in that both metals are highest in Nangruma when organized by community. Trends for copper and zinc are not very different when organized by soil type. Copper is highest in logged area and zinc is highest in mine tailings and also in logged area (Figure 4.3a).

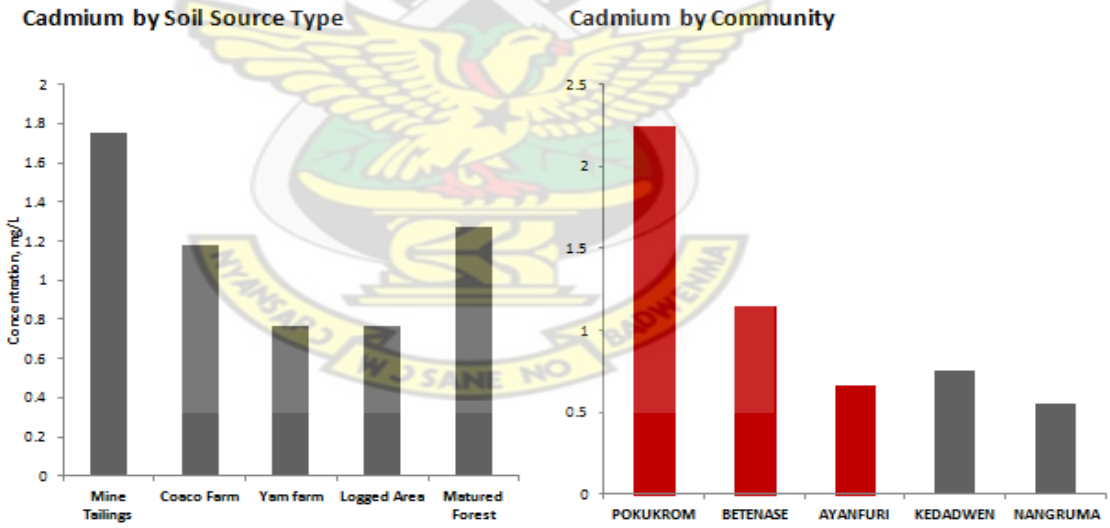


(4.3a) **(4.3b)**

Fig. 4.3 Plots of copper in soil during the wet season, a) source of soil, and b) community. Red indicates an endemic community and black non-endemic community.

The lowest copper concentration was obtained for cocoa farm soil samples; while the highest was obtained for logged area soil samples. The difference in copper between

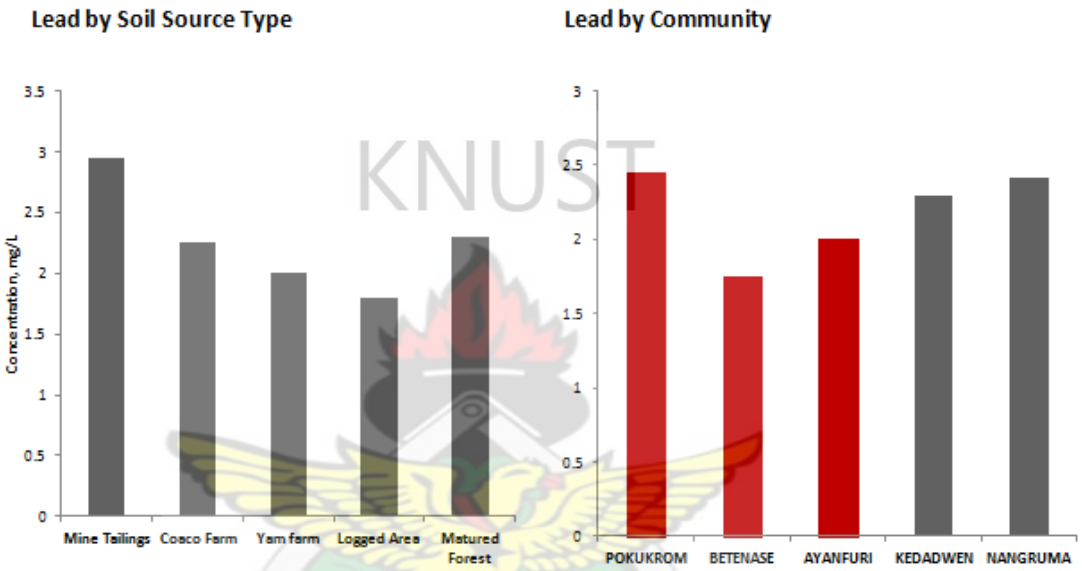
the soil samples was not significant according to ANOVA. When organized by community, Betenase had the lowest copper content; while Nangruma had the highest content (Figure 4.3b). Trend of copper in soil is in the order: 5058.00 mg/kg for matured forest, >3662.50 mg/kg for cocoa farm >3020.00 mg/kg for logged area. Cadmium and lead show similar trends when organized by soil type and community. Both metals are high in mine tailings when organized by soil type (Figure 4.4a) and high in Pokukrom when organized by community (Figure 4.4b). The concentration of cadmium in soil types samples were in the order Mine tailings > matured forest > cocoa farm > logged area > yam farm with no significant difference between them according to ANOVA. The concentration of cadmium based on communities was in the order: Pokukrom > Betenase > Kedadwen> Ayanfuri > Nangruma with significant differences between them according to ANOVA.



(4.4a) **(4.4b)**

Fig.4.4 Plots of cadmium in soil during the wet season, a) source of soil, and b) community. Red indicates an endemic community and black non-endemic community.

