SPATIAL ANALYSIS OF FACTORS IMPLICATED IN *MYCOBACTERIUM ULCERANS* INFECTION IN GHANA

ALFRED ALLAN DUKER

Promotors:

Prof. Dr. Ir. A. Stein, Professor of Spatial Statistics, ITC and Wageningen University
Prof. Dr. M. Hale, Professor of Economic Geology, ITC and Utrecht University

Co-promotor:

Dr. John Carranza, Assistant Professor, Department of Earth Systems Analysis, ITC, Enschede

Examining committee:

Prof. Dr. Ir. A. Veldkamp (Wageningen Universiteit)

Prof. Dr. N. Nagelkerke (University of United Arab Emirates)

Prof. Dr. Ohene-Adjei (Kwame Nkrumah University of Science and Technology, Kumasi, Ghana)

Prof. Dr. G. Jacks (Royal Institute of Technology, Stockholm, Sweden)

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Contents

Dedicated to the memory of my late father and mother

Abstract

Buruli ulcer (BU), the common terminology for the disease caused by *Mycobacterium ulcerans* (MU) infection manifests as disfiguring skin ulceration which is difficult to treat. In its advanced stage the disease does not respond to drugs and requires surgery, often limb amputation. It sometimes results in death. It is most widespread in West Africa.

In Ghana, the first BU case was reported in 1971 and between 1991 and 1997 more than 2000 case were reported. Approximately 6000 cases were recorded in a national survey in 1999. Of the 110 districts in Ghana, at least 90 of them were found to have BU cases of which Amansie West District (the study area) had the highest rate with a prevalence of 150.8 per 100,000. The district is made up of about 310 settlements with a total population of about 108,726. High incidence of BU occurs in settlements in close proximity to the Offin River.

Infection is acquired through MU in the natural environment. Knowledge gaps about the exact mode of transmission and factors that pre-dispose to infection motivate this study. It employs a spatial approach to the relation between BU and postulated risk factors in part of the Amansie West District of Ghana.

Arsenic is implicated in several types of skin diseases including skin cancers. This may be due to its immunosuppressive affects, which enhance susceptibility to infection. This study takes as risks factors different pathways of potential exposure to enhanced levels of arsenic in the environment.

Arsenic concentrations in stream sediment were used to infer relative arsenic levels in surface waters and floodplain soils. The incidence of BU per settlement was then related to the arsenic levels of these domains by proximity analysis. The results showed that mean BU prevalence in settlements along arsenic-enriched drainages and within arsenic-enriched farmlands is greater than elsewhere. However, mean BU prevalence is greater along arsenic-enriched drainages (0.7%) than within arsenic-enriched farmlands (0.6%).

Arsenic concentrations in surface (stream) waters and ground (wells) waters were determined for abstraction points used by inhabitants of settlements of the study area. These arsenic data and BU prevalence per settlement were employed

in exposure-response modelling using linear regression. The results showed that BU prevalence has a significant positive relationship with arsenic levels in surface water (R^2 = 0.82, $p < 0.05$) but not with arsenic levels in groundwater (R^2 = 0.10, p = 0.60).

Arsenic liberation into the environment by oxidation of arsenopyrite during artisanal gold mining renders minesites a postulated risk factor for BU. Cases increased since the promulgation in 1989 of the Small Scale Mining Law, which legalized and increased such mining. The results of proximity analysis showed that most settlements relatively near to artisanal minesites exhibited disproportionately higher BU incidence (R^2 = 0.48, $p < 0.01$) than elsewhere in the study area (R^2 = 0.00, p = 0.90).

An integrated spatial-statistical model showed that settlement elevation, inferred arsenic floodplain soils, and distance to minesites were significantly correlated with BU. Surface water has a positive association with disease but not at statistically significant level. The results suggest that chronic ingestion of elevated concentrations of arsenic in drinking water and of arsenic taken up by foodcrops produced in arsenic-enriched soils adversely impact human health, perhaps by disruption of immune mechanisms, compromising defence against opportunistic infections such as MU.

Samenvatting

Buruli ulcer (BU) is de naam voor een ziekte die veroorzaakt wordt door een infectie met *Mycobacterium ulcerans* (MU). Deze ziekte wordt zichtbaar als een vergroeiing van de huid in de vorm van zweren. De ziekte is moeilijk te behandelen. De ziekte in een gevorderd stadium is immuun voor medicijnen en kan alleen via een chirurgische ingreep worden behandeld, vaak enkel via amputatie van ledematen. Soms is de dood het gevolg. Buruli Ulcer komt in West Afrika veel voor.

Nadat het eerste geval van BU in Ghana werd gerapporteerd in 1971, is het aantal gevallen tussen 1991 en 1997 gestegen boven de 2000. Ongeveer 6000 gevallen zijn geregistreerd gedurende een landelijke inventarisatie in 1999. Tenminste 90 van de 110 districten in Ghana kenden gevallen van BU, waarvan het Amansie West District (het studiegebied van deze thesis) het hoogste ziektecijfer kende: 150.8 per 100,000 inwoners. Het district bestaat uit ongeveer 310 bewoningsplaatsen, met een totale populatie van ongeveer 108,726. Een verhoogd BU ziektecijfer is geconstateerd in bewoningsplaatsen dicht bij de rivier de Offin.

Deze studie is geïnspireerd op het bestaan van duidelijk gaten in de kennis met betrekking tot de wijze van overdracht en de factoren die de gevoeligheid voor de ziekte verstreken. Een ruimtelijke benadering is gekozen voor het modelleren van de relatie tussen BU en vermoedelijke risico factoren in een gedeelte van het Amansie West district in Ghana.

Arsenicum is een bekende agens voor verschillende soorten huidziektes, waarbij ook verschillende huid kankers horen. Dit kan veroorzaakt worden door zijn immunosuppressieve eigenschappen, die de gevoeligheid voor de ziekte verhogen. Deze studie beschouwt verschillende oorzakelijke paden van mogelijke blootstelling aan verhoogde arsenicum waarden in het milieu.

Concentraties van arsenicum in het sediment van de Offin rivier zijn gebruikt om de arsenicum waarden in het oppervlaktewater en de bodems van de rivierbedding af te leiden. BU ziektecijfers in de bewoningsplaatsen zijn vervolgens gerelateerd aan arsenicum waarden zoals die in de nabije omgeving zijn vastgesteld, door middel van een nabijheidanalyse. De studie laat zien dat

het gemiddelde ziektecijfer van BU hoger is in bewoningsplaatsen bij afwateringskanalen die hoge arsenicum gehaltes kennen of die te midden van landbouwgronden liggen die verrijkt zijn met arsenicum. Ook blijkt het arsenicum gehalte hoger te zijn langs de kanalen (0.7 %) dan temidden van de verrijkte bouwlanden (0.6 %).

Concentraties van arsenicum in (stromend) oppervlaktewater en in stilstaand water (waterputten) zijn bepaald op die plaatsen waar de bewoners van de bewoningsplaatsen hun drink- en gebruikswater verzamelen. Deze arsenicum waarden zijn gerelateerd aan het BU ziektecijfer in een blootstelling–effect model, waarbij gebruik gemaakt is van lineaire regressie. De resultaten laten zien dat het BU ziektecijfer een significante positieve relatie heeft met de concentratie aan arsenicum in oppervlakte water ($R^2 = 0.82$, $p < 0.05$) maar niet met arsenicum concentraties in het grondwater ($R^2 = 0.10$, $p = 0.60$).

Het vrijkomen van arsenicum in het milieu via oxidatie van arsenopyrite tijdens het kleinschalig mijnen van goud vormt een tweede potentiële risicofactor voor het ontstaan van BU. Het aantal gevallen van BU is toegenomen sinds de Wet op het Kleinschalig Mijnen van kracht is geworden in 1989, die deze vorm van mijnbouw heeft gestimuleerd. De resultaten van de nabijheidanalyse laten zien dat de meeste bewoningsplaatsen die zich in de nabijheid van zo'n mijn bevinden een duidelijk hogere BU ziektecijfer kennen ($R^2 = 0.48$, $p < 0.01$) dan elders in het studiegebied ($R^2 = 0.00$, $p = 0.90$).

Een geïntegreerd ruimtelijk statistisch model laat tenslotte zien dat de hoogte (m) van de bewoningsplaatsen, het arsenicum in de bodems van de rivierbeddingen en de afstand tot een mijn alle een significante relatie vertonen het BU ziektecijfer. Arsenicum concentraties in het oppervlakte water hebben een positieve, maar statistisch niet-significante relatie met het ziektecijfer. De resultaten laten zien dat langdurige inname van drinkwater met een verhoogde arsenicum concentratie of van groenten en gewassen, die in gebieden met een verhoogde arsenicum concentratie groeien, een negatief effect hebben op de gezondheid van de mens. Wellicht gebeurt dit via een verstoring van het immuniteit mechanisme, waarbij de weerstand tegen MU infecties afneemt.

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I am greatly indebted to Prof. Dr. Martin Hale who in the first place guided me to obtain funding for my studies from the Ghana Government. His cooperation, motivation and direction have brought me to the completion of this thesis. His personal relationship gave me courage and excellent opportunity to pursue to the end, and was always eager to have me in his office for the discussion of any problem.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Problem definition

Buruli ulcer (BU) is a skin disease caused by *Mycobacterium ulcerans* (MU). The bacteria often destroys skin, underlying tissues, muscles and bones and when untreated leaves the victim disfigured (Figure 1.1). It can also lead to permanent disabilities such as restricted movement of affected limbs.

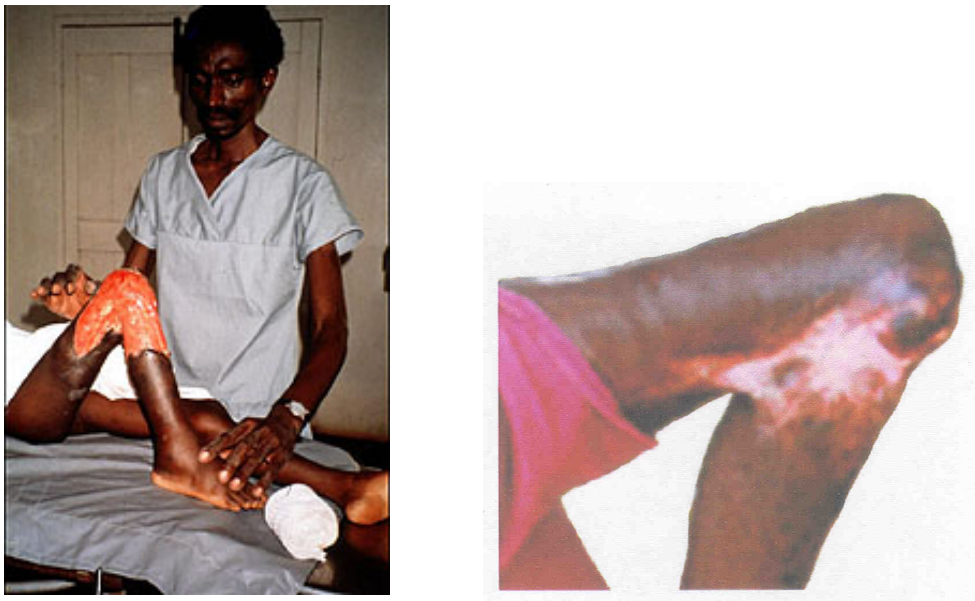

Figure. 1.1. BU: (left) an ulcerative lesion (WHO Fact Sheet 2000); (right) postulcerative sequelae: contracture deformity of knee joint (photo by Kanga and Kacou, 2001).

Outbreaks of BU have in many cases been attributed to environmental disturbances such as flooding, deforestation, and construction of dams or damming of rivers. Because BU is associated with riverine and swampy areas (Portaels, 1989; Johnson et al., 1999) several attempts to find its reservoir have been made. Cultures of water, soil, fish, rodents, biting flies and reptiles from BU-endemic areas failed to yield MU (Barker, 1972; Portaels, 1989; Roberts and Hirst, 1997), though testing of water samples by polymerase chain reaction (PCR) found MU DNA (Ross et al., 1997; Roberts and Hirst, 1997). Aquatic insects have also been linked with BU outbreaks (Portaels et al., 1999;

Marsoliers et al., 2002) and very recently fish has been found to be a potential reservoir for MU (Eddyani et al., 2004). However, the mode of BU transmission remains unclear.

The disease is predominantly tropical, (although a few cases have occurred in temperate areas). It has been reported in at least 27 countries (Hayman and Asiedu, 2000) (Figure 1.2). In some African countries and in particular rural areas the number of infections per settlement can be daunting. In such areas, high infection rates coupled with the lack of knowledge about the mode of transmission of the disease have made it a threat to public health.

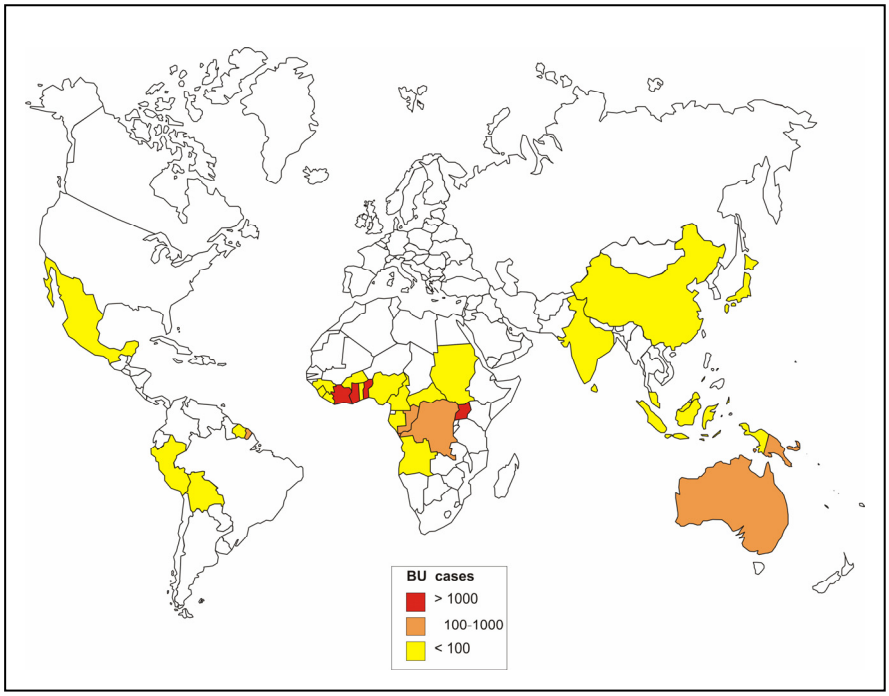

Figure 1.2. Map of the world showing countries where Buruli ulcer is present. Modified from Hayman and Asiedu (2000).

Patients, unfortunately, seek medical attention at the late stages of the disease when drugs are virtually ineffective. Surgery (to remove the lesion) then

remains the only alternative treatment. But surgery, which sometimes includes a skin graft, may involve amputation and consequently lengthy hospitalization, (sometimes as long as 18 months), life-long disability and economic liability to society.

In Ghana where the average income is less than one dollar per day the cost of BU treatment has been estimated to be as much as $783 per patient (Asiedu and Etuaful, 2000). Despite this expensive treatment, it is known that the disease resurfaces in 30% of surgery cases (WHO, 2002). Although early medical attention reduces the cost of treatment it is both medically and economically desirable to search for preventive measures by finding an answer to the transmission dilemma.

1.2 Main research questions

BU is associated with riverine and poorly-drained areas. Other factors associated with exposure to these environments could contribute to the risk of infection. Provided these are nearby sources, riverine and poorly-drained environments can be locations of arsenic enrichments; the resulting increased arsenic levels can affect human health.

The main route of arsenic exposure is via arsenic ingestion (i.e., through water and food). The clearance, however, of arsenic is rapid except for keratin-rich tissues such as skin, hair, and nails. The high affinity of arsenic for sulfhydryl groups makes keratin-rich cells (e.g., epidermal keratinocytes) a sensitive target for arsenic-induced toxicity, leading to dermal lesions including skin cancer, which could be on any part of the body just as BU. It is therefore hypothesized that there may be a link between arsenic and BU. For the purpose of this research this hypothesis is set out as follows:

- is there a causative or contributory link between arsenic in the environment and BU infection, and

- can this link be recognized by analysis of spatial data in a suitable study area?

1.3 Aims and objectives

The research is aimed at contributing to the understanding of the epidemiology of BU with the following objectives:

(1) identify, from published literature, the possible contributory role of arsenic in the environment with regards to the transmission of BU; and

(2) explore the spatial relationship between arsenic in the environment and BU incidence.

1.4 Selection of study area

In Ghana, the first case of BU was reported in 1971 and, between 1991 and 1997, more than 2000 cases have been reported (Grosset et al., 2000). Approximately 6000 cases were recorded in a national survey in 1999. BU is the second most widespread mycobacterium infection in the country with an overall prevalence of 22.7 per 100,000. Cases have been identified in all ten regions of the country and in 90 out of 110 districts (Amofah et al., 2002). The Ashanti Region (which accounts for 10.2% of the land area and 19.1% of the population of Ghana) is the worst affected, accounting for 60% of all reported cases, with the Amansie West District (of the Ashanti Region) having the highest prevalence of 150.8 per 100,000 (Amofah et al., 2002). In the Amansie West District high incidence of BU occurs in communities in close proximity to the Offin River. Reports also show that 44% of BU patients are farmers (Amofah et al., 1993). This district is therefore placed in a suitable setting for studying the relation between BU and environmental factors that may potentially contribute to infection.

The Amansie West District (Figure 1.2) lies between latitudes 6°00'N and 6°45'N and longitudes 1°30'W and 2°15'W and covers an area of about 1,136 km^2. The District is underlain by Lower Proterozoic volcanic greenstones with intervening sedimentary rocks and granitoid intrusions (Robb et al., 1999). The District is drained by the Offin and Oda rivers and the landscape varies from gentle to broken. Vegetation thrives on ferric fluvisols (the major soil types), which have been developed through yearly rainfall ranging from 125 to 200 cm and temperatures of 22 to 30º C. Vegetation is secondary forests, thicket,

swamp and forb regrowth (i.e., soft-stemmed leafy herbs, mostly the weeds, which appear on farms and have to be cut regularly).

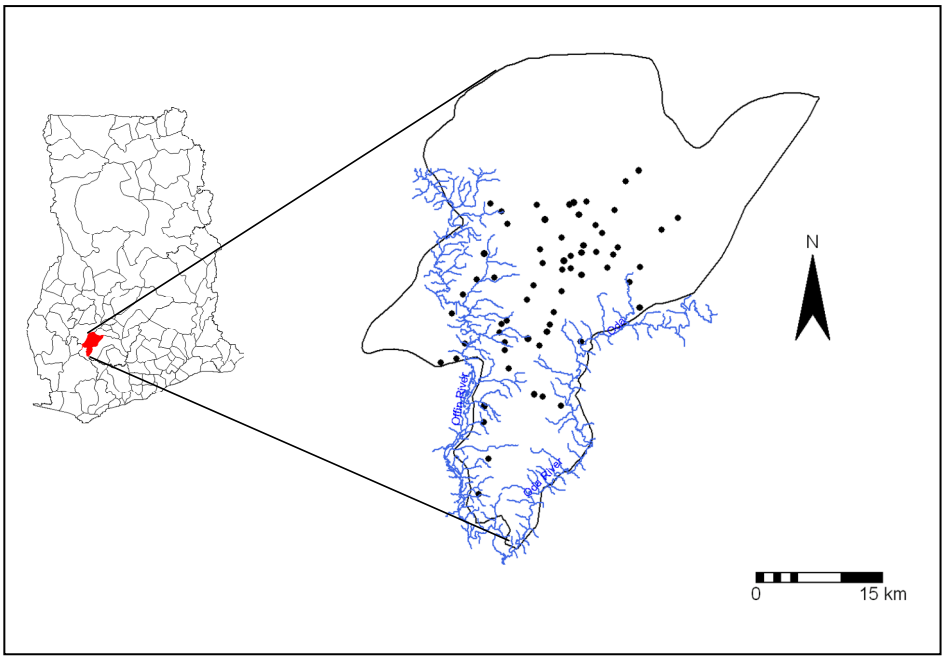

Figure 1.3. Location of the Amansie West District, Ghana

Of the 310 settlements in the Amansie West District, 19 have a population of 1000 or more, and their total population in 2000 was 108,726. There are approximately equal percentages of males and females. In terms of occupation, about 70% are farmers and 22% are engaged in legal and 'galamsey' (or illegal) gold mining.

Since the national BU case search in 1999, the Amansie West District Health Administration and the mission hospital (St. Martin's Hospital) at Agroyesum have consistently collected data on BU. The data is available to World Health Organisation (WHO) and other researchers. In other places, data is mainly hospital-based and may not be very dependable since most BU patients are

unwilling to seek medical attention for several reasons including the fear of surgery. This quality of the BU data makes research in the district favourable.

1.5 Research Methodology

The research methodology is in two parts, namely:

(a) conceptual modelling, in which published literature is studied to identify possible contributions of arsenic in the environment to the emergence and increased rate of incidence in BU; and

(b) spatial modelling, in which exploratory spatial analysis is used to support the conceptual model(s).

The conceptual modelling rests on the hypothesized relation between BU and arsenic in the environment. Characterization of locations in terms of their arsenic geochemistry concern both anthropogenic activities and natural processes. Point sources of arsenic enrichment have a wide impact in the event of wider distribution in and by transport media such as groundwater and surface water. Arsenic in water and stream sediments may contaminate both drinking water and agricultural soils in which foodcrops are cultivated. Soil contamination typically leads to plant and animal tissue contamination, depending also on the bioavailability of the contaminant as well as the bioaccumulation capability of the species concerned. Since local inhabitants largely depend upon local water and foodcrops for their livelihood, ingestion of arsenic is likely, and may pose a risk to their health. These concepts underlie a spatial approach to risk assessment using the available and relevant data.

1.5.1 Datasets for spatial analysis

Topographic data input to GIS were derived from digitized topographic maps at scale of 1: 50,000 covering the study area. These digitized data consist of screen digitized segments (elevation contours, hydrography) and vector point locations of settlements in the universal transverse Mercator (UTM) system. Elevation contours and drainage channels were digitized and converted from vector (segment) to raster format. Each segment was assigned its elevation value as a spatial attribute. Interpolation was carried out to generate a digital elevation

model (DEM), which would be integrated into the modelling process to obtain a catchment basin map. Settlement locations were digitized as points to generate a point map of settlements in a UTM coordinate system. Each point was assigned its name as spatial attribute.

Incidence of BU for each settlement was obtained from two sources: the 1999 national case search from the Korle-BU Teaching Hospital, Accra, Ghana; and for the years 2000-2002, from the Amansie West Health Administration, Ashanti Region, Ghana. Settlement population estimates for 2000 were obtained from the Ministry of Local Government and Rural Development, Accra. Prevalence per settlement is expressed as incidence for a specified period (1999 or 1999-2002) per head of population 2000. Both incidence and prevalence for each settlement are stored as settlement attributes.

