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Arsenic in Water and Soil: A Possible Contributory Factor in *Mycobacterium ulcerans* Infection in Buruli Ulcer Endemic Areas

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ABSTRACT

A study was conducted to determine the arsenic, pH, electrical conductivity and total dissolved solid (TDS) levels for streams and soil around, in Buruli Ulcer endemic and non endemic communities in the Amansie West District of the Ashanti Region of Ghana over a period of 12 months. Results from analysis of arsenic and other related physicochemical parameters revealed that, mean arsenic concentration for the streams in the entire sampling community was (0.8058 mg L^{-1}). Though this figure exceeded the World Health Organization (WHO) recommended guideline for drinking water, it was within the Ghana Environmental Protection Agency (GEPA) permissible guideline. However, mean arsenic concentration for the entire sampling communities were higher for soil (1.464 mg kg^{-1}) compared to that of its stream (0.8058 mg L^{-1}). Electrical conductivity when monitored over the period also revealed a mean value of 139.4 μS cm^{-1}. Mean Total Dissolved Solids recorded for the stream during the study stood at 69.6 mg L^{-1} and was much lower than both WHO and GEPA. When the levels of arsenic during the study were analysed based on endemicity, it was revealed that arsenic concentration for streams in BU endemic communities were higher (0.8720 mg L^{-1}; ±0.4235) compared to their non-endemic counterparts (0.739 mg L^{-1}; ±0.4188). Mean levels of arsenic in the soil when stratified based on endemicity revealed that, endemic levels (1.820 mg kg^{-1}) were not only higher than that of the non-endemic (1.108 mg kg^{-1}) but also statistically significant ($p = 0.0452$). Results from this work revealed that streams and soils in endemic communities within the Amansie west district were generally high for arsenic contamination compared to their non-endemic counterpart. It is an undeniable fact that long-term exposures to arsenic via drinking water are known to cause a number of arsenic related diseases including cancer of the skin among many others. In the absence of clear cut pathogenesis to *M. ulcerans* infections, these elevated arsenic levels in Buruli Ulcer endemic community cannot be ignored. These results might confirm some aspect of earlier works linking arsenic to a possible BU infection though further research is urgently needed to unravel the mystery surrounding the possible mode of transmission of this neglected yet treatable disease.

Key words: Arsenic, Buruli ulcer, *M. ulcerans,* Amansie West District, galamsey operators