The level of lead in the soil samples were in the order: Mine tailings> matured forest> cocoa farm> yam farm> logged area (Figure 4.5a), with no difference between them according to ANOVA. The level of lead based on communities is in the order: Pokukrom>Nangruma> Kedadwen> Ayanfuri> Betenase (Figure 4.5b) with significant differences between them according to ANOVA.

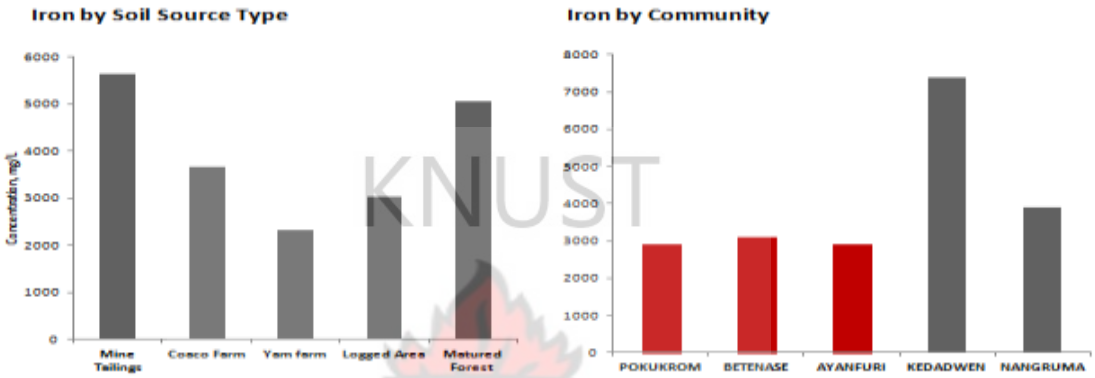


(4.5a) **(4.5b)**

Fig. 4.5Plots of lead in soil during the wet season, a) source of soil, and b) community. Red indicates an endemic community and black non-endemic community.

Iron had a mean range of 2317.60 -5642.50 mg/kg. The lowest iron level of 2317.60 mg/kg was obtained for yam farm soil samples; while the highest iron level of 5642.50 mg/kg was obtained for mine tailings samples (Figure 4.6a).No significant difference was observed when the levels in soil types were compared according to ANOVA. Iron exhibits much the same trend as cadmium and lead when organized by soil type but different when organized by community, in that, iron is highest in

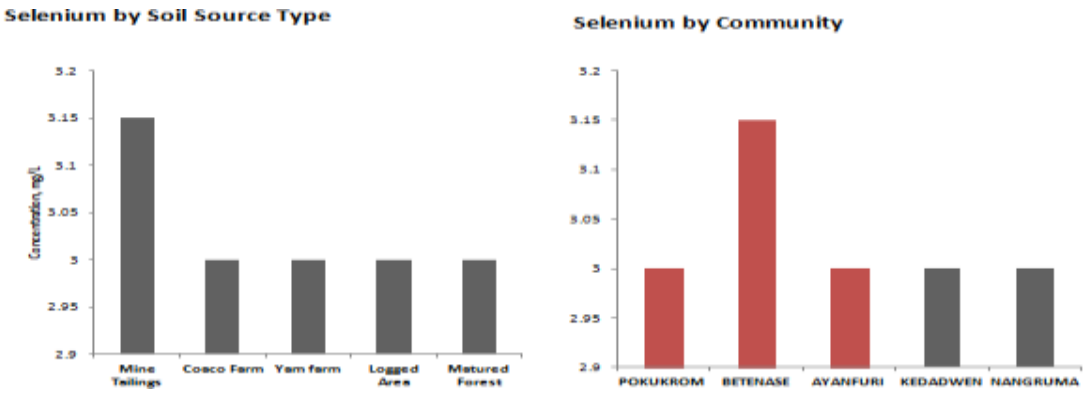
mine tailings and in samples from Kedadwen when organized by soil type and community respectively (Figure 4.6b). Trend of iron levels when organized by community is: 7377.00mg/kg for Kedadwen >3897.50mg/kg for Nangruma> 3106.50mg/kg for Betenase > 2915.00mg/kg for Ayanfuri > 2891.60mg/kg for Pokukrom (Figure 4.6b) but the differences were not significant according to ANOVA.



(4.6a) **(4.6b)**

Fig. 4.6 Plots of iron in soil during the wet season, a) source of soil, and b) community. Red indicates an endemic community and black non-endemic community.

The trend for iron is seen for selenium when organized by soil type, in that, selenium is highest in mine tailings (Figure 4.7a), but trends for iron and selenium are different when organized by community. Selenium contents in the rest of the soil type samples are similar if not the same as cocoa farm=logged area=matured forest=yam farm (Figure 4.7b), and as expected the difference was not significant according to ANOVA.



(4.7a) **(4.7b)**

Fig. 4.7 Plots of selenium in soil during the wet season, a) soil source type, and b) community. Red indicates an endemic community and black non-endemic community.

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

The analysis of results in the study have found no significant differences in pH values between water bodies or between communities. The lower pH values in southern Ghana suggest that if pH does affect the viability of *M. ulcerans*, then southern Ghana may be a favourable growing environment for *M. ulcerans* than Northern Ghana, because *M. ulcerans* is assumed to thrive in environments with low pH. Based on the water samples analyzed for trace metal concentrations, high levels of most of the trace metals were found in galamsey pits and BU hot spots compared to the other water bodies, though the differences were not significant. Differences in trace metals concentration between communities in endemic and non-endemic communities were not significant, though Nangruma, a non-endemic community in the Northern Region recorded the highest metal concentration throughout the study. The presence of arsenic within the environment could serve as a threat for BU incidence. Arsenic in water from galamsey pits and BU hot spots could support the growth of *M. ulcerans*, while arsenic in drinking water could suppress immune systems, making the population more susceptible to BU.