Stream sediment geochemical data were obtained at map scale of 1: 62,500 from the Geological Survey Department, Accra, Ghana. The geochemical data consist of sample point locations and arsenic values, which were determined by inductively coupled plasma mass spectrometry (ICP-MS) analysis. This data pertains to the central and part of the south of the district consisting of about 1125 samples, representing an area of about 760 km^2.

Water samples were collected in duplicate from representative groundwater and surface water abstraction points. The samples were filtered using a 0.45 micron filter and acidified to prevent absorption of dissolved ions or colloids and to prevent the growth of algae, and stored in plastic bottles. The samples were analyzed for arsenic using neutron activation. The positions of abstraction points were located using a GPS. These locations were digitized as points to generate two separate point maps of groundwater and surface water in a UTM coordinate system.

The ASTER imagery was georeferenced to a topographic map, resampled and stretched in order to enhance ground features. This was aimed at identifying disturbed ground (i.e., minesites) and classifying pixels into landcover information.

1.5.2 Data quality

Data quality with respect to digital datasets is defined as, "that part of the data statement that contains information that describes the source of observation or materials, data acquisition and compilation methods, conversions, transformations, analyses and derivations that the data has been subjected to, and the assumptions and criteria applied at any stage of its life" (Clarke and Clark, 1995). Data quality, therefore, is linked with analysis of the data sets. While the accuracy of results depends on quality of the analysis it equally depends on the quality of the data. The USEPA (1994) states that all collected data have error and that no-one can be absolutely certain about the data. This implies that data must be monitored right from the beginning of their collection through their delivery, storage, integration, retrieval and analysis.

Reduction of data quality can be seen in various stages during the combination of sources of information. Digitization uncertainty is present. The digital elevation data are then used in turn within a statistical model. Similarly, positional uncertainty in location of the settlement is ignored. The reason is obvious, as far as the concept of a settlement is in principle somewhat vague, but well-understood within Ghana. Arsenic determinations are not precise and contain an error component. Some BU cases reported in the study area may have been contracted elsewhere and vice-versa. Better understanding of data quality may possibly lead to some refinements, although it is doubtful whether it would lead to significant changes of results.

1.5.3 Spatial modelling

The spatial data analysis and modelling can be seen in the context of a geographic information system (GIS). Several layers of information can be identified. Basic layers are the digital elevation, spectral data from ASTER imagery, location of settlements and location of geochemical samples. The settlements have as non-spatial attributes the number of inhabitants and BU incidence. There are several layers of geochemical samples, namely stream sediments, surface waters and groundwaters. Each sample has as non-spatial attribute its arsenic concentration. Other layers are created by interpretation of the ASTER imagery, for example, the distribution of farmland and the location and extent of artisanal minesites.

The importance of the GIS lies in its ability to retrieve appropriate datasets, analyze the data in them and to integrate the data in them. Thus, human health risk analysis in a GIS uses integration of disease risk (incidence or prevalence) with postulated risk factors or explanatory variables. In this study, BU is modelled using as explanatory variables arsenic in stream sediments, surface waters and groundwaters; and specific sources of arsenic such as farmlands and minesites are also considered.

In this study, the modelling developed within the GIS environment has, as its location specificity, the settlement (Figure 1.4). Data of population and BU incidence are intrinsically tied to settlements. Data of arsenic concentrations in surface water and groundwater are tied to settlements by virtue of the fact that samples were taken at abstraction sites routinely used by the inhabitants of each settlement. Related data not intrinsically tied to the settlement have to be transferred to the location of the settlement using a statistical function. This applies to elevation data and arsenic concentrations in stream sediments. Other spatial data such as farmlands and minesites are related to settlements through proximity functions.

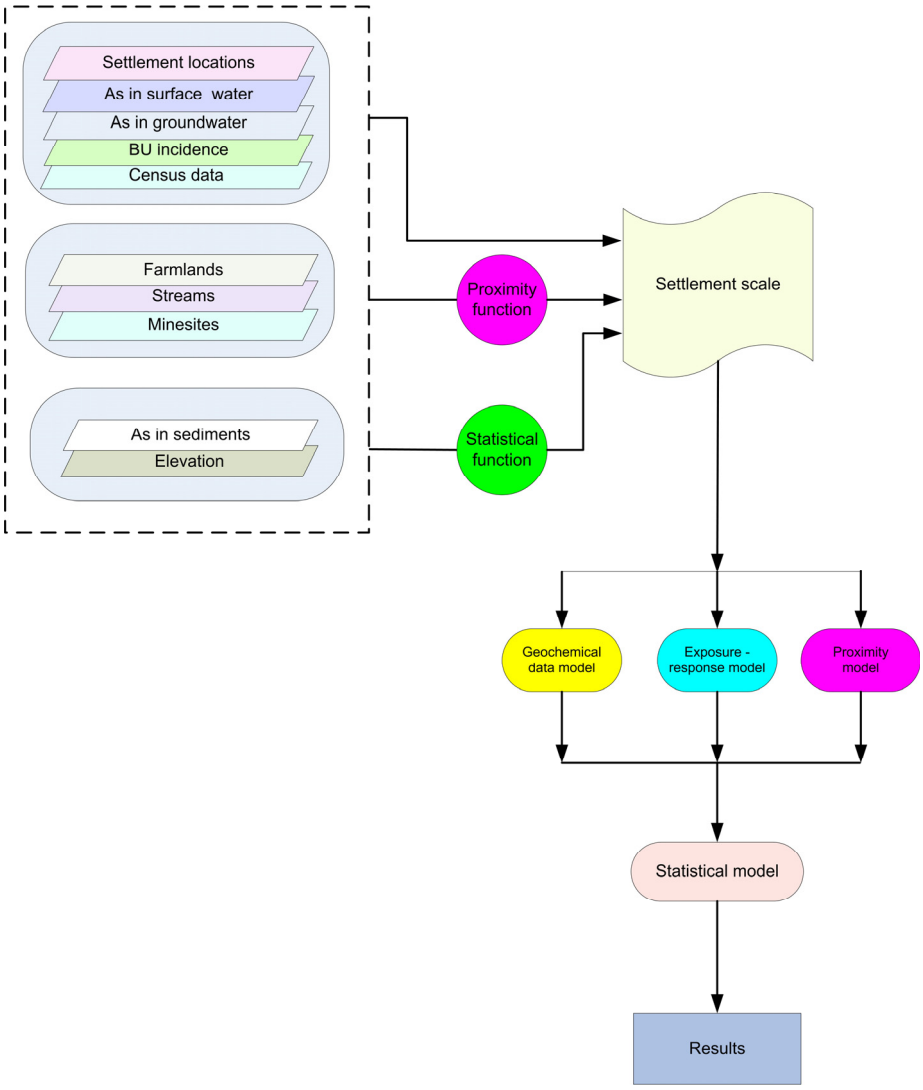

Figure 1.4. GIS data and modelling procedure

1.7 Outline of Thesis

Chapter 1 is an introduction to the research that provides a background and problem definition, research objectives, the study area and the research methodology.

Chapter 2 reviews pathways of MU infection with some emphasis on immune down-regulation and subsequent susceptibility to bacterial infections.

Chapter 3 reviews arsenic mobilization in the environment and its impact on health, with some emphasis on induced cytokine expression by arsenic and thus susceptibility to pathogenic infection.

Chapter 4 uses geomorphologic techniques to convert arsenic concentrations in stream sediments into catchment basins. Proximity analysis is carried out to determine the spatial relations between anomalous areas, or arsenic-enriched domains, and BU distribution to assess the role of arsenic in BU prevalence in the study area.

Chapter 5 looks at potable water pathways by which the population may be exposed to arsenic. Using arsenic concentrations in surface water and groundwater, an exposure-response relationship with BU is modelled to assess the influence of arsenic concentration levels in drinking water on BU infection.

Chapter 6 goes on to look at the role of artisanal gold mining in the study area. The chapter carries out proximity analysis to determine spatial relationship between BU cases and minesites.

Chapter 7 uses spatial statistics to quantify and analyse the impact of the environmental risk factors in chapters 5-7 and brings them into a coherent framework.

Chapter 8 draws together the results and conclusions of preceding chapters and offers recommendations for future studies.

CHAPTER 2

PATHWAYS OF MYCOBACTERIUM ULCERANS INFECTION

This chapter is based on

Duker, A. A. and Hale, M. Pathways of *Mycobacterium ulcerans* infection **(in preparation).**

The chapter presents a general overview from published literature on *Mycobacterium ulcerans* (MU), its infection, geographical distribution and environments of Buruli ulcer (BU) outbreaks with some emphasis on the possibility of a defect in the individual's immune system that could enhance BU development.

2.1 Introduction

Buruli ulcer (BU) is a skin disease caused by *Mycobacterium ulcerans* (MU) and is rated as the third most common mycobacterial infection after tuberculosis and leprosy (Jossé et al., 1995). It begins with a painless nodule or papule in the skin and, without appropriate therapy, causes massive skin ulceration, which often results in grossly deforming sequelae (Connor et al., 1976). The disease has been reported in many countries, in particular the countries of West Africa, Australia, Papua New Guinea, Indonesia, Malaysia, Mexico, Bolivia, French Guiana, Surinam and Peru. In many of these countries, BU is known to afflict impoverished inhabitants living in remote areas where amenities of modern medical science are not available or are expensive (Guédénon et al., 1995). Of the BU-affected inhabitants in certain countries, many are children. For example in Ghana, about 76% of BU patients from Affram valley in the Eastern Region prior to 1989 were younger than 20 years (Van der Werf et al., 1989) and about 70% of BU patients from Ashanti Region prior to 1998 were younger than 15 years (Asiedu and Etuaful, 1998). The epidemiology of the disease is poorly understood, but the increasing incidence of BU in certain parts of the world, particularly in West Africa, led the World Health Organisation (WHO) to recognize it as an emerging disease and called for urgent action to control it (WHO, 1998a).

2.2 Microbiology of MU

MU is a slow-growing organism and is not easily contained by normal standard biochemical tests. It grows slowly at both 25ºC and 37ºC. It grows optimally under microaerophilic conditions (Jenkins et al., 1982) at 25ºC and 28˚C than at higher temperatures (Tsukamura, 1983).

Molecular analysis indicates that MU is almost identical to another mycobacterium, *M. marinum* (Portaels et al., 1996) and the next closest being *M. tuberculosis* (Tonjum et al., 1998) but the diseases each of these mycobacteria cause are different.

2.3 Clinical presentation

Primarily, BU is a disease of the skin and can be characterized as a non-ulcerative (papules, nodules, plaques and oedematous form) and ulcerative disease. The disease begins as a firm, painless nodule, often leading to an oedema. The focus of infection is the subcutaneous tissue (Van der Werf et al., 1999). When the skin ulcerates it shows an extensive zone of necrotic subcutaneous fat (panniculus), which very often might have undergone extensive lateral destruction, undermining the dermal edges of the ulcer (Hayman and McQueen, 1985). There are some variations in the clinical presentation of BU. For example, in Australia the disease begins as a papule or pimple in the skin with no involvement of the subcutaneous fat (Van der Werf et al., 1999), whereas in Africa the subcutaneous fat is involved in the infection. Sometimes the entire trunk or limb may undergo serious necrosis of massive areas of the skin (Abalos et al., 2000). Some patients, especially in Africa, may develop osteomyelitis (Van der Werf et al., 1999) (a gross deformation) that may necessitate amputation (Connor et al., 1976).

2.4 Pathology and immune response

The progression of BU is characterized not only by damage to skin, nerves and blood vessels but also by absence of inflammatory response during the early and acute phases of the disease (Hayman, 1993; Pimsler et al., 1988; Hayman and McQueen, 1985; Connor and Lunn, 1966, 1965; Dodge, 1964). This suggests that the bacteria secrete an immunosuppressive toxin (Hockmeyer et al., 1978). This toxin was later found and named “mycolactone” (George et al., 1999). This toxin is normally at or close to the site of infection. By suppressing the production of interleukin-2 (IL-2) and tumour necrosis factor (TNF), T-helper-1 (Th1) (the pro-inflammatory immune response) is down-regulated (Pahlevan et al., 1999); hence, the development of the disease. Development of MU infection therefore has much to do with the state of the individual’s immune response (Van der Werf et al., 1999).

Studies by Gooding et al. (2002, 2001) indicate that those developing BU may already have either (a) immune deviation resulting in down-regulation of Th1 and increased Th2 (humoral immune response) or (b) have inherent defect in the immune system that leads to failure to develop any strong response to mycobacterial antigens. He further found that the toxin of MU alone could not induce systemic immunosuppression. Johnson et al. (1999) support the fact that the toxin elaborated by MU results in the development of BU, but state that it may not be the only virulence factor in the pathogenesis of infection. Stienstra et al. (2001) suggest that host susceptibility factors, which could be genetic or environmental, need be explored in order to understand the reason for the development of BU. A study by Barker and Ninkibigaya, (1972) in which case control studies showed no differences in activity between BU patients and control, suggests that infection by MU could be due to the extent of an individual's exposure and response to MU infection, thus reinforcing the outcome of studies by Gooding et al. (2002, 2001).

2.5 Epidemiology

2.5.1 Geographic distribution

MacCallum published the first clinical description of MU disease in a young child in Bainsdale, Australia, in 1940 (MacCallum et al., 1948). The authors then called it "Bainsdale ulcer". Since that first report in Australia the disease still remains in the country with Douglas Shire having the highest incidence (Smith, 1997; Jenkin et al., 2002; Gooding et al., 2002).

Many cases have since been reported in other countries of the world (Hayman and Asiedu, 2000) with most cases in the tropics, especially in rural Africa and catastrophically in West Africa (Nigeria, Benin, Togo, Ghana, Burkina Faso, Côte d'Ivoire and Liberia) (Monson et al., 1984; Cornet et al., 1992; Amofah et al., 1993; Amofah et al., 1995; Jossé et al., 1995; Marston et al., 1995; Meyers et al., 1996; Aguiar and Stenou, 1997). Even before MacCallum's first publication, the disease was already known in Africa (Meyers, 1995). Several cases were reported from the Congo (Portaels, 1973) but it was in Uganda where the disease was named "Buruli ulcer" by Clancey et al. (1961) after the Buruli County, where there was a large number of cases during the late 1960s

and early 1970s (Uganda Buruli Group, 1971; Clancey, 1964; Dodge, 1964; Dodge and Lunn, 1962; Clancey et al., 1961).

Other BU endemic areas of the tropical world include Papua New Guinea (Radford, 1974a; 1974b), Malaysia (Petit et al., 1966), Northern Sumatra (Shattock, 1965), Mexico (Aguilar et al., 1953), Bolivia (Lindo and Daniels, 1974), Peru (Radford, 1974a), Surinam (Portaels, 1989), French Guiana (Grosshans and Pradinaud, 1979) and Sri Lanka (Seevanayagam and Hayman, 1992). Few cases have been reported in the non-tropical world. Cases are reported from South Australia (Hayman, 1993), Japan (Tsukamura and Mikoshiba, 1982) and China (Faber et al., 2000).

The outbreaks, especially those in developing countries, have increased knowledge of MU infections. However, the precise mode of transmission of BU both in humans and animals remains elusive.

2.5.2 Transmission hypothesis

The mode of MU infection is described by two hypotheses. The first hypothesis is that trauma to the skin by a contaminated environment (e.g., soil, water, vegetation, insect vector) is one mode of transmission (Portaels et al., 2001). The second hypothesis is that, as MU has been shown to be aerosolized from suspensions of tap water (Hayman, 1991) it could be inhaled or ingested (Johnson et al., 1999; Hayman, 1991; Connor and Lunn, 1965) and then reactivated in low temperature areas of the body at the sites of trauma. The second hypothesis is demonstrated in an extensive outbreak among residents of Philip Island (Ross et al., 1997; Stinear et al., 2000). Of the two hypotheses, however, the first seems to be more favoured (Radford, 1974a; Portaels, 1995; Hayman, 1991; Johnson et al., 1999).

Infection by MU occurs commonly in areas related to rivers, swampy terrain or lacustrine systems (Portaels, 1995). There have, however, been reports of endemic areas not associated with relatively large water masses (Christie, 1987). Animals (e.g., koalas) in Australia have been known to be infected (Mitchell et al., 1987) and it is thought that this could be from an environmental source. Observations also indicate that increased incidence of MU infections occur due to anthropogenic activities (Portaels, 1995). New endemic areas are

associated with recent disturbances such as flooding (Barker, 1973; Hayman, 1991), mining (Aguiar et al., 1997), logging of rain forest (Hayman, 1991) and damming of rivers (Aujoulat et al., 1996; Van der Werf et al., 1999). However, it seems that the socio-economic environment of endemic communities is also an important factor to be considered with regards to MU infection.

2.6 Environments of BU outbreaks

2.6.1 Socio-economic environment

Malnutrition impairs several aspects of host defense (e.g., T-lymphocyte function) (Dai et al., 1998); and several aspects of cell-mediated immunity are sensitive to nutritional effects (McMurray et al., 1990; McMurray, 1984; Suskind, 1977). Calder and Jackson (2000) indicate that undernutrition and infection are the main causes of morbidity and mortality in the developing world. Undernutrition, which includes protein-energy malnutrition and miconutrient deficiencies, helps to defect the host's immune defense mechanisms against pathogens; and once infected the malnourished status of the subject is aggravated. Increased infections may be attributed to specific nutritional deficiencies (Scrimshaw et al., 1968). Unfortunately, protein-energy malnutrition, for example, may consequently cause the deficiency of a micronutrient (i.e., zinc) and result in the susceptibility of the subject to toxin-producing bacteria (Wapnir, 2000). It has been suggested that the synergy of poverty, ignorance, poor hygiene, lack of good water supplies, poor housing, lack of modern health facilities, cultural practices and certain social setups create a poor nutritional environment as well as enhance exposure to pathogenic assaults (Calder and Jackson, 2000). Such an environment especially is more detrimental to children (WHO, 1998b) as infection with them becomes more severe.

Several studies (e.g., Aujoulat et al., 2003; WHO, 2003; Stienstra et al., 2002; WHO Fact Sheet, 2000; Bär et al., 1998; Asiedu and Etuaful, 1998; Portaels, 1995) have found that BU is prevalent among poor populations in remote rural areas where modern medical amenities are lacking. BU has often been referred to as the 'disease of the poor' (WHO Fact Sheet, 2000). In this environment of poverty the necessary proteins and micronutrients are lacking in staple food of endemic communities (Aujoulat et al., 2003) and due to lack of finances BU

patients do not seek medical attention until it becomes the only option (Stienstra et al., 2002; Asiedu and Etuaful, 1998). There have been instances where BU patients were diagnosed with hypoproteinaemia (Bär et al., 1998), anaemia (Pszolla et al., 2003), some form of severe illness (e.g., sickle-cell anaemia) (Portaels, *pers. comm.*) or nutrition-related illness. Such severe illness coupled with MU toxin may enhance immunosuppression and subsequently dissemination and spread of MU (Johnson et al., 2002; Pimsler et al., 1988).

2.6.2 Riverine and volcanic environments

The earliest report (in 1957) of BU in Papua New Guinea occurred after the eruption of Mount Lamington in 1951. Following the eruption, floods of the Sepik and Kumusi Rivers devastated the area and most BU infections were found in settlements along the inundated portions of the rivers (Radford, 1974b). Studies of Portaels (1989) in central Africa suggest that MU, like other mycobacterial species, is present in water but in very low concentrations. There has also been evidence of the disease associated with slow-flowing or stagnant water (Portaels, 1989). There are reported infections occurring near rivers in Uganda (Barker, 1973; Uganda Buruli Group, 1971), Côte d'Ivoire (Marston et al., 1995) and Ghana (Amofah et al., 1993; Mensah-Quainoo, 1998).

It has been suggested that insects (e.g., firefly larvae, *Naucoridae, Belostomatidae*) could be involved in the transmission of MU infection since they prey on water-filtering organisms, which might have concentrated MU (Portaels et al., 2001; Portaels et al., 1999). Marsolliers et al. (2002) subsequently carried out an experimental study to show that not only did this insect concentrate MU in its salivary gland but also that its bite transmitted infection to mice.

2.6.3 Agricultural environments

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 and Carswell (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; Monson et al., 1984). The Benin incidence of BU, especially around Zangnanado, could be related to recent construction of canals for irrigation purposes for rice cultivation (Portaels, *pers. Comm*.).

2.6.4 Lakes and reservoir environments

Aujoulat et al. (1996) indicate that, in Côte d'Ivoire, increased incidence of BU was very much related to areas around dammed rivers. 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 a 2.5-ha 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; Monson et al., 1984). 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 (with the highest occurring about 7 km north of the impoundment) than in the downstream part south of the impoundment where settlements were on higher elevations.

2.6.5 Swamps and related environments

Many of the MU infections occurred after flooding. Bainsdale, Australia, experienced its worst floods on record in 1935 (Hayman, 1998) 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, 1974b; 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 a nearby swamp were 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) and Uganda (Barker, 1971, 1973).

2.7 Seasonal variation and MU infections

Some authors (e.g., Revill and Barker, 1972; Meyers et al., 1996) have referred to the seasonal dimension of MU infections. For example, in Australia, it was noted that the disease appeared at the end of the autumn or winter (Hayman, 1985); and in Papua New Guinea and Cameroun, it was observed that incidence of the disease increased during the dry season (Radford, 1974b; Ravisse, 1977). In Uganda, reports from two separate studies showed that the peak incidence of the disease occurred in low rainfall months between May and September (Uganda Buruli Group, 1971; Revill and Barker, 1972). In Ghana, the peak incidence of the onset of symptoms was in September and October (Amofah et al., 1993); and similarly in Côte d'Ivoire, Marston et al. (1995) found the peak incidence in the same months. Thus, there seems to be a temporal relationship between BU incidences and relatively dry periods.

2.8 Treatment and control

A wide range of drugs (e.g., ethambutol, rifampicin, and clarithromycin) has been used against BU (Feldman and Karlson, 1957; Pattyn and Ermengem, 1968; Lunn and Rees, 1964; Thangaraj et al., 2000) and found to be effective *in*

vitro but not *in vivo* (Johnson et al., 1999) probably due to the poor penetration of drugs into necrotic tissue (Goutzmanis and Gilbert, 1995). Portaels et al. (1998) found clarithromycin to be effective against several MU strains and suggested that the drug could be used for the treatment of early lesions. Other treatment options indicate that infection is impaired at oxygen partial pressures > 2.5 kPa probably because low oxygen concentration enhances growth of MU and that low oxygen concentrations prevail in necrotic tissues (Palomino et al., 1998). However, considering the fact that most patients are poor, this treatment may be expensive (van der Werf et al., 1999). In view of the ineffectiveness of most antimicrobial drugs *in vivo* and the high cost of other treatment options, surgery remains the primary treatment for BU.

