OCCURRENCE OF GLYPHOSATE IN GROUNDWATER AND SURFACE WATER WITHIN FARMING COMMUNITIES IN CENTRAL TONGU DISTRICT, GHANA

By

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DECLARATION

I, AFEMEKU, CONFIDENCE MAWUFEMOR, do hereby declare that except for references to other studies (duly acknowledged in this work); this thesis submitted to the School of Graduate Studies (SGS), University of Ghana, Legon is the result of original research I carried out at the School of Nuclear and Allied Sciences (SNAS). This thesis has not been presented for the award of any degree in this university or elsewhere.

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DEDICATION

Dedicated to my family and friends.

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LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
AMPA	Aminomethylphosphonic Acid
AT	Average Time
BH	Borehole
BW	Body Weight
C	Concentration
CDI	Chronic Daily Intake
CWSA	Community Water and Sanitation Agency
Dpot	Potential Dose
EC	Electrical Conductivity
ED	Exposure Duration
EF	Exposure Frequency
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immuno-Sensitive Assay
EPA	Environmental Protection Agency
EU	European Union
EURL	European Union Residue Laboratory
FAO	Food and Agricultural Organization
FD	Fluorescence Detection
FMOC-Cl	9-fluorenylmethylchloroformate
FPD	Flame Photometric Detector
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GLY	Glyphosate

GM	Genetically modified
GR	Glyphosate-resistant
GSA	Ghana Standard Authority
GSS	Ghana Statistical Service
HCA	Hierarchical Cluster Analysis
HPLC	High Performance Liquid Chromatography
HPLC-FD	High Performance Liquid Chromatography – Fluorescence Detector
HQ	Hazard Quotient
IARC	International Agency Research in Cancer
IC	Ion Chromatography
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
IDA	Irrigation Development Authority
IngR	Ingestion Rate
JICA	Japan International Cooperation Agency
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography Coupled to Tandem Mass Spectrometry
LD	Lethal Dose
LOD	Limit of Detection
LOQ	Limit of Quantification
MiDA	Millennium Development Authority
MLGRD	Ministry of Local Government and Rural Development
MoFA	Ministry of Food and Agriculture
MRL	Maximum Residue Limits
MS	Mass Spectrometry
OP	Organophosphates

PCA	Principal Component Analysis
PDI	Potential Daily Intake
PFJ	Planting for Food and Jobs
POEA	Polyoxyethylene Amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
QuPPe	Quick, Polar, Pesticides
R_fD	Reference Dose
SDG	Sustainable Development Goal
SPE	Solid Phase Extraction
SRM	Single Residue Method
SW	Surface Water
TDS	Total Dissolved Solid
UN	United Nations
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WHO	World Health Organization

ABSTRACT

The study investigated the occurrence/presence of glyphosate residues in groundwater and surface water from selected farming (Pepper, Maize and Cassava) communities within the Central Tongu district of Ghana. The study also evaluated the potential health risk posed to children and adults through drinking of ground and surface water. This was achieved through: **(i)** assessment of the levels of glyphosate residues in groundwater (Boreholes) and surface water within farming communities of Central Tongu; **(ii)** appraisal of physical parameters of the waters, as well as assessment of their influence on the levels of glyphosate residues via Principal Component Analysis (PCA); **(iii)** investigation of the relationship between glyphosate levels and water resources within Central Tongu district using Hierarchical Cluster Analysis (HCA); and, **(iv)** estimation of the Human Health Risk associated with consumption of groundwater (Borehole) and surface water by inhabitants (children and adults). Glyphosate residue levels were determined by LC-MS/MS; after extraction of glyphosate residues from water using dichloromethane. pH, Temperature, Salinity, Conductivity and Total Dissolved Solids (TDS) were measured on-site using LaMotte PockeTester Multi-meter. The pH of the borehole samples ranged from slightly acidic (5.69) to near neutral (7.4); whiles the pH of the surface water samples ranged from near neutral (7.35) to weakly basic (8.0). Temperature of borehole samples was between 29.7 to 33.5 . The temperature of the surface water samples was also between 30.5 and 31.8 . Conductivity of surface water ranged from 1025 to 1175 µS/cm; with that for groundwater samples (Borehole) ranging from 1350 - 11320 µS/cm. Salinity ranged from 450 to 570 mg/L; and 640 and 6640 mg/L for surface water and groundwater (Borehole) respectively. TDS for surface water ranged from 670 to 850 mg/L; and 920 to 7780 mg/L for borehole samples. Levels of glyphosate residues (Groundwater: **2.57 - 9.21 µg/L**; Surface water: **25.10 - 42.50 µg/L**) obtained were below the 280 µg/L Recommended Guideline Value for Canada; and the USEPA Maximum Allowed

Concentration of 700 µg/L. However, the levels of glyphosate residue obtained were higher than the recommended 0.1 µg/L glyphosate residue in drinking water by the European Union. pH was identified as the sole physicochemical parameter influencing glyphosate residues in the water resources (through Principal Component Analysis [PCA]). Glyphosate residues were high in slightly basic water samples; and low in slightly acidic water samples. Hierarchical Cluster Analysis (HCA) also established close association between levels of glyphosate and the type of Water resources. The levels of glyphosate in groundwater were relatively low compared to the high levels of glyphosate in surface water. Assessment of Non-carcinogenic Risk using Hazard Quotient (HQ) for acute and chronic exposures revealed some level of risk associated with the consumption of groundwater and surface water from the selected farming communities in the Central Tongu district. For acute exposure, the HQ for groundwater ranged from 0.18 to 0.65 for adults; 0.36 - 0.80 and 1.19 - 1.29 (for two communities; Bakpa Dzave and Kanikope) for children. For surface water, HQ ranged from 1.77 - 3.01 (Adults) and 3.51 - 5.95 (Children). For chronic exposure, the HQ for groundwater ranged from 0.05 - 0.19 (Adults) and 0.10 - 0.37 (Children); whiles the HQ for surface water ranged from 0.51 - 0.86 and 1.00 - 1.70 for adults and children respectively. Groundwater/borehole water did not pose any potential health risk to both adults and children from chronic exposure. Though surface water did not pose any potential health risk chronically to adults; children are at risk. For acute exposure, borehole water did not pose a potential health risk to both adults and children; notwithstanding, children in Bakpa-Dzave and Kanikope were at risk. Acute exposure to surface water poses a potential health risk to both adults and children.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Glyphosate, {2-[(phosphonomethyl) amino] acetic acid} or [N-(phosphonomethyl) glycine], a systemic, broad-spectrum herbicide is the world's popular chemical herbicide. Glyphosate was synthesized in 1950. Roundup^{TM}, a glyphosate containing herbicide was then developed (Franz et al., 1997). This was done by the Mosanto Corporation in the 1970 (Baylis, 2000). These Glyphosate – containing herbicides are widely applied in weed clearing on farms. They are also used in forestry, parks, public places and gardens (Sirinathsinghji, 2014). Glyphosate's main effect is to block an enzyme: 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) that aid plants to manufacture amino acids and proteins. Blockage of the enzyme results in the death of plants within few days (Hoagland and Duke, 1982).

Globally, farmers apply more than a billion pounds of pesticides every year during land clearing for agricultural activities especially during crop cultivation. The applied agrochemical aids in weed control resulting in high crop yield (Benbrook, 2016). Notwithstanding, their vital role, the application of these agrochemicals has potential adverse effects on the environment (water resources etc.) and human health (Fleming, 1987).

Pesticides enter the hydrologic system from point sources (associated with specific points of discharge), and from non-point sources, which are diffuse and widely dispersed. Non-point sources are the dominant sources of pesticides found in ditches, lakes, and estuaries streams, rivers (surface water) and groundwater. Some non-point sources have also been identified. They include runoff

to streams from agriculture and urban land. They can also include seepage through soil and rock layers. These move into underground water sources; with Aquifers as examples (USGS, 2019).

About 97% of the available freshwater on earth is ground water. This forms an important component of the water cycle (Delluer, 1999). It plays major roles to life. It is used in agriculture, industry and for domestic use. It has also found use in maintaining soil moisture. They are also used in maintaining wetlands and stream flows around the world (Oladeji et al., 2012).

It has been estimated that in China, groundwater makes up about 70% of water sources. It also accounts for 40% of the total water used for domestic and irrigation purposes (Qiu, 2010). More than one-third of the global population depends on groundwater as their major source of potable water (Nickson et al., 2005).

Groundwater in Ghana serves as the major source of water for its indigenes of rural communities. People in emerging urban communities also use groundwater (Duah and Xu, 2006). The quality of groundwater is important to these communities. This is because it is the main source of water for them. They drink and use it for other domestic activities. The United Nations Sustainable Development (UN SDG) Goal 6 *"Availability and Sustainable Management of Water for All"* is a global clarion call to ensure the availability and sustainable management of water sources (UN, 2015). Farming communities within the Central Tongu District rely chiefly on Groundwater (drinking and other purposes). Surface water is the source of drinking water for few communities within the district.

Contamination of limited water resources has become one of the most serious environmental, social, economic, and political challenges, especially in developing countries. It is estimated that

87% of farmers in Ghana use pesticides to control pest and disease on vegetables (Dinham, 2003). It has been estimated that 44% out of the 87% pesticides used are herbicides. Insecticides also make up 33%. Fungicides also account for 23% (Ntow et al., 2006).

Herbicides are an undeniable part of modern agriculture, used to control weeds from flower gardens to agricultural crops. Although often taken for granted, without these important products, food production would decline, many fruits and vegetables would be in short supply and prices would rise (Paloma, 2011).

As the effectiveness of these herbicides is realized, farmers have increased its application proportionately to meet their production target without giving due cognizance to the side effect of the herbicide in the environment and its potential of contaminating ground water and other water bodies (Ayansina et al., 2003).

Herbicides currently constitute the largest pesticides consumed in Ghana compared to insecticides and fungicides, in terms of quantity (Frimpong-Anin et al., 2017). Agrochemical vendors and farmers generally regard herbicides as relatively non-poisonous to humans and thus do not normally put on protective gear when handling or using it (Frimpong-Anin et al., 2017). Another area where herbicide residue is crucial but again has not attracted attention is pollution of water bodies. Herbicides are easily washed away by rain runo or through irrigation waters or drift into water bodies, especially when time lapse between spraying and rainfall is short. It may also leach into underground water (Ruiz-Toledo, 2014). All these are exacerbated by excessive dose, short post application to rainfall interval (usually less than 2/4hrs) improper disposal of empty containers and washing of equipment (Fianko et al., 2011).

The physicochemical properties of pesticide compounds play active roles in water. Properties such as solubility in water and organic compounds are vital. Their octanol-water partition coefficient also plays a part in their activities. These parameters determine the leaching characteristics of the chemicals into surface water (McBean, 2015). The chemicals undergo decomposition processes. These are in relation to their chemical stability. This makes the metabolites of these compounds important (Szekacs et al., 2015).

Glyphosate [N-(phosphonomethyl) glycine] and glyphosinate are non-selective herbicides widely used globally, being routinely applied to control weeds in both agricultural and urban setting (Duke and Powles, 2009)**.** The sodium salt of glyphosate can act as a plant growth regulator and accelerate ripening of specific crops (USEPA, 1993). They bind with soil components such as clay and minerals. They are thus thought to be immobile in the soils. They are however unstable. This happens when they are bio-transformed into aminomethylphosphonic acid in the soil (Rose et al., 2016). They have low cost of production. They are also known to be low in mammalian toxicity. They have high polarity and are water-soluble. This makes then have high leaching potential (Baylis, 2000; Busse et al., 2001).