Concentrations of arsenic, cadmium, copper, iron, lead, and zinc are highest in galamsey pits and BU hot spots, and elevated in Betenase and Nangruma over other communities, which may be attributed to the geology or land use patterns. These trace metals may contribute to the favourable environment for *M. ulcerans* growth.

Although high levels of nitrate and phosphate have been linked to buruli ulcer diseases, there is no significant difference in nitrate concentrations between endemic and non-endemic communities in this study. However, phosphate concentrations are

higher in endemic communities than in non-endemic communities. Thus phosphate could be a contributory factor toward the growth of *M. ulcerans* in the environment.

The results obtained from this study indicate that the concentration of trace metals in water vary significantly not only by water body type, but is influenced in a remarkable degree by the seasons. By comparing the accumulation of trace metal in water seasonally, it can be concluded that trace metals are highly accumulated in the wet season compared to the dry season. Since elevated trace metal levels are associated with *M. Ulcerans* infection, the wet season has a relationship with BU incidence.

Results of trace metals levels in the different soil type samples revealed that mine tailings contained the highest metal concentration throughout the study. It was also revealed that Nangruma among the rest of the communities recorded the highest levels of almost all the metals analyzed in the soil samples.

5.2. RECOMMENDATION

The results of this study are a step toward determining the relationship between water quality, soil chemistry and the incidence of BU, however, more work is necessary; samples were collected in only five communities, and sampling sites within those communities were limited to a subset of the total water bodies in each community. Weekly or monthly measurements of water chemistry would provide invaluable information about the temporal variability of water bodies in these study communities; however, frequent sampling at these sites can be difficult due to limited infrastructure. Measurements of water chemistry in the wake of extreme rainfall could also be useful; extreme rainfall and associated flooding may increase the number and size of water bodies that could harbour *M. ulcerans*. Samples should also be collected for *M. ulcerans* isolation in the same location as water and soil samples were collected; this is difficult because of the storage requirements for microbiological samples and the remoteness of the study communities. Despite the limited scope of this study due to the difficulties of sampling, this work is an important step toward identifying the environmental niche of *M. ulcerans.*

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APPENDIX

Physicochemical parameters and levels of some trace metals in water samples during the dry season

SAMPLE ID	COMMUNITY	TYPE OF SOURCE	pH	DO (mg/L)	COND. (µS/cm)	Turb
A1-GP	POKUKROM	GALAMSEY PIT	6.80	3.16	124.90	50.60
A2-BH	POKUKROM	WELL	5.60	1.80	137.20	0.20
A3-W	POKUKROM	WELL	4.56	3.44	324.00	0.90
A4-WB	POKUKROM	STREAM	5.92	2.40	103.60	46.18
A5-WB	POKUKROM	STREAM	5.87	2.36	101.30	41.61
A6-ST	POKUKROM	RIVER	6.47	2.78	135.00	56.00
A7-BH	POKUKROM	STREAM	5.93	2.80	261.60	16.32
A8-WB	POKUKROM	STREAM	6.37	2.47	40.30	100.00
A9-ST	POKUKROM	STREAM	5.67	1.66	52.50	17.43
BT2-BH	BETENASI	BOREHOLE	5.71	1.51	61.60	ND
BT3-ST	BETENASI	STREAM	6.13	0.96	28.60	26.26
BT4-ST	BETENASI	STREAM	4.90	1.25	31.60	83.00
SB2-BH	BETENASI	BOREHOLE	5.91	2.2	136.1	33.45
KD1-R	KEDADWEN	RIVER	7.30	4.54	91.10	15.24
KD3-SW	KEDADWEN	SWAMP	6.18	2.12	80.80	21.72
KD4-BH	KEDADWEN	BOREHOLE	5.63	0.66	300.00	14.81
KD5-SW	KEDADWEN	SWAMP	6.12	0.32	97.70	19.59
KD6-BH	KEDADWEN	BOREHOLE	5.47	1.12	186.10	30.34
KD 7-GP	KEDADWEN	GALAMSEY PIT	6.20	2.31	228.90	32.40
NG 1-GP	NANGRUMA	GALAMSEY PIT	7.52	5.61	93.80	45.71
NG 2-SW	NANGRUMA	SWAMP	7.66	4.32	105.70	32.56
NG 3-BH	NANGRUMA	BOREHOLE	6.72	2.12	1332.00	12.61
AF 2-W	AYENFURI	WELL	5.74	0.74	108.50	33.10
AF 3-W	AYENFURI	WELL	5.38	3.90	179.00	21.50
AF 4-W	AYENFURI	WELL	5.58	0.37	80.60	4.10
AF 5-W	AYENFURI	WELL	5.86	2.05	198.30	24.29
AF1-GP	AYENFURI	GALAMSEY PIT	6.26	0.12	98.4	12.31