Excision of preulcerative lesions (papules and nodules) is an important means of managing BU. It is, however, important that the removal of necrotic tissues be extended as far as to healthy tissues in order to prevent any bacillus remaining to cause further infection. The skin is closed after excision is completed. In the case of extensive ulcerative lesions, skin grafting may be needed.

In spite of all these forms of treatment, several individuals (especially of the rural population) in endemic areas consider hospital treatment expensive and sometimes ineffective and will not attend or report to the hospital when infected (Aujoulat et al., 2003; Amofah et al., 1993). In the light of this situation other control measures must be taken.

The difficulty in finding alternatives is the fact that the means of transmission is yet unclear. One of the precautionary measures that has been recommended is limiting contact with the environmental source of MU (Portaels et al., 2001), which could be difficult for rural communities in the short run if it affects their source of water or farmlands. The wearing of trousers, boots and long sleeved shirts when on farms and swamps is said to have helped (Marston et al., 1995). However, this is only a partial solution since infection can affect every part of the body including the face (Amofah et al., 1993). Vaccination of Bacille Calmette-Guérin (BCG), which partially protects from BU for few months (Smith et al., 1977), could be implemented. Therefore, until knowledge of the mode of transmission is attained, there can be no clear preventive measures.

However, education, especially of the rural population where the disease is endemic, is important and the partial solutions or preventive measures can be used in the interim. The form of education must first focus on early detection of nodules by individuals so that they can report to the nearest clinic or hospital for removal. Another aspect of education is to stress the fact that BU is a result of a mycobacterial infection like any other disease and not as a result of sorcery (Aujoulat et al., 2003). As BU mainly afflicts relatively poor communities and countries, the additional strain imposed on limited medical services is also an important consideration. When the disease reaches the ulceration stage, confinement in a hospital bed is protracted and in itself an economic burden (Asiedu and Etuaful, 1998).

2.9 Summary

- MU is normally a slow-growing mycobacterium but grows luxuriantly at 25ºC and 28ºC under microaerophilic conditions.

- BU is a skin disease and is characterized as non-ulcerated and ulcerative disease. The focus of the disease is the subcutaneous tissue. MU secretes a toxin, which destroys the skin, nerves and blood vessels.

- The toxin elaborated by MU suppresses the immune inflammatory response. The toxin alone, however, does not induce systemic immunosuppression and therefore any such situation in an individual suggests host susceptibility factors that must be explored.

- Geographically the disease is generally prevalent in the tropics with West Africa having a very high incidence. Few cases, however, have been reported in the non-tropical world.

- Because the exact mode of transmission is unknown, it has been hypothesized that infection could be via trauma to the skin by a contaminated environment or that MU is inhaled or ingested, later to be reactivated in sites of trauma.

- Commonly MU host environments are related to rivers, swampy terrain, lacustrine or reservoir systems or through environmental disturbances such as flooding, mining, logging of rain forest and damming of rivers.

- The disease has a seasonal dimension and incidences seem to have a temporal relationship with relatively dry periods.

- Since most endemic areas are rural populations who are poor and consider hospital treatment expensive, and since even the mode of transmission of the disease is unknown, alternative interim precautionary measures through education (of the rural population) are seen as a way to reduce human misery and economic burden imposed by the disease.

CHAPTER 3

ARSENIC GEOCHEMISTRY AND HEALTH

This chapter is based on

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Duker, A. A. and Hale, M. Environmental arsenic: a contributory factor in *Mycobacterium ulcerans* infection? (**in preparation**).

This chapter presents a review of toxic effects of arsenic and the way these can lead to opportunistic infections. It discusses further environments where, when and how arsenic may be enriched naturally or anthropogenically, spatial coincidences of BU-endemic areas and arsenic-enriched areas and temporal coincidences of BU-infection rates. Finally, some measures for treatment of arsenic poisoning are recommended.

3.1 Introduction

Arsenic occurs naturally and ranks as 20th most abundant trace element in the earth's crust (NRC, 1977) and is widely distributed in the environment. Its association with some non-weathering-resistant mineral deposits (e.g., sulphide minerals) has contributed to its release in large amounts into the environment (Murdoch and Clair, 1986).

Arsenic exists mainly in three valency states (i.e., -3, +3, +5). The trivalent arsenic (As^{3+}) and the pentavalent arsenic (As^{5+}) are widely present in natural waters (Feng et al., 2001) and are soluble over a wide range of pH and Eh conditions (Bell, 1998). In oxidizing environmental conditions As^{5+} species are more stable and predominant, whereas in reducing environmental conditions As^{3+} species are predominant. The trivalent compounds are generally more toxic than the pentavalent compounds (Smedley et al., 1996; Cervantes et al., 1994). The most toxic of them all is arsine gas (AsH_3) (Buchet and Lauwerys, 1983; Leonard, 1991). Organic arsenical compounds exist but these are generally low but not irrelevant toxicological significance (Gebel, 2000; Gochfeld, 1995). Under anaerobic conditions, arsenite can be reduced to arsine by microorganisms in soil (Bachofen et al., 1995; Gao and Burau, 1997; Cheng and Focht, 1979). Arsenic species may be methylated as monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and trimethylarsine oxide (TMAO) by microorganisms (Ridley et al., 1977; Woolson, 1977; Cullen and Reimer, 1989; Gadd, 1993), humans and animals (Styblo et al., 2000; Buchet and Lauwery, 1985; Buchet et al., 1984; Buchet et al., 1981).

Arsenic is used in hardening of alloys and in production of semiconductors, pigments, glass manufacturing, pesticides, rodenticides and fungicides (Hathaway et al., 1991). It is also used as an ingredient of drugs for the treatment of some diseases (e.g., sleeping sickness, chronic myeloid leukemia)

(Nevens et al., 1990; Luh et al., 1973). Because of its usefulness and exploitation, arsenic contamination is now widespread in the environment. In as much as toxic compounds of arsenic could occur naturally or anthropogenically in the environment, an understanding of its toxic effects is warranted.

3.2 Kinetics and metabolism of arsenic

Ingested elemental arsenic is considered less toxic, poorly absorbed and largely eliminated unchanged from the human body. However, soluble arsenic compounds are absorbed from the gastrointestinal tract (Hindmarsh and McCurdy, 1986) and eliminated via the kidney (Buchet et al., 1981; Luten et al., 1982; Tam et al., 1982). Trivalent arsenic (As^{3+}) is removed from the body through urinary excretion of non-methylated and methylated arsenic (As^{5+} and As^{3+}). Methylated arsenic species are inorganic forms of As^{3+} and As^{5+} sequentially reduced *in vivo* (Vahter and Enval 1983; Winski and Carter, 1995), and are detoxified in the liver to MMAA and DMAA. Both *in vivo* and *in vitro* studies show that these 'detoxified' species (MMAA and DMAA) are both toxic to humans and animals (Ochi et al., 1996; Petrick et al., 2001, 2000; Styblo et al., 2000; Kaise et al., 1989). The significance of the methylation of arsenic, which in the past was considered solely as a detoxification process has recently changed. Studies (e.g., Aposhian et al., 2000) have shown that trivalent methylated arsenicals (i.e., MMAA and DMAA) have been detected in urine samples of individuals exposed to inorganic arsenic. Animal studies and *in vitro* studies in human cells showed that these methylated compounds are more toxic or carcinogenic than the corresponding inorganic arsenic (i.e., As^{3+}) (Yamanaka et al., 1991; Yamanaka and Okada, 1994; Styblo et al., 2000; Del Razo et al., 2001). This suggests that methylation may not only be a detoxification process but may also enhance toxicity and/or carcinogenesis. Farmer and Johnson (1990) report that about 40-60% of arsenic may be retained in the body even after exposure cessation and that this may be accumulated in the skin, hair, nails, muscle and small amounts in teeth and bones (ATSDR, 1990; Ishinishi et al., 1986).

3.3 Arsenic toxicity

Arsenic (e.g., As^{3+}) can be toxic through its interaction with sulfhydryl groups of proteins and enzymes (to denature the proteins and enzymes within the cells)

(Gebel, 2000; Graeme and Pollack, 1998) and through an increase of reactive oxygen species in the cells, consequently causing cell damage (Ahmad et al., 2000; Wang et al., 1996; Chen et al., 1998; Nies, 1999). Arsenic can interfere with essential enzymatic functions and transcriptional events in cells, leading ultimately to a "multitude of multisystemic non-cancer effects that might ensue" (NRC, 1999). For example, oxidative stress induced by trivalent methylated arsenicals inhibits glutathione (GSH) reductase (Styblo et al., 1997) and thioredoxin reductase (Lin et al., 1999) with subsequent impairment of cellular protective mechanism against oxidants. While depletion of cellular GSH sensitizes cells to arsenicals and may also contribute to cell transformation (Shimizu et al., 1998), thioredoxin depletion affects gene expression due to the fact that it modulates DNA binding activity of some transcriptional factors (Matthews et al., 1992; Arrigo, 1999; Powis et al., 2000). Arsenite is known to inhibit more than 200 enzymes in the body (Abernathy et al., 1999) and, because arsenate has a similar structure as phosphate, it can substitute for phosphorus in the body, which can lead to replacement of phosphorus in the bone for many years (Arena and Drew, 1986; Ellenhorn and Barceloux, 1988). Since arsenate is hydrolyzed easily (in the cell) it prevents subsequent transfer of phosphate to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) (the energy currency of the cell) and thus depletes the cell of its energy (Winship, 1984). Arsine, the most toxic of the arsenicals (Buchet and Lauwerys, 1983; Leonard, 1991), is known to cause hemolysis of red blood cells, leading to hemolytic anaemia, which is primarily responsible for the development of oliguria renal failure (Fowler and Weissberg, 1974; Fowler, 1977). It has been suggested that arsine interaction with sulfhydryl group of proteins and enzymes (Levinsky et al., 1970) may be responsible for inhibition of erythrocyte sodium-potassium pump. It also is known that arsenic decreases DNA repair process (Brockmoller et al., 2000) and, hence, enhances susceptibility to cancer (e.g., skin cancer) (Wei et al., 1994) and noncancer-related diseases (Feng et al., 2001).

3.4 Chronic effects of arsenic

Arsenic toxicity affects a wide variety of organisms including humans (Cervantes et al., 1994). Chronic arsenic effects in humans have been well documented and reviewed (e.g., Webb, 1966; Klevay, 1976; Pershagen, 1983).

Organs most affected are those involved with arsenic in absorption, accumulation and/or excretion. These organs are the gastrointestinal tract, circulatory system, liver, kidney, skin, tissues very sensitive to arsenic and those tissues secondarily affected (e.g., heart) (Squibb and Fowler, 1983). Signs of chronic arsenic toxicity include dermal lesions (e.g., hyperpigmentation, hyperkeratosis, desquamation and loss of hair (Zaloga et al., 1985)), peripheral neuropathy, skin cancer and peripheral vascular disease. These signs have been observed mostly in populations whose drinking water contains arsenic (Tseng, 1977; Tseng et al., 1968; Zaldivar, 1980; Zaldivar and Ghai, 1980; Cebrián et al., 1983; Smith et al., 2000). Among these symptoms, dermal lesions were most dominant, and were also known to occur within a period of about five years. The skin is known to localize and store arsenic because of its high content of keratin, which contains several sulfhydryl groups to which As^{3+} may bind (Kitchin, 2001) and may be the reason for its sensitivity to the toxic effects of arsenic.

A study of Tseng (1977) in the Province of Taiwan (China) established a clear dose-response relationship between arsenic and dermal lesions, Blackfoot disease (a peripheral vascular disorder) and skin cancer. From several studies (e.g., Chen and Wu, 1962; Chi and Blackwell, 1968; Tseng, 1977; Chen et al., 1988a; Engel et al., 1994), it has been established that peripheral vascular diseases are associated with arsenic in well water in Taiwan. However, vascular disease has also been reported among German vintners (Grobe, 1976) and inhabitants of Antofagasta in Chile (Borgono et al., 1977). Skin cancers including in-situ cell carcinoma (or Bowen's disease), invasive cell carcinoma and multiple basal cell carcinomas are all known to be associated with chronic arsenic exposure (Shneidman and Belizaire, 1986; Tseng et al., 1968; Yeh et al., 1968; ATSDR, 1990). Chen et al. (1995) observed that hypertension was linked to long-term arsenic ingestion as well as cerebrovascular disease (i.e., cerebral infection). Other effects are hematopoietic depression, anhydremia (due to loss of fluid from blood into tissue and the gastrointestinal tract), liver damage characterized by jaundice, portal cirrhosis and ascites, sensory disturbance and peripheral neuritis, anorexia and loss of weight (Webb, 1966). Moreover, the ability of arsenic to draw iron from ferritin (Ahmad et al., 2000) could enhance the adhesion of bacteria to human tissues (Bullen, 1981; Sugarman, 1980).

Although the effects of arsenic, as recounted above, result in several kinds of diseases, it may also impact adversely on the immune system, which may predispose to viral/bacterial infections. Several of such diseases resulting from alterations of the immunologic surveillance may not have been known to be due to arsenic and therefore may not have been attributed to arsenic effects. Some probing into this area is therefore appropriate.

3.5 Immune system response

3.5.1 Animal studies

Aranyi et al. (1985) studied the immune effects of arsenic in animals and reported that inhalation of arsenic trioxide by mice caused an increased susceptibility to respiratory bacterial pathogens (i.e., *Klebsella pneumonia*) apparently via injury to alveolar macrophages. A report by Hatch et al. (1985) also indicated significant increases in mortality in mice which were infected with bacterial pathogens after intratracheal injection of sodium arsenate. Gainer and Pry (1972) reported of inhibition of interferon action by arsenic in mice (as compared to controls) when mice were dosed with sodium arsenite prior to viral inoculation. Blakely et al. (1980) also found that levels of arsenite (0.5-10 ppm) in drinking water produced immunosuppressive effects in mice, which demonstrated an inhibitory effect on the immune system. Arsenic was found to defect antigen processing of splenic macrophages with consequent defective protective mechanism of helper T cells (Lewis et al., 1998a, b) and alterations of the humoral response parameters (e.g., IgM) (Sikorski et al., 1989, 1991; Lewis et al., 1996). Studies have shown that immunosuppression of arsenic enhances susceptibility to infections (Dai et al., 1999; Lantz et al., 1994; Aranyi et al., 1985; Vos, 1977; Gainer and Pry, 1972). Furthermore, studies by Kaltreider et al. (2001) showed that arsenic disrupts the hormone glucocorticoid, which helps to regulate the immune system, central nervous system, and changes in blood, bone and kidneys as well as the body's use of sugars, starches, fats and proteins.

3.5.2 Human studies

A study by Gonseblatt et al. (1994), using arsenic-exposed subjects whose drinking water contained a mean arsenic concentration of 412 $\mu g l^{-1}$ and controls

whose drinking water had a mean arsenic concentration of 37 μgl^{-1}, found impairment in the immune response of the arsenic-exposed subjects. Samet et al. (1998) found that the transcriptional factors (c-Jun, ATF-2, substrate of JNK and p38) were markedly phosphorylated in BEAS cells when treated with As^{3+}, with MAPKs inducing interleukin-8 (IL-8) protein expression. They speculated that such activation was likely to result in cellular responses such as growth proliferation, apoptosis and modulated inflammatory expression. Harrison and McCoy (2001) suggested that apoptosis might be an important mechanism for arsenic-induced immunosuppression. However, when apoptosis malfunctions it leads to several kinds of diseases including cancer (Miller and Max, 1998). Furthermore, Frenkel et al. (2002) report that arsenic impairs the immune system.

A study by Rosales-Castillo et al. (2004) in the Lagunera Region of Mexico assessed the relationship between chronic arsenic exposure, human papilloma virus (HPV), contact and non-melanoma skin cancer (NMSC) and concluded that viral infection (i.e., HPV) could constitute an additional risk factor for the development of NMSC among populations chronically exposed to arsenic. Other researchers (Grimmel et al. 1988; Gerdsen et al., 2000) had previously found HPV in arsenic-induced lesions such as squamous cell carcinoma and keratoses. The above findings may be viewed from the background that arsenic may cause a defect in cell-mediated immune function, compromising its protective mechanism and thus enhancing viral intervention.

Arsenic also causes anaemia (ATSDR, 2000; Parish et al., 1979), the severity of which indicates the extent of disruption to normal regulatory mechanisms exerted by the macrophages and the T-cells (Sathe et al., 1990; Bogner et al., 1990; Gascon et al., 1993); and also acts as an antagonist to selenium (Se), affecting its metabolism *in vivo* (Miyazaki et al., 2003; Schrauzer, 1987). In Taiwan, Lin and Yang (1988) studied patients with Blackfoot disease (BFD), a disease associated with arsenic (Tseng, 1977, 1989; Tseng et al., 1968, 1995, 1996; Chen et al., 1988b; Chi and Blackwell, 1968), and reported lower concentrations of Se and Zn in urine and blood of BFD patients than controls. The deficiency of micronutients (e.g., Se, Zn) is associated with malnutrition (Houssaïni et al., 1997) and may also alter the immune function (Good et al., 1980). Zinc, for example, is essential for normal immune function (Good et al.,

1980; Vruwink et al., 1993; McMurray et al., 1990; McMurray and Yetley, 1983). Selenium occurs in the enzyme Glutathione peroxidase in human erythrocytes (Auasthi et al., 1975). It is suggested that the enzyme Glutathione peroxidase together with vitamin E protect cell membrane against oxidative damage (Gibson and Scythes, 1984). Selenium therefore may act to negate the toxic effect of arsenic, which not only induces oxidative stress (Miyazaki et al., 2003) but also is capable of down-regulating inflammatory cytokines (e.g., Th-1) (Frenkel et al., 2002; Vega et al., 1999; Gainer and Fry, 1972). Guanquing (1979) suggested that Keshan disease, which is prevalent in some areas of China and associated with selenium deficiency (Chen et al., 1980; Xu et al., 1985), could be a viral disease exacerbated by selenium deficiency or low selenium and low proteins. The deficiency of these micronutrients may therefore predispose to immunoincompetence (Golden et al., 1978) and could lead to susceptibility to opportunistic pathogens to establish infection (Cunningham-Rundles and Lin, 1998).

3.6 Arsenic and microorganisms

Certain microbes can adapt to arsenic toxicity (Cervantes et al., 1994; Silver et al., 1993) and a wide range of microorganism can thrive in arsenic-enriched environments (Ahmann et al., 1994; Macrae and Edwards, 1972; Laverman, 1995). In order to thrive and function in this (arsenic) environment, several microorganisms have developed resistance to arsenic toxicity (Nakahara et al., 1977). The resistance is due to reduced uptake of arsenate and increased concentrations of phosphate transport into bacterial cells (Harold and Baarda, 1966; Bennett and Malamy, 1970; Willsky and Malamy, 1980). This phenomenon of decreased arsenate and increased phosphate uptake results from intracellular competition between arsenate and phosphate (Thiel, 1988). In addition to being resistant to arsenic toxicity, certain microorganisms are able to reduce the less toxic arsenate form to the more toxic arsenite (Andreae, 1978, 1979; Ji and Silver, 1995; Nies and Silver, 1995; Rensing et al., 1999). The reduction is an energy-generating process for the microorganism (Ilyaletdinov and Abdrashtova, 1981); however, the impact of such arsenic geochemistry in anoxic systems cannot be underestimated, especially with respect to arsenic mobilization (Ahmann et al., 1997; Cummings et al., 1999).

3.7 Arsenic-enriched environments and BU infections

Arsenic is concentrated by natural processes in certain environments and naturally-occurring microorganisms play an essential role in the environmental fate of arsenic in relation to mechanisms of arsenic transformations (e.g., soluble and insoluble forms, toxic and nontoxic forms) (Nealson, 1997; Lovley, 1997; Banfield et al., 1998). Human activities have exacerbated arsenic contamination in the environment (Bell, 1998). Examples of human activities that have adversely affected the environment are mining, waste disposal, indiscriminate use of fertilizers, pesticides, herbicides, manufacturing and chemical spillage. In 1979, for example, the total amount of arsenic released into the environment (in the U.S.) as a result of anthropogenic activities was estimated to be 5.3 x 10^6 kg (US EPA, 1982), of which 81% was deposited on land. Jahan et al. (2002) report that in the state of Victoria (Australia), mining of gold had caused an estimated 30,000 tonnes of arsenic to be redistributed to the surface across the landscape through erosion into streams and rivers. Many incidents of arsenic contamination of the environment have been reported in several countries of the world. The situation can have significant adverse influence on health due to arsenic uptake in water and food especially by developing and rural populations who depend on local sources of food and water. Therefore any arsenic geochemical anomaly may impact negatively on health (Plant et al., 1996). Some examples of arsenic-enriched environments are described below.

3.7.1 Riverine and volcanic environments

Several studies (e.g., Nriagu, 1979, 1989; Nriagu and Pacyna, 1988; Lantzy and Mackenzie, 1979) have shown that volcanoes are important natural sources of arsenic especially in the southern hemisphere (Nriagu, 1989; Nriagu and Pacyna, 1988). Under high temperatures (e.g., volcanic eruptions), arsenic is very mobile in the fluid phase and may also be present in fumaroles as sublimates and incrustations (Signorelli, 1993).

The explosive eruption of Mt. Lamington in 1951 (in Papua New Guinea) was also followed by mudflows that lasted until 1956 (Simkin and Siebert, 1994; Taylor, 1957). Floods of the Sepik and Kumusi Rivers and devastation followed the eruption. The earliest report of *Mycobacterium ulcerans* infection was in

1957. Infections were found mainly in settlements along the inundated portions of these rivers (Radford, 1974b). Lack of scarring coupled with absence of the disease in the older people was an indication that the disease was recent. Although no data on arsenic in the flood waters were determined, volcanic ashes could have generated high arsenic concentrations in the floodwaters (Smedley and Kinniburgh, 2002; Nicolli et al., 1989) and could have also generated low pH in surface and groundwaters.

3.7.2 Mining-related environments

Mining activities cause arsenic to be released in high concentrations from oxidized sulphide minerals (Smedley and Kinniburgh, 2002). This has resulted in high concentrations of arsenic in surface water (Smedley et al., 1996; Williams et al., 1996; Azcue et al., 1994), groundwater (Smedley and Kinniburgh, 2002; Del Razo et al., 1990; Armienta et al., 1997), soil and vegetation (Amasa, 1975).

The extensive Archaen West African Craton, stretching from Ghana to Sierra Leone, is underlain by the Birimian Formation (including greenstone belts), which consists of folded and metamorphosed sediments and volcanics intruded by suites of granites, associated with which are gold-bearing sulphide mineralizations (Mining Journal, 2000). In Ghana, and other parts of West Africa, gold occurs in quarts veins accompanied by arsenopyrite and pyrite (Bowell, 1992; Wright et al., 1985).