Microbial breakdown of glyphosate produces Aminomethylphosphonic acid (AMPA) which is more toxic and persistent product and non-toxic products- Sacrocine and Glycine. Glyphosate is considered to decay fast in soil, with some reported half-lives (DT_{50}) < 5 days and (DT_{90}) < 10days (ie, the time required for 50% and 90% of a compound to disappear from the soil) (Bento et al., 2016).

In 2015, a monograph issued on basis of scientific evidence concluded, “Glyphosate is probably carcinogenic to humans” (IARC, 2015). This means that this popular organophosphate compound, which is found in most agro-chemicals including the popular Ghanaian brand ‘Edwuma Wura’,

'Nwura wura', 'Landlord,' among others, potentially causes cancer. Exposure to glyphosate is linked to Chronic Kidney Diseases (CKD) and over eight types of Lymphoma (Cocco et al., 2013).

1.2 Problem Statement

Agriculture is the leading/dominant economic sector and the main source of livelihood for the people of the Central Tongu District. Major crops cultivated include; maize, cassava and pepper. Rice and tomatoes are other crops produced by farmers in the district. Farm products from the district are primarily marketed in the cities, and exported to some West African countries. Due to the thriving nature of agriculture in the district, farmers over the years have continuously increased the acreages of land cultivated; resulting in extensive use of human labour for land clearing/preparation for crop cultivation. Due to rising cost, the use of human labour has largely paved way for the use of herbicides (mostly glyphosate-based) in land clearing. In addition, to land clearing, glyphosate-based herbicides are applied in fields to control pests, diseases and weeds; thus, preventing them from reducing the crop production potential. This is as a consequence of the invasion by many common weed species due to favorable environmental conditions (abundant rainfall, adequate sunlight, fertile soil) prevailing in the district.

In view of the large quantities of herbicides applied by the farmers in the Central Tongu district, a reasonable amount of the applied herbicides does not reach the target; but ends up contaminating environmental resources (such as surface and groundwater) due to its ability to leach into the soil profile until reaching the water table and the well.

The population of Central Tongu district depends largely on surface and ground water for drinking and other activities. Glyphosate-based herbicides metabolizes in surface and groundwater into its major metabolite, AMPA. This metabolite is persistent and more toxic in groundwater. This poses a major health risk to the population (children and adults) since it is a probable carcinogen (IARC, 2015). Consequently, it has become imperative for constant monitoring of the levels of glyphosate (as well as its metabolite, AMPA) in surface and groundwater within the district in order to ascertain their wholesomeness. In addition, data generated could aid policy formulation or review on the use of glyphosate-based herbicides in agriculture within the Central Tongu district and its environs.

1.3 Research Objectives

1.3.1 Overall Objective

The overall aim of this work is to investigate the occurrence/presence of glyphosate residues in groundwater and surface water from selected farming communities within the Central Tongu District; and to evaluate the health risk posed to children and adults.

1.3.2 Specific Objectives

The specific objectives of the study are:

(i) To assess the levels of glyphosate residues in groundwater (boreholes) and surface Water within farming communities in Central Tongu;

(ii) To appraise the physical parameters of the waters, and assess their influence on the levels of glyphosate residues;

(iii) To investigate the relationship between glyphosate residue levels and type of water resource; and,

(iv) To estimate the Human Health Risk associated with consumption of groundwater (borehole) and surface water by inhabitants of the farming communities in Central Tongu.

1.4 Justification

The Central Tongu vicinity is predominantly agrarian and therefore majority of the indigenes are mostly farmers who entirely rely on pesticides to combat pest and diseases, which affect the quality of drinking water and soils in the area. Even though, with the high use of pesticides in the area, little is known about the levels of their residues in water and soils in the district (GSS, 2014).

Due to the adverse human health effects caused by pesticides, there is an uncertainty with relation to long-term low dose exposure effect on the environment as well as humans.

Investigation of glyphosate residue levels in the environment has become imperative; to provide the necessary data that could be used by health professionals to protect people in the area. Consequently, the study endeavors to address this concern as well as provide data on glyphosate residue levels of groundwater and surface water in the communities.

CHAPTER TWO

LITERATURE REVIEW

This Chapter provides a review of pertinent literature related to the work/research undertaken. The review commences with information on glyphosate and ends with analytical methodology.

2.1 Glyphosate

2.1.1 General Description

[(phosphonomethyl)amino]acetic acid (commonly called Glyphosate [GLY]) is a herbicide (systemic and broad-spectrum). Accordingly, GLY moves through entire plant, and it kills any plant not genetically modified to resist it. Its main effect is to block the enzyme, *5-enolpyruvylshikimate-3-phosphate synthase* (EPSPS) that plants need to produce amino acids and proteins (van Stempvoort et al., 2014). Glyphosate has a molecular formula, $C_3H_8NO_5P$; and structurally represented by (Fig.2.1):

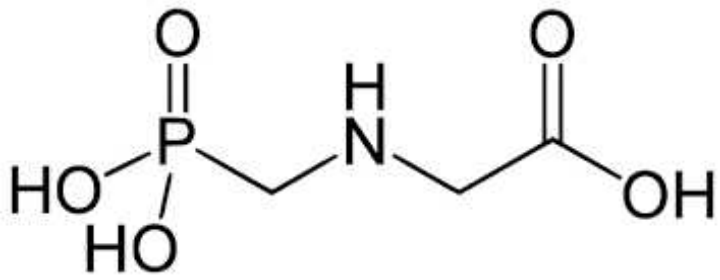

Fig.2.1: Chemical Structure of Glyphosate

A pharmaceutical company originally developed Glyphosate. Its weed-killing properties were however, patented by a US company by Mosanto in the 1970s. The patent by Mosanto nonetheless expired in 1991 outside of the United States of America. In the United States of America however, the patent expired in 2000. This has made other pesticide companies market their glyphosate-based

products. This has led to the sale of hundreds of glyphosate-containing herbicides the world over (Hanke et al., 2010).

In herbicide formulations, the pure state of glyphosate is not used; rather its combination with an alkali to form a salt is used. Ammonium, trimethylsulphonium and isopropyl-ammonium (IPA) are the major alkalis used. IPA is a major chemical used in dye making. Other substances were included in the glyphosate production process. To increase glyphosate's ability to penetrate plant cells, Surfactants are also used. The right quantity of the chemicals used remains a trade secret. The only active ingredient in all these is glyphosate; and always listed on the products' label. This makes it difficult to find out the names of the other constituents used in the chemical (FoEE, 2013).

2.1.2 Biochemistry of Glyphosate

Glyphosate biodegrades by two pathways [Fig. 2.2]: **(i)** Sarcosine; **(ii)** the Aminomethylphosphonic acid (AMPA) (Karpouzas & Singh, 2006; Borggaard & Gimsing, 2008). Pathway (ii) is regarded as the prominent degradation path in soils with AMPA, the main product of degradation (Al-Rajab and Schiavon, 2010). Glyphosate breakdown through microbial action (Sarcocine) is associated with cleavage of glyphosate's C-P link through C-P lyase; with the resultant release of PO_4^{3-} as well as Sarcosine (Karpouzas & Singh, 2006). Further transformation of Sarcosine to glycine follows (microorganisms use the glycine formed for biosynthesis of protein) [Borggaard & Gimsing, 2008].

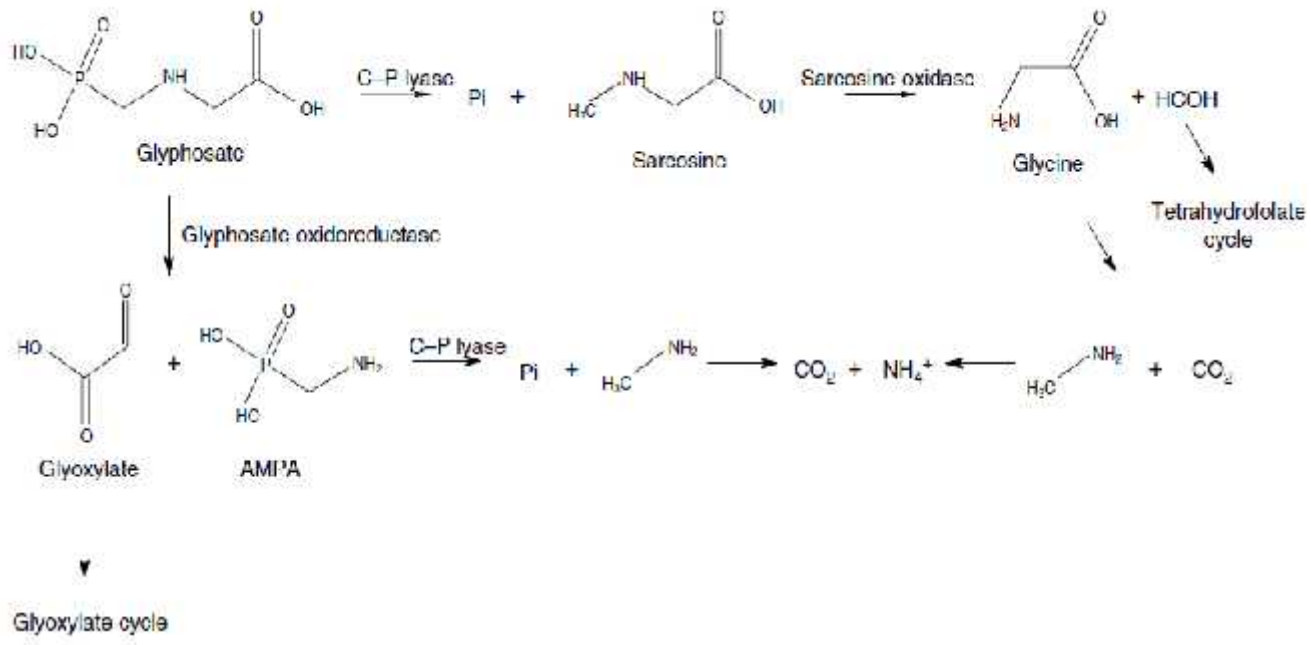

Fig. 2.2: Glyphosate degradation pathways (Sacrosine and AMPA) in Soil

Glyphosate is degraded in the AMPA pathway. This is done by the cleavage of the C-N bond. This resulted in the release of AMPA and glyoxylate (Borggaard and Grimsing, 2008; Karpouzas and Singh, 2006). The AMPA is dephosphorylated by the C-P lyase. This results in the formation of an inorganic phosphate and methylamine. This is then mineralized into CO_2 (Borggaard and Grimsing, 2008; Karpouzas and Singh, 2006).

2.1.3 Glyphosate Formulated Herbicide Application

Applied in both Agricultural and non-agricultural activities for weed clearing/control; Glyphosate formulated herbicides have been used for decades (Benbrook, 2016; Duke and Powles, 2008).

For non-agricultural applications, herbicides with glyphosate formulations are generally for weeds control on urban roads, amusement/community playgrounds, private homes, landscaping designing and horticulture (Tang et al., 2015). For agricultural applications, glyphosate formulated herbicides are used for pre-and post-planting weed control (Singh et al., 2011; Maqueda et al.,

2017). Also, used as desiccant during pre-harvesting of crops (bean and grain) [Goffnett et al., 2016]. Glyphosate formulated herbicides are applied/used throughout the planting season (during crops growing period) and during pre-planting period (mostly a current/recent agricultural practice; that is cultivation of glyphosate resilient crops without soil-tillage) [Baylis, 2000; Benbrook, 2016].