Continuation

SO_4^{2-} (mg/L)	SO_2^{-} (mg/L)	PO_4^{3-} (mg/L)	F- (mg/L)	NH_3 (mg/L)	Alk (mg/L)	Cl- (mg/L)	T. hard (mg/L)
120.00	0.10	5.60	ND	0.26	40.00	2.66	70.00
3.00	0.06	4.10	0.40	ND	32.50	2.84	85.00
3.00	0.06	ND	0.35	ND	10.00	2.84	55.00
25.00	0.06	2.10	ND	0.01	32.50	2.49	56.50
19.00	0.06	15.00	ND	ND	40.00	1.78	59.50
20.00	0.06	19.00	0.10	0.04	50.00	1.60	60.00
20.00	0.07	12.00	0.60	ND	52.50	2.84	150.00
87.00	0.12	18.00	ND	ND	27.50	1.78	112.00
ND	0.05	0.70	ND	ND	25.00	1.78	105.00
ND	0.60	0.70	0.20	ND	20.00	2.13	65.00
13.00	0.07	18.00	0.20	ND	30.00	2.31	61.50
ND	0.06	ND	0.60	ND	10.00	1.95	80.00
13.00	0.11	21.00	0.40	0.21	80.00	10.10	46.96
24.00	0.05	12.00	ND	0.03	90.00	1.80	43.92
50.00	0.08	11.00	0.60	0.01	47.00	1.42	173.40
16.00	0.06	7.50	0.20	0.01	65.00	6.03	283.52
14.00	0.03	7.50	0.55	0.06	47.00	2.13	136.32
24.00	0.01	14.00	0.60	0.05	47.00	4.97	85.40
40.00	0.02	8.70	0.55	0.01	13.00	2.84	172.60
14.00	0.01	21.00	0.40	0.01	60.00	2.84	36.92
7.00	0.07	4.10	1.00	ND	21.00	2.13	47.40
48.00	0.05	5.20	0.35	0.28	223.00	9.23	541.08
24.00	0.10	16.00	0.60	0.01	90.00	14.90	171.16
19.00	0.10	18.00	0.10	0.06	85.00	27.70	30.36
20.00	0.14	44.00	0.05	0.06	168.00	15.10	27.12
14.00	0.13	21.00	0.10	0.01	90.00	20.20	26.56
52	0.07	6.4	1.1	0.08	125.00	5.30	90.76

Continuation

Ca (mg/L)	Mn (mg/L)	As (mg/L)	Se (mg/L)	Zn (mg/L)	Fe (mg/L)	Cd (mg/L)	Pb (mg/L)	Cu (mg/L)
0.540	0.024	0.004	ND	0.024	1.783	ND	ND	0.024
0.616	0.021	0.001	ND	0.017	0.539	ND	ND	0.036
0.365	0.021	ND	0.020	0.016	0.040	ND	ND	0.018
0.400	0.006	0.011	ND	0.022	2.679	ND	ND	0.021
0.300	0.004	0.008	ND	0.030	2.909	ND	ND	0.092
0.450	0.004	0.010	ND	0.016	2.248	ND	ND	0.021
1.150	0.011	0.001	ND	0.024	0.993	ND	ND	0.027
0.750	0.018	0.005	0.006	0.016	3.039	ND	ND	0.028
0.650	0.003	0.003	ND	0.024	2.017	ND	ND	0.020
0.550	0.030	ND	ND	0.013	0.044	ND	ND	0.052
0.450	0.006	0.002	0.009	0.027	2.423	ND	ND	0.023
0.650	0.011	ND	ND	0.014	ND	ND	ND	0.004
0.280	0.007	0.102	ND	ND	0.021	ND	ND	0.014
0.380	0.008	0.011	ND	ND	22.633	ND	ND	0.092
1.850	0.030	0.005	ND	ND	1.052	ND	ND	0.009
2.780	0.016	0.023	ND	ND	17.026	ND	ND	0.196
1.480	0.009	0.072	ND	ND	20.553	ND	ND	0.040
0.850	0.016	0.034	ND	ND	0.460	ND	ND	0.028
1.400	0.019	ND	ND	ND	ND	ND	ND	ND
0.130	0.016	0.013	ND	ND	22.630	ND	ND	0.074
0.350	0.006	0.013	ND	ND	0.011	ND	ND	0.006
1.870	0.011	0.176	ND	ND	0.349	ND	ND	0.014
1.740	0.015	0.006	ND	ND	2.121	ND	ND	0.010
0.040	0.012	0.034	ND	ND	5.216	ND	ND	0.017
0.180	0.030	0.013	ND	ND	2.711	ND	ND	0.009
0.090	0.022	0.219	ND	ND	0.611	ND	ND	0.027
0.89	0.00	0.03	ND	ND	24.36	ND	ND	0.296

Physicochemical parameters and levels of some trace metals in water samples during the wet season