Reports (e.g., KENOR ASA, 1995) indicate that gold mining increased considerably in the period 1980-1990 in the West African region. Studies of Portaels (1998) also show that since 1980 new foci of BU have emerged in West Africa. Indeed West Africa seems to have borne the brunt of BU infections in the last 15 years (Aguiar and Stenou, 1997; Amofah, 1995; Amofah et al., 1993; Marston et al., 1995; Monson et al., 1984). There was a sharp increase in the incidence of BU in Ghana, with about 2000 cases recorded in the period 1993-1997 (Grosset et al., 2000), coincident with the initial wave of legal registration of artisanal miners (1992-1996).

3.7.3 Agricultural environments

Although the dominant source of arsenic in soils is parent rock (Smedley and Kinniburgh, 2002), pesticides and phosphates can substantially enhance arsenic concentration in soils. Arsenic has been used and is still used as pesticides, insecticides and in cattle and sheep dips (Azcue and Nriagu, 1994), and for control of moth in fruit crops (Buchanan, 1977; Yoon and Kim, 1977). Between the 1920s and the 1980s, 1647 sheep and cattle dips (to control tick) were built by the Government of Australia. The dips were situated every couple of miles and stretched from tropical Queensland to New South Wales. The soil around the dips was found to be contaminated with arsenic; levels measured in some of the disposal pits were up to about 3000 ppm (Loyd-Smith and Wickens, 2000).

Groundwater may be contaminated by arsenic through agricultural applications by leaching through soils and fissures of rocks, especially when applied during the dry season when net movement of water is downwards. Surface water contamination by arsenic via surface runoff may also be linked to the local rainfall as well as adsorption of arsenic onto soil.

Arsenate portrays certain characteristics of phosphates through its absorption by ligand exchange on hydrous iron and aluminium oxide (Davies and Jones, 1988). Hence, arsenic accumulates in soil, contaminates both surface water and groundwater (Lloyd-Smith and Wickens, 2000), is taken up by plants and is then entrenched in mammalian/insectivore food chain (Green et al., 2001). Irrigation, especially with wastewaters, can cause a problem of build-up of mobile and potentially toxic metals (e.g., arsenic) in soils and in surface runoff (Siegel, 2002). It was reported in Australia that BU cases appeared in 1995 following the creation of a golf terrain irrigated by used water (Johnson et al., 1996). In the Amansie West District of Ghana where BU is endemic and 44% of the patients are farmers (Amofah et al., 1993), irrigation of vegetable crops, especially in the dry season, is by surface waters (Pearson, 2001) containing 252 to 535 $\mu g l^{-1}$ arsenic (Duker, unpublished data).

3.7.4 Lakes and reservoir environments

Arsenic concentrations in lakes compared to those in rivers or streams may be lower due to the adsorption of arsenic on iron oxides in neutral to alkaline

conditions. Geothermal activities or inputs into some lakes or reservoirs, however, may contribute to the high dissolved arsenic concentrations (Maest et al., 1992; Aggett and Kriegman, 1988). Porewaters from shallow anoxic sediments in a lake situated in a geothermal region in New Zealand contained up to 6430 µg l^{-1} of arsenic (Aggett and Kriegman, 1988), which comprised mostly As^{3+} that had diffused across the sediment/lake interface and which had accumulated along with dissolved Fe and Mn in the hypolimnion. Geothermal activity beneath the lakes of the East African Rift Valley (BGS, 2000; Tole, 2002) could also lead to similar arsenic enrichment in the lake waters (no data are as yet available). Lake Kyoga is one such lake, and several cases of BU occurred around its edge (Barker, 1971).

Low water flow and water impoundments enhance enrichments of arsenic (Smedley and Kinniburgh, 2002; Nimick et al., 1998). The increase in As^{3+} and mobility (McLaren and Kim, 1995) may be linked to the depletion of O_2 levels especially in the bottom of lakes due to microbial reduction (Azcue and Nriagu, 1995). Buruli ulcer infections have been reported in lake/reservoir environments (Hayman, 1985; Monson et al., 1984; Hayman and Huygens, 1982). Although there is no corresponding report on arsenic data, there was a report in Nigeria of BU incidence among Caucasians on the campus of Ibadan University (Oluwasani et al., 1976), which is adjacent to a small stream that was dammed to make a 2.5-hectare artificial lake. In Liberia, there were reports of BU cases after a dam construction following the introduction of swamp rice to replace upland rice (Monson et al., 1984; Ziefer et al., 1981). In Cote d'Ivoire, a boy residing beside Lake Kossou, an artificial lake in the centre of the country was reported as infected with BU (Peraudin et al., 1980). In Ghana, BU is clustered along the Densu River, mostly in rural settlements (Mensah-Quainoo, 1998). Levels of arsenic concentration measured from 100 m and 12 km below the Weija Dam (an impoundment on the Densu River that stores water for the western part of Accra) were 19,100 µgl^{-1} and 14,000 µgl^{-1} respectively (Armah et al., 1998). BU occurred in settlements both upstream and downstream of the impoundment. Upstream and along the impoundment where wetlands have been created, BU incidences were higher (with the highest occurring about 7 km upstream of the impoundment), than downstream of the impoundment where settlements were mostly at higher elevations.

3.7.5 Swamps and related environments

Flooding induces (anaerobic) reducing conditions in soils (Deuel and Swoboda, 1972; Hess and Blanchar, 1977; McGeegan and Naylor, 1994; Reynolds et al., 1999). Under this condition, As^{5+} is reduced to As^{3+} and adsorbed As^{5+} is released as As^{3+} (Reynolds et al., 1999; Masscheleyn et al., 1991; Rochette et al., 1998). Floods carry along sediments and/or contaminants that have been stored (for short periods, several decades or even a millennium) in river beds and other sediments preserved in local low-energy environments such as behind bedrock obstructions in valley floor or alcoves developed in valley walls. Floods and/or storm waters can therefore carry along metals that contaminate the environment (Miller, 1997). For example, alluvial soils in Thailand could contain up to 5000 mgg^{-1} of arsenic (Thornton and Farago, 1997). Such contaminations through flooding increase and become severe with time and pose health hazard both to wildlife and humans (Bickford et al., 1999; Stoughton and Marcus, 2000).

Swamps, alluvial and deltaic environments are mostly characterized by reducing conditions, which cause high arsenic concentrations in groundwater (Smedley and Kinniburgh, 2002). In these environments, aquifer sediments do not allow air to enter (the aquifer) and coupled with the fact that recent sediments contain organic matter (which uses available oxygen), result in the development of reducing conditions (Smedley and Kinniburgh, 2002). Such conditions, which favour mobilization of arsenic, are also found in wetlands. Wetlands are effective filters for metal-containing water due to the high metal-binding affinity of their soils (Beining and Otte, 1996). In these environments, reducing conditions result in increased concentration of arsenic in solution, which are dominated by As^{3+} species. The most serious occurrences (involving large populations) of arsenic exposure in groundwater in alluvial and deltaic environment with resultant health problems are in Bangladesh and West Bengal (India) (BGS and DPHE, 2001; DPHE/BGS/MML, 1999; Smedley and Kinniburgh, 2002).

Several references have been made to renewed outbreaks of BU after flood events (Meyer et al., 1996; Barker, 1974; Portaels, 1989, 1995; Radford, 1997b). The appearance of the first patient in 1939 in Australia was after the

occurrence of the worst flooding on record in the district of Bainsdale in 1935 (Hayman, 1987). The outbreak of BU cases in the Busoga district of Uganda was related to an unprecedented flooding from 1962-1964 (Barker, 1971). In Cameroun, sharp increases of BU cases occurred after the flooding of the Nyong River (Ravisse, 1977). These and others have been mentioned in the previous chapter.

3.8 Seasonal variation

It is noteworthy that those arsenic-enriched and BU endemic areas mentioned above are mostly in tropical (to sub-tropical) countries. In these countries, there is a characteristic alternation of dry and wet seasons and this seasonal variation is influential to arsenic enrichment in the environment and could be a factor in BU infection.

Seasonal variation affects, in general, metal concentration and particularly arsenic speciation both in water and soil, apparently due to biologic uptake (Andreae, 1978, 1979). Temperature changes, particularly dry spells, may help to potentiate metal toxicity (Savage et al., 2000). A drop in water levels in certain parts of the tropical world during dry seasons exposes arsenic-enriched substrate to air and oxidation (Rodríguez et al., 2004; Akai et al., 2004). For arsenopyrite, the aqueous oxidation by dissolved oxygen is described by the following equation:

$$4FeAsS + 3O_2 + 6H_2O = 4Fe^{2+} + 4AsO_4^{3-} + 4SO_2^{2-} + 12H^+$$

Based on many studies (e.g., Thornton and Farago, 1997; Andreae, 1978, 1979; Savage et al., 2000; Sohrinet al., 1997; Michel et al., 1999) the arsenic cycle could be described as follows. The dry period is a preparatory stage in which arsenic-rich beds (pyrites/arsenopyrites) are exposed to air and oxidized. The rains during the wet season solubilize oxidized arsenic or secondary minerals and disseminate arsenic into the ecosystems through floods or storm waters. The concentration and thus toxic effects of arsenic is after the recession of the floods (i.e., the dry season). In Papua New Guinea and Cameroun, for example, it was observed that the disease was on the increase during the dry season (Radford, 1974b; Ravisse, 1977) at which time the toxicity of arsenic (increased

proportion of As^{3+} to As^{5+}) may be enhanced by the high temperatures and extreme evaporation of the season (Maest et al., 1992).

Amofah et al. (1993) and Marston et al. (1995) found that the peak incidence of onset of the symptoms of BU in Ghana and Côte d'Ivoire respectively, was towards the beginning of the dry season. In a separate study in an arsenic-enriched environment, Sarkodie et al. (1997) also found that the peak period in which subsistence crops and fern contained the highest concentration of both species of arsenic (As^{3+}, As^{5+}) was the beginning of the dry season. This may imply bioaccumulation of arsenic in human tissues through ingestion of arsenic-enriched food and water, which could cause, for example, immune dysfunction (Vega et al., 1999; Lantz et al., 1994; Ostrosky-Wegman et al., 1991) and thereby susceptibility to bacterial infection (Stienstra et al., 2001). Several authors have referred to the seasonal dimension of BU infections (Meyers et al., 1996; Revill and Barker, 1972). Thus, there appears to be a relationship between BU incidences and relatively dry periods during which arsenic toxicity is elevated.

3.9 Treatment and control

A range of chelating agents has been used in counter-acting arsenic poisoning. These include D-penicillamine, dimercaprol or British Antilewisite (BAL), dimercaptosuccinic acid (DMSA) and dimercaptopanesulfonic acid (DMPS). These, in general, have been reported to be ineffective against arsenic poisoning (Hall, 2002). Others (e.g., Saha et al., 1999) report the success of these agents but not after complications have developed. Wax and Thornton (2000) also reported one case of success in the use of DMPS against peripheral neuropathy. The cost, however, of these chelating agents may not be affordable by most people (Saha et al., 1999). (Arsenic scourge seems to occur among rural populations of the developing world).

The best control measure may be to implement preventive rather than curative measures. This implies adhering to the WHO minimum regulatory limit of 10 μgl^{-1} of arsenic in water, improving water quality or finding alternative water supply (e.g., rain water harvesting) for the population to prevent altogether arsenic poisoning or morbidity. These measures should be coupled with regular testing of wells (Hall, 2002) to detect any increase in arsenic concentration.

Where farmlands are affected by arsenic contamination, soil remediation may be implemented.

3.10 Summary

- Arsenic occurs naturally in the earth's crust and is widely distributed in the environment. The trivalent species is more toxic than the pentavalent, and arsine gas is the most toxic arsenic compound.

- Arsenic is promptly absorbed through the gastrointestinal tract and excreted in the urine as a mixture of As^{3+}, As^{5+}, MMAA and DMAA. Some arsenic may, however, remain bound to certain tissues.

- Toxicity of arsenic may be due to its reaction to sulfhydryl groups of proteins and enzymes, subsequently inhibiting functions of sensitive enzymes and ultimately leading to a "multitude of multisystemic non-cancer effects".

- Organs most affected by arsenic toxicity are the gastrointestinal tract, circulatory system, liver, kidney, nervous system other sensitive tissues and the heart. The skin localizes and stores arsenic because of its high keratin content and this may be the reason for its high sensitivity to arsenic.

- Impairment of the immune system by arsenicals through chronic ingestion may imply susceptibility to opportunistic pathogens to establish infection.

- A wide range of microorganisms can thrive in arsenic-enriched environments; having developed resistance mechanisms to function in that environment. Activities of these microorganisms enhance arsenic mobilization in the environment.

- There have been many incidents of arsenic contamination in the environment with resultant adverse health effects in several countries of the world. The causes of such contamination vary from agriculture and/or irrigation, geological changes, mining, natural intervention (such as floods) and groundwater exploitation.

- Seasonal variation also affects arsenic speciation both in water and soil, (often due to microbial intervention). Situation during such times, especially in dry periods enhance arsenic toxicity.

- Chelating agents are known to be ineffective against arsenic poisoning especially in cases of malignancy.

- Adhering to WHO regulatory minimum limits, improving water quality or finding alternative water supply (e.g., rain water) may be the best control measure against arsenic poisoning or morbidity.

CHAPTER 4

SPATIAL DEPENDENCY OF BURULI ULCER PREVALENCE ON ARSENIC-ENRICHED DOMAINS: IMPLICATIONS FOR ARSENIC MEDIATION IN *MYCOBACTERIUM ULCERANS* INFECTION

This chapter is based on

Duker, A. A., Carranza, E. J. M., Hale, M. 2004. Spatial dependency of Buruli ulcer prevalence on arsenic-enriched domains in Amansie West District, Ghana: implications for arsenic mediation in *Mycobacterium ulcerans* infection. *Int J. Health Geogr* 3: 9

The chapter explores the potential mediation by arsenic of MU infection in parts of the Amansie West District by determining the spatial relationship between arsenic-enriched domains and prevalence of BU.

4.1 Introduction

Buruli ulcer (BU) is a skin disease which usually begins as a painless nodule or papule and may progress to massive skin ulceration. If untreated BU may lead to extensive soft tissue destruction, with inflammation extending to deep fascia. The parts of the body most affected by BU are the extremities. In recent years, there has been increased incidence of BU in West Africa (Table 1.1) (Johnson et al., 1999), in particular Benin, Burkina Faso, Cote d'Ivoire, Ghana, Guinea, Liberia and Togo, and in Mexico, French Guyana, Papua New Guinea and Australia.

Table 1.1. 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) and Meyer et al. (1996)*; Amofah et al. (2002) and Aujoulat et al. (2003)**.

The causative agent of BU is *Mycobacterium ulcerans* (MU), which was first described in Bainsdale, Australia, in 1948 (Portaels et al., 1996). From the

medical point of view, MU is among the group of mycobacteria that are potentially pathogenic in humans and animals under special circumstances (Portaels, 1995). It is suggested MU enters through a small break or trauma in the skin because it is not known to penetrate through intact or healthy skin (Portaels et al., 2001; Johnson et al., 1999). Portaels et al. (1999) and Marsolliers et al. (2002) have suggested that insects may be involved in the transmission of the disease because insects found in the roots of trees tested positive with the mycobacterium. However, the reservoir of MU and the mode of transmission of BU are still unclear (Hayman, 1991; Meyers, 1995; Mitchell et al., 1994; Portaels, 1978; Portaels, 1995).

Epidemiological data suggest that environmental factors such as climate, soil, geology, geochemistry, etc. may indirectly influence or contribute to MU infection (Portaels, 1995). In addition, the frequencies of some diseases caused by mycobacteria indicate that species are distributed geographically (Falkinham et al., 1980). For example, MU has been observed mainly in the tropics (Portaels, 1995) especially in anthropogenically-polluted areas (Tacquet et al., 1973).

Since MU is known to be present in nature although its reservoir is not known and since the epidemiology of BU is still unclear, there is a need to have a better understanding of environmental, ecological, and behavioural factors that predispose to infection. Spatial analysis potentially contributes important information leading to the understanding of the epidemiology and etiology of BU.

4.2 Conceptual framework of the study

4.2.1 Research hypotheses

It has been consistently theorized that BU is acquired when MU enters the body through a skin rupture (Meyer et al., 1974; Van der Werf et al., 1999). However, several people who were affected by the disease do not recall having any break or trauma in their skin prior to being infected (Mensah-Quainoo, 1998). A possible alternative is entry through non-ruptured but unusually unhealthy or thin skin.

Several dermatological diseases (e.g., Bowen's disease, hyperkeratosis, hyperpigmentation) are related to arsenic ingestion and exposure (Gorby, 1994). Bioaccumulation of arsenic in the fatty tissues of the skin (Isensee et al., 1973), due to its high lipid solubility (Schoolmeester and White, 1980; Mahieu et al., 1981), may provide a favourable environment for MU in the skin because arsenic is known to help microorganisms grow (Ahmann et al., 1994). It can be hypothesized, therefore, that (a) arsenic induces MU adhesion to human tissues and (b) arsenic influences the ability of MU to establish BU.

In a case study, Amofah et al. (1993) reported that about 44% of the BU patients in the Amansie West District were farmers whilst about 54% were school children. In Ghana many children help their parents on farms. Not only do farmers and children come in contact with natural drainage areas on their journeys to and from their farmlands, but also the farms are located near water bodies or drainage systems for obvious irrigation purposes (Pearson, 2001). If farmlands and surface drainage channels are contributory factors to BU, farmlands and surface drainage channels enriched in arsenic may contribute to still higher prevalence of BU.

4.2.2 Research methodology

Spatial analysis of data provides opportunities for epidemiologists to study associations between environmental factors and spatial distribution of diseases (Glass et al., 1995). A geographic information system (GIS) is capable of analyzing and integrating large quantities of geographically distributed data as well as linking geographic data to non-geographic data to generate information useful in further scientific (or medical) research and in decision-making.

In this study, topographic map data, stream sediment geochemical data for arsenic, ASTER satellite imagery, locations of settlements and their population data and BU cases were the basic data inputs into the GIS. Spatial data processing was carried out (a) to delineate arsenic-enriched catchment basins based on arsenic concentrations in stream sediment samples, (b) to delineate farmlands from ASTER satellite imagery and determine arsenic-enriched farmlands based on catchment basin data and (c) to extract drainage channels from the topographic map and determine arsenic-enriched drainage channels based on arsenic-enriched catchment basins. Proximity analysis was undertaken

to determine spatial relationships between BU-affected settlements and the arsenic-enriched farmlands and drainage channels determined from the data inputs.

4.3 Materials and methods

4.3.1 Sources of data

The following are the sources of spatial data input to the GIS.

- Incidence of BU per settlement in 1999, obtained from Korle-Bu Teaching Hospital, Accra, Ghana.
- Settlement population estimates for 2000, projected by the Ministry of local government and rural development.
- Topographic map (Sheet 0602C1, 1974, at a scale of 1: 50,000), a single sheet covering the study area, obtained from the Survey Department, Accra, Ghana.
- Location map (at scale of 1: 62,500) of stream sediment samples collected in part of the Amansie West District in 1992 and list of arsenic concentrations determined in these samples, obtained from the Geological Survey Department, Accra, Ghana.
- Boundary map (at scale of 1: 250,000) of the district, obtained from the Amansie West District Administration.
- ASTER imagery (level 1B) acquired on 15/01/2002, obtained from the US Geological Survey.
- Landuse/landcover map of Ghana (traced on Landsat TM data of 1998 and published in the same year), obtained from the Remote Sensing Application Unit (RSAU), University of Ghana, Legon.

The GIS operations were carried out in three principal steps: (1) spatial data capture; (2) generation of spatial factor maps; and (c) spatial data analysis. The GIS operations were carried out using ILWIS (Integrated Land and Water

Information Systems), a GIS software package developed by the International Institute for Geo-information Science and Earth Observation (ITC) in the Netherlands.

4.3.2 Spatial data capture

The different analog maps were scanned then georeferenced (by defining the x and y coordinates of the corner points of the maps) into a UTM coordinate system. From the scanned map, spatial data were captured by screen digitizing. From the topographic map, rivers, streams and gullies were digitised as line segments as were elevation contours. The boundaries of the district were digitised as line segments and then polygonized. The locations of centres of 61 settlements (identifiable on the topographic map) were digitised as points and the BU incidence in 1999 was recorded as spatial attribute of each settlement.

From the stream sediment sample location map, the locations of the samples were digitised as points and the arsenic concentrations (in ppm) were recorded as a spatial attribute of each sample. The ASTER imagery was also georeferenced to the same coordinate system using eight reference points (tie points), which were selected in the image and which could be identified in the topographic map. Using an affine transformation, a root mean square error (RMSE) of 0.58 pixel was obtained in georeferencing the ASTER imagery.

For each of the settlements with incidence of BU the percentage prevalence of BU was calculated. Prevalence expresses cases of a disease in terms of the proportion of the population afflicted at a specified time (Colton, 1974). It is expressed here as the number of BU cases in a settlement in 1999 divided by the estimated population in 2000 multiplied by 100 to yield a percentage.

4.3.3 Spatial factor maps

The spatial factor maps generated from the stream sediment geochemistry data for use in the spatial analysis were: (a) map of arsenic-enriched catchment basins; (b) map of arsenic-enriched farmlands; (c) map of arsenic-enriched drainage channels.

The stream sediment geochemical data for arsenic were initially analysed statistically to determine a threshold value that divides the data into background (normal) classes and anomalous classes of arsenic concentrations. The data are lognormally distributed. After considering the toxicity of arsenic and its poisoning in drinking water and soil, especially in the tropics, a geometric median of 8.0 ppm As and standard deviation of 2.8 ppm As were obtained. The threshold value was therefore set at 20 ppm As (i.e., approximately the median plus one standard deviation (Salminen and Tarvainen (1997)). The spatial distribution of arsenic was then mapped through the generation of a catchment basin anomaly map in which a sample catchment basin is assigned the geochemical attribute of the corresponding sample (Bonham-Carter et al., 1987; Carranza et al., 1997). Generation of sample catchment basins involved the following steps (using ILWIS):

- creation of a raster digital elevation model (DEM) through interpolation of elevation contours;
- generation of raster map of drainage lines; and
- calculation of sample catchment basin boundaries via an iterative calculation procedure involving the DEM and the raster map of drainage lines.

The catchment basin map of arsenic concentrations was then classified into a binary map showing arsenic-normal areas (with $\leq$ 20 ppm As) and arsenic-enriched areas (with > 20 ppm As) as shown in Figure 4.1. Approximately 24% of the study area is occupied by arsenic-enriched catchment basins.