INTRODUCTION

Mycobacterium ulcerans infection otherwise known as Buruli ulcer is an ulcerative disease of the skin, tissue and sometimes bone. The disease is found in rural areas located near wetlands (ponds, swamps, marshes, impoundments, backwaters) and slow-moving rivers, especially in areas prone to flooding (Walsh *et al*., 2008). Logistically, the disease is very difficult to detect, as a result, prevalence rates are likely to be underestimated. Even within endemic areas, significant inter-village variability in prevalence data exists which may be attributable to geographic proximity to water (Williamson *et al*., 2008). The association between *M. ulcerans* and an aquatic reservoir is supported by epidemiological and environmental sampling data. Recent landscape-based studies have strengthened this association and confirm a link between *M. ulcerans* transmission and areas of decreased urbanization (Brou *et al*., 2008). Despite these data, the mechanism of transmission remains unclear. It has been postulated that transmission may occur after skin contaminated with environmental material harboring the organism is penetrated by trauma or following an insect bite Although direct culture of *M. ulcerans* has been obtained from the *Gerris* water strider found in endemic areas (Portaels *et al*., 2008), this predatory aquatic insect is not known to bite humans, supporting other entomological studies which have not confirmed this as the primary vector of transmission (Wagner *et al*., 2008). Cases have also been reported from at least 32 countries in Africa (mainly west), Australia, Southeast Asia, China, Central and South America and the Western Pacific (Merritt *et al*., 2010). A number of cases have been reported in non-endemic areas of North of America and Europe as a sequel to international travel (Ezzedine *et al*., 2009). Although research has been documented for risk factors for developing BU, the perceptions of these risk factors by disease endemic dwellers somehow seem to differ from what is known in literature (Gyasi *et al*., 2011). Arsenic (As) is ubiquitous, naturally occurring element present in food, soil, air and water (Abernathy *et al*., 1999). Since Hippocrates's time, it has been used in medicinal and homicidal preparations. In addition, occupational and environmental exposure occasionally causes toxic manifestations (Lalwani *et al*., 2006). All human beings are exposed to it in one form or other. The toxicology of As is a complex phenomenon as acute As poisoning is believed to occur due the consumption of contaminated food and water (Bose *et al*., 2011). About 64% of total intake is accounted for by organic arsenic such as arsenocholine and arsenobetaine which are relatively nontoxic and rapidly excreted unchanged in urine. Inorganic arsenic which is more toxic accumulates in various body tissues such as liver, kidney, gastrointestinal tract, spleen. Pentavalent arsenic undergoes methylation in liver and forms Monomethylarsonic Acid (MMA) and Dimethylarsinic Acid (DMA) which are less reactive with tissue constituents, less toxic and more readily excreted in urine. But once this process gets saturated, there is accumulation of arsenic in tissues leading to health hazards (Lalwani *et al*., 2006). Cancer risk from drinking water containing arsenic at 500 mg L^{-1} may be as high as one in 100 (NRC, 2001). Chronic exposure of arsenic through drinking water is known in different continents. In India, natural exposure of man to arsenic through drinking water of wells, hand pumps and springs in Chandigarh and its surrounding areas was first highlighted in 1976 (Bhattacharya *et al*., 2009). Arsenic level exceeding WHO permissible limit has been reported in ground water of West Bengal, some areas of Bihar and Madhya Pradesh in Bangladesh (Chakraborti *et al*., 2003). This was when a newspaper in that country reported peoples feet and limbs were being eating away in a fashion similar to BU. However, diagnostic test presented the absence of *M. ulcerans*. In a work published in 2004 using spatial dependency, samples of water from arsenic enriched domains and farmlands in the Amansie West district (part of which has a high prevalence of BU) was carried out, it was hypothesized that arsenic in drinking water indirectly may contributes to Buruli ulcer infection (Duker *et al.*, 2004). It is therefore imperative to monitor the levels of arsenic and other heavy metals over a period. This work is a pilot study which is a prelude to a main research aimed at investigating the influence of arsenic in the environment to the development of Buruli Ulcer with the help of an animal model. The main objective of this work were (1) to monitor streams and soils used by inhabitants of Buruli Ulcer endemic communities for arsenic contamination (2) compare these levels based on endemicity and (3) to assess whether or not there are variations in these heavy metals based on location.

MATERIALS AND METHODS

Study area and population: The Amansie West District of the Ashanti Region of Ghana lies between latitudes 6°00'N and 6°45'N and longitudes 1°30'W and 2°15'W (covering an area of about 1,136 km^2) has high incidence of BU (Fig. 1). The District is drained by the Offin and Oda rivers and the landscape varies from gentle to broken. Most of the communities occur in close proximity to the Offin River and many of its tributaries. 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 30°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). Of the 310 settlements in the 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 illegal (galamsey)