SAMPLE ID	WATER BODY	COMMUINTY	pH	DO (mg/L)	COND. (uS/cm)	TURB. (NTU)
P-BW-1	Well	Pokukrom	5.41	0	206.7	<8
P-BW-2	Well	Pokukrom	5.64	0	284.1	<8
P-BW-3	Well	Pokukrom	4.35	0	81.5	<8
P-BW-4	Well	Pokukrom	5.16	0	155.7	<8
P-BW-5	Well	Pokukrom	4.21	0	146	<8
P-RS-1	River	Pokukrom	6.16	0	92.8	100
P-RS-2	River	Pokukrom	6.52	0	252.2	90
P-GP-1	Galamsey	Pokukrom	6.82	0.23	129.9	>240
P-GP-2	Galamsey	Pokukrom	6.28	0.01	117.8	>240
P-GP-3	Galamsey	Pokukrom	7.01	0	145.5	150
P-GP-4	Galamsey	Pokukrom	6.91	0.27	120.3	90
P-BU-1	HotSpot	Pokukrom	6.79	0	231.3	>240
P-SW-1	Swamp	Pokukrom	6.41	0.01	192.9	30
P-SW-2	Swamp	Pokukrom	5.58	0	95.2	>240
B-BW-1	Well	Betenase	3.89	0	34.4	9
B-BW-2	Well	Betenase	4.38	0	64.6	<8
B-RS-1	River	Betenase	5.24	0	90.9	14
B-RS-2	River	Betenase	5.41	0	89.4	<8
B-GP-1	Galamsey	Betenase	5.43	0	65.9	>240
B-GP-2	Galamsey	Betenase	5.15	0	75.9	>240
B-GP-3	Galamsey	Betenase	5.87	0	56.6	>240
B-GP-4	Galamsey	Betenase	5.31	0	67.8	>240
B-BU-1	HotSpot	Betenase	6.21	0.01	323	>240
B-BU-2	HotSpot	Betenase	6.34	0.02	425	>240
B-SW-1	Swamp	Betenase	5.74	0	36.7	24
B-SW-2	Swamp	Betenase	5.6	0	119.6	>240
K-BW-1	Well	Kedadwen	5.86	0	201.4	<8
K-BW-2	Well	Kedadwen	4.27	0	50.6	<8
K-BW-3	Well	Kedadwen	4.02	0	218.7	30
K-BW-4	Well	Kedadwen	3.57	0	648	<8
K-BW-5	Well	Kedadwen	5.05	0	324	<8
K-BW-6	Well	Kedadwen	4.84	0	232.5	<8
K-RS-1	River	Kedadwen	7.63	0.2	86.6	<8
K-RS-2	River	Kedadwen	6.21	0.89	72.1	<8
K-RS-3	River	Kedadwen	5.81	3.8	88.6	90
K-GP-1	Galamsey	Kedadwen	6.43	0	44.2	>240
K-GP-2	Galamsey	Kedadwen	6.64	0	328	>240

Continuation

K-GP-3	Galamsey	Kedadwen	6.47	0	408	>240
K-GP-4	Galamsey	Kedadwen	4.91	0	221.2	>240
K-SW-1	Swamp	Kedadwen	6.04	0.04	386	<8
K-SW-2	Swamp	Kedadwen	5.63	0	159.1	30
A-BW-1	Borehole	Ayanfuri	6.15	3.59	278.8	<8
A-BW-2	Borehole	Ayanfuri	5.84	0.47	281.6	<8
A-BW-3	Borehole	Ayanfuri	6.02	1.96	280	<8
A-BW-4	Borehole	Ayanfuri	5.86	1.02	281.5	<8
A-BW-5	Borehole	Ayanfuri	5.75	1.73	282	<8
A-BW-6	Borehole	Ayanfuri	6.03	0.78	280.7	<8
A-RS-1	River	Ayanfuri	6.41	0.4	250.5	15
A-RS-2	River	Ayanfuri	5.86	0.52	48.9	14
A-RS-3	River	Ayanfuri	6.11	1.41	61.9	35
A-RS-4	River	Ayanfuri	5.99	0.06	115.9	<8
A-GP-1	Galamsey	Ayanfuri	4.32	0.95	51.4	>240
A-GP-2	Galamsey	Ayanfuri	5.53	0.01	72.8	>240
A-GP-3	Galamsey	Ayanfuri	6.02	1.46	0	>240
A-GP-4	Galamsey	Ayanfuri	6.11	0.15	44.5	>240
A-SW-1	Swamp	Ayanfuri	6.65	0.6	39.7	17
A-SW-2	Swamp	Ayanfuri	6.46	0.11	196.6	21
N-BW-1	Borehole	Nangruma	0	0	0	<8
N-RS-1	River	Nangruma	0	0	0	>240
N-RS-2	River	Nangruma	0	0	0	>240
N-RS-3	River	Nangruma	6.27	0	0	<8
N-RS-4	River	Nangruma	0	0	0	>240
N-GP-1	Galamsey	Nangruma	7.7	0	140.4	>240
N-GP-2	Galamsey	Nangruma	0	0	0	>240
N-GP-3	Galamsey	Nangruma	0	0	0	>240
N-GP-4	Galamsey	Nangruma	7.4	0	135.2	>240
N-SW-2	Swamp	Nangruma	0	0	0	65

Physicochemical parameters and levels of some trace metals in water samples during the wet season

Cl - (mg/L)	TOT. HARD. (mg/L)	PO_4^{3-}(mg/L)	NH_3 (mg/L)	F- (mg/L)	Mn (mg/L)
3.39	70.89	9.77	0.049	1.44	0.006
4.81	99.29	6.27	0	0	0.005
3.39	70.89	3.97	0	0.04	0.011
4.1	85.09	20.87	0.229	0.89	0.017
1.97	42.49	3.97	0.009	1.24	0.004
2.68	56.69	1.67	0	0.09	0.006
2.68	56.69	2.77	0	0.24	0.016
1.97	42.49	7.37	0	0.29	0.003
2.68	56.69	10.87	0	0	0.004
2.68	56.69	2.77	0	1.24	0.005
1.26	28.29	3.77	0	0	0.015
4.81	99.29	11.87	0.049	0	0.011
1.97	42.49	8.57	0	0	0.005
1.26	28.29	15.87	0.079	1.39	0.006
1.26	28.29	9.77	0	1.49	0.006
1.97	42.49	3.97	0	1.29	0.006
11.2	227.09	6.27	0	0	0.002
1.97	42.49	18.87	0.139	1.34	0.008
2.68	56.69	6.27	0.009	1.29	0.003
105.63	2115.69	9.77	0	0	0.005
2.68	56.69	25.87	0.119	0.02	0.003
1.97	42.49	3.97	0	0	0.004
1.26	28.29	0.57	0	0	0.005
1.26	28.29	2.77	0	0	0
1.26	28.29	6.27	0	0	0.003
1.97	42.49	3.97	0	0	0.003