2.1.4 Glyphosate and AMPA in the Environment

Though generally glyphosate and its metabolite, AMPA, are least expected to be present in adrift environment, a number of studies have reported their transport off-site. Occurrence of glyphosate in surface water, suspended matter and stream-bed sediments have been reported (Aparicio et al., 2013; Primost et al., 2017; Peruzzo et al., 2008; Okada et al., 2018; Battaglin et al., 2014). There have been reports of the presence of glyphosate and its main metabolite in groundwater, atmospheric dust and rainwater (Brüsch, 2006; Scribber et al., 2007; Chang et al., 2011; Sanchis et al., 2012; Humphries et al., 2005). This is against their backdrop as having low leaching and volatility.

2.1.5 Problems Associated with Glyphosate Formulated Herbicides (Environmental Health)

Due to its perceived low/no environmental and human health concerns, glyphosate is main times regarded as environmentally friendly (Williams et al., 2000; Giesy et al., 2000; Cox, 2004).

These are because of some properties that glyphosate possess. It is known to be non-volatile, has rapid biodegradation and strong soil particles adsorption; with Shikimate pathway action as favorite (non-existent in humans as well as animals) [USEPA, 1993; Borggaard and Gimsing,

2008; Duke and Powles, 2008]. The main metabolites of glyphosate as well as commercial formulations of glyphosate exhibit the same properties (Williams et al., 2000).

A number of studies have highlighted glyphosate's adverse effects on the environment and on human health. Societal concerns about the safety of glyphosate have increased considerably (van Bruggen et al., 2018). Commercial glyphosate-containing products have higher toxicity. Their toxicity is higher than that of the raw glyphosate. Commercial glyphosate-containing product like polyethoxylated tallowamine (POEA) surfactants are examples (Howe et al., 2004; Peixoto, 2005; Bonfanti et al., 2018).

2.1.6 Glyphosate Formulated Herbicides and its Human Health Impacts

Glyphosate and its metabolites are considered non-toxic and of no adverse human health impacts as available evidence suggests low absorption (oral and dermal) which are basically excreted (faeces and urine) un-metabolized; confirming the non-bioaccumulation of glyphosate in body tissues (Williams et al., 2000). Furthermore, glyphosate is considered to be non-carcinogenic, non-neurotoxic, non-mutagenic, non-genotoxic, and non-teratogenic since there is no clear proof to this effect (USEPA, 1993).

Nevertheless regarded as a˝probably carcinogenic to human˝; leading to increased societal concerns and disagreements among regulatory bodies (IARC, 2015). EFSA, the European Food Safety Authority, is however, in disagreement (EFSA, 2015). In the review study by Mesnage et al. (2015) on glyphosate formulations toxic effect, they concluded that below regulatory limits, formulations could be toxic (chronic).

Numerous studies have shown that glyphosate formulations may have adverse human health effects. Studies have revealed a link between glyphosate and increasing number of miscarriages, respiratory diseases and dermatological effects (Camacho & Mejia, 2017). It also affects human reproduction and developmental foetus (Benachour et al., 2007).

Studies by Jayasumana et al. (2015) in Sri Lankan Farmers associated increased frequency of chronic kidney disease among farmers with exposure to glyphosate (drinking of water from contaminated wells as well as spraying of glyphosate herbicides without protective clothing/equipment). According to Kruger et al. (2014), chronically ill humans showed higher glyphosate in their urine compared to healthy humans.

2.1.7 Monitoring of Glyphosate in the Environment

Glyphosate exhibit unique characteristics in soil compared to other pesticide active ingredients. Predominantly, apolar group pesticides bind to soil organic (Borggaard & Gimsing, 2008). Glyphosate has amphoteric character (zwitterionic), offering analytical challenge to Analytical chemists. Due to this chemical behavior of glyphosate and its metabolite, routine analytical methods are unable to detect them with desired sensitivity. The analytical challenge in glyphosate determination is the reason for its rare monitoring in the environment (Payne, 1992).

During the mid 1990's, Glyphosate was among pesticides of concern in surface water in Europe's Mediterranean region (Barcelo and Hennion, 1997). Glyphosate and AMPA detected in water from two small tributaries of Ruhr River in North-Rhine-Westphalia, Germany; with levels around 590 ng/L was reported (Skark et al., 1998). A monitoring study in Norway found glyphosate and

AMPA in surface water. Glyphosate as well as its metabolite, AMPA were identified in about 54% of 540 samples of surface water collected between 1995 and 1999. Highest concentration detected was 0.93ug/L (Mean: 0.13ug/L glyphosate) [Ludvigsen and Lode, 2001].

2.1.8 Previous Work Done in Ghana on Pesticide Residues in the Aquatic Ecosystem

A study by Acquaah (1997) on the levels of pesticides in water samples from Akumadan, a well-known vegetable farming community in the northwestern part of the Ashanti Region of Ghana revealed the presence of significant levels of pesticide residues. The pesticides detected (µg/L) were; lindane (9.5); α-endosulfan (62.3); β-endosulfan (31.4) and endosulfan sulfate (30.8). Lindane and endosulfan in water from the Volta Lake (Ghana) were in concentrations: ≤ 0.008 µg/L and 0.036 µg/L, respectively; and ≤ 2.3 µg/L and 0.36 µg/L respectively in sediments. Dichlorodiphenyltrichloroethane and dichlorodiphenyldichloroethylene in sediment samples (≤ 9.0 µg/L and 52.3 µg/L, respectively) were reported [Ntow, 2005].

Kuranchie-Mensah et al. (2012) also assessed the levels of organochlorine pesticide residues in sediment and water from the Densu river basin, Ghana. The highest detected concentration of organochlorine in water was endosulfan sulfate with mean concentration of 0.185 µg/L. Water from Lake Bosomtwi (Ashanti region of Ghana) was found to be mildly contaminated with lindane (0.071 µg/L), DDT (0.012 µg/L), DDE (0.061 µg/L) and endosulfan according to a study by Acquaah, (1997).

A study conducted on water from some rivers in the Ashanti region of Ghana: river 'Oda' (Ejisu-Besease), River Kowire (Agogo) and River Twetwe (Akumadan) revealed the presence of two pesticides (lindane and endosulfan). The respective levels detected for lindane and endosulfan

were 19.4 and 12.4 µg/L (Oda River), 16.4 and 17.9 µg/L (Kowiæ River) 20.5 and 21.4 µg/L (Atwetwe River) [Acquaah and Frempong, 1998].

Akoto et al. (2016) assessed the levels of organochlorine (OC) and organophosphorus (OP) pesticide residues in fish, sediments and water and the health risk associated with the consumption of the fish from the Tono Reservoir, Northern Ghana. Organochlorine and organophophorus pesticides (after ultrasound sonication and Soxhlet extraction of pesticides) respectively were detected by Gas Chromatography equipped with electron capture detector (GC-ECD) and pulse flame photometric detector (GC-PFP). For water, the concentrations of pesticides reported were below the detection limit. Mean concentrations of organochlorine pesticide (OCP) residues in fish ranged (µg/g): 0.017-0.17 (*Sarotherodon galilaeus*), 0.043-0.30 (*Clarias anguillaris*), 0.027-0.243 (*Schilbe intermedius*) and 0.097-0.263 (*Marcusenius senegalensis*). The study (for sediments) identified Aldrin as the pesticide with the highest level (0.090 µg/g); while the lowest level (0.047 µg/g) was p,p′-DDD. Health risk estimation revealed that aldrin in *Marcusenius senegalensis* had great potential for systemic toxicity to consumers.

Another study conducted by Fosu-Mensah et al. (2016) in four water bodies [Chemu lagoon (Tema), Korle lagoon (Accra), Fosu lagoon (Cape Coast) and the Etsii lagoon (Abandzi)] in Southern Ghana (Accra and Central regions). The objective was to ascertain the presence and subsequent amount of four organochlorine pesticides: dichlorodiphenyl dichloroethylene (2,4'-DDE), 4,4'-dichlorodiphenyldichloroethane (4,4'-DDD), p,p'-dichloro- diphenyltrichloroethane [p,p'-DDT (r) (i) (f)] and Propiconazol (f); as well as four organophosphorous pesticides: Fenitrothion (i), Chlorpyrifos (i), Dichlorvos (a) (i) and Diazinon (a) (i) Liquid-liquid and liquid-solid extraction technique was employed to extract pesticide residues in water and fish samples,

respectively, using 1:1 (v/v) ethyl acetate/dichloromethane mixture before being analyzed by gas chromatography. The highest level of pesticide contaminations was recorded in the Chemu lagoon as compared to the Korle lagoon and Fosu lagoon, with the Etsii lagoon showing the least contamination. The total average pesticide residues in water samples from the four lagoons: Chemu, Korle, Fosu and Etsii are 2.6384 mg/L, 0.4992 mg/L, 0.3045 mg/L and 1.3629 mg/L, respectively. The total average pesticide residues obtained in fish samples (*Sarotherodon melaanothern*) from the Fosu and Etsii lagoons are 0.0155 mg/kg and 0.0088 mg/kg, respectively. This shows some level of exposure of pesticide, which would be harmful to human.

2.2 Statistics of Glyphosate Import and Use in Ghana

In 2013, glyphosate was the world's largest selling herbicide, with Chinese manufacturers as the world's largest producers of glyphosate and its precursors (Duke and Powles, 2008). Currently marketed under numerous trade names by more than 50 companies in several hundreds of crop protection products around the world. More than 160 countries have approved uses of glyphosate-based herbicide products (Monsanto, 2003).

In Ghana, common glyphosate-based herbicides are marketed under trade names such as GlyKing Eduodzi Ridout® (Glyphosate 480 g/L, Soluble Liquid) and Ridover Eduodzi® (Glyphosate Ammonium 75.7%, Soluble Granular) [RACL, 2019]. Others brands are sold with local names such as 'Asase wura', 'Edwuma wura', 'Nwura wura', 'LandLord', 'Power' and 'Sarosate' (Crozier et al., 2018).

Glyphosate is the best-selling herbicide active ingredient on the Ghanaian market. As at December 2014, over twenty (20) different herbicide active ingredients have registered with the Ghana Environmental Protection Agency [with 24.3 % being glyphosate] (EPA, 2014). In 2014, Ghana

imported a total of 4,578,396.38 kg of solid glyphosate and 18,395,719 L of liquid glyphosate. In 2015, the figures increased by about 74.4 % (17,876,234.21 kg) for solid glyphosate and approximately 57.3 % (43,078,776 L) for liquid Glyphosate (EPA, 2015).

2.3 Analytical Techniques Used in Glyphosate and AMPA Analysis

Due to low levels set by countries and international bodies for glyphosate in water, soil and food, glyphosate-based herbicides finds extensive application globally. Determination of glyphosate residues in such matrices requires the use of extremely sensitive analytical methods. Analytical techniques applied for separation and quantification of glyphosate residues in various matrices includes, Capillary Electrophoresis (CE), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), and Ion Chromatography (IC) are prominent separation techniques reported used (Struger et al., 2008; Botta et al., 2009).

2.3.1 Chromatographic Methods

To separate and quantify glyphosate mixtures into their components, chromatographic techniques (HPLC, LC, GC, IC) are used to allow each part to be analyzed separately (Zelenkova and Vinokurova, 2008). On the other hand, Mass Spectrometry can detect separated components after chromatographic separations.