A supervised classification of ASTER imagery was carried out to distinguish between the major landcover/landuse classes known in the area. These landcover classes are (a) forest areas, (b) residential areas or settlements (bare of vegetation), and (c) farmlands. Using the available landuse/landcover map and topographic map as references, training pixels of known landuse/landcover classes were selected using a colour composite of ASTER bands 2, 3 and 4. These three bands gave the highest optimal index factor (OIF), which indicates the combination of three spectral bands that provide optimum information about landcover (Jensen, 1986).

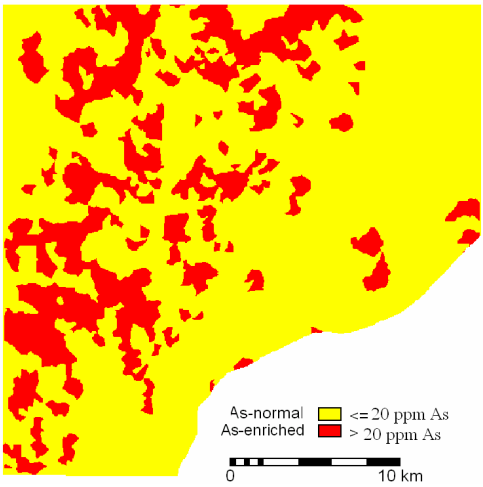

Figure 4.1. Binary map of catchment basin

The box classifier (Lillesand and Kiefer, 2000) was chosen for the image classification. The classified image (Figure 4.2), which was also validated in the field, has an overall accuracy of at least 91% with reference to the landcover/landuse map. The classified image indicates that about 91% of the area is farmland.

To determine arsenic-enriched farmlands, a Boolean AND operation was performed by crossing the catchment basin anomaly map and the classified landcover/landuse image. About 21% of the total area of farmlands in the classified image is arsenic-enriched.

A Boolean AND operation was performed by crossing the catchment basin anomaly map and the raster map of drainage lines. About 22% of the total length of drainage lines is indicated to be arsenic-enriched.

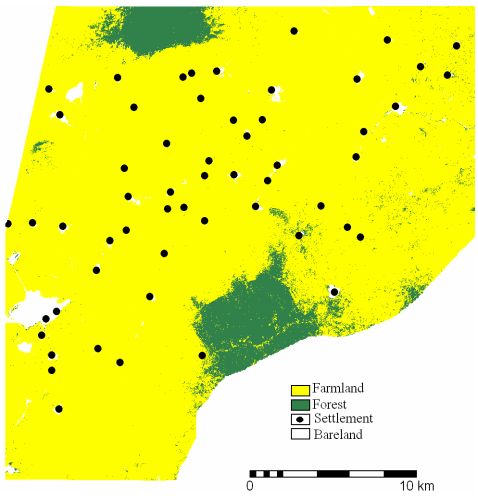

Figure 4.2. Landuse/landcover map

4.3.4 Spatial data analysis

The inhabitants of a settlement earn their livelihoods by exploiting the resources of the surrounding land. This land influences their exposure to infections and to environmental factors that dispose to infections. Proximity analysis was therefore used to determine spatial relationships between BU prevalence per settlement and (i) arsenic-enriched farmlands and (ii) arsenic-enriched portions of the drainage system. The proximity analysis was carried in two principal steps. First, maps of distances from arsenic-enriched farmlands and arsenic-enriched portions of the drainage system were generated. Second, the point map of BU prevalence per settlement was overlaid on (or crossed with) each of these maps.

A buffer is a zone of specified distance around a selected map feature. A GIS creates buffer zones around selected map features such as arsenic-enriched farmlands and arsenic-enriched portions of drainage systems. Around each of

these, buffers were set at intervals of 100 m up to 1000 m. Each buffer zone map was crossed with BU prevalence data of settlement to determine how many of these fall within and outside of the buffer.

At each increasing interval of 100 m, a test of the significance of the difference of the mean BU prevalence within the buffer and outside of the buffer is made using the t-statistic:

$$t_{ij} = (\bar{x}_i - \bar{x}_j) / \sqrt{s_p^2 (1/n_i + 1/n_j)}$$

where i is the BU prevalence inside the buffer zone, and j is the BU prevalence outside the buffer zone, $\bar{x}_i$, $\bar{x}_j$ are the sample means for the i^{th} and j^{th} buffer, s_p^2 is the pooled sample variance, n_i and n_j are the sample sizes from population i and j respectively. It is best in this hypothesis that buffers i and j have the same mean. Using t_{ij} and degrees of freedom given by $n_i + n_j - 2$, a t distribution provides the probability, p that the means are different.

4.4 Results

For the buffers tested, p values range from 0.09 to 0.46 (Table 4.1).

Table 4.1. Drainage and farmland buffer distances and p-values.

Buffer distance (m)	100	200	300	400	500	1000
p-value (drainage channels)	0.09	0.12	0.17	0.33	0.33	0.13
p-value (farmlands)	0.46	0.19	0.21	0.09	0.16	0.17

The buffers with the lowest p-values (i.e., 0.09) are 100 m for drainage channels and 400 m for farmlands. With these buffers 24 of the 61 settlements (i.e., 39%) fall within 100 m of arsenic-enriched drainage channels (Figure 4.3a) and 41 of the 61 settlements (i.e., 67%) fall within 400 m of As-enriched farmlands (Figure 4.3b). The mean BU prevalence within and beyond the drainage buffer are 0.70% and 0.35% respectively whereas the mean BU prevalence inside and outside the farmland buffer are 0.60% and 0.25% respectively. Thus the

naturally smaller number of settlements within the drainage buffer (i.e., 24) has a slightly higher BU prevalence than the relatively larger number of settlements within the farmland buffer (i.e., 41).

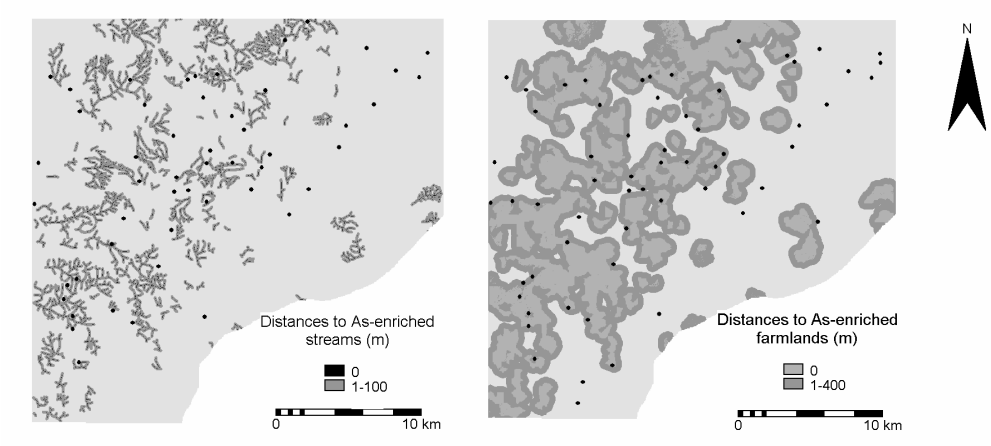

Figure 4.3. Distances to (left) arsenic-enriched drainage channels; (right) arsenic-enriched farmlands.

4.5 Discussion

Siting of rural settlements in the study area is based primarily on proximity to and availability of water for drinking and other domestic purposes. In spite of the availability of groundwater many inhabitants use (and even prefer) surface water. Consequently, many settlements are located within the optimum buffer distance of 100 m from drainage channels. Where water is abstracted from drainage channels enriched in arsenic, chronic ingestion of arsenic-enriched water through drinking and cooking is likely. This renders the inhabitants susceptible to several kinds of diseases (Abernathy et al., 1999; NRC, 1999) including BU. Amofah et al. (1993) studied 90 BU patients and found that 52 used surface water as the source of their drinking water. The result of the statistical analysis corroborates this observation: BU prevalence is highest where the inhabitants have ready access to domestic water supplies from arsenic-enriched surface drainage.

Subsistence farmlands, especially those that depend partially on irrigation, tend to be located along stream floodplains. Soils in these floodplains have a high cation exchange capacity (Adu, 1992) so that, where streams carry high concentration of arsenic, there is accumulation of arsenic in the soils of the floodplains. These high concentrations of arsenic are in part taken up by the foodcrops grown there (Sarkodie et al., 1997; Alam et al., 2003; Warren et al., 2003). The results of the statistical analysis suggest that a high proportion of settlements with high BU prevalence exploit such floodplain farmlands enriched in arsenic.

Through consumption of arsenic-enriched drinking water and arsenic-enriched foodcrops, inhabitants in some settlements in the Amansie West District are prone to chronic ingestion of higher-than-average (but sub-toxic) levels of arsenic. Arsenic could predispose to defect the immune system (Lantz et al., 1994). Subjects exposed to high levels of arsenic concentrations were reported to have had impaired immune response (Gonseblatt et al., 1994). Immunosuppression due to arsenic has ben found to defect antigen processing of splenic macrophages with consequent defective mechanism of helper T-cells (Lewis et al., 1998a, 1998b). Down-regulation of the immune system is known to be a risk factor for the development of BU (Stienstra et al., 2001; Van der Werf et al., 1999). Several studies (e.g., Rosales-Castillo et al., 2004; Gerdsen et al., 2000; Aranyi et al., 1985) have reported of impaired resistance to viral/bacterial infection via arsenic ingestion.

4.6 Conclusions

The results of this study reveal spatial dependency of BU prevalence upon proximity to drainage channels and farmlands containing > 20 ppm arsenic. Proximity implies chronic exposure to and/or ingestion of elevated concentrations of arsenic, which influences susceptibility to infection.

CHAPTER 5

SPATIAL RELATION BETWEEN ARSENIC IN DRINKING WATER AND *MYCOBACTERIUM ULCERANS* INFECTION

This chapter is based on

Duker, A. A., Carranza, E. J. M., Hale, M. 2005. Relation between drinking water and *Mycobacterium ulcerans* infection in the Amansie West District, Ghana. *Mineralog Mag* (**in press**).

Based on the findings of chapter 4, this chapter investigates the application of an exposure-response model to determine any relationship between arsenic in potable water and BU development.

5.1 Introduction

Arsenic is widely distributed throughout the earth's crust and occurs in the natural environment as a main constituent of more than 200 mineral species (Thornton, 1996). Through natural weathering and dissolution of these minerals dissolved arsenic enters potable water sources. For some time the World Health Organization (WHO) recommended that the maximum arsenic concentration in potable water should be 50 μgl^{-1}. However, about ten years ago it reduced its recommended maximum to 10 μgl^{-1} (WHO, 1993). The European Union (EU) maintained the standard of 50 μgl^{-1} until 1998, and since then it has adopted the new WHO standard. Since compliance will be a very costly proposition, most countries (especially developing countries) still maintain the 50 μgl^{-1} maximum.

Many cases involving acute arsenic poisoning in well water have been documented, e.g., in Bangladesh (Mudur, 2000), West Bengal (Das et al., 1996), Canada (Grantham and Jones, 1977), Chile (Borgono and Greiber, 1972; Zaldivar, 1974; Smith et al., 1998), Argentina (Astolfi, 1971; Hopenhayn-Rich et al., 1996), Taiwan (Tseng et al., 1968). The human health effects of chronic arsenic exposure include skin lesions and problems with the circulatory system, including numbness in the extremities. Several studies have established that high arsenic concentration in drinking water is associated with various kinds of skin cancers as well as cancers of the liver, bladder respiratory and gastrointestinal tracts (US EPA, 1988; Reymann et al., 1978; Chen et al., 1985, 1986). Arsenic exposure is known to interfere with the actions of several enzymes in the body (Abernathy et al., 1999) and hence essential actions in the cells, which may consequently lead to several kinds of diseases (NRC, 1999).

The adverse health effects of arsenic might also indirectly influence bacterial or viral infections (Rosales-Castillo et al., 2004; Gerdsen et al., 2000; Grimmel et al., 1988; Harada, 1996). For instance, *in vitro* studies carried out by Ahmad et al. (2000) indicate that arsenic is capable of releasing iron from human liver ferritin and horse spleen ferritin, which led the authors to hypothesize that this

could also occur *in vivo*. The release of iron by arsenic is thought to enhance bacteria adhesion to human tissues and influence their ability to establish infection (Rodriguez and Smith, 2003; Weinberg, 1978; Sugarman, 1980). Gerdsen et al. (2000) reported of the detection of a virus (atypical human papillomavirus) in skin biopsies of patients affected with arsenical keratosis. This may be considered as a result of impairment in cell-mediated immune function, which could also result in defective protective mechanism in the immune function, and thus enhance viral/bacterial infection. It raises potential risk in the development of BU. In their study of cytokine profiles of BU patients, Gooding et al. (2001, 2002) report that the immunosuppressive properties of the mycolactone secreted by MU alone are not likely to account for the induced systemic effects in BU patients and that this could be attributed to immune defect predisposing to MU infection. Inhabitants of the study area depend on groundwater and/or surface water for drinking and for other domestic purposes. Many inhabitants, however, use surface water mostly because it is easily available and partly because they have become accustomed to the taste. Those using surface water for drinking try to fetch the available water even when it is muddied, as is common when streams begin to dry up during the dry season (November-March).

The common diseases in the area, as reflected in order of cases at the only district hospital, are malaria, anaemia, gastroenteritis, renal diseases, skin diseases including Buruli ulcer, diabetes mellitus, hypertension, septicaemia, hepatitis and pneumonia (Etuaful, pers. comm., 2001). Severe anaemia and malnutrition are common in children. The previous chapter indicated that BU prevalence in settlements close to arsenic-enriched drainages is greater than elsewhere.

5.2 Materials and methods

5.2.1 BU data

The following are data inputs for the calculation and mapping of BU prevalence per settlement.

- Buruli ulcer cases in 1999 from the National Search, Korle-BU Teaching Hospital, Accra, Ghana.

- Buruli ulcer cases for the years 2000-2002 from the District Health Administration, Amansie West District, Ashanti Region, Ghana.

- Topographic maps (sheets 0602C1, C2; 0603D2, D4 of 1974 at a scale of 1:50000) showing the location of settlements in the study area, obtained from the Survey Dept., Accra, Ghana.

- Boundary map (at scale of 1: 250,000) of the district obtained from the Amansie West District Administration.

- Settlement population estimates for 2000, as projected by the Ministry of Local Government and Rural Development.

5.2.2 Arsenic data

Water samples were collected at a total of 76 sites (generally occurring in the settlements) where the local inhabitants abstract water. Of these samples, 39 were groundwater and 37 were surface water. All samples were collected in February 2003. This time of the year marks the dry season, when streams begin to dry up and the water table falls, thus raising concentrations of dissolved solids including arsenic. Surface water abstraction points were often dug out in sites close to streams so as to contain water even during the dry season.

A Global Positioning System (GPS) was used to determine the geographic coordinates of the sample sites. On-site analysis consisted of measuring temperature, pH and electrical conductivity. Samples were filtered through 0.45 μm filter to remove particulates into a 500 ml plastic bottle and acidified with nitric acid to a pH < 2. Subsequently the samples were analysed by neutron activation by the National Nuclear Research Institute Laboratory, Ghana, for As.

5.2.3 Spatial Data Capture

The spatial data were managed and analysed using the GIS software package ILWIS (Integrated Land and Water Information System). The analogue maps were raster scanned and georeferenced, from which spatial data were screen-digitized. Streams and the boundary of the district were digitized as line

segments. The locations of settlements, groundwater and surface water sample sites were digitized as points. The four spatial data layers (point locations of settlements, point locations of surface water samples, point locations of groundwater samples and line segments of water courses) were overlaid to create a map view of the study area (Figure 5.1).

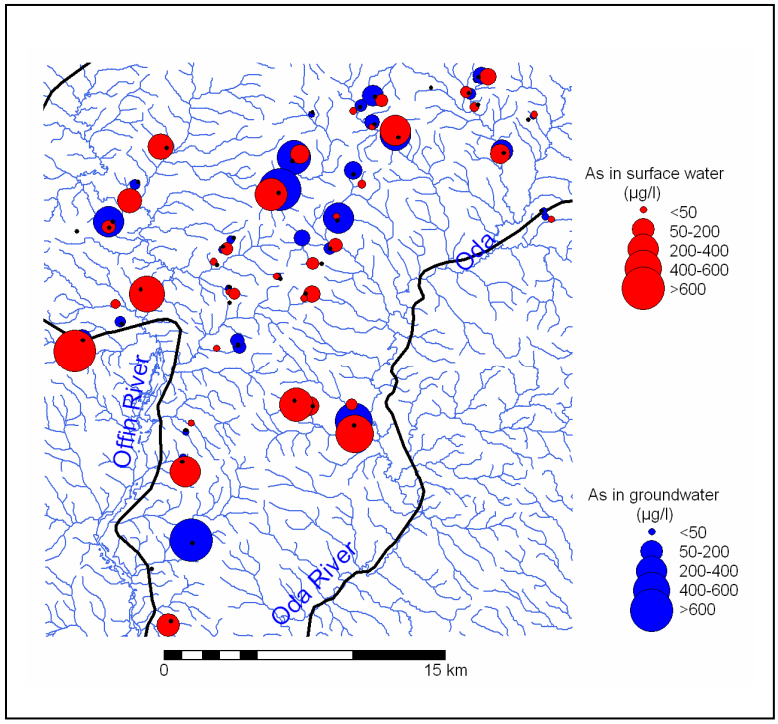

Figure 5.1. Arsenic levels in groundwater and surface water at potable water abstraction points in the study area.

Arsenic concentration levels in both groundwater and surface water are interpolated using Topo to raster method, which creates a simple surface and does not create artificial peaks. The segment map of the Offin/Oda Rivers and their tributaries were overlayed to determine the relationship of the basins with the arsenic concentration levels (Figure 5.2).

Population estimates and incidences of BU for the period 1999-2002 were recorded as spatial attributes of the settlements. Arsenic concentrations in groundwater and surface water were recorded as spatial attributes of the sample sites.

It is also hypothesized in this study that anthropogenic activities in either of the Basins generate high arsenic concentrations levels in both surface water and groundwater, and that this reduces further away from the river channels. This hypothesis is tested by crossing the rasterized point maps of groundwater and surface water with the rasterized maps of the Offin/Oda channels and regressing the arsenic concentration levels on distance in order to determine the relationship of arsenic (in groundwater and surface water) with distance from the river channels as well as derive (if any) a relationship with BU.

5.2.4 Spatial data analysis

To assess potential likelihood of health disorders (i.e., response) occurring due to exposure to prevailing environmental conditions (e.g., arsenic levels), there is the need to model an exposure-response relationship (Edler et al., 2002; Evans et al., 2001). In this case, the exposure-response relationship to be modelled is amount of response (measured as BU prevalence) as influenced by potential exposure to arsenic in surface and/or groundwater. First sub-populations using water with specified arsenic concentrations are determined. Then the number of BU cases within each sub-population is used to determine the prevalence of BU (in percentage) per sub-population. Prevalence per sub-population is plotted against midpoint of intervals of arsenic concentration (e.g., Yang et al., 2002) and linear regression is used to model an exposure-response relationship.

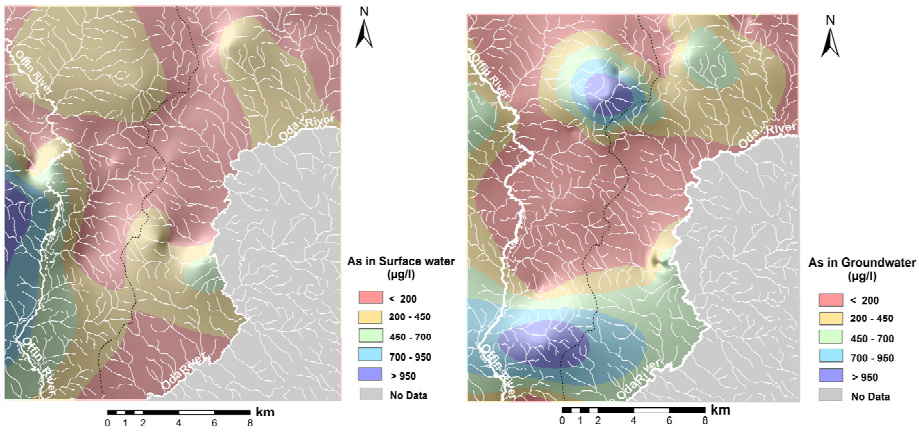

Figure 5.2. Spatial relations of arsenic in (left) surface water; (right) groundwater and the Offin/Oda Basins with light black line as watershed between the basins.

5.3 Results

5.3.1 Analysis of arsenic levels in groundwater and surface water

The range of arsenic concentrations in groundwater and surface water are 10-1200 μgl^{-1} and 5-2900 μgl^{-1} respectively (Table 5.1). Of the 39 samples of groundwater, 56% and 90% exceeded the 50 μgl^{-1} and 10 μgl^{-1}, respectively, of Ghana and WHO guidelines maxima for arsenic. Of the 37 samples of surface water 65% and 84% also exceeded the 50 μgl^{-1} and 10 μgl^{-1}, respectively, of Ghana and WHO guidelines maxima for arsenic.

Table 5.1. Statistical summary of arsenic in surface water and groundwater.

	Min.	Median	Mean	Stdev.	Max.
As (μgl^{-1}) in surface water (SW)	5.0	74.0	221.8	481.1	2900.0
As (μgl^{-1}) in groundwater (GW)	10.0	59.0	170.6	269.5	1200.0

5.3.2 Data distribution by basin

Analysis of the relationship between BU and distance from the main Offin River channel showed a strong inverse relationship (i.e., $R^2 = 0.27$, $p < 0.001$). Arsenic in surface water and distance from the Offin River channel showed also an inverse relationship ($R^2 = 0.03$, $p = 0.29$), whilst that of groundwater showed no relationship ($R^2 = 0.00$, $p = 0.74$); indicative of some relationship between arsenic in surface water and BU (Figure 5.3 (top)).

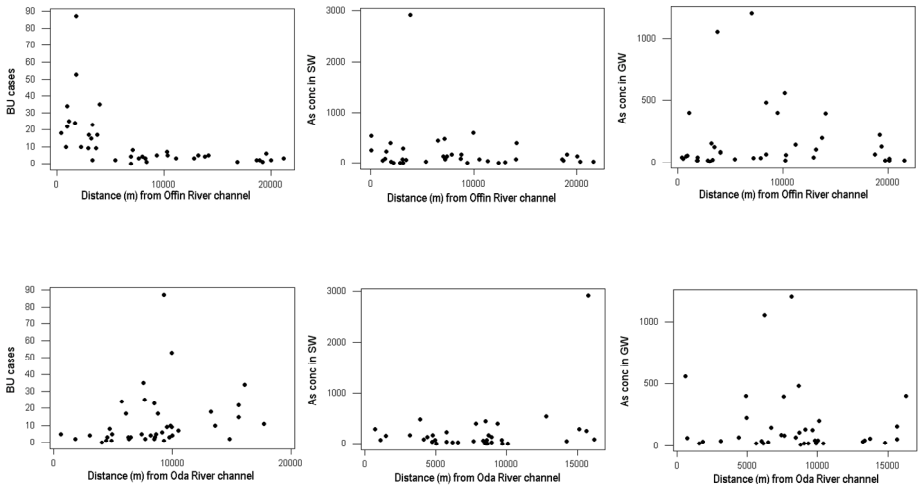

Figure 5.3. Spatial relations of BU and As with the Offin and Oda Basins in the area.