Physicochemical parameters and levels of some trace metals in water samples during the wet season

ALK. (mg/L)	Cl - (mg/L)	TOT. HARD. (mg/L)	PO_4^{3-} (mg/L)	NH_3 (mg/L)	F- (mg/L)	Mn (mg/L)
40	2.68	56.69	6.27	0	0	0.003
60	6.23	127.69	19.87	0.339	0.99	0.006
70	4.1	85.09	14.87	0.139	1.29	0.017
160	2.68	56.69	18.87	0.199	1.39	0.017
110	4.1	85.09	5.07	0.179	0	0.008
300	2.68	56.69	22.87	0.469	1.09	0.005
60	11.2	227.09	11.87	0.309	1.19	0.023
240	1.97	42.49	11.87	0.209	0	0.006
130	3.39	70.89	7.37	0.059	0	0.001
200	2.68	56.69	14.87	0.069	0	0.005
350	2.68	56.69	11.87	0.069	0	0.004
250	9.78	198.69	13.87	0.349	1.29	0.022
150	2.68	56.69	17.87	0.139	1.29	0.007
60	6.23	127.69	1.27	0	0	0.003
10	1.97	42.49	11.87	0.059	0.04	0.008
20	1.97	42.49	6.27	0	0	0.003
50	1.97	42.49	14.87	0.039	0.49	0.005
60	1.97	42.49	2.77	0.029	0.19	0.001
220	4.1	85.09	15.87	0.489	1.19	0.007
90	4.1	85.09	2.77	0	0	0
250	8.36	170.29	9.77	0.029	0	0.039
400	4.1	85.09	15.87	0.259	0	0.004
190	4.81	99.29	8.57	0.019	0.19	0.012
280	58.06	1164.29	5.07	0.109	0.04	0.006
35	3.39	70.89	9.77	0.209	0.09	0.005
50	3.39	70.89	8.57	0.029	0.39	0.003
110	1.26	28.29	0.57	0	0.24	0
20	1.97	42.49	2.77	0.009	0.09	0.005
50	6.23	127.69	1.67	0	0	0.008
10	9.07	184.49	1.67	0.009	0.19	0.015
60	5.52	113.49	2.77	0	0.04	0.001
40	4.81	99.29	13.87	0.179	1.09	0.022
60	1.26	28.29	0.57	0.009	0.04	0.004
40	2.68	56.69	2.77	0	-0.01	0.002
40	1.26	28.29	14.87	0.009	0.04	0.004
150	1.26	28.29	6.27	0.009	1.44	0.005
250	2.68	56.69	0.57	0	-0.01	0.005
150	2.68	56.69	10.87	0.249	-0.01	0.007
20	1.97	42.49	6.27	0.009	0.24	0.001
160	1.26	28.29	6.27	0.009	1.14	0.005

Continuation

SO_4^{2-} (mg/L)	SO_2^{-} (mg/L)	As (mg/L)	Se (mg/L)	Zn (mg/L)	Fe (mg/L)	Cd (mg/L)	Pb (mg/L)	Cu (mg/L)
7.83	0.009	0.003	0.001	0.04	0.097	0.001	0.009	0.016
9.83	0.009	0.017	0.001	0.04	23.997	0.012	0.009	0.016
13.83	0.009	0.003	0.001	0.04	0.197	0.001	0.009	0.016
15.83	0.009	0.019	0.001	0.04	0.097	0.001	0.009	0.016
17.83	0.009	0.005	0.001	0.07	0.297	0.001	0.009	0.016
15.83	0.009	0.025	0.001	0.04	7.997	0.001	0.009	0.016
13.83	0.009	0.079	0.028	0.04	21.497	0.001	0.009	0.016
15.83	0.009	0.459	0.008	0.24	179.997	0.014	0.069	0.366
11.83	0.009	0.739	0.002	1.11	509.997	0.039	0.629	0.736
16.83	0.009	0.015	0.001	0.04	6.197	0.001	0.009	0.016
13.83	0.009	0.005	0.001	0.04	6.797	0.001	0.009	0.016
17.83	0.009	2.999	0.014	2.9	1639.997	0.119	0.809	0.856
12.83	0.009	0.011	0.001	0.04	4.997	0.001	0.009	0.016
20.83	0.009	0.043	0.001	0.04	12.497	0.001	0.009	0.016
16.83	0.009	0.001	0.001	0.04	0.097	0.001	0.009	0.016
8.83	0.009	0.001	0.001	0.04	0.097	0.001	0.009	0.016
9.83	0.009	0.005	0.001	0.04	4.997	0.001	0.009	0.016
12.83	0.009	0.003	0.001	0.04	25.997	0.001	0.009	0.016
20.83	0.009	0.259	0.008	0.79	343.997	0.023	0.239	0.436
15.83	0.009	0.159	0.004	0.78	331.997	0.022	0.229	0.406
16.83	0.009	0.339	0.008	1.37	727.997	0.051	0.539	0.936
17.83	0.009	0.259	0.014	0.66	363.997	0.025	0.319	0.446
14.83	0.009	0.031	0.002	0.41	38.297	0.001	0.039	0.046
9.83	0.009	0.045	0.001	0.7	73.897	0.004	0.069	0.076
15.83	0.009	0.003	0.001	0.04	4.597	0.001	0.009	0.016
6.83	0.019	0.001	0.001	0.04	10.297	0.001	0.009	0.016
2.83	0.009	0.001	0.001	0.04	0.097	0.001	0.009	0.016
7.83	0.009	0.009	0.001	0.04	0.197	0.001	0.009	0.016
8.83	0.009	0.001	0.001	0.04	0.597	0.001	0.009	0.016
19.83	0.009	0.003	0.001	0.04	0.097	0.001	0.009	0.016
7.83	0.009	0.005	0.001	0.06	0.097	0.001	0.009	0.016
9.83	0.009	0.025	0.001	0.04	0.097	0.001	0.009	0.016
2.83	0.009	0.005	0.001	0.04	1.797	0.001	0.009	0.016
12.83	0.009	0.001	0.001	0.04	23.997	0.001	0.009	0.016
17.83	0.009	0.007	0.001	0.04	6.297	0.001	0.009	0.016
55.83	0.009	0.005	0.001	0.04	6.897	0.001	0.009	0.016