2.3.1.1 Liquid chromatographic Technique

This technique is one of the prominent techniques for glyphosate separation. A derivatization process is required; approaches like pre-column and post-column have been used (Hogen-doorn et al., 1999; Patsias et al., 2001). LC is used after derivatization in conjunction with fluorescence and

UV/visible (LC-UV/Vis) detection, as well as LC-FLD (Nedelkoska and Low, 2004). LC with direct sample injection, column derivatization (post) and fluorescence detection without concentration is the recommended USEPA method for GLY determination in drinking water. P-toluenesul-fonyl chloride, o-nitroben-zenesulfonyl chloride and 2, 5-dimethoxy benzenesulfonyl chloride are derivatization reagents for UV detection. Pre-column, 9-fluorenylmethyl chloroformate (FMOC-Cl) derivatization and fluorimetric detection are adopted LC techniques for GLY. 9-fluorenylmethyl chloroformate (FMOC-Cl) and o-phthalaldehyde (OPA) were used in post-column FLD detection (Nedelkoska and Low, 2004; Hidalgo et al., 2004).

Owing to the complexity of regulating the reaction in the post-column HPLC reflux system, derivitization by pre-column is accurate than the post-column. Limit of detection (LOD) for derivitization by pre-column is 0.2ng/L (water) and 0.02mg/kg (soil); derivitization (post column) is 2.0ng/mL in aqueous samples. Liquid chromatography is a fast and sensitive method for GLY residues detection; it however, requires processes of derivitization and high-end equipment (Sancho et al., 1996).

2.3.1.2 Gas Chromatography (GC)

Application of gas chromatographic technique is scarce due to the complex derivatization process, with evaporation enhanced by esterification and acylation. Gas chromatographic separation mostly transforms the analyte into volatile form. It also produces a stable derivative. This is after the pre-column derivatization of glyphosate (Tadeo et al., 2000; Borjesson and Torstensson, 2000). As a flame photometric detector (GC/FPD) flame ionization detector (GC/FID), electron capture detector (GC/ECD) as well as nitrogen phosphorus detector (GC/NPD); the molecules (P, C, H) present in glyphosate enables the use of allied analytical techniques.

Frequently used derivitization reagents include: N-methyl-N-tert butyldimethylsilicontri-fluoroacetamide, dimethylformamide, trifluoroacetic anhydride, 4,4,4-trifluoro-1-butanol isopropyl chloroformate, diazomethane Trifluoroacetic acid, trifluoroacetic anhydride, trimethyl orthoformate, propionic anhydride and methanol (Ding et al., 2015; Kudzin et al., 2003; Kataoka et al., 1996; Tsunoda, 1993).

Less harmful solvents such as methanol, acetone and ethyl acetate in place of carcinogenic dichloromethane, chloroform and neurotoxic n-hexane as solvents [eluents] (Tseng et al., 2004). LC and GC can therefore evaluate derivatives of GLY in a responsive and selective manner, besides producing unstable products; the steps to turn GLY into a detectable substance are very complicated.

2.3.2 Chromatographic Separation Coupled with Mass Spectrometry

Based on the relatively high and improved sensitivities of LC- and HPLC- Mass Spectrometry, they are useful for separation and detection of glyphosate residues in environmental matrices. Reduced analysis time but relatively high detection limit achieved with Liquid Chromatograph-Solid Phase Elution have been reported (Delmonico et al., 2014). Liquid Chromatography coupled with Electrospray Ionization-Mass Spectrometry (LC/ESI-MS) has also been applied (Sato et al., 2001).

Another analytical technique applied is liquid chromatography coupled with mass spectrometry-mass spectrometry, LC-MS/MS. This technique presents derivitization with substantially increased sensitivity. The technique integrates 2-mass analyzers into one instrument in which the first MS acts as filter for the precursor ion with subsequent high-energy fragmentation; and the second MS analyzer filters ions generated by fragmentation. Apart from noise reduction, the other

advantage of the application of MS/MS is enhanced sensitivity. It has been stated that GLY is sufficiently detected by the LC-MS/MS process; notwithstanding, it takes longer balance time, less robustness and shortens lifetime of the column (Valle et al., 2019).

Good results are obtainable with LC-MS / MS, though metal ions, sample preservation and storage time may have affected the efficiency of the process. However, the LC-MS/MS offers easier preparation of samples, easier operation, quicker and more sensitive, although LC/FLD needs less costly equipment (Valle et al., 2019). Using this technique, repetitive research is achievable without laborious instrumental adjustments. From the findings, LC-MS/MS is applicable to oil plants, for analysis of GLY residues of these compounds. For rapid detection of GLY (a polar pesticide), flow injection tandem mass spectrometry (MS/MS) has been studied (Mol and van Dam, 2014).

The quest for an analysis without derivatization procedures has led to the development of alternative techniques for determination of GLY residues through fast-chromatography and sensitive detection. Post basic extraction (liquid-liquid) and calibration curves prepared in the matrix; followed by precipitation of proteins with organic solvent (sample complexity minimization) [Martins-Júnior et al., 2011].

To analyze potable water, surface water and wastewater with good LOD, a validated fully-automated SPE-LC-ESI-MS/MS have been reported (Vreeken et al., 1998). Likewise, in soil and water samples, selective but responsive SPE-LC-ESI-MS/MS online approach gave amazing detection for GLY, reaching as low as 50 ng/g (soil) and 0.0005 ng/mL (H_2O) [Ibáñez et al., 2005].

It is also interesting to highlight that its application to real-world samples failed to use labeled GLY as internal standard and to apply methods such as SPE-LC-ESI/MS/MS. As further studies

are needed to decide if acidification is a general approach that should be extended to analysis of water samples, the nature of the shaped complex has not been clarified (Ibáñez et al., 2006). Another technique requiring derivatization to impart GLY volatility (Kudzin et al., 2003) is GC/MS. GLY has been detected using three GC / MS related technologies: GC-CI-MS, GC-FID (flame ionization detector)-MS and GC-EI (electron impact)-MS. Techniques typically time-wasting, repetitive and require a considerable multiple manipulation of the sample. Though the techniques are highly sensitive and have the ability to detect very low GLY residue levels, they require the use of advanced equipment, as well as laborious.

CHAPTER THREE

MATERIALS AND METHODS

This Chapter consists of five (5) sections [Sections 3.1 to 3.5]. Section 3.1 describes the study area. Section 3.2 is a description of the collection of water samples from the Central Tongu district. The third part of this Chapter (Section 3.3) is a description of the determination of glyphosate in groundwater and surface water. Section 3.4 is the statistical analysis of data generated from the study. The final part (Section 3.5) focuses on human health risk assessment.

3.1 Study Area

3.1.1 The Central Tongu District

Central Tongu forms part of the twenty-five (25) Municipalities and Districts in the Volta Region of Ghana. The district has its administrative capital at Adidome. The Central Tongu district (5°47 N 6°0 N and 0°5 E to 0°45 E), with a size of 1,460 square kilometres was carved out of the former North Tongu District Council in 1989 by Legislative Instrument (LI.15). The district (Fig.3.1) shares boundaries with Akatsi East and Akatsi West to the east, to the south with South Tongu district and Ada East district respectively, to the west with Dangme West district and to the north with North Tongu district (GSS, 2014).

The population of Central Tongu district according to the 2010 Population and Housing Census is 59,411 representing 2.8 % of the region's total population. The district has a household population of 158764 with a total number of 12,996 households. Households in the district derive their drinking water from diverse sources but the five main sources are river/stream, well, standpipes, dugout and borehole, which together constitute the main sources for 91.2 % of households. Most

of the communities within the sampling site rely on public boreholes as source of water for domestic use (GSS, 2014).

3.1.2 Physical Features

3.1.2.1 Flora

Central Tongu is within the Tropical Savannah Grassland zone. The Volta River runs along the district with many communities dotted along the banks of the river. This makes the district a suitable place for large-scale farming. Agriculture is the leading sector in the district's economy, and dominated by small-scale unorganized farmers who depend mainly on simple, labor-intensive production techniques (GSS, 2014).

3.1.2.2 Geology and Mineral Composition

Central Tongu belongs to the Dahomeyan formation (geological) with Black Earths and Tropical Grey soil composition (medium-moderately coarse alluvial soils in Volta River adjoining areas). Under these soil categories are heavier clayey soils (predominant in Central Tongu). The nature of the soil suits rice farming. Towns within the Central Tongu; namely, Mafi-Kumase, Adidome, BakpaAvedo, Anfoe, Sasekpe and Kpedzeglo have sandy loams/moderately coarse soils. These soil type drains easily and favorable for Agriculture. Sand, Clay and Oyster shells are major mineral deposits (GSS, 2014).

Temperatures (monthly average) range from 22 ^{o}C to 32 ^{o}C generally high temperatures all year round. Notwithstanding, dry season average temperatures are high, reducing cultivation of crops to minimal levels. Rainfall pattern is bimodal with the main season commencing in March to June; while the minor season starts from August and ends in November. The period between December and February constitutes the dry season (lowest rainfall period; leading to scarce agricultural

activities). Highest and lowest rainfall occurs in June and December respectively; with 1250 mm as average annual rainfall (MoFA, 2020).

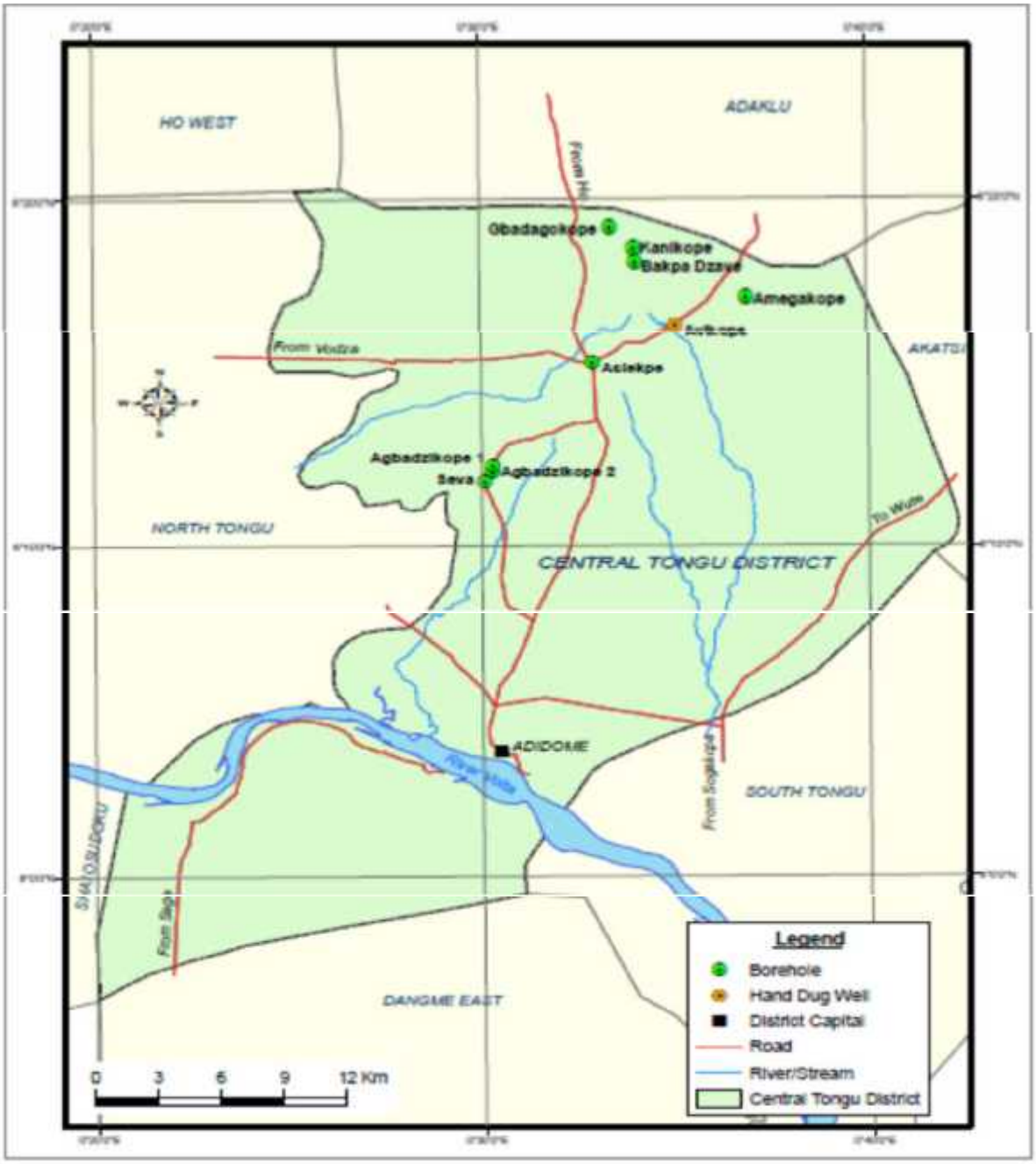

Fig. 3.1: Map of Central Tongu District (Avi et al., 2019**)**

3.1.2.3 Economic Importance of Farming Activities in the Central Tongu District

Agriculture, a leading sector of the district's economy, is dominated by small scale unorganized farmers who depend mainly on natural rainfall, which patterns are uneven and uses simple intensive production techniques. The major crops cultivated include; maize, cassava (Fig. 3.2c), rice, including vegetables such as pepper (Fig. 3.2a, b) and tomatoes (NDPC, 2017).