On the contrary, relationship between BU and distance from the Oda River channel has a direct positive relationship (i.e., $R^2 = 0.06$, $p = 0.11$). Arsenic in surface water and distance from the Oda River channel also showed similar direct positive relationship ($R^2 = 0.11$, $p < 0.05$), whilst groundwater showed an inverse relationship ($R^2 = 0.01$, $p = 0.54$). This is an indication that both high incidence of BU and elevated arsenic in surface water occur at some distance from the Oda River channel (Figure 5.3 bottom).

5.3.3 Exposure-response of water-arsenic and BU prevalence

Both groundwater and surface water are used for drinking in the study area; therefore the calculated percentage prevalence values for groundwater use and surface water use for the period 1999-2002 is used to obtain models for the study area in general. The relationship between BU prevalence and arsenic in surface water is a significant positive relation (i.e., $R^2 = 0.82$, $p = 0.04$). The relationship, however, between BU prevalence and arsenic in groundwater (i.e., $R^2 = 0.10$, $p = 0.60$) is negative and not significant (Figure 5.4). The models, generated from the data of Tables 5.2 and 5.3 have a *y-axis* representing percentage prevalence of BU and *x-axis* the concentration of arsenic in water.

Table 5.2. Exposure-response relationship between BU prevalence and arsenic in groundwater.

Water-As (µg/l)	Sub-population	BU cases	Prevalence (%)
<50	11219	239	2.13
50-199	9334	114	1.22
200-399	5590	49	0.88
400-599	1180	3	0.25
>599	1232	21	1.70
Total	28555	426	1.49

Table 5.3. Exposure response relationship between BU prevalence and arsenic in surface water.

Water-As (µg/l)	Sub-population	BU cases	Prevalence (%)
<50	9661	178	1.84
50-199	10285	100	0.97
200-399	6031	122	2.02
400-599	365	18	4.93
>599	300	15	5.00
Total	26642	433	1.63

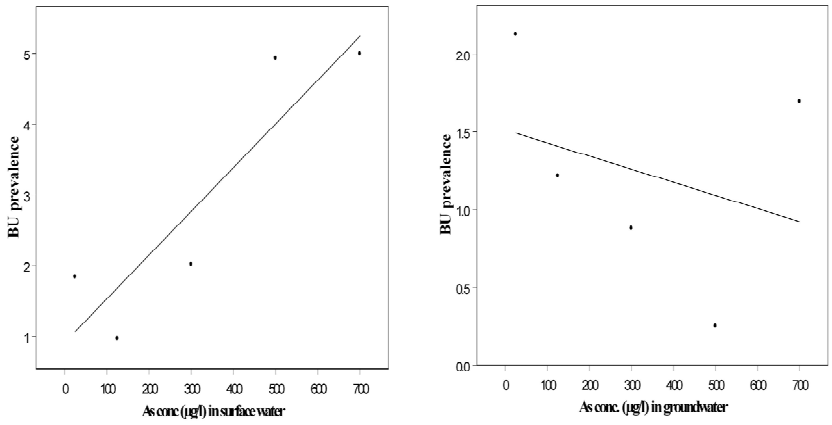

Figure 5.4. Exposure-respose curve for (left) surface water; and (right) groundwater.

5.4 Discussion

High arsenic concentration levels in both groundwater and surface water are much more related to the Offin Basin than the Oda Basin (groundwater wells are constructed in lowlying areas and/or close to river channels). Buruli ulcer is also clearly related to the Offin Basin rather than the Oda Basin. Although statistical analysis between arsenic (in both groundwater and surface water) with respect to distance from the Offin River channel is not significant, that of arsenic in surface water shows a slight positive relationship when compared to that of groundwater.

Generally, arsenic concentrations in surface water are low. This may be due to arsenic adsorption on to Fe-oxyhydroxide; an effective mechanism of arsenic attenuation (Roddick-Lanzilotta et al., 2001; Swedlung and Webster, 1999). However, there were some cases of high arsenic concentration (i.e., 200-2900 μgl^{-1}) in the southern portion of the Offin Basin that may be attributable to dissolved arsenic from stream-washed sulphide materials dissolved by acid generated by anthropogenically disturbed areas, coupled by lack of rainfall and evaporative concentration at this period of sampling (i.e., dry season). Elevated arsenic concentration in groundwater reaches its highest level in the north-central and southern parts of the study area.

Although most settlements have access to both groundwater and surface water, exposure-response models indicate that arsenic concentrations in surface water have a significant positive exposure-response relationship with BU in the study area. With this relationship it may be possible to suggest that other exposure factors (e.g., dietary and inhalation) may also contribute to the incidence of BU in the area. Streams in the area have been noted to have higher As^{3+}/As^{5+} than those of deeper groundwater due to reduction of As^{5+} to As^{3+} by bacterial activity (Smedley et al., 1996), and which is likely to be higher in the Offin River upon which several settlements depend as source of drinking water. Anthropogenic activities in the Offin Basin have for several years helped to discharge sediments into the main channel, causing broad meandering of its valley. The accumulation of the buried sediment load could create a reducing condition, which may trigger the release of the more toxic species of arsenic. On the contrary, there was a negative exposure-response relationship between

BU and arsenic concentration in groundwater. The negative relationship in groundwater could be attributed to both lower concentrations of arsenic and the species of arsenic (As^{3+}, As^{5+}) in water since As^{3+} is known to be 60 times more toxic than the As^{5+} (Smedley et al., 1996). Such relationship has been studied elsewhere (Lin and Tang, 1999; Lin et al., 2002; WHO, 2001) and may form a basis for further study. The exposure-response models in the study area show no threshold, implying that there is no safe limit of arsenic in relation to MU infections, especially when considered in the light of co-factors (e. g., severe anaemia, and severe malnutrition) in the area.

Malnutrition, coupled with anaemia, down-regulates much more the host defense against both pathogenic and opportunistic pathogenic assaults (Sakamoto et al., 1998; McMurray et al., 1990; McMurray, 1984) and exacerbates the severity of the resulting disease (Mve-Obiang et al., 2003; Bär et al., 1998; Pszolla et al., 2003). This may suggest a relationship between severe anaemia, severe malnutrition, which are the two top killer diseases prevalent among children in the study area (Etuaful *pers. Comm*., 2001) and arsenic found in water. It is important to note that severe health effects due to chronic ingestion of arsenic via drinking water have largely been reported in populations of low socio-economic status and poor nutrition (US EPA, 1988). Interestingly, the majority of those affected by BU are children (Van der Werf et al., 1999; Weir, 2002); and in the study area children constitute about 54% of those affected by BU (Amofah et al., 1993).

Prior to 1992, the communities of the region entirely depended on surface water and this might have had its cumulative effect as indicated also by the study of Barker (1973), which indicated that the disease was frequent among inhabitants who use surface water for drinking and for other domestic purposes. A study of 90 BU patients by Amofah et al. (1993) showed that about 58% of them used surface water and 18% used ponds. However, ponds were dug at sites close to streams and received stream inflow.

5.5 Conclusion

The results show that the drinking water sources in the study area have concentration levels of arsenic that are deleterious to health especially with

regards to skin lesions. Arsenic concentrations in surface water as compared to groundwater were significantly positively associated with BU.

Although arsenic in groundwater is generally more elevated, arsenic in surface water has more extreme values. BU prevalence is predicted by arsenic in surface water but not by arsenic in groundwater.

Chronic arsenic ingestion may help to impair the immune system indirectly through co-factors (e.g., severe anemia, severe malnutrition) or directly decrease immune surveillance, which may then compromise the host defense mechanisms against opportunistic pathogenic infections. The result of this study, despite the small sample size, suggests an association between arsenic exposure and BU incidence in the area. However, studies with a larger sample size are needed to confirm this association.

CHAPTER 6

SPATIAL RELATION BETWEEN MINING AND *MYCOBACTERIUM ULCERANS* INFECTION

This chapter is based on

Duker, A. A. and Hale, M. Spatial relation between mining and *Mycobacterium ulcerans* infection in the Amansie West District, Ghana. Submitted to *J Appl Earth Sci (Trans Inst Min Metall B)*.

The chapter aims at determining the spatial relationship between artisanal mining of gold, (which is an intervention in the natural environment) and BU by applying proximity and statistical analysis.

6.1 Introduction

Much of Ghana is underlain by Archaean and early Proterozoic rocks of the Man-Leo Shield, part of a former craton spanning western Africa and northeastern South America. In western Ghana the shield is characterised by many north-northeaststriking belts of early Proterozoic metamorphic rocks comprising Birimian (2.1 Ga) metasediments and metavolcanics overlain by Tarkwaian (2.0 Ga) fluviatile clastics (fine-grained phyllites, feldspathic quartzites, grits and conglomerates), intruded by granitoids and dolerites. These belts of Birimian and Tarkwaian rocks, which have been progressively deformed, culminating in overturning and over-thrusting, host almost all of the known gold deposits of Ghana. The Ashanti Belt of southwestern Ghana, though measuring only 250 km long by about 50 km wide, hosts many arsenical lode gold vein and disseminated deposits. The gold occurs as native gold and in sulphide minerals, primarily arsenopyrite.

Gold has been mined in Ghana for at least one thousand years. In former times the Ashanti goldfields were the source of the wealth of the Ashanti kings. Today the gold mining sector in Ghana is one of the most important and fast growing industries, earning foreign exchange for the country. It exerts strong physical, socio-economic and cultural benefits on the nation and particularly on the local residents. The surge in gold mining has resulted in increased gold production, which has established Ghana as the second largest gold producer in Africa, after South Africa (Iddrisu, 1996). Gold mining is carried out on both industrial and artisanal scales. The Ashanti Belt alone has seven industrial gold mines and numerous artisanal mines.

Prior to the enactment of Ghana's Small Scale Mining Law of 1989, artisanal mining was illegal. Nevertheless, illegal gold mining or 'galamsey' had for many years been highly lucrative though small-scale activity. Between 1992 and 1996 these existing artisanal miners and several further groups of entrepreneurs were legally registered. Exploitation of gold is carried out along

natural surface drainage channels, where the miners dig pits or trenches that eventually fill with stagnating water. This disturbance of rivers and streams often contaminates the water downstream, on which the various communities depend, including those in which the artisanal miners live (Smedley et al., 1996).

Whilst artisanal mining has become an important economic sector in Ghana, lack of training, resources and environmental awareness amongst artisanal miners leads not only to environmental damage but also to health hazards. Coincident with the initial wave of legal registration of artisanal miners (1992-1996), Ghana experienced a sharp increase in the incidence of Buruli ulcer, with about 2000 cases recorded in the period 1993-1997 (Grosset et al., 2000) and the Amansie West District in the Ashanti region worst affected (Amofah et al., 2002).

This chapter explores a possible link between BU and artisanal gold mining. Using as a study area part of the Amansie West District, it considers the spatial relation between artisanal minesites and BU. It relates elevated arsenic in the environment to the oxidation of aresnopyrite through mining and the resulting dispersion of arsenic in the drainage systems. Then it assesses the spatial relation between arsenic and BU.

Artisanal mining has several evident environmental impacts, any or all of which could influence human health. Arsenic is investigated here because, elsewhere, increased risk of skin, in particular pigmentation disorders and keratosis, have been linked to long-term exposure to low doses of arsenic (Tseng et al., 1968). Other serious diseases associated with arsenic are dermatological problems (e.g., skin cancer, Bowen's disease) (Chen et al., 1988a; Chen and Wang, 1990), cardiovascular diseases (Blackfoot disease, Raynaud's syndrome, hypertension, gangrene) (Tseng, 1977; Chen et al., 1995; Chowdhury et al., 1999), as well as neurological, respiratory and hepatic diseases and diabetes mellitus (Chen et al., 1997a; Tseng et al., 2000; Lai et al., 1994). Arsenic interferes with several enzymes in the body (Abernathy et al., 1999; NRC, 1999) and could predispose to defects in the immune system (Harrison and McCoy, 2001; US.EPA Office of Water, 2001; Lantz, 1994; Frenkel et al., 2002), lowering resistance to bacterial/mycobacterial infections (Stienstra et al., 2001).

6.2 Materials and methods

6.2.1 Sources of data

- Boundary map (at scale 1: 250000) of the Amansie West District, obtained from the District administration. After scanning, the district boundary was screen digitized as line segments and polygonized, and the locations of settlements were digitized as points.

- Topographic map (at scale 1: 50000), which was scanned and from which streams and rivers were digitized as line segments.

- Landcover/landuse map of Ghana (traced on Landsat TM data of 1998 and published in the same year), obtained from the Remote Sensing Application Unit (RSAU), University of Ghana, Legon.

- BU incidence for settlements in the study area: (1) for 1999 from the Korle-BU Teaching Hospital, Accra, Ghana; and (2) for 2000-2002 from the District Health Administration, Amansie West District, Ashanti Region, Ghana. These data are combined and attributed to settlements.

- ASTER imagery (level 1B) acquired on 15/01/2002, obtained from the USGS. The imagery was georeferenced (using affine transformation) to the UTM coordinate system using reference points selected in the image and which could be identified in the topographic map. Root mean square error (RMSE) was 0.58 in georeferencing. The georeferenced image is further resampled using the bilinear interpolation method. With the available landuse/landcover map and topographic map as references, a supervised classification was performed using bands 2, 3, and 4 (which gave the highest optimal index factor), and the resulting image was stretched to enhance the delineation of disturbed ground or minesites.

- Arsenic concentrations of 1125 stream sediment samples collected in 1992. The sample locations were digitized as points (from stream sediment location map) and the arsenic concentration (ppm) recorded as spatial attribute of each location.

6.2.2 Method

Spatial data analysis was carried out using ILWIS (Integrated Land and Water Information Systems), a GIS software package developed by the International Institute for Geo-information Science and Earth Observation (ITC) in the Netherlands. The spatial data consists of points, segments and polygons, which are allocated by means of (x, y) UTM coordinates, while the attribute data are stored as records in a relational data base. The exploration of the spatial relations between minesites, arsenic and BU relies on distance calculation and buffer zones.

The incidence data for BU are available per settlement. The arsenic data, available for stream sediment sample locations throughout the study area, were used to estimate the arsenic burden per settlement. The calculation was performed using weighted inverse distance interpolation of all arsenic concentrations within a search radius of 1 km around a settlement (a radius chosen so that search radii do not overlap). It is the simplest interpolation method, which applies weights that are a decreasing function of distance. It does not make assumptions about spatial relationships (except that nearby points are more closely related than distant points to the value of the interpolate location). Since this study aims at determining the influence the metalloid may have on the inhabitants of the settlements within 1 km radius it has not been necessary to look into other methods. The interpolation equation is expressed mathematically as follows:

$$As_0 = \frac{\sum_{i=1}^{N(As_0)} 1/d_i^{\lambda} As_i}{\sum_{i=1}^{N(As_0)} 1/d_i^{\lambda}}$$

where As_0 is the estimated concentration at the centre (x_0, y_0) of the settlement, As_i is a neighbouring arsenic concentration at (x_i, y_i), d_i is the distance between the points (x_0, y_0) and (x_i, y_i); λ is the power (where in this case $\lambda = 2$), and $N(As_0)$ is the number of data points in the neighbourhood. This method of calculation recognizes that the population of a settlement is exposed (e.g., through drinking water, foodcrop consumption and occupational activities) to

arsenic in its surrounding environment, and that its arsenic exposure is approximately proportional to the arsenic concentrations of stream sediments substantially derived from the soils and groundwater of that environment.

A buffer zone is a polygon with boundaries at a specified distance around a pixel or a number of pixels that constitute a selected map feature. Buffer zones were used as a basis for exploring the spatial relation between minesites and the attributes of settlements. Buffer zone maps were generated around minesites interpreted from the classified ASTER imagery at ten intervals of 200 m up to 2000 m. This range was chosen because buffer distances <200 m would classify only settlements at minesites inside the buffer whilst buffer distances >2000 m would include almost all settlements in the study area inside the buffer zone. Each buffer zone map was crossed with mean BU incidence per settlement to determine the buffer distance at which the discrimination between arsenic burdens inside and outside the buffer is optimal. For each of the ten buffer zone maps a test of significance of constant incidence was calculated using the t-statistic:

$$t_{ij} = \frac{\bar{x}_i - \bar{x}_j}{\sqrt{s_p^2 (\frac{1}{n_i} + \frac{1}{n_j})}}$$

where *i* is the BU incidence inside the buffer zone, *j* is the BU incidence outside the buffer zone, $\bar{x}_i$ and $\bar{x}_j$ are sample means, s_p^2 is the pooled sample variance, n_i and n_j are the sample sizes from population *i and j*. Each buffer zone map was also crossed with estimated arsenic burden per settlement to determine the buffer distance at which the discrimination between arsenic burdens inside and outside the buffer is optimal. For each of the ten buffer zone maps a test of significance of constant arsenic burden was calculated using the t-statistic, where in this case *i* is the arsenic burden inside the buffer zone, *j* is the arsenic burden outside the buffer zone.

Using the optimal buffer distance for twin attribute discrimination, dependency of BU incidence on arsenic burden was evaluated by linear regression for settlements inside the buffer and settlements outside the buffer. Finally the

spatial relation between BU incidence and arsenic burden was mapped after classifying the variables according to thresholds extracted from the regression analysis and the scientific literature.

6.3 Results

For the 39 settlements in the study area, the BU incidence per settlement is 0-87 cases and the estimated arsenic burden per settlement is 2-100 ppm. The results of the t-tests for significant spatial discrimination of BU incidence per settlement per settlement and estimated arsenic burden per settlement are shown in Figure 6.1. The maximum peak t-test values show that the optimum spatial discrimination for BU incidence occurs at 1000 m from minesites and the optimum spatial discrimination for arsenic burden is just slightly less. The probability of these distributions occurring by chance is 0.2% in the case of BU incidence and 0.1% in the case of arsenic burden. The common spatial discrimination buffer was chosen at 1000 m, resulting in 15 settlements within the buffer and 24 settlements beyond the buffer.

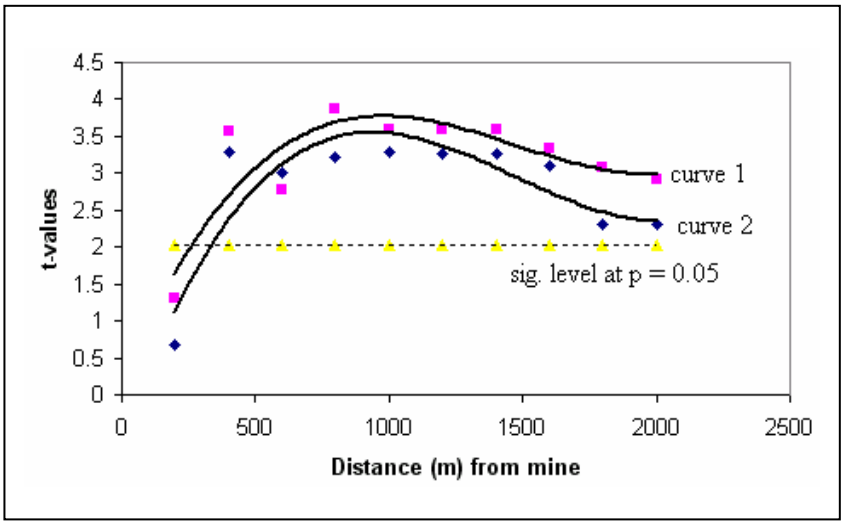

Figure 6.1. Quantitative assessment of discrimination obtained by applying experimental buffer zones around minesites: curve 1= arsenic burden; curve 2= BU incidence

For the settlements within the buffer, BU incidence ranges from three to 87 cases per settlement and arsenic burdens range from seven to 100 ppm.

Regression of BU incidence on arsenic burden yields a best-fit line with a positive slope (R^2 = 0.48. p < 0.01) (Figure 6.2 (left)), indicating a linear dependency of BU incidence on arsenic burden. For settlements beyond the buffer, BU incidence ranges from zero to eight cases per settlement and arsenic burdens range from two to 47 ppm. Regression yielded a best-fit line with zero slope (R^2 = 0.00, p = 0.90) (Figure 6.2 (right)) suggesting that BU incidence here is not dependent on arsenic burden.

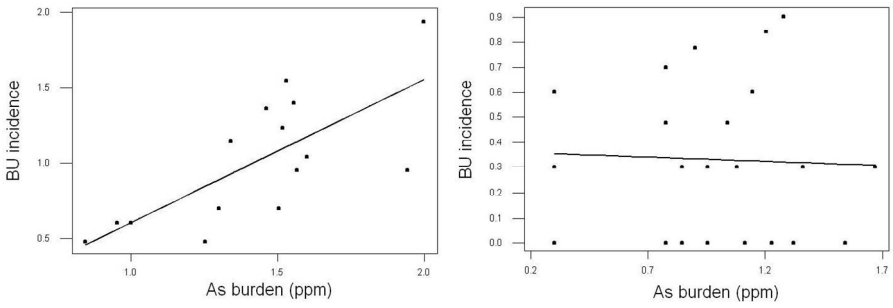

Figure 6.2. Log-log plots of BU incidence per settlement on arsenic burden per settlement: (left) within minesite buffer zone; (right) beyond minesite buffer zone.

Whilst regression analysis makes clear the different influence of arsenic burden on BU incidence within and beyond the buffer, the ranges of each variable within and beyond the buffer clearly overlap. In order to map effectively the relation between BU incidence and arsenic burden throughout the study area, a bi-partite classification of each variable made using independent criteria. Arsenic burdens are classified above or below a threshold of 20 ppm, which is used elsewhere for food sensitivity to arsenic (USEPA, 1993) and as a health hazard in residential areas (CCME, 1991). For BU incidence, the threshold is eight cases per settlement, the highest incidence beyond the 1000 m buffer around minesites in the study area. On the resulting map is shown in Figure 6.3.

By virtue of the threshold criterion, all settlements with a high BU incidence (>8 cases) fall within the buffer zone, which also includes four settlements with low BU incidence (<8 cases). All of these settlements also exhibit a high arsenic burden (>20 ppm), whereas most of those within the buffer zones with a low BU incidence exhibit a low arsenic burden. This is consistent with the relation found by regression analysis. The sole exception is one settlement which has low BU incidence but high arsenic burden.