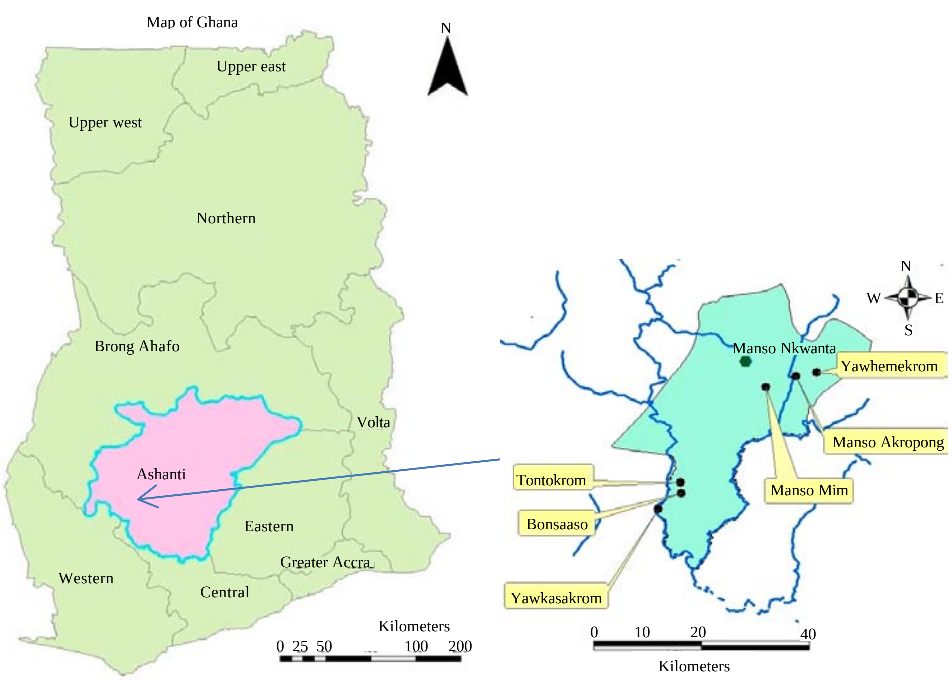

Fig. 1: A Map of Ghana (Left) and global positioning system (GPS) of sampling sites in the Amansie west district (right) in the Ashanti region

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. This District is therefore placed in a suitable setting for studying the relation between BU and environmental factors that may potentially contribute to infection.

Selection of study communities: Six communities were systematically selected based on reports of BU in the community in the last three years. Endemic communities chosen were Yawkasakrom, Bonsaaso and Tontokrom whiles the non-endemic communities were Manso Mim, Manso Akropong and Yawhemekrom.

Study design: An analytical study was employed where data was collected and analyzed with physicochemical parameter probes (Eutech, CyberScan, PC 300 series) and Varian 220 (SpectrAA 220) (http://www.eutechinst.com/pdt-para-ph-cyberscanph300.html).

Sample collection: Water and soil samples from streams where study participants wash hands and feet on their way to their house from farms and galamsey sites were collected from October 2010 to August 2011. With the help of Global Position System (GPS 76, Garmin, 2001) sampling sites were sighted.

Water sampling: After agitating the water for about 10 seconds, water at a depth of 20-30 cm was collected from the streams into 500 mL sterile transparent plastic bottles. With a field conductivity probe, conductivity, total dissolved solids and pH were measured on site. Samples for arsenic detection were also acidified with 5 mL concentrated nitric acid for every 500 mL sample on site. They were tightly covered and stored in a cool ice chest and hurriedly carried to the laboratory.

Soil sampling: Soil samples were taken from the banks of streams in sampling communities where water was collected. Composite sampling which involved taking soil and underwater sediments from 10 different spots (at depths of 10 cm) within 3 m radius, mixing them together after which about 10 g was also taken into sterile plastic bags, kept in a cool ice chest and quickly transported to the laboratory.

Laboratory treatment of samples

Digestion of water: Five hundred milliliters (500 mL) of acidified water collected from streams in the six study communities, 5 mL of concentrated nitric acid were added, placed on heated water bath till the samples volume reduced to 50 mL. Samples were then filtered through a 45 micron filter paper into sterile container and stored for onward analysis with the Varian 220 (SpectrAA 220) at Anglo Gold, Obuasi, Ghana.

Digestion of soil: To 1 g of the soil sample, 30 mL of digestion ions in the ratio of 9: 4 (Nitric acid: Per chloric acid) was added. The resulting supernatant was digested on a heated water bath until the soil became clear whiles ensuring that, the soil did not dry out in the conical flask by toping up occasionally with distilled water when volume decreased below 20 mL. Upon obtaining a clear solution, the resultant solution was topped up to 50 mL, filtered and stored for onward analysis Varian 220 (SpectrAA 220).

Determination of arsenic: Arsenic in water and soil after digestion was determined by the injection of hydride generation Atomic Absorption Spectrophotometer. All values for arsenic (water and soil) were initially analysed in parts per million (ppm) and later changed to SI units depending on which material that was being analysed.

Data analysis: Data obtained was analyzed using GraphPadPrism-5.0 software (Motulsky, 1999). Means were compared using unpaired student t-test (95% confidence intervals) and p value$\leq$0.05 were considered statistically significant.