Fig. 3.2 (a, b & c): Some Food crops cultivated in the Central Tongu District

3.1.2.4 Agro Processing and Export

Agro processing provides value addition to agricultural commodities to increase its marketability. Agricultural produce are marketed in the raw state, especially vegetables. Cassava maybe processed into Gari, Kokonte (dried cassava flour), Starch and Dough. Maize being a staple food is used in the raw powdery form or as fermented dough. Pepper is parboiled, dried and sold in sacks.

The raw and processed products are sold in local markets or nearby markets (such as Mafi-Kumase, Juapong, Adidome and Battor markets). The district also links economically with major marketing centres in the neighboring districts and towns. Furthermore, there is trading activities between the district, Ho municipality, Akatsi, Ketu South, Ketu North, Accra, Ashaiman, Tema and the Republic of Togo (MoFA, 2020).

3.2 Collection of Water Samples from the Central Tongu District

3.2.1 Selected Communities

Eight (8) farming areas/communities and one (1) non-farming area in the Central Tongu District in the Volta Region were used for the study. Areas selected included: Mafi-Agbadzikope, Mafi-Amegakope, Mafi-Bakpa Dzave, Mafi-Gbadagokope, Mafi-Kanikope, Mafi-Seva, Mafi-Tsawla, Mafi-Asiekpe and Mafi-Avikope. These areas were selected due to the intensity of farming activities; and reliance of the selected farming communities on public boreholes and surface water (Fig. 3.3) as the primary source of water for domestic activities (GSS, 2014).

Fig. 3.3 (a, b, c & d): Some Water Resources in the selected communities within the Central Tongu District

3.2.2 Sampling Technique and Size

The study applied Purposive Sampling technique. This technique allows the researcher to choose communities and persons that are relevant to the research and are easily available to the Researcher. Water samples from existing boreholes and surface water resources in nine (9) farming communities were collected for physical parameters and glyphosate residue analysis respectively (November 2019 to January 2020). A total of fifteen (15) water samples; consisting of nine (9) borehole water samples, four (4) surface water samples, one (1) sample from a hand-dug well and one (1) water sample non-farming area (control) were collected for the study.

For Groundwater sampling, water from the borehole was pumped out for approximately 5-min to clear stagnant/still water from the pipes prior to water sample collection. Washing/cleaning (3 times) of Polypropylene bottles to be used for water samples collection was done with pumped borehole water. Surface water (Grab samples) were taken/collected using polypropylene bottles; the bottles were submerging to a depth of about 10 cm below the surface and filled completely/fully with the water (Lawrence, 1996). The water samples were transported under hermitic conditions to the Pesticide Residue Laboratory at Ghana Standard Authority, Accra; and were stored at $^{-}4$ °C and $^{-}18$ °C until further analysis of Glyphosate residue.

3.2.3 Physical Analysis of Water Samples (On-Site/Field Measurements)

Physical parameters such as pH, Temperature, Conductivity, Total Dissolve Solids and Salinity were measured in-situ using a LaMotte **1766 pH/Salinity/TDS/Conductivity/Temperature PockeTester (Tracer™)**.

The Garmin eTrex® Vista (USA) GPS handheld device was used to establish the GPS Coordinates of the sampling locations (Fig. 3.4).

Fig. 3.4 Garmin eTrex® Vista used for taking GPS Coordinates of Sampling Locations

Calibration of LaMotte PockeTester (pH/Conductivity/TDS/Salinity Meter)

pH and Conductivity Standard Buffer solutions were used for the calibration of the Multi pH meter (Fig.3.5). Subsequently, the calibrated meter was immersed into the water samples for pH and conductivity determinations.

Three sample cups were washed thoroughly with distilled water and labeled pH 4.0, pH 7.0 and pH 10.0. The cups were filled with 20 mL distilled water and one buffer tablet of pH units 4.0, 7.0 and 10.0 each were added respectively into the labeled cups. The tablets were dissolved to obtain buffer solutions of pH 4.0; 7.0; 10.0. The multi pH meter was then set to calibrate and the pH of the buffer solutions taken respectively. Concurrently, Physical parameters were taken.

Collected samples on ice were stored in a thermally insulated large polyethylene container, and transported to the laboratory in Accra [Ghana Standards Authority (GSA) Pesticide Residue Laboratories] for determination of glyphosate levels in water samples.

Fig. 3.5 LaMotte PockeTester used for measurement of physical parameters

3.3 Determination of Glyphosate Residue in Water Samples

Chemicals and Reagents Used for Analysis

Certified glyphosate reference standards (Dr. Ehrenstorfer GmbH, Augsburg, Germany) with certified purity of 99% were used for identification and quantification of glyphosate. The standards were stored in a freezer to minimize degradation.

Pesticides-grade Dichloromethane (CH_2Cl_2, 99%, d = 1.32 g/mL, Park Scientific, Northampton, U.K); **HPLC PLUS Gradient grade of Methanol** (CH_3OH, 99.9%, 1.11 vs air, Park Scientific, Northampton, U.K); **anhydrous Sodium Sulphate** (Na_2SO_4, 99.5%, Park Scientific, Northampton, U.K); **Gradient-grade Acetone** (C_3H_6O, 99.9%, 2 vs air, Park Scientific, Northampton, U.K), and **anhydrous Sodium Chloride** (NaCl, 98%, Park Scientific, Northampton, U.K). Phenomenex of USA supplied **Silica Gel** (SiO_2, 55m, 1000 mg/ 6 mL, Teflon® Tubes) adsorbent.

Standard Solutions

Stock solution (1000 µg/L) of the Glyphosate standard were prepared by weighing 1 g of the Glyphosate into a glass beaker (250 ml) and then dissolving with methanol. Solutions were quantitatively transferred with a funnel into 1 L volumetric flasks and made up to the 1 L mark with methanol. Working standards (10 µg/L; 20 µg/L; 50 µg/L; 100 µg/L; and, 200 µg/L) were prepared by appropriate dilutions of the stock glyphosate solution; with mixing by vortex (Thermolyne MaxiMix). Working/operational standards are always prepared fresh; and filtered through a Whatman® 0.45 µm nylon for liquid chromatographic (LC) separation.

Apparatus/Instrumentation

Vortex Mixer (Thermolyne MaxiMix-Plus, Thermo Fisher, Barnstead, USA) used for mixing working solutions; **Büchi Rotary Evaporator** (Rotavapor® R-300, USA) was used for drying extracts; **Liquid Chromatogram** (Agilent Infinity 1290, USA) coupled to an **Agilent G424A Quaternary Pump**, **Temperature Control Compartment** and equipped **with G4226A auto-sampler** used for the identification, detection and quantification of glyphosate residues.

3.3.1 Sample Preparation and Extraction of Glyphosate Residues

The QuPPe Method (Quick Polar Pesticides Method), an European Union Reference Laboratories for Residues of Pesticides requiring Single Residue Methods (EURL-SRM) was employed in its slightly modified form (Ghana Standard Authority's (GSA) Pesticides Residue Laboratory modified Protocol). QuPPe allows the analysis of a number of high-polar pesticides non-amenable to common multi-residue methods (e.g., QuEChERS). The method involves extraction with acidified methanol and LC-MS/MS measurement (Anastassiades et al., 2015).

3.3.1.1 Preparation of Water Samples

Whatman® 0.45 µm nylon filters were used for pre-filtering of samples to remove debris and suspended materials. Appropriate representative sub-samples (~500 mL) were taken after filtration for analysis. Fig. 3.6 is the general scheme (flow chart) for measurement of physical parameters, extraction and detection of glyphosate residues from water; followed by detailed description of experimental procedure.