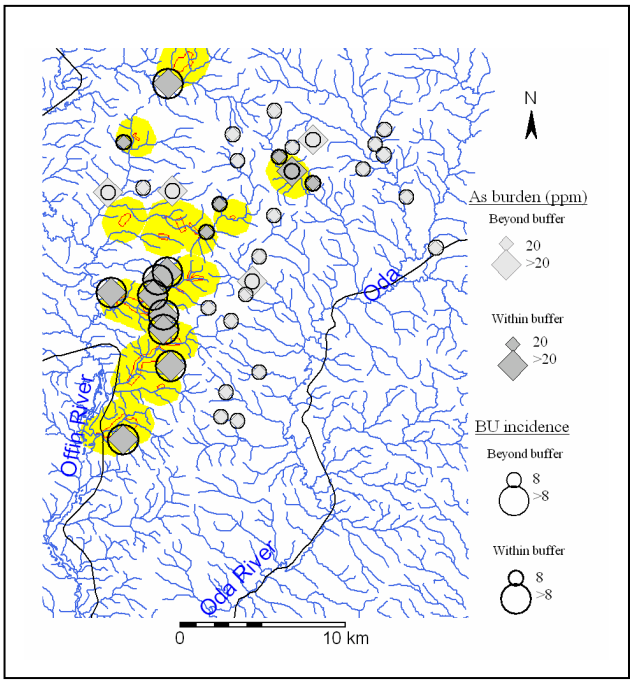

Figure 6.3. Spatial relations of BU and arsenic burdens inside yellow buffer (dark grey diamond in dark circles) and outside buffer (light grey diamond in light grey circles).

The threshold criterion also means that all settlements beyond the buffer have a low BU incidence (<8 cases). Most of these settlements exhibit a low arsenic burden (<20 ppm), although four exhibit a high arsenic burden.

Most minesites and settlements with high BU incidence and high arsenic burdens lie on tributaries of the Offin River, which drains the west of the study area. In the case of the Oda River, which drains the east of the study area, there is a minesite on only one tributary and settlements have low BU incidence and, mainly, low arsenic burdens.

Discussion

In the Amansie West District, disturbance of fluvial sediments by artisinal gold mining in drainage channels accelerates the oxidation of arsenopyrite associated with the gold. The oxidation reaction generates a low pH environment and liberates ferrous and arsenate ions:

$$4FeAsS + 13O_2 + 6H_2O = 4Fe^{2+} + 4AsO_4^{3-} + 12H^+$$

The stream water into which the ferrous and arsenate ions are liberated constitutes a supply of drinking water for the artisinal miners and the inhabitants of nearby settlements. Ahmad et al. (2000) found that artisanal miners in the study area had high levels of arsenic (i.e., 1.4-3.4 $\mu g g^{-1}$) in hair samples as compared to controls. According to Hindmarsh et al. (1977) this level of arsenic equates with drinking water with an arsenic concentration of about 400 $\mu g l^{-1}$. This is consistent with concentrations in water of up to 535 $\mu g l^{-1}$ As reported in chapter 5 in the drainage system of the study area.

High rainfall in the study area results in the downstream transport of substantial fluxes of arsenic from artisanal mine workings (Craw et al., 2004). As the acidic stream water from a minesite mixes downstream with natural water of higher pH, arsenate is increasingly adsorbed onto the surfaces of clays and iron-manganese colloids in stream sediments, resulting in lower arsenic concentrations in drinking water. The arsenic concentrations within the sediments typically exceed 20 ppm within the 1000 m buffer zone set around minesites. In times of flooding, which is frequent in the study area, these arsenic-bearing sediments are deposited on river floodplains, which constitute the farmland soils of the local inhabitants. Foodcrops grown on arsenic-enriched soils tend to take up arsenic (Sarkodie et al., 1997; Alam et al., 2003). Furthermore, when prolonged flooding produces anaerobic and reducing conditions in soils, readily-available soluble arsenic in the form of As^{3+} is

liberated (Deuel and Swoboda, 1972; Hess and Blanchar, 1977; McGeehan and Naylor, 1994; Mok and Wai, 1994; Reynolds et al., 1999). A study of Masscheleyn et al. (1991) showed that following flooding and reduction of arsenic-contaminated soil, it was found that, (1) arsenic was released first before the dissolution of Fe and that (2) as Fe-oxide dissolved more arsenic (As^{3+} and As^{5+}) were released. McGeehan (1996) also knew that in flooded soils As^{5+} species were reduced to As^{3+}, but was unsure as to whether they occurred in the soil solution or on soil particles.

The inhabitants of settlements near to minesites are exposed to arsenic not only through arsenic dispersion in solution but also through airborne dispersion of arsenic (Hinwood et al., 2004; Kavanagh et al., 1997). Amasa (1975), for example, reported of arsenic and sulphur poisoning 8 km north of Obuasi (a goldmining town in Ghana), resulting in chronic eye inflammation, destruction of vegetation and uptake (of arsenic) in foodcrops and drinking water. The arsenic-bearing dust created by mining operations is inevitably inhaled and transported from the lungs through the mucocilliary process to the gut and then mixed with saliva and swallowed (Ruttenber and Kimbrough, 1995). Smelting or roasting arsenopyrite-bearing ores liberates As_2O_3 into the air, some of which is inhaled and some of which dispersed by the wind to be deposited in the neighbouring environment (Smedley et al., 1996).

Arsenic toxicity is well established and in the study area there have been several cases of arsenic poisoning resulting from ingestion of contaminated drinking water (Smith et al., 2000). Ingestion of relatively high concentrations of arsenic inactivates enzymes (Abernathy et al., 1999), interferes with metabolic processes (Quig, 1998) and induces immune suppression (Gonseblatt et al., 1994; Blakely et al., 1980). Suppression of immunity impairs resistance to bacterial infections (Gerdsen et al., 2000; Karcher et al., 1999; Hatch et al., 1985; Aranyi et al., 1985) and is considered a risk factor for the development of BU (Stientra et al., 2001; van der Werf et al., 1999). Spatial data analysis has revealed some 250 cases of BU within a 1000 m buffer zone around minesites, compared to 62 cases elsewhere in the study area and, within the buffer zone, a positive linear relation of BU incidence per settlement and arsenic burden per settlement. It seems, therefore, that BU incidence in settlements within the 1000

m buffer zone around minesites might be linked to arsenic-induced suppression of the immune system of the inhabitants.

Conclusion

Within the limitations of exploratory data analysis and the size of the datasets used in this study, there is a reasonable indication that elevated levels of arsenic in the environment are a contributory factor in BU incidence. In the study area, arsenic minerals area associated with gold ore and arsenic is dispersed in the surrounding environment as a result of artisanal mining. Settlements in the vicinity of many minesites have elevated environmental arsenic burdens accompanied by a high incidence of BU. It is therefore conjectured that artisanal mining is implicated in BU incidence.

In the study area, the tributaries of the Oda River are devoid of minesite, and the Oda Basin has mainly low arsenic burdens and low BU incidence. By contrast, several minesites, high arsenic burdens and high BU incidence are found on tributaries of the Offin River. Artisanal mining takes place in river beds and hence arsenic is released into the natural drainage. It is dispersed downstream and, in periods of flood, introduces arsenic into soils of the floodplains on which foodcrops are cultivated. In this way the contribution of arsenic to BU infection might spread significant distances downstream of minesites.

This chapter has investigated only mining-related arsenic as an environmental influence upon BU incidence. Further rigorous research is needed to determine whether other sources of arsenic in the environment and what other environmental factors influence BU incidence. The findings from spatial data investigations can be only indicative, and require confirmation by environmental and epidemiological research.

CHAPTER 7

A STATISTICAL MODEL FOR SPATIAL PATTERNS OF *MYCOBACTERIUM ULCERANS* INFECTION

This chapter is based on

Duker, A. A., Stein, A., Hale, M. 2005. A statistical model for spatial patterns of Buruli ulcer in the Amansie West District, Ghana. *Int J Appl Earth Observ Geoinfo* (**in review**).

Using spatial statistics this chapter models the data based on conditional autoregressive (CAR) model in order to quantify and/or analyze the impact of risk factors in chapter 5 to chapter 7 on BU prevalence, and further bring the preceding chapters into coherence.

7.1 Introduction

Buruli ulcer (BU) is a mycobacterium infection. Subsequent complications may include contractural deformities, amputation of limbs, loss of organs (e.g., eye, breast, genitalia) (Asiedu and Portaels, 2000) and (rarely) death. Currently surgery remains the only means of treatment, but this has not been successful in all cases. Due to limited systematic data collection little is known of the global burden of the disease and true prevalence rates may be underestimated (Hayman and Asiedu, 2000).

Several authors have reported that BU infection is influenced by the environment (Johnson et al., 1999; Portaels et al., 2001; Ross et al., 1997; Johnson et al., 1995) and, more than thirty years ago, Barker (1973) reported that BU incidence is higher amongst inhabitants who use surface water rather than water from deep wells. High BU incidence tends to be clustered along or near surface drainage courses (Mensah-Quainoo, 1998; Radford, 1974b; Barker, 1974; Lunn et al., 1965). Several studies have associated swampy lowland areas with increased incidence of BU (e.g., Barker, 1973, 1972; Pradinaud et al., 1974; Ravisse, 1977). These environments are prone to flooding and development of anaerobic conditions (McGeehan and Naylor, 1994; McGeehan, 1996; Reynolds et al., 1999).

Studies by Lin et al. (2004) have shown that prevalence of endemic diseases is highly correlated with environmental risk factors such as water and soil. For example, health may be affected by ingesting food or water contaminated with arsenic. Arsenic may bioaccumulate in tissues, which may subsequently inhibit the functioning of several sulfhydryl-bearing enzymes in the body (Abernathy et al., 1999; NRC, 1999). Besides, other researchers (eg., Rosales-Castillo et al., 2002; Gerdsen et al., 2000; Grimmel et al., 1988) have found viral infection in arsenic-induced lesions, which may imply a defect in cell-mediated immune

function with consequent enhanced risk of infection (Aranyi, 1985; Gainer and Pry, 1972; Lantz et al., 1994).

It is therefore hypothesized that arsenic carried in the surface drainage system and/or groundwater contributes to BU incidence in parts of Amansie West District. Exploitation of arsenopyrite-bearing gold deposits including historic emissions of As_2O_3 from mine operations (Smedley et al., 1996) may make additional arsenic available for dispersion in the surface drainage system and/or groundwater. Arsenic so dispersed enters the potable water supply and is deposited on floodplains and farmlands (Masscheleyn et al., 1991) where foodcrops take up the arsenic from soils (Alam et al., 2003; Warren et al., 2003; Sarkodie et al., 1997). Spatial pattern analysis of BU incidence, drainage courses and arsenic in the environment may provide clues about the contributory factors influencing infection and provide additional information to guide further investigation of the disease.

Relating a disease to the environment, however, demands that the distribution is not purely due to chance (Oliver et al., 1992). Several investigators have sought clusters in incidences of diseases such as cancer and leukaemia (e.g., Craft and Birch, 1983; Urquhart et al., 1984; Roman et al., 1987; Gardner et al., 1990). Investigators have applied Poisson probability mapping, nearest neighbour analysis and a 'geographical analysis machine' (Cuzick and Edwards, 1990; Openshaw et al., 1988; Muir et al., 1990), but their studies were inconclusive due to limitations in the methods (Oliver et al., 1992). It is therefore important to investigate and apply methods that can model patterns of disease incidence and further help to track the underlying process of infection. Geostatistics, for instance, has found applications in epidemiology (Carrat and Valleron, 1992; Oliver et al., 1992; Rushton et al., 1996; Torok et al., 1997; Kleinschmidt et al., 2000). In addition spatial autoregressive models offer considerable improvement over geostatistics, as they produce less smoothing than geostatistical models (Cressie et al., 1998; Bell and Broemeling, 2000; Mollie and Richardson, 1991; Carlin and Banerjee, 2003; Bernardinelli and Montomoli, 1992; Heisterkamp et al., 2000). In this study, therefore, we concentrate on spatial autoregressive models.

7.2 Materials and methods

7.2.1 Sources of data

The following sources of data related to the study area have been used. These data were digitised prior to data analysis.

- Topographic map (at scale 1: 50000) showing elevation contours, rivers and streams, settlements and their names, obtained from the Survey Department Accra, Ghana.

- ASTER imagery georeferenced to the topographic map resampled and stretched to enhance ground features.

- Settlement population estimates for 2000, projected by the Ministry of local government and rural development.

- BU incidence (cases) per settlement from the 1999 national case search, kindly supplied by the Korle-BU Teaching Hospital, Accra, Ghana.

- BU incidence (cases) per settlement for the years 2000-2002, kindly supplied by the District Health Administration, Amansie West District, Ghana.

- Location map (at scale 1:62,500) of 1125 stream sediment samples (in which about 300 were used for calculation). The samples were collected in 1992 by the Geological Survey Department, Accra, Ghana; consist of wet and dry sandy sediments as well as wet organic sediments collected along drainage channels at intervals of about 400 m. Some 300 samples sites fall within the study area.

- Location map (1:50,000) of 37 surface water samples and 37 deep well (groundwater) samples collected in clean plastic bottles and acidified with nitric acid to pH < 2 in order to free complexed metals in January-February 2003.

- List of arsenic concentrations in the minus 80-mesh fraction for the 1125 stream sediment samples mentioned above, determined by the Geological Survey Department, Accra, Ghana, using inductively-coupled plasma mass spectrometry (ICP-MS).

- List of arsenic concentrations in the 37 surface water samples and 37 groundwater samples mentioned above, determined by the National Nuclear Research Institute Laboratory, Ghana, using neutron activation analysis.

7.2.2 Study parameters

Modelling is carried out with two BU datasets; this approach is used as a check on the consistency of the results (rather than to recognize differences). One BU dataset is BU incidence per settlement from the 1999 national case search of Ghana and the second is the combined data of the national case search and BU incidence per settlement for the year 2000-2002 from the Amansie West District Health Administration. Census data together with data of BU incidence allow the calculation of BU prevalence per settlement. BU incidence for each settlement for the year 1999 (national case search dataset) and for the period 1999-2002 (the combined dataset) were divided by the population per settlement and multiplied by 100 to obtain the percentage prevalence, $Prev_{99}$ and $Prev_4$ (Figure. 7.1).

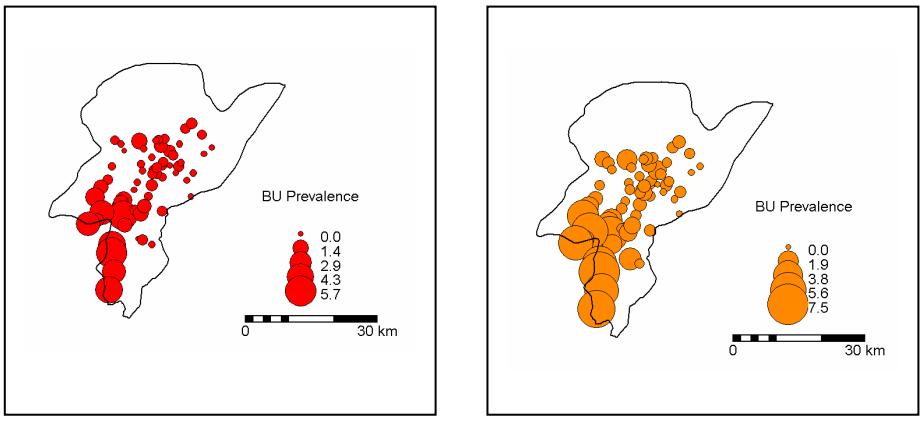

Figure 7.1. BU prevalence in settlements in parts of the Amansie West District, Ghana: (left) in 1999 ($Prev_{99}$*);* and (right) for the period 1999-2002 ($Prev_4$)

Other parameters used in modelling are also extracted or re-calculated to a per settlement basis (Table 7.1). Elevation (h) at which each settlement is situated and its distance (d_m) to the nearest minesite were extracted from the topographic map and ASTER imagery respectively. Arsenic concentrations in surface water ($As_s^{(w)}$) and groundwater ($As_g^{(w)}$) were attributed to the settlements at which the samples were taken. Surrogate concentrations of arsenic in soil around (and within) a settlement are estimated from the concentrations of arsenic in stream-sediment derived by erosion of the soils of the catchment area.

Table 7.1. Input attributes to CAR modelling

Attributes per settlement	Units	Minimum	Maximum	Mean
Inhabitants (census 2000)	no.	50	2774	866
BU incidence 1999	cases	1	74	6
BU incidence 1999-2002	cases	1	87	9
As (mean) in soil $As_{mean}^{(s)}$	ppm	2	95	25
As (max) in soil $As_{\max}^{(s)}$	ppm	2	400	90
As in surface water $As_s^{(w)}$	μgl^{-1}	5	2900	248
As in groundwater $As_g^{(w)}$	μgl^{-1}	10	1200	172
Elevation asl, h	m	152	274	200
Distance from minesite, d_m	km	0.2	17.4	7.4

To avoid overlaps, a spatial analysis is carried out to find a distance within which no other settlement location is encountered. The probability at which a settlement will not encounter another within a distance of 1 km is 0.29. Therefore, using the arsenic concentrations in the stream sediment samples within 1 km radius of each settlement, the mean soil-arsenic ($As_{mean}^{(s)}$) and maximum soil-arsenic ($As_{\max}^{(s)}$) were calculated per settlement. The attribute ($As_{mean}^{(s)}$) is the mean of the number of all arsenic concentrations found within a

1 km radius of a settlement, whilst the attribute ($As_{\max}^{(s)}$) is the single highest arsenic value found within a 1 km radius of a settlement.

7.2.3 Neighbour model

Since it is hypothesized that BU infections are related to surface drainage channels and/or groundwater aquifers shared by several settlements (Radford, 1974a; Barker, 1974; Meyers et al., 1974; Lytton and Lavett, 1974; Oluwasani et al., 1976) modelling disease prevalence in one settlement is influenced by its neighbours. The implication of this dependence is spatial autocorrelation, whereby adjacent locations or neighbours tend to have similar risk factors while non-neighbours do not.

If the neighbourhood is small estimated parameter tends to become unstable (Manton et al., 1989). This requires incorporation of a model estimator that interacts with neighbours to improve disease prevalence estimates. A choice of the neighbour structure is then required. Neighbours in this case are defined as regions that border each other, or as regions within a fixed distance of 15 km of each other (Kaluzny et al., 1998).

7.2.4 Modelling

The modelling process is parameterised to incorporate two levels of variation or structures.

- Large-scale trend in the mean attributable to spatial location or other explanatory variables (or large-scale mean structure), and

- Small-scale spatial variability due to interaction with neighbours (or interaction spatial correlation structure).

A spatial model is considered, where BU is modelled by a spatial variable $B(\mathbf{s})$, depending upon location $\mathbf{s}$. If n locations are considered, then $B(\mathbf{s}_i)$ is a collection of n random variables at sites $\mathbf{s}_i$, $i = 1, 2, \ldots n$. The model takes the form

$$B(\mathbf{s}_i) = \mu_B(\mathbf{s}_i) + \delta \qquad (1)$$

where $\mu_B(\mathbf{s}_i)$ is the expected BU value at $\mathbf{s}_i$ and δ is an error term, $\delta \sim N(0,\Sigma)$ with Σ the covariance matrix. The large-scale variation is modelled by $\mu_B(\mathbf{s})$, either constant or linearly depending upon covariates, whereas the small-scale variation is modelled by an autoregressive model for Σ.

Autoregressive models assume that the response $B(\mathbf{s})$ is a function of both explanatory variables and values of $B(\mathbf{s})$ at neighbouring locations. Two types of autoregressive models are commonly used in spatial statistics: the conditional spatial autoregressive model (CAR) and the simultaneous spatial autoregressive model (SAR). Both CAR and SAR models correspond to autoregressive procedures in time series analysis (Ripley, 1981). Both models have often been used to fit the same response distributions (Lichstein et al., 2002). Here we choose for the CAR model. CAR ranks among the most important models widely used to represent spatial correlations in disease mapping (Clayton and Kaldor, 1987; Cressie and Chan, 1989; Cressie, 1993; Waller et al., 1997).

The general equation of the CAR model is expressed as:

$$E[B(\mathbf{s}_i)|\, b(\mathbf{s}_j)_{j\neq i}\,] = \mu_B(\mathbf{s}_i) + \rho \sum_{j=1}^{n} w_{ij}\,(b(\mathbf{s}_j) - \mu_B(\mathbf{s}_j)). \qquad (2)$$

where ρ is a correlation coefficients and the w_{ij} are weights relating the variable $B(\mathbf{s})$ at the i^{th} location to itself and the variable at the other locations. In a matrix/vector notation, (2) equals:

$$\mathbf{B}(\mathbf{s}) - \boldsymbol{\mu}_B(\mathbf{s}) = \rho\, \mathbf{N}\, (\mathbf{B}(\mathbf{s}) - \boldsymbol{\mu}_B(\mathbf{s})) + \mathbf{v}(\mathbf{s}), \qquad (3)$$

where $\mathbf{N}$ is an $n \times n$ matrix with (i,j)-th element equal to w_{ij}, and $\mathbf{v}(\mathbf{s}) = (\mathbf{I} - \rho\mathbf{N})(\mathbf{B}(\mathbf{s}) - \boldsymbol{\mu}_B(\mathbf{s}))$ are the residuals. The CAR model has the following covariance matrix:

$$\Sigma = (\mathbf{I} - \rho\mathbf{N})^{-1}\mathbf{D}\sigma^2 \qquad (4)$$

where σ is a scalar parameters, and $\mathbf{D}$ is a diagonal matrix. Parameters ρ and σ are estimated using spatial regression.

The set of weights is usually restricted to a neighborhood Ω_i of the ith observation point. The CAR model then has as its properties that the matrix **N** is symmetric and has zeros at the diagonal: $w_{ij} = w_{ji}$, $w_{ii} = 0$, and $w_{ik} = 0$ if k is not in Ω_i. This shows that the residuals **v**(**s**) are not related to B(**s**).

For the CAR model the matrix (**I** – ρ**N**) must be invertible. Therefore, ρ must be restricted between $\lambda_{\min}^{-1}$ and $\lambda_{\max}^{-1}$ where $\lambda_{\min} < 0$ and $\lambda_{\max} > 0$ are the smallest and largest eigenvalues of **N**.

Modelling with CAR proceeds as follows: Suppose that settlement i is surrounded by settlements j, which have a higher BU prevalence than expected, then i will also tend to have higher prevalence. Based on the hypothesis that BU prevalence is related to surface drainage and/or groundwater aquifer shared by neighbouring settlements, this framework becomes suitable for the modelling of BU infections in the study area.