RESULTS

Results from analysis of arsenic and other related physicochemical parameters revealed that, mean arsenic concentration for the streams in the entire sampling community stood at (0.8058 mg L^{-1}, Range: 0-1458). Though this figure exceeded the World Health Organization (WHO) recommended guideline for drinking water, it was within the Ghana Environmental Protection Agency (GEPA) permissible guideline as shown in Table 4. However, mean arsenic concentration was higher for soil (1.464 mg kg^{-1}) compared to that of the stream (0.8058 mg L^{-1}) as shown in Table 1. Electrical conductivity when monitored over the period also revealed a mean value of 139.4 µS cm^{-1}. Mean Total Acidity analysis for the streams in the sampling communities also revealed a mean pH of 6.6, standard deviation and standard error of mean of (0.389) (0.06479), respectively shown in Table 1.

When the levels of arsenic during the study were analysed based on endemicity, it was revealed that arsenic concentration of streams in BU endemic communities were higher (0.8720±0.4235 mg L^{-1}) compared to their non-endemic counterparts (0.739±0.4188 mg L^{-1}) as shown in Table 2. Mean levels of arsenic in the soil when stratified based on endemicity revealed that, endemic levels (1.820 mg kg^{-1}) were not only higher than that of the non-endemic (1.108 mg kg^{-1}) but also statistically significant ($p = 0.0452$) as shown in Table 2. These figures

Table 1: The concentration of arsenic (water and soil) with some physicochemical parameters in the Amansie West District of Ghana

Parameters	Mean	Range	SD	SEM
Arsenic (streams) mg L^{-1}	0.8058	0-1.458	0.4205	0.07009
Arsenic (soils) mg kg^{-1}	1.464	0-2.987	1.075	0.1792
Conductivity (µS L^{-1})	139.4	68.8-545.6	124.6	20.77
Total dissolved solids (mg L^{-1})	69.5	34.4-271.3	61.77	10.29
pH	6.6	6.0-6.7	0.389	0.06479

SD: Standard deviation, SEM: Standard error of mean, Note: Arsenic levels <0.05 in Table 2 have been rounded up to 0 for the sake of statistical analysis, Comparison for arsenic in water and soil were done on the basis that all initial concentration for both material were in parts per million (ppm), This was equivalent to mg L^{-1} for water and mg kg^{-1} for soil

Table 2: Stratification of arsenic and some physicochemical parameters in the Amansie west district based on endemicity

Parameters	BU endemic communities	Non-endemic communities
Arsenic (mg L^{-1}) stream	0.87±0.42	0.74±0.42^{ns}
Arsenic (mg kg^{-1}) soil	1.82±1.08	1.11±0.98†
Total dissolved solid (mg L^{-1})	82.89±84.57	56.1±18.01^{ns}
Conductivity (µS cm^{-1})	166.4±140.6	112.4±36.23^{ns}
pH of stream water	6.5±0.22	6.9±0.39†††

Values are Mean±SD (N = 36), Levels of significance were determined using students unpaired t-test, $^{ns}p>0.05$, $^{\dagger}p = 0.05$, $^{\dagger\dagger\dagger}p = 0.001$

Table 3: Levels of Arsenic in soil and stream in some communities in the Amansie west district

Community	Arsenic (soil) mg kg^{-1}	Arsenic (stream) mg L^{-1}	p-value
Yawkasakrom (Aprapim stream)	0.842±0.4006	1.059±0.6964	0.5232
Bonsaaso (Bonsaan stream)	0.904±0.4485	1.666±1.2910	0.2018
Tontokrom (Asuobum stream)	0.870±0.4961	2.734±0.2225	<0.0001
Manso Mim (Asuapre stream)	0.694±0.3694	0.756±0.6013	0.8331
Manso Akropong (Nwene stream)	1.028±0.4704	0.835±0.7837	0.6163
Yawhemekrom (Abesua stream)	0.497±0.2569	1.734±1.2510	0.0391

Words in Parenthesis, next to the communities refers the names of streams where water and soil samples were taken, Values are as Mean±SD, p-value <0.05 is considered statistically significant at 95% confidence interval

Table 4: Ghana environmental protection agency and WHO permissible guidelines for drinking water and for soil in agricultural field

Parameter	GEPA	WHO
Arsenic(mg L^{-1}) water	1	0.01
Arsenic(mg kg^{-1}) soil	-	20
Total dissolved solid (mg L^{-1})	1000	1000
Conductivity (μS cm^{-1})	-	-
pH	6.5-8.5	6.5-8.5

were within the WHO arsenic permissible guidelines for agricultural field (Table 4). Analysis of mean total dissolved solids and electrical conductivity of streams of the combined communities based on endemicity also revealed that, levels were higher in the endemic communities compared to their non-endemic counterpart as shown in Table 2. Analysis of acidity levels of the streams in the sampling communities based on whether or not a community was considered endemic also revealed that mean pH was however lower in BU endemic communities (6.5) compared to their non-endemic communities and this was significant (p = 0.0005).