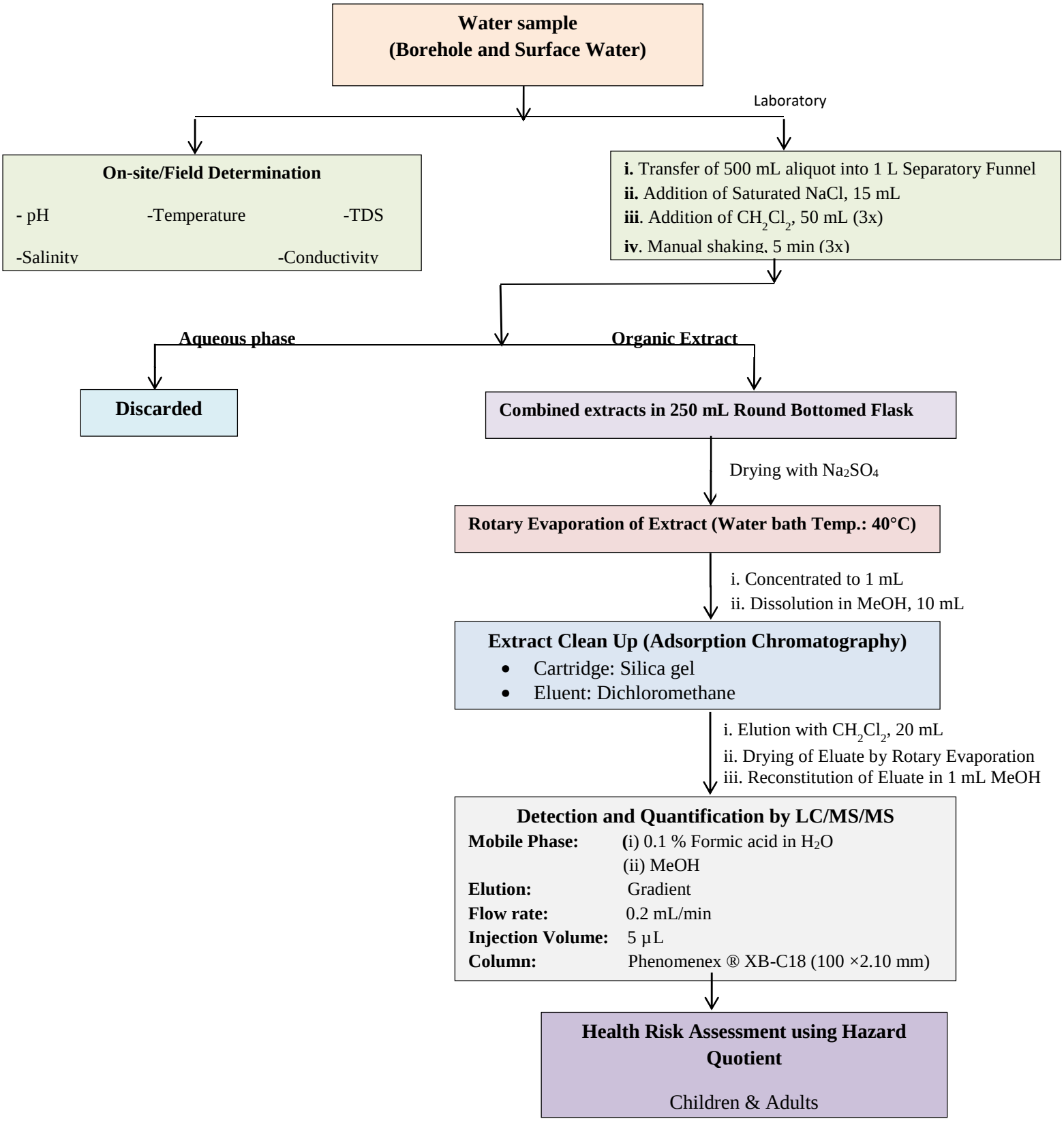

Fig. 3.6: Scheme for analysis of water sample

3.3.2 Extraction of Glyphosate Residue

After filtration, 500 mL of water sample was transferred into a 1 L capacity separatory funnel and 15 mL of saturated sodium chloride (NaCl) solution added. The solution was partitioned with 50 mL of dichloromethane (CH_2Cl_2) (thrice), each time shaking the separatory funnel vigorously for 5 minutes and releasing the pressure intermittently. The dichloromethane (CH_2Cl_2) extract was drained after allowing the layers to settle and separate.

Combined extracts of dichloromethane (CH_2Cl_2) layers were collected and dried over anhydrous sodium sulphate (Na_2SO_4) through Whatman® 0.45 µm nylon filter paper to remove water (if any). The collected extracts were concentrated to dryness (~1 mL) by rotary evaporation (R-300, BÜCHI, USA). Concentrated extracts were subsequently transferred with a Pasteur pipette into vials, rinsing the round-bottomed flask with 10 mL methanol (CH_3OH) and subjected to clean up.

3.3.2.1 Clean-up/Extract purification using 'Silica Solid-Phase Elution (SPE) cartridge'

Polypropylene cartridge columns packed with 1 g silica gel (pre-oven-activated for 10-hours at 130 ºC) was used for extract clean-up (Adsorption Chromatographic separation) [Fig. 3.7].

About 2 g Na_2SO_4 (anhydrous) was placed above the silica gel and conditioned by elution with CH_2Cl_2 (6 mL). Extracts (concentrated) were percolated through the conditioned silica-packed-Na_2SO_4-topped column; with a 100 mL round bottom flask placed under the column for eluent collection. The sorbed glyphosate residues in the SPE cartridge were eluted with 20 mL dichloromethane (CH_2Cl_2). The eluates collected were subsequently dried by rotary evaporation.

Fig. 3.7: Silica gel Clean-up

3.3.2.2 Evaporation of Eluate to Dryness and Reconstitution of Eluate

The eluates were concentrated to near-dryness at 40 ºC by rotary evaporation (R-300 BÜCHI Ratovapor®) [Fig. 3.8]. Quantitative transfer followed re-dissolution of concentrates in methanol (CH3OH; 1 mL) into 2 mL standard vials for Liquid Chromatographic-Triple Quadrupole Mass Spectrometric analysis. During the period of analysis, extracts were kept frozen.

Fig. 3.8: Rotary Evaporator used for evaporating Extracts to dryness

3.3.3 Identification and Quantification of Glyphosate

An Agilent 1290 Infinity Liquid Chromatogram coupled to an Agilent G424A Quaternary Pump, Temperature Control Compartment and equipped with G4226A auto-sampler was used for the identification of the Glyphosate (Fig. 3.9).

Fig. 3.9: Agilent 1290 Infinity series LC-MS/MS facility in the Pesticide Residue Laboratory of Ghana Standard Authority used for identification and quantification of Glyphosate residues

The identification of analytes was based on their retention times to the retention times of the internal standard. Table 3.1 gives the chromatographic conditions used for glyphosate levels determination.

Table 3.1: Chromatographic Conditions and flow program for glyphosate residues

Parameters	Conditions	
Analytical Column:	Phenomenex ® Kenetex 2.6 µ XB-C18 (100 x 2.10 mm)	
Guard Column:	Agilent Eclipse C18 (2.1x5 mm, 1.8 µm)	
Temperature:	40 °C	
Flow:	0.2 mL/min	
Post Time:	2 minutes	
Injection Volume:	5 µL	
Run Time:	22 minutes	
Elution:	Gradient:	
Eluting Solvents:	Solvent A:	0.1% Formic Acid
	Solvent B:	Methanol
Program:	Time (mins)	Solvent B (%)
	1. 0.0	0
	2. 3.0	80
	3. 14.0	80
	4. 14.5	0
	5. 22.0	22

Identification of glyphosate was based on comparison of its retention time to that of the internal standards in each sample; as well as comparing the ratio/proportion of quantitation daughter-ion to confirming daughter-ion for glyphosate. The levels of glyphosate residue in water samples were calculated through determination of ratio/proportion of area response produced via quantitation daughter-ion of glyphosate to the peak area response produced by quantitation daughter-ion of the internal standard. The residual glyphosate level (µg/L) was calculated using Equation 3.1:

$$Residual\ \ level_{[Sample]}\ («g/L) \quad = \quad \frac{Peak\ \ Area_{(Sam)} \cdot \quad V_{Std} \cdot \quad V_{Extract}}{Peak\ \ Area_{(Std)} \cdot \quad V_{Sam} \cdot \quad V_{Sam}} \cdot C_{Std} \qquad 3.1$$

Where:

Peak Area $_{(sam)}$ = peak Area of sample injected (mv);

Peak Area $_{(std)}$ = peak Area of standard injected (mv);

V_{std} = volume of sample injected (mL); and,

V_{sam} = volume of standard injected (mL).

C_{std} = concentration of standard (µg/L), and

V_{sam} = volume of sample taken (mL)

3.3.4 Quality Control and Validation of Analytical Method

Glassware were subjected to rigorously cleaned with detergent, distilled water and thorough analytical-grade acetone rinsing; as well as overnight oven-drying (150 ºC), cooling and storage in super-clean cabinets. Other quality assurance measures applied in the laboratory included monitoring of Reagent blanks, recovery of spiked standards, analysis of procedural blanks, linearity and monitoring of detector response were additional quality control measures applied. Blanks were used to avoid laboratory contamination and analytical interferences, and the target compound was detected in a quantifiable value.

Method performance was assessed using spiked water samples (spiking level at 10 µg/L), and the method was shown to have good precision and high recoveries. Results of method performance are presented in Chapter 4.2.1. Percentage recoveries in spiked samples were 70 – 120% hence the results of the study were not corrected for recoveries since all were within the normal acceptable range of 65 - 120% (FAO/WHO, 2007). The Percentage Recovery data obtained attest to the reproducibility of the method.

3.4 Statistical Analysis of Data and Interpretation

ANOVA & Pearson's Correlation

Microsoft Excel software was used for graphical presentation of data obtained, as well as relationships between them. The version 20.0 software [Statistical Package for Social Sciences (SPSS)] was applied in the generation of the means of physical parameters of water and glyphosate residue levels. Test for significant differences and similarities between physical parameters and glyphosate residues in water from the study area, was accomplished with 1-way analysis of variance (ANOVA). Degree of relationship between Physical parameters of water and detected Glyphosate was undertaken with Pearson Correlation Analysis [at 95% confidence level ($p < 0.05$)].

Principal Component Analysis and Hierarchical Cluster Analysis (HCA)

Principal Component Analysis (PCA) through Varimax rotation was applied to ascertain the relationship and effect of the physicochemical parameters on the glyphosate residue; using the Averages (Mean) of the physicochemical parameters and glyphosate residue data. The application of varimax rotation was because orthogonal rotation reduces the number of variables with high loading on every component, thereby facilitating PCA results interpretation (Frimpong et al., 2013). Hierarchical cluster was used in the identification of diverse groups, clustering with samples of similar glyphosate residues levels, as well as deduction of the hypothetical sources of Glyphosate.

3.5 Human Health Risk Assessment

Hazard Quotient (HQ) was used to assess the non-carcinogenic health risk through exposure to glyphosate (ingestion/intake) in borehole and wurface water. Hazard Quotient (HQ) is the fraction of Average Daily Intake (ADI) to Reference Dose (R_fD). HQ indicates whether the degree of exposure is greater or less than the R_fD. HQ $\geq$ 1, exposure surpasses the R_fD; and therefore, potential detrimental health risk (IRIS, 1994). The study assessed acute and chronic HQ.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter presents the results and discussion of the findings of the study. The results and discussion cover physical parameters, levels of glyphosate in groundwater and surface water from the studied communities. The chapter ends with the presentation and discussion of on the assessment of human health risk associated with the consumption of groundwater (borehole) and surface water.

4.1 Physical Parameters

This aspect of the study entails the measurement of physical parameters namely pH, temperature, conductivity, salinity and total dissolved solids (TDS). Results (Table 4.1) of the assessed physical parameters for boreholes and surface water at the studied communities in the Central Tongu district follows.

Generally, conductivity, salinity and total dissolved solids assessed in groundwater (borehole) were higher than surface water. This may be attributed to the fact that surface water has limited or no contact with rocks and particles may settle underneath as sediments whereas groundwater flows slowly thus long contact time for water-rock interaction, resulting in dissolution of minerals (Edmunds and Smedley, 2013).