45.83	0.009	0.199	0.004	1.42	1109.997	0.076	0.319	1.636
18.83	0.009	0.199	0.01	0.23	1279.997	0.099	0.389	1.696
7.83	0.009	0.053	0.002	0.32	355.997	0.023	0.939	0.536
13.83	0.009	0.009	0.001	0.04	16.997	0.001	0.009	0.016
17.83	0.009	0.003	0.001	0.04	10.097	0.001	0.009	0.016
8.83	0.009	0.005	0.001	0.17	0.197	0.001	0.009	0.016
8.83	0.009	0.003	0.001	0.14	0.097	0.001	0.009	0.016
11.83	0.009	0.001	0.001	0.14	0.197	0.001	0.009	0.016
11.83	0.009	0.005	0.001	0.21	0.597	0.001	0.009	0.036
8.83	0.009	0.001	0.001	0.15	1.597	0.001	0.009	0.016
16.83	0.009	0.001	0.001	0.23	0.197	0.001	0.009	0.026
12.83	0.009	0.011	0.001	0.04	3.897	0.001	0.009	0.016
8.83	0.009	29.999	0.018	1.56	2409.997	0.199	2.479	2.006
12.83	0.009	0.007	0.001	0.04	3.697	0.001	0.009	0.016
8.83	0.009	0.021	0.001	0.04	24.997	0.001	0.009	0.016
18.83	0.009	113.999	0.022	0.51	2219.997	0.179	1.099	0.496
12.83	0.009	29.999	0.018	1.56	2409.997	0.199	2.479	2.006
15.83	0.009	0.359	0.001	0.04	14.197	0.001	0.009	0.016
11.83	0.009	0.179	0.001	0.04	8.797	0.001	0.009	0.016
15.83	0.009	0.029	0.001	0.04	2.297	0.001	0.009	0.016
12.83	0.009	0.017	0.004	0.04	4.497	0.001	0.009	0.016
9.83	0.009	0.001	0.001	0.04	0.697	0.001	0.009	0.016
16.83	0.009	3.399	0.001	0.64	213.997	0.014	0.129	0.216
9.83	0.009	0.119	0.001	0.04	11.997	0.001	0.009	0.016
13.83	0.009	0.015	0.001	0.04	0.897	0.001	0.009	0.016
13.83	0.009	19.599	0.001	5.81	78.297	0.007	0.089	0.096
13.83	0.009	339.999	0.002	1.85	569.997	0.039	0.919	0.786
4.83	0.009	37.999	0.001	1.99	639.997	0.042	0.359	0.676
8.83	0.009	193.999	0.001	1.08	481.997	0.033	0.109	0.536
9.83	0.009	23.999	0.001	0.13	129.997	0.007	0.029	0.096
11.83	0.009	0.419	0.001	0.04	3.997	0.001	0.009	0.016

Trace metal content of soil samples

Sample ID	Type of Source	Community	CADNIUM	COPPER	IRON	LEAD	ZINC	ARSENIC	SELENIUM
P-MF-1	Mature Forest	Pokukrom	0.80	14.00	3800.00	2.0	5.10	3.00	<3
P-MF-2	Mature Forest	Pokukrom	6.80	37.00	5800.00	3.0	9.80	<2	<3
P-LA-1	Logged Area	Pokukrom	0.50	8.10	5300.00	2.0	4.30	<2	<3
P-LA-2	Logged Area	Pokukrom	4.30	15.00	6300.00	3.0	7.10	4.00	<3
P-CO-1	Cocoa	Pokukrom	0.50	30.00	4300.00	2.0	5.70	<2	<3
P-CO-2	Cocoa	Pokukrom	0.30	12.00	2500.00	2.0	5.00	<2	<3
P-YF-1	Yam Field	Pokukrom	2.30	44.00	4900.00	2.0	7.60	<2	<3
P-YF-2	Yam Field	Pokukrom	0.80	64.00	4400.00	4.0	11.00	3.00	<3
P-MT-1	Mine Tailings	Pokukrom	0.80	5.90	1000.00	3.0	5.10	<2	<3
P-MT-2	Mine Tailings	Pokukrom	0.40	7.70	260.00	2.0	3.30	<2	<3
P-MT-3	Mine Tailings	Pokukrom	<0.3	12.00	200.00	3.0	2.90	<2	<3
P-MT-4	Mine Tailings	Pokukrom	<0.3	16.00	340.00	<1	3.90	<2	<3
B-MF-1	Mature Forest	Betenase	0.40	6.70	4800.00	3.0	4.80	<2	<3
B-MF-2	Mature Forest	Betenase	0.80	7.20	1900.00	1.0	3.10	<2	<3
B-LA-1	Logged Area	Betenase	<0.3	5.90	1600.00	1.0	3.60	<2	<3
B-LA-2	Logged Area	Betenase	0.40	7.30	2500.00	2.0	4.80	<2	<3
B-CO-1	Cocoa	Betenase	0.60	5.50	2600.00	2.0	4.70	<2	<3
B-CO-2	Cocoa	Betenase	5.50	3.40	1900.00	2.0	17.00	<2	<3
B-YF-1	Yam Field	Betenase	<0.3	3.60	3100.00	1.0	3.40	<2	<3
B-YF-2	Yam Field	Betenase	<0.3	6.70	990.00	1.0	3.80	<2	<3
B-MT-1	Mine Tailings	Betenase	0.60	2.80	2800.00	2.0	3.90	2.00	<3