BU communities stratification based on levels of arsenic in streams and its surrounding soils also showed that, apart from Manso Akropong which had its levels of arsenic in surrounding soils (1.028 mg kg^{-1}) greater than stream (0.835 mg L^{-1}), all other communities for the study were greater for levels of arsenic in streams compared to their surrounding soils as shown in Table 3. This was however not statistically significant. (p = 0.616). Mean arsenic levels in Aprapim stream (Yawkasakrom) was higher (1.059±0.6964 mg L^{-1}) compared to soils near the stream (0.842 mg kg^{-1}) and this was also not statistically significant (p = 0.5232) as shown in Table 3. However, the figure exceeded both WHO and GEPA guidelines for drinking water as shown in Table 4. When mean levels of arsenic in streams and its surrounding banks in the study area were analysed for Tontokrom, it revealed that Asuobum stream (Tontokrom) had higher mean arsenic level (2.734±0.2225 mg L^{-1}) compared to soil from its surrounding bank (0.8702±0.4961 mg kg^{-1}) and this was statistically significant (p<0.0001) (Table 2). For all communities involved in the study, soils around the banks of Abesua stream (Yawhemekrom) had the lowest mean arsenic levels (0.4972±0.2569 mg L^{-1}) compared to the rest of the communities and was far lower than levels in soil around the banks Abesua stream (Yawhemekrom) (1.734±1.2510 mg kg^{-1}).

DISCUSSION

From the study, elevated levels of arsenic in the soils coupled with slightly acidity of streams from the study community could be attributed to the anthropogenic sources of the heavy metal. This was consistent with a study carried out by a group of researchers in a work published in 1998

(Carpenter *et al.*, 1998) where both anthropogenic pressures and natural processes accounted for arsenic degradation in soil, surface water and groundwater quality (Carpenter *et al.*, 1998). Earlier work in arsenic enriched farmlands using spatial dependencies (Duker *et al.*, 2004) hypothesized that arsenic could compromise ones immunity in the presence of *Mycobacterium ulcerans* could trigger Buruli Ulcer infection. The relatively higher mean arsenic concentration for the soil compared to that of the stream could be due to the fact that artisanal small scale miners otherwise known as “Galamsey operators” use extensively a variety of chemicals including arsenic. It is also a common practice by farmers in the district in the use of arsenic contaminated water in cultivating their lands and arsenic based agrochemicals in treating their agricultural field. In view of the above, the heavy metal could easily be trapped into near-by drainage channels and soil during floods on their way into the streams. These polluted soil and streams are often the only sources of water used for farming and other domestic activities including cooking and drinking. This result was consistent with a work carried out in the Gangetic floodplains of Bangladesh in 2004 (Islam *et al.*, 2004) where water polluted with arsenic when used for irrigation pollutes their soil. Food produce from these contaminated arsenic sources when consumed are most likely to be eliminated through urine. However, if arsenic ingestion exceeds rates at which they are excreted, they tend to accumulates in individuals who are being exposed and this could make them susceptible to a lot of tropical disease of which BU could be a part in the presence of *M. ulcerans*. The fact still remains that among toxicities produced by different elements, arsenic toxicity associated with human health is now a burning problem throughout the world, especially in India (Halder *et al.*, 2007). Total dissolved solids of streams were low for most of the communities visited and they were all below the GEPA and WHO permissible guidelines for drinking water. Interactions with the local folks during visits to the sampling community by the research team revealed the streams are being used for agriculture purposes and for domestic chores sometimes when boreholes breakdown. Communities and opinion leaders have always ensured they are kept aesthetically clean especially in BU non endemic communities because of its usage.