Table 4.1: Results of Physical analysis of water samples (Mean)

Sampling Location (Mafi)	Borehole Water					Surface Water				
	pH	Temp. °C	Cond µS/cm	Sal ppm	TDS ppm	pH	Temp. °C	Cond µS/cm	Sal ppm	TDS ppm
Agbadzikope **1**	7.32	34.0	10570	6090	7420	7.35	30.5	1175	570	850
Agbadzikope **2**	7.11	33.0	9650	5520	6670					
Bakpa Dzave	7.40	32.5	8290	4500	6900	7.90	31.8	1040	450	670
Gbadagokope	7.10	32.6	9020	4900	6260					
Kanikope **1**	7.11	33.5	11320	6640	7780					
Kanikope **2**	**6.90**	32.5	9910	5460	6900					
Amegakope	7.31	29.7	1305	640	920					
Asiekpe	**6.70**	30.0	1520	560	1100					
Seva	**5.69**	31.4	7560	3120	5900					
Avikope	7.15	30.5	1650	756	1480	7.35	30.9	980	460	655
Tsawla						**8.00**	31.7	1025	490	680
*Tsawla						**8.38**	31.7	**227**	**580**	**100**
WHO	**6.5-8.5**		**250**	**250**	**1000**					

* Stand pipe water collected at Tsawla with Adidome as origin

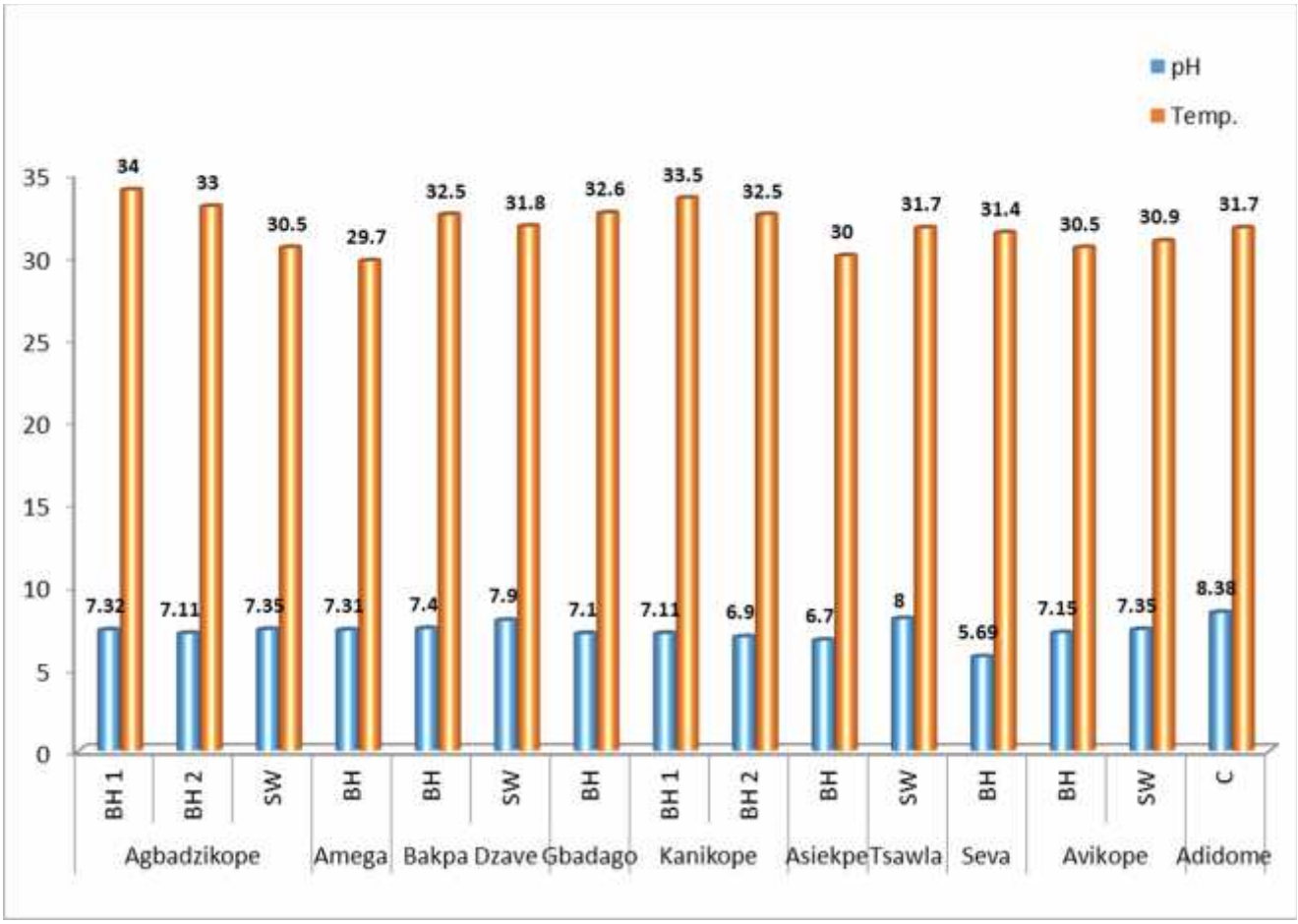

Fig. 4.1: pH and Temperature of Water Samples from the various Communities

4.1.1 pH of Water Samples

Measured pH values obtained are presented in Table 4.1 and Fig. 4.1. Overall, the pH ranged from acidic (5.69) to basic (8.34) for all the water samples (both borehole and surface water). The pH of the borehole samples ranged from slightly acidic (5.69) to near neutral (7.4); whiles the pH of the surface water samples ranged from near neutral (7.35) to weakly basic (8.0). Water samples from all study sites within Central Tongu except Seva, had pH values within the 6.5-8.5 WHO acceptable pH range [drinking and domestic chores] (WHO, 2004). Remarkable to marginal acidic pH values recorded at Seva (5.69), Asiekpe (6.70) and Kanikope **2** (6.90) may be attributed to the

levels of acidic cations in nearby soil or relatively high organic matter levels in soil zones whose oxidation releases CO_2; which in turn return reacts with H_2O forming H_2CO_3 (Langmuir, 1997; Kortatsi, 2007).

According to Nkansah et al. (2010), water pH ≤ 6.5 are considered potentially harmful for human consumption due to its associated health problems (acidosis and adverse effects on lymphatic and digestive systems). The capacity of the water to react with geological materials results in the leaching of toxic metals (trace) into the water. Such waters are potentially harmful for consumption by humans (Kortatsi, 2007). Consequently, groundwater samples with acidic pH are susceptible to trace metal pollution if these metals are present in the rock matrix through which the water percolates. The range of pH recorded in this study (5.69-8.35) was differed slightly from the pH range of 6.42-7.40 obtained in a similar study (Avi et al., 2019) within the Central Tongu district. However, there were overlaps in the pH values obtained for both studies.

4.1.2 Temperature of Water Samples

Temperatures of water samples are presented in Table 4.1 and Fig. 4.1. The temperature ranged between 29.7 to 33.5 for all water samples. The temperature of the borehole samples was between 29.7 to 33.5 . The temperature of the surface water samples was also between 30.5 and 31.8 . The first borehole from 'Kanikope' recorded the highest temperature (33.5). The lowest temperature recording in borehole was at 'Amegakope' (29.7). Thus, borehole samples had relatively lower temperatures.

Temperature is an important biologically significant factor, which plays an important role in the metabolic activities of organisms as well as the physical and chemical characteristics of water (Murhekar, 2011; Sanjay, 2014). The WHO permissible temperature range for drinking water is between 22 and 29 (WHO, 1998; Addo et al., 2011). Prevailing weather conditions during the period of investigation and groundwater influx could account for the variations in the temperature of water (Sirajudeen and Mubashir, 2013). Additionally, this could possibly be due to the different sources of collected water samples as well as the time of sampling. Notably, the pH units measured in this study were non-dependent of the temperature of the water sample.

4.1.3 Conductivity of Water Samples

Measured conductivities are presented in Table 4.1 and Fig. 4.2. The conductivity values ranged from 1025 to 11320 µS/cm for all the water samples analyzed. Generally, the conductivity values for the groundwater samples (borehole) were higher than the conductivity values of the surface water samples. The conductivity values for the surface water ranged from 1025 to 1175 µS/cm whiles the conductivity values of the groundwater samples (borehole) ranged from 1350 - 11320 µS/cm.

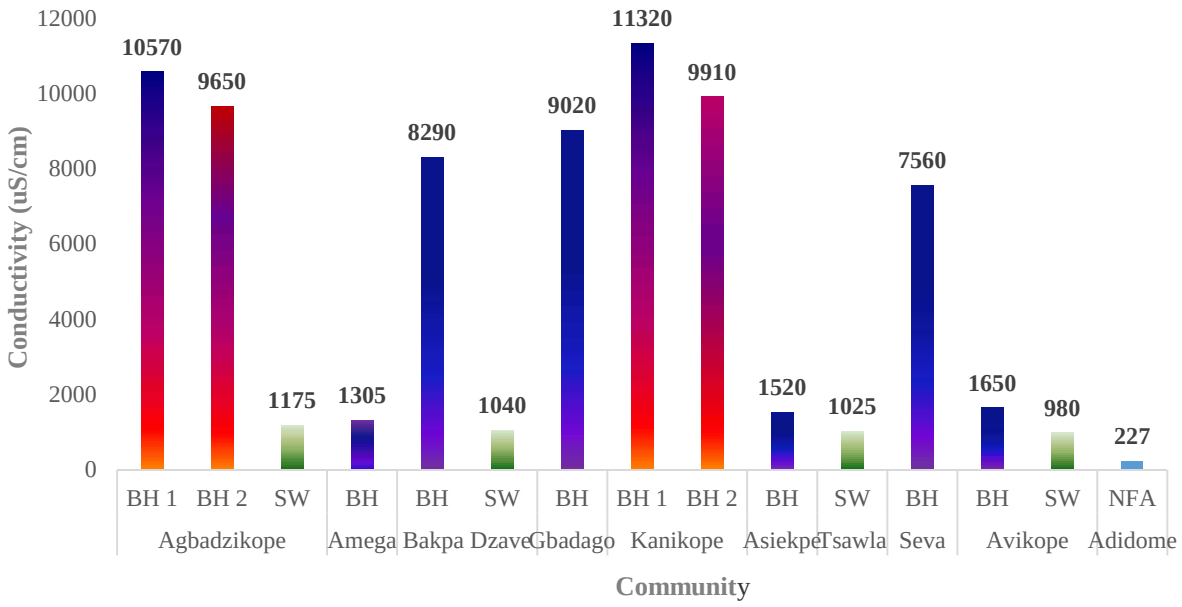

Fig. 4.2: Conductivity of Water Samples from the various Communities

To assess the concentration of dissolved solids (total) and main ions in water, measurement of water conductivity (electrical) and temperature are vital (Dahiya and Kaur, 1999; Amoako et al., 2011; Gyamfi et al., 2012). The conductivity values of the borehole samples recorded for all the sites (except Seva: 7560 µS/cm) in this study were above the WHO (2004) allowable/tolerable limit of 1000 µS/cm for potable water. Highest conductivity value observed in this study (11, 320 µS/cm) is higher than the highest conductivity value (4453.3 µS/cm) by Avi et al., (2019) in borehole water, Central Tongu district. Moreover, the high conductivity in majority of the water samples analyzed indicates that the water is able to react with the rock matrix to equilibration; indicating high resident times as observed by Kortatsi (2007). This may also be because the water from the boreholes has high concentration of dissolved ions.

4.1.4 Salinity of Water Samples

Salinity measurements for the water samples are presented in Table 4.1 and Fig. 4.3. The values for salinity ranged from 450 to 6640 mg/L for all water samples. Comparatively, surface water had lower salinity values than the borehole samples. The salinity values for the surface water ranged from 450 to 570 mg/L whereas the salinity of the groundwater samples (borehole) ranged from 640 and 6640 mg/L.