Continuation

B-MT-2	Mine Tailings	Betenase	0.40	5.10	3100.00	2.0	4.30	<2	6.00
B-MT-3	Mine Tailings	Betenase	<0.3	17.0	650.00	2.0	7.20	<2	<3
B-MT-4	Mine Tailings	Betenase	4.50	7.60	1800.00	3.0	6.90	<2	<3
K-MF-1	Mature Forest	Kedadwen	1.30	16.00	19000.00	3.0	8.60	<2	<3
K-MF-2	Mature Forest	Kedadwen	0.50	3.20	2700.00	2.0	3.50	<2	<3
K-LA-1	Logged Area	Kedadwen	<0.3	4.10	2600.00	1.0	3.60	<2	<3
K-LA-2	Logged Area	Kedadwen	<0.3	8.40	1300.00	<1	3.60	<2	<3
K-CO-1	Cocoa	Kedadwen	0.30	12.00	2100.00	4.0	6.80	<2	<3
K-CO-2	Cocoa	Kedadwen	0.60	18.00	9700.00	3.0	11.00	<2	<3
K-YF-1	Yam Field	Kedadwen	0.70	6.60	3300.00	2.0	5.30	<2	<3
K-YF-2	Yam Field	Kedadwen	<0.3	9.40	670.00	<1	2.60	10.00	<3
K-MT-1	Mine Tailings	Kedadwen	0.80	15.00	1100.00	3.0	6.30	17.00	<3
K-MT-2	Mine Tailings	Kedadwen	2.30	34.00	24000.00	5.0	15.00	<2	<3
K-MT-3	Mine Tailings	Kedadwen	2.50	43.00	37000.00	10.0	23.00	<2	<3
K-MT-4	Mine Tailings	Kedadwen	0.80	13.00	2700.00	2.0	9.10	<2	<3
A-MF-1	Mature Forest	Ayanfuri	0.80	16.00	1000.00	3.0	6.20	18.00	<3
A-MF-2	Mature Forest	Ayanfuri	<0.3	43.00	2100.00	3.0	4.20	38.00	<3
A-LA-1	Logged Area	Ayanfuri	<0.3	6.90	4000.00	2.0	4.00	<2	<3
A-LA-2	Logged Area	Ayanfuri	0.70	8.80	3200.00	1.0	4.80	<2	<3
A-CO-1	Cocoa	Ayanfuri	0.50	8.10	4100.00	2.0	3.80	3.00	<3
A-CO-2	Cocoa	Ayanfuri	1.60	7.20	2100.00	1.0	7.50	<2	<3
A-YF-1	Yam Field	Ayanfuri	1.10	6.80	2800.00	2.0	8.00	2.00	<3
A-YF-2	Yam Field	Ayanfuri	1.20	18.00	8000.00	3.0	11.00	480.00	<3
A-MT-1	Mine Tailings	Ayanfuri	0.40	10.00	3900.00	2.0	6.50	33.00	<3

A-MT-2	Mine Tailings	Ayanfuri	<0.3	18.00	1400.00	1.0	3.20	12.00	<3
A-MT-3	Mine Tailings	Ayanfuri	<0.3	11.00	2000.00	1.0	5.30	12.00	<3
A-MT-4	Mine Tailings	Ayanfuri	<0.3	7.90	2600.00	2.0	4.50	21.00	<3
N-MF-1	Mature Forest	Nangruma	0.60	17.00	8500.00	1.0	9.70	<2	<3
N-MF-2	Mature Forest	Nangruma	0.40	5.30	980.00	2.0	5.10	<2	<3
N-LA-1	Logged Area	Nangruma	<0.3	130.00	2100.00	4.0	37.00	2.00	<3
N-LA-2	Logged Area	Nangruma	<0.3	8.40	1300.00	1.0	4.30	<2	<3
N-YF-1	Yam Field	Nangruma	<0.3	9.70	1400.00	3.0	5.10	<2	<3
N-YF-2	Yam Field	Nangruma	0.40	6.30	2900.00	1.0	4.60	<2	<3
N-MT-1	Mine Tailings	Nangruma	0.60	8.00	3700.00	3.0	5.70	<2	<3
N-MT-2	Mine Tailings	Nangruma	1.10	11.00	9400.00	4.0	8.20	110.00	<3
N-MT-3	Mine Tailings	Nangruma	0.90	18.00	13000.00	6.0	34.00	1100.00	<3
N-MT-4	Mine Tailings	Nangruma	1.60	7.20	1900.00	2.0	7.00	<2	<3