Acknowledgement of higher arsenic concentrations in stream as well as soil predominant with BU endemic communities compared to their non-endemic counterparts was to be anticipated. Most endemic communities within the Amansie West District are saturated with the activities of artisanal small scale miners in their quest to amass wealth and would employ any means to undertake their business. In the process, chemicals, some of which are arsenic based are employed. This was consistent with a study carried out by Armah and a group of workers in areas around Tarkwa in western part of Ghana (Ato *et al.*, 2010) where the use of chemicals by artisanal small scale miners has contributed to the point sources of surface water contamination. Visit by the research team to the Amansie west district revealed most streams which acts as the main sources of water for farmers and even for human consumption during times that their bore holes break down especially in BU endemic communities were often polluted by Galamsey operators. The activities of this small scale artisanal miners requires a lot of water for washing their ores so these streams are diverted to their excavated pits with the help of powered pumping machines. This often constitutes a point source of arsenic contamination. Our study revealed a generally, a high TDS, acidity and electrical conductivity of streams in the endemic communities were higher compared to those in the non-communities (Table 2). In the wake of the enigma surrounding the pathogenesis of Buruli Ulcer, attempts by opinion leaders in the district has been intensified to discourage community dwellers from sitting all occupational activities away from their streams and rivers. But for “Galamsey operations”, whose activities are rather on theascendancy in the endemic communities, avoidance of these water bodies is a luxury they cannot afford. With the use of earth moving excavators, huge trenches are dug after which mechanized water pumping machines washing their precious mineral. In the process, the effluent ends up in streams polluting them in the process. Elevated physicochemical parameters could therefore be associated with the introduction of debris to streams as a result of small scale mining activities and this is consistent with a work carried out by Akpoveta and his team, assessing the quality of borehole water used in the vicinities of Benin, Edo State and Agbor, Delta State of Nigeria (Akpoveta *et al*., 2011). The higher level of arsenic in the streams of all but one community (Manso Akropong) compared to their soil could be as a result of reasons already explained in the preceding paragraph. Visit by the researchers to the study community during sampling revealed streams being surrounding by pockets of mining tailings as a result of the activities of artisanal small scale miners. Once streams were sited downstream, effluents from these tailings occasionally found their way into the stream. Tontokrom, the community with the highest BU prevalence in the district coincidentally recording the highest arsenic level in its Asuobum stream in Tontokrom could also be attributed to the topography of its bank. Lying in a deep valley, the Asuabum stream is the recipient of most of the mining waste washed from up-hill during the washing of dug out soil believes to contain mineral deposit. These findings were in line with results of research carried out by a group of researchers in the Amansie West District in a work published in 2004 (Duker *et al*., 2004) that linked arsenic in arsenic enriched farmlands to a possible *M. ulcerans* infection. This stream until the emergence of boreholes in recent times was the main source of water for both domestic and agricultural purposes, although some inhabitants downstream still utilize as their source of drinking water. The lowest arsenic concentration of the Abesua stream (Yawhemekrom) could be attributed to the absence of artisanal small scale miners within this community. The Abesua stream during most part of the year during sampling depicted the cleanliness of its banks. Records from the Amansie West district health directorate at the time of the study revealed that, Yawhemekrom is not endemic for Buruli Ulcer. Inhabitants of Manso Akropong a non-endemic community like Yawhemekrom also did not engage in any form of galamsey activity at the time of sample collection. The higher arsenic concentration in the soil (Manso Akropong) however could be due to arsenic based agrochemical washed from near-by agricultural field and this was consistent with a publication by Peryea, in 1998 (http://soils.tfrec.wsu.edu/leadhistory.htm) where the use of arsenical insecticide in treating agricultural fields in Australia in 1997 had contributed to the pollution of its surrounding water bodies.

CONCLUSION

Results from this study revealed that streams and soils in endemic communities with in the Amansie west district were generally high for arsenic contamination. It is an undeniable fact that long-term exposures to arsenic via drinking water are known to cause a number of arsenic related diseases including cancer of the skin among many others. In the absence of clear cut pathogenesis to *M. ulcerans* infections, these elevated arsenic levels in Buruli Ulcer endemic community cannot be ignored. These results might confirm some aspect of earlier works linking arsenic to a possible BU infection though further research is urgently needed to unravel the mystery surrounding the possible mode of transmission of this neglected yet treatable disease.

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