Based on Cressie's distance decay correlation function (Cressie, 1993) locations with shorter distances are likely to have more effect than those further away. Weights $\left(w_{ij}\right)$ are supposed to decrease with increasing distance between $\mathbf{s}_i$ and $\mathbf{s}_j$. The matrix **D** is set by comparing the distance d_{ij} between pairs of observations $\mathbf{s}_i$ and $\mathbf{s}_j$ to d_i^k, which is the distance from i and its k^{th} nearest neighbour. Distances beyond this threshold are set to zero. In order to prevent an observation from predicting itself d_{ii} is also set to zero (i.e., $d_{ii} = 0$). A weight of $1/d_{ij}$ to observations is assigned whenever d_{ij} is greater than zero; i.e., $0 < d_{ij} < d_i^k \leftrightarrow \mathrm{D} = 1/d_{ij}$.

Three different weights, (i.e., $w_{ij} = 1$, $1/d_{ij}$, $1/(d_{ij})^2$) may be fitted. In the present study, however, where the effect of neighbourhood locations depends upon distances, a choice $w_{ij} = 1$ does not apply. As only minor differences occurred between $1/d_{ij}$ and $1/(d_{ij})^2$, we have used $1/(d_{ij})^2$ as a weight.

An autoregressive CAR model has been fitted to explain the variation of BU within the study area. Both the log-likelihood (LL) of expected incidence or prevalence and the residual standard error (RSE) were used as measures of association. Two models were fitted. The first model (Model 1) aimed to explain the spatial pattern of BU by arsenic in soil. The second model (Model 2) aimed to explain the spatial pattern of BU by arsenic in water. The two models are as follows:

$$\text{Model 1: (BU)} = \beta_0 + \beta_1 As^{(s)}_{mean} + \beta_2 As^{(s)}_{\max} + \beta_3 d_m + \beta_4 h + \varepsilon_1 \qquad (5)$$

$$\text{Model 2: (BU)} = \gamma_0 + \gamma_1 As^{(w)}_{s} + \gamma_2 As^{(w)}_{g} + \gamma_3 d_m + \gamma_4 h + \varepsilon_2 \qquad (6)$$

where $\beta_0 \ldots\ldots \beta_4$, $\gamma_0 \ldots . \gamma_4$ are parameters to be fitted and ε_1, ε_2 are the two error terms of the models, d_m is the distance to the mine and h is the elevation.

For the first model 49 settlements were available, and for the second model 37 settlements. No attempt was made to combine the two models because water is characteristically unstable and often seasonal or ephemeral, whereas soil is relatively stable. In addition, effects of the two variables with regards to disease causation are not comparable. First, a full model is built including all explanatory variables. This was subsequently reduced maintaining the statistically-significant variables. Throughout this paper the term 'significant' unless otherwise stated implies a probability of 5% or less (i.e., $P \leq 0.05$).

The software used for this spatial modelling included S-PLUS and S + Spatial Stats. The GIS operations were carried out using ILWIS (Integrated Land and Water Information Systems).

7.3 Results

7.3.1 Model 1: arsenic in soil

The results for modelling the influence of arsenic in soil on BU infection are summarized in Table 7.2.

Table 7.2. Model 1 CAR intercept and slope coefficients

		$Prev_{99}$		$Prev_4$
Intercept	β_0	Not significant	β_0	Not significant
$As^{(s)}_{mean}$	β_1	0.0422	β_1	0.0427
$As^{(s)}_{\max}$	β_2	-0.0053	β_2	-0.0052
d_m	β_3	Not significant	β_3	Not significant
h	β_4	Not significant	β_4	Not significant
LL		-71.98		-76.99
RSE		0.6351		0.7048

No significant differences occur between $Prev_{99}$ and $Prev_4$, as evidenced by the relatively similar values in each model for LL and RSE. This indicates that environmental factors influencing BU changed little during the four-year period (1999-2002).

In both $Prev_{99}$ and $Prev_4$, BU has a significant positive association with $As^{(s)}_{mean}$ whereas it has significant negative association with $As^{(s)}_{\max}$. In simple linear regressions, the coefficient of BU on $As^{(s)}_{mean}$ is 0.042 whereas the coefficient of BU on $As^{(s)}_{\max}$ is –0.005. This suggests that an increase of As concentrations over the entire area contributes to an increase in BU whereas a higher but more localized As concentration does not. Both d_m and h are negatively associated with the disease but the coefficients are not significantly different from zero ($\alpha = 0.05$).

The quantile-quantile plots (QQ plots) of residuals show rather poor fits in the tails (Figure. 7.2(top)). This could be the effect of skewing due to settlements with a small number of inhabitants but high prevalence, especially located near the boundary of the study area that have limited spatial interaction with neighbours.

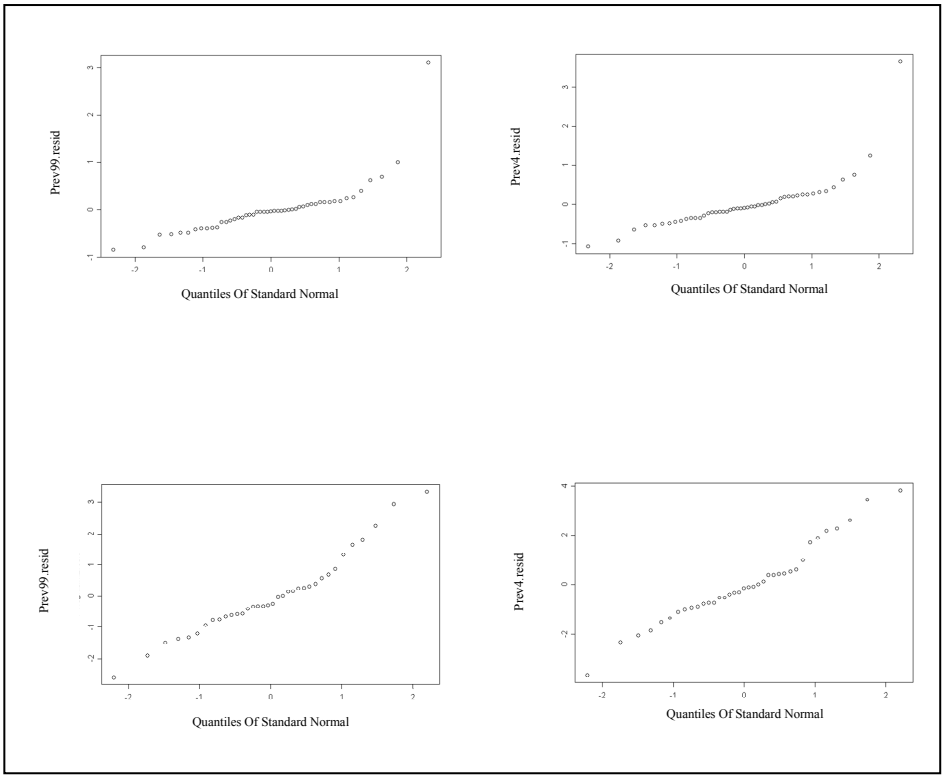

Figure 7.2. Plots of quantiles of standard normal vrs residuals for (top) Model 1soil; and (bottom) Model 2. water.

7.3.2 Model 2: arsenic in water

The results for modelling the influence of arsenic in water on BU infection are summarized in Table 7.3**.** No significant differences occur between $Prev_{99}$ and $Prev_4$, as evidenced by the relatively similar values in each model for LL and RSE. This indicates that environmental factors influencing BU changed little over the four-year period 1999-2002. In both $Prev_{99}$ and $Prev_4$ BU has a significant negative association with both h and d_m. This indicates that the disease is inversely related to As in water dispersing from minesites. BU has a

negative association with $As_g^{(w)}$ and a positive association with $As_s^{(w)}$ but the coefficients are not significantly different from zero.

Table 7.3. Model 2 CAR intercept and slope coefficients

		$Prev_{99}$		$Prev_4$
Intercept	γ_0	6.2121	γ_0	9.6425
$As_s^{(w)}$	γ_1	Not significant	γ_1	Not significant
$As_g^{(w)}$	γ_2	Not significant	γ_2	Not significant
d_m	γ_3	-0.1032	γ_3	-0.1349
h	γ_4	-0.0235	γ_4	-0.0383
LL		-76.05		-84.71
RSE		1.3381		1.6920

The QQ plots show that $Prev_{99}$ and $Prev_4$ for water tend to have better fits than those for soil (Figure. 7.2(bottom)). However, the fit at the tails is generally a little problematic.

7.4 Discussion

Model 1 tests the hypothesis that $As_{\max}^{(s)}$ influences BU through arsenic bioaccumulation in foodcrops (Sarkodie et al., 1997; Alam et al., 2003; Warren et al., 2003) and subsequent bioaccumulation in human tissues. Most farmland is situated close to streams or rivers and foodcrops are mainly cultivated on floodplains owing to their flatness, fertility and ease of irrigation during dry periods. The floodplains themselves comprise mainly recent sediments transported down streams and rivers and deposited on the adjacent land during flood events. If the suspended load includes arsenic-bearing minerals, these too are deposited on the floodplains during flood events. The general hypothesis is supported by the results of Model 1, which reveals that BU has a significant

positive association with $As_{mean}^{(s)}$. However, the significant negative association with $As_{\max}^{(s)}$ is seemingly grounds for questioning acceptance of the hypothesis.

A high $As_{mean}^{(s)}$ is interpreted as suggesting elevated As concentrations throughout much or all of the floodplains within the 1 km radius used here to define the spatial extent of a settlement. Thus these As-enriched floodplains provide much of the foodcrops consumed by the inhabitants. The high $As_{\max}^{(s)}$ locations are more restricted in spatial extent and therefore influence only part of the agricultural land and foodcrop consumption. More importantly, high $As_{\max}^{(s)}$ may be a measure or reflection of more general contamination (e.g., from mining), which has rendered land unsuitable for agriculture. Lack of foodcrop production at high $As_{\max}^{(s)}$ locations explains the negative association between BU and $As_{\max}^{(s)}$.

Lack of a spatial association in Model 1 between BU and proximity to minesites, d_m, and between BU and changes in elevation, *h*, reflects the greater importance of the availability of floodplains on which the suspended load of streams and rivers can be deposited. Arsenic released through mining into the suspended load of a stream occupying a narrow valley and experiencing a relatively large elevation fall over a comparatively short distance is not deposited. It may travel a considerable distance until it enters a more mature section with more gradual elevation change and the development of floodplains. This change in fluvial environment may well be paralleled by a change in the geochemical environment, from predominantly oxidizing in the fast-flowing sectors to mildly reducing in sluggish and perhaps swampy floodplains. Such a geochemical change reduces relatively stable As^{5+} in mineral and amorphous phases to more mobile and more toxic As^{3+} that is more readily taken up by foodcrops (Sarkodie et al., 1997; Mattusch et al., 2000; Abedin et al., 2002). There are reports of BU in the vicinity of swamps (Barker, 1973; Barker and Carswell, 1973; Ravisse, 1977).

Arsenic adversely affects the human immune system (Vega et al., 1999; US EPA Office of Water, 2001; Frenkel et al., 2002) leading to several kinds of

diseases (Abernathy et al., 1999; NRC, 1999). Studies (e.g., Gainer, 1972; Gainer and Pry, 1972) have shown that arsenicals enhanced viral infections because it inhibited interferon formation. Blakeley et al. (1980) also demonstrated the immunosuppressive effect of dissolved arsenic on mice. Using arsenic-exposed subjects (who use drinking water with mean arsenic concentration of 412 μgl^{-1}) and controls (who use drinking water with mean arsenic concentration of 37 μgl^{-1}), Gonseblatt et al. (1994) found impairment in the immune response of the arsenic-exposed subjects; down-regulation of the immune system is known to be an environmental risk factor for the development of BU (Van der Werf et al., 1999; Stienstra et al., 2001).

Model 2 includes testing the hypothesis that $As_s^{(w)}$ and/or $As_g^{(w)}$ influence BU through use as potable or irrigation water, but the results fail to reveal a direct significant association. The results do show a significant negative association with d_m, distance from minesites, which can also be stated as a significant positive association with proximity to minesites. Of course one or several parameters associated with working at or living in settlements near to minesites, but not included in the model, could account for this association (and the poor fit of QQ plots indicated the need for additional explanatory variables). However, potable water may well be implicated. Gold mining in the study area takes place exclusively in or near surface drainage channels and hence there is propensity for soluble contaminants released by mining to enter the local groundwater and surface water, either of which might be used as potable water supply at minesites and in nearby settlements. There are reports of health problems elsewhere down-drainage of minesites (Williams et al., 1996; Ogola et al., 2002). A survey of the potable water supply of 90 BU patients in Ghana (Amofah et al., 1993) showed that the majority relied on surface water but a significant majority used shallow groundwater.

Several cases of arsenic poisoning have resulted from ingestion of contaminated drinking water (Smith et al., 2000). Severe health effects due to arsenic-contaminated drinking water have mainly been reported in populations of low socio-economic status and poor nutrition (US EPA, 1988). When arsenic exposure is high and nutritional condition is poor, the disposal of highly-

reactive methylated arsenic species in the body is also poor (Marafante and Vahter, 1986; Vahter and Marafante, 1987).

7.5 Conclusion

Statistical modelling using a spatial neighbour method reveals an association between (a) the mean As content of the soil and the spatial distribution of BU and (b) the distance to sites of gold mining and the spatial distribution of BU. It is deduced that contributory factors to BU infection include regular exposure to arsenic-contaminated soil, chronic ingestion of foodcrops grown on arsenic-contaminated soil, and/or regular consumption of foodcrops grown on arsenic-contaminated soil, and proximity to minesites. The role of arsenic is that of an immuno-depressant, raising susceptibility to opportunistic pathogens like MU.

Further modelling would benefit from additional inputs to identify other factors contributing to MU infection. Broader socio-economic data and improved geochemical coverage would allow considerable refinements of the model used here.

CHAPTER 8

SYNTHESIS

8.1 Introduction

In the foregoing chapters, it is deduced that arsenic could play a contributory role in the development of a mycobacterial disease. Increased incidence and resurgence of BU in the world in general and Ghana in particular are of great concern to the World Health Organisation (WHO, 2002) as well as to the Government of Ghana. This concern, underlain by the disease's unclear mode of transmission and its uncontrolled rampage in recent years especially in the West African Region, motivated this research.

The approach to the research relies upon the application of a geographic information system (GIS). GIS has encouraged and enhanced elaborate spatial analyses of disease occurrence or patterns. GIS has also been applied in various environmental fields of science such as land and its subsurface, ecological and biological systems (e.g., pesticides and immune competence, toxic chemicals and low birth weight, radiation and leukemia, lead blood levels and autism or learning disabilities). GIS is capable of integrating both geographic and non-geographic data to estimate, for example, levels of toxic exposure of individuals within defined geographic regions. Such estimates can be used to suggest and support hypotheses in relation to the causative and/or contributory factors of disease (Nizeyimana et al., 1996; Marilyn et al., 1998). Since GIS can be used to investigate relationships between spatial variables in the search for causal or contributory factors in relation to disease distribution, it has become a valuable tool for epidemiologists in the study of association between environmental factors and spatial distribution of diseases.

Modern society is confronted with serious challenges with regards illnesses related to heavy-metal/metalloid sensitivity. People become ill due to environmental metal/metalloid exposures. Contamination of the environment by metals and metalloids is increasing due to anthropogenic activities (e.g., mining, manufacturing, damming of rivers, deforestation) (Johnson et al., 1999; Becker and McCoy, 2003; Flora, 2000). The resulting exposures tend not only to affect and disable organs of the body (Chen et al., 1992; Smith et al., 1992; Cuzick et al., 1992; Tang et al., 1996; McCabe and Lawrence, 1991; Venogopal and Luckey, 1978) but also may interfere with organs responsible for immune response. Their effect may often alter immune surveillance and homeostasis

with either cell-mediated immunity depressed and humoral immunity elevated or vice versa (Heo et al., 1997; McCabe and Lawrence, 1991; Heo et al., 1996; Guanquing, 1979) and subsequently lead to ill health.

One such metalloid is arsenic, which binds to sulfhydryl groups of proteins and enzymes (Gebel, 2000; Abernathy et al., 1999), paralyzes them, depletes cellular stores of antioxidant glutathione and/or induces oxidative stress with subsequent damage to cells (Ahmad et al., 2000). Although chronic arsenic exposures are often masked by microbial infections (Harada, 1996), studies have shown that both animals and humans exposed to arsenic have had immune dysfunction including humoral and cell-mediated immunity (Aranyi et al., 1985; Gainer and Fry, 1972; Blakeley et al., 1980; Vega et al., 1999; Kaltreider et al., 2001; Gonseblatt et al., 1994; Frenkel et al., 2002; Sikorski et al., 1989, 1991). Impairment of the cell-mediated immunity by arsenic results in defective protective mechanism of the immune system thus enhancing viral/bacterial infections (Aranyi et al., 1985; Gainer and Fry, 1972; Karcher et al., 1999). Studies have detected viruses in skin biopsies of patients affected with arsenic-induced malignant lesions (keratoses, squamous cell carcinoma) (Gerdsen et al., 2000; Grimmel et al., 1988). In their study of subjects chronically exposed to arsenic, Rosales-Castillo et al. (2004) concluded that viral infection (i.e., human papillomasvirus) could be an additional risk in the development of non-melanoma skin cancer (NMSC). This risk factor may underscore the fact that arsenic defects the immune function, predisposes chronically-exposed subjects to infection and thus raises serious concerns about its potential contributory role in the development of a mycobacterial disease.

The area chosen for this study is parts of the Offin and Oda river systems in the Amansie West District of central Ghana. Parts of the area are underlain by gold-arsenopyrite mineralized rocks and placer accumulations are exploited by artisanal miners. In the study area high incidence and prevalence of BU is found in the Offin River basin, where mining activities, that could contaminate the environment are concentrated. The main objective of this study was to use spatial analysis to determine relationship between BU and arsenic.

8.2 Stream sediment geochemical data transformed into arsenic-enriched domains

This method was based on stream sediment catchment basin model to identify the extentions of geochemical anomalies and their spatial relationships to disease incidence or prevalence in the study area through proximity analysis. The results showed that:

- Higher prevalence of BU is found in proximity to arsenic-enriched domains than elsewhere; an indication that arsenic-enriched domains could be a factor to BU incidence in the area.

- However, BU prevalence in settlements in arsenic-enriched drainage is greater than that of arsenic-enriched farmlands.

8.3 Arsenic concentration in drinking water and exposure-response model

An exposure-response model was used to show the general relationship between increased exposures of environmental pollutant (arsenic in water) and increased response of health disorder (BU). The results showed that:

- BU shows strong evidence of association with arsenic in surface water.

- BU does not show association with arsenic in groundwater.

- Both high incidence of BU and elevated arsenic in surface water are found in the Offin Basin. On the contrary high incidence and elevated arsenic in surface water are further away from the Oda Basin.

- The result suggests that there is a greater tendency of the population to prefer surface water rather than groundwater.

8.4 Minesites and BU in proximity analysis

Proximity analysis also illustrated the spatial relationship between BU and minesites. Minesites were a proxy measure for arsenic exposure (exploitation of gold is carried along river profiles, exposing sulphide minerals to sub-aerial

oxidation and disseminating them into rivers during rainy season). The results were as follows:

- Settlements near to artisanal minesites have higher BU incidence, suggesting a spatial relationship between BU and artisanal mining. Elevated levels of arsenic tend to be coincident with artisanal mining, thus there is also a relation between BU and arsenic.

8.5 Spatial regression analysis

Finally, spatial statistical analysis is employed to generate linear combination of risk factors that best explain the spatial variation in BU prevalence. Weights and cut-off values were chosen for the regression analysis after exploring their sensitivities to the data. The use of this methodology was aimed at bringing the other methods outlined above into a coherent framework. The regression analysis gave the following results:

- BU is positively associated with regionally-elevated (rather than locally anomalous) arsenic in soils and at statistically significant level.

- Both distance to artisanal minesites (which are in the river valleys) and elevation at which settlements were situated were significantly negatively associated with BU prevalence.

- BU was positively associated with surface water but not at a statistically significant level.

8.6 Main conclusions

Based on the results from each method used to achieve the objective the following conclusions have also been drawn:

- High BU prevalence rate was found to occur in arsenic-enriched domains suggesting that arsenic-enriched domains are a factor for the development of BU. BU prevalence, however, is higher along arsenic-enriched drainages than in arsenic-enriched farmlands.

- Arsenic-contaminated surface water tends to have a significant correlation with BU, and therefore MU infections are better predicted by arsenic-contaminated surface water rather than by arsenic-contaminated groundwater.

- Settlements near artisanal mining activities tend to have higher BU incidence because artisanal mining contributes to the release of arsenic into natural drainage used as potable water and for irrigation of farmlands where foodcrops are grown for consumption.

- Contributory factors therefore to BU infection include chronic ingestion of arsenic-contaminated drinking water, chronic ingestion of foodcrops grown on arsenic-contaminated soil coupled with the irrigation of arsenic-contaminated water.

- This research corroborates medical studies reporting that exposure to arsenic impairs cell-mediated immunity, and thereby enhances the risk of viral/bacterial infections such as MU infections.

8.7 Recommendations

Although the methods used established a relationship between arsenic and BU much can still be achieved to help obtain a better understanding of the epidemiology of BU. The following suggestions can therefore be helpful for future research, relying primarily on spatial data.

- Large geochemical datasets (stream sediments, water and soils) should be collected to cover areas with combinations of high BU incidence, low BU incidence, high arsenic concentrations and low arsenic concentrations. It would be more appropriate for water samples to be collected at monthly intervals throughout the year.

- Samples should be analysed for many additional elements, arsenic determinations could be speciated and dissolved arsenic should be distinguished from suspended arsenic in waters.

- When arsenic exposure is high and nutritional condition is poor, the disposal of highly-reactive methylated arsenic species in the body is also poor. Therefore broader socio-economic data must be studied and integrated into spatial modeling since this plays a role in a person's physiologic response to chemical exposures.

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Resumé

Alfred Allan Duker was born on the 17th of July 1953 at Krofu in the Central Region, Ghana. He obtained his Bachelor of Science (Bsc.) degree in Geodetic Engineering in 1979 from the School of Engineering, University of Science and Technology (UST), Kumasi, Ghana. He worked in the Department of Geodetic Engineering as a Teaching Assistant from 1980 until 1982.

He obtained his Master of Science (Msc.) degree in 1984 from the International Institute for Geo-information Science and Earth Observation (ITC), Enschede; and went back to work in the Department of Geodetic Engineering as a Lecturer.

In 1989 he returned to Toulouse, France where he obtained a postgraduate diploma (Diplôme d'Etude Supérieure Spécialisée) in remote sensing in 1990 from the University of Paris VI and Marie Curie, France. He has since worked in the School of Engineering of the University of Science and Technology, Kumasi, as a Senior Lecturer.

In July 2000, he obtained funding from the Government of Ghana and support from the Ghana Chamber of Mines to begin his PhD research at ITC and Wageningen University in the Netherlands.