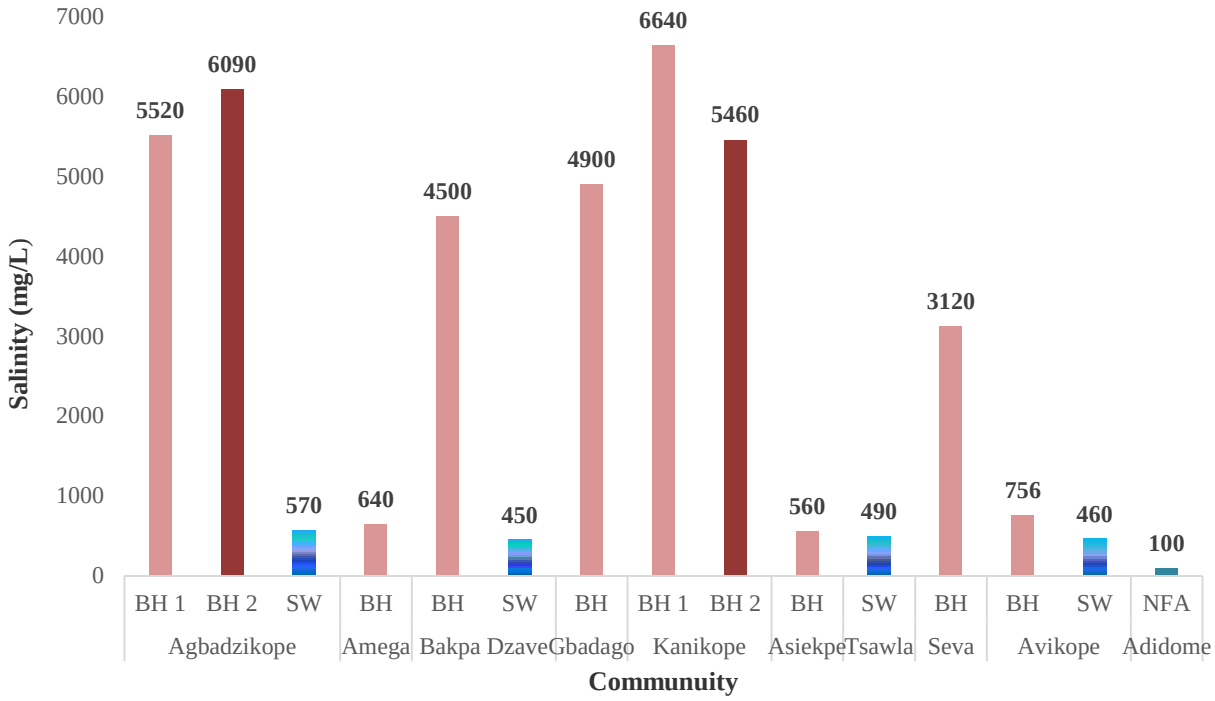

Fig. 4.3: Salinity of Water Samples from the various Communities

Salinity is generally essential for human health and metabolism (Safe Drinking Water Comm., 1980). Although these substances are not necessarily harmful to health, they may give rise to complaints from consumers because of the aesthetic effects they produce (Kortatsi, 2007). The Salinity values recorded for all the sampling sites were higher than WHO (1998) allowable limit of 250 mg/L for potable water.

4.1.5 Total Dissolved Solids of Water Samples

Total dissolved solids (TDS) content of the water samples are presented in Table 4.1 and Fig. 4.4. The TDS values ranged from 655 to 7780 mg/L for all analyzed water samples. The TDS values for the surface water ranged from 670 to 850 mg/L. TDS of surface water were within the WHO recommended standard (1000 mg/L). The TDS of the borehole samples were between 920 and 7780 mg/L. Borehole in Amegakope recorded the least TDS (920). This was within the WHO recommended standard. Other borehole samples recorded values (5900-7780 mg/L) which were higher by a factor of five (5) to seven (7) that of the WHO standard (1000 mg/L).

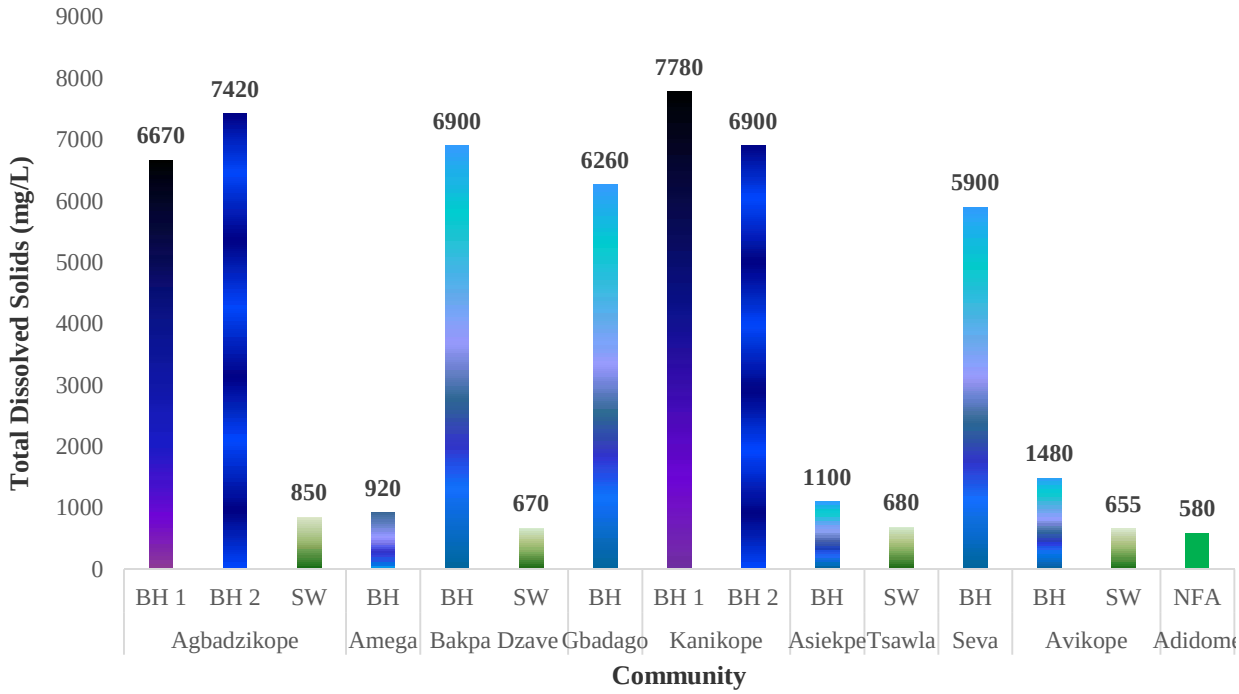

Fig. 4.4: Total Dissolved Solids of Water Samples from the various Communities

Total dissolved solids (TDS) are a measure of the total organic and inorganic substances dissolved in water. Murhekar (2011), as well as Sirajudeen & Mubashir (2013) reported that total dissolved solids (TDS) indicate the salinity behavior of groundwater. According to Tay (2007), total dissolved solids is a common indicator for polluted waters. A similar study by Avi et al. (2019) reported TDS values of 172.90 mg/L to 1,061.33 mg/L in borehole samples from some parts of the Central Tongu district. The reported values were lower than values obtained in this study. An elevated concentration of TDS above 1000 mg/L is an indication of high mineral content. This gives water an unpalatable taste, bad odour, displeasing colour and may contribute to induced physiological reaction when consumed (Spellman and Drinan, 2000).

4.2 Occurrence of Glyphosate in Water Resources (Surface and Groundwater)

4.2.1 Quality Assurance/Quality Control for Glyphosate Determination

The analytical performance of the chromatographic separation was assessed using spiked water samples (spiking level at 10 µg/L), and the method was shown to have good precision and high recoveries. Percentage recoveries in spiked samples were 70 – 120% hence the results of the study were not corrected for recoveries; since all were within the normal acceptable range of 65 - 120% (FAO/WHO, 2007). The percentage recovery data obtained attest to the reproducibility of the method. The performance of the chromatographic response was also checked (Fig. 4.5).

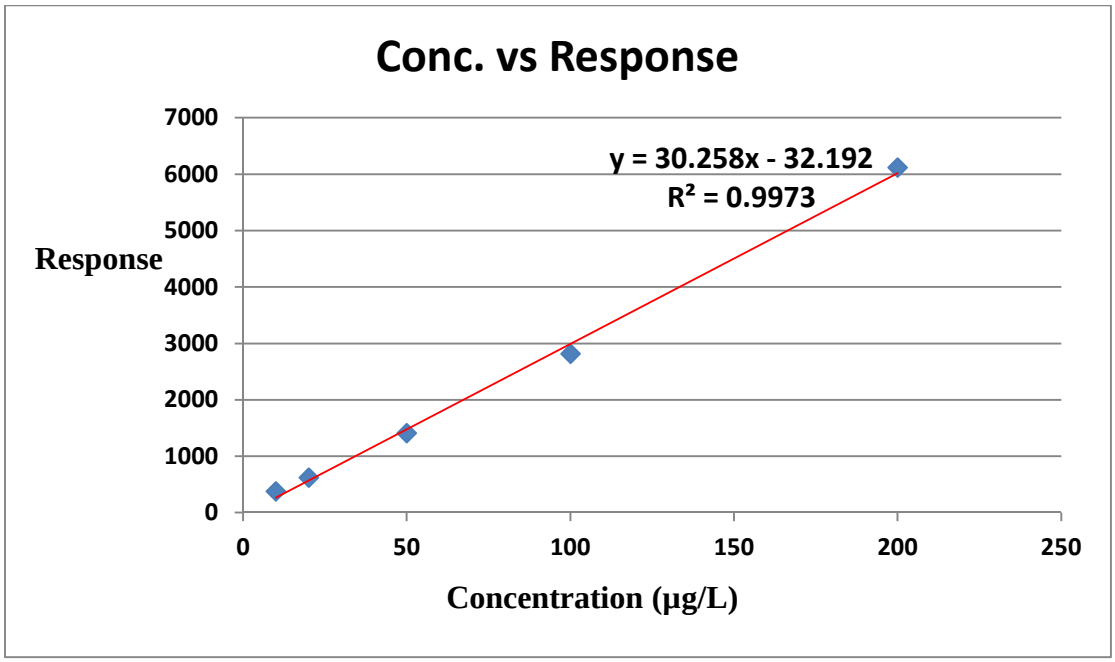

Fig. 4.5: Performance of Chromatographic Response

4.2.2 Glyphosate Residues in Central Tongu Water Resources

The levels of glyphosate residues in the groundwater and surface water samples from the Central Tongu district are presented in Table 4.2.

Table 4.2: Levels of glyphosate in water samples

Sampling Location (Mafi)	Levels of Glyphosate (µg/L)			
	Borehole Water		Surface Water	
	Mean±SD	Range	Mean±SD	Range
Agbadzikope 1	4.53±0.02	4.51-4.55	42.5±0.13	42.35-42.60
Agbadzikope 2	4.45±0.01	4.45-4.47		
Bakpa Dzave	8.52±0.08	8.45-8.60	25.2± 0.06	25.14-25.25
Amegakope	2.57±0.02	2.55-2.58		
Gbadagokope	6.13±0.06	6.09-6.20		
Kanikope 1	9.21±0.01	9.21-9.22		
Kanikope 2	9.01±0.02	8.99-9.05		
Asiekpe	5.69±0.01	5.68-5.70		
Seva	4.61±0.01	4.60-4.62		
Avikope	2.89±0.01	2.88-2.90	31.1±0.05	31.05-31.15
*Tsawla			1.82±0.02	1.80-1.84
Tsawla			25.05±0.45	24.55-25.50

* Stand pipe water collected at Tsawla with Adidome as origin
- Data are presented as Mean ±Standard Deviation (Mean±SD) of three (3) replicate measurements

Glyphosate residues were detected in water samples (groundwater and surface water) collected from communities used for the study (Table 4.2). The concentrations of glyphosate residues in both borehole and surface water ranged from 2.57 to 42.5 µg/L. Glyphosate residues were higher in surface water (25.05 – 42.5 µg/L) than groundwater (2.57 – 9.21 µg/L). The lowest glyphosate concentration was recorded in surface water at Tsawla (25.05 µg/L) and the highest in surface water sample at Agbadzikope 2 (42.5 µg/L) (Fig. 4.6). The relatively high levels of glyphosate in surface water could be a result of the continuous glyphosate-based herbicide input, spray drift and runoffs attributable to intensive agricultural activities within Central Tongu. The high concentrations of glyphosate found in surface water sampled from the Central Tongu district is attributable to the closeness of the sampling locations to farms. A number of studies are in agreement with this assertion; and has demonstrated that levels of glyphosate in transgenic soybeans increase in plantations (Peruzzo et al., 2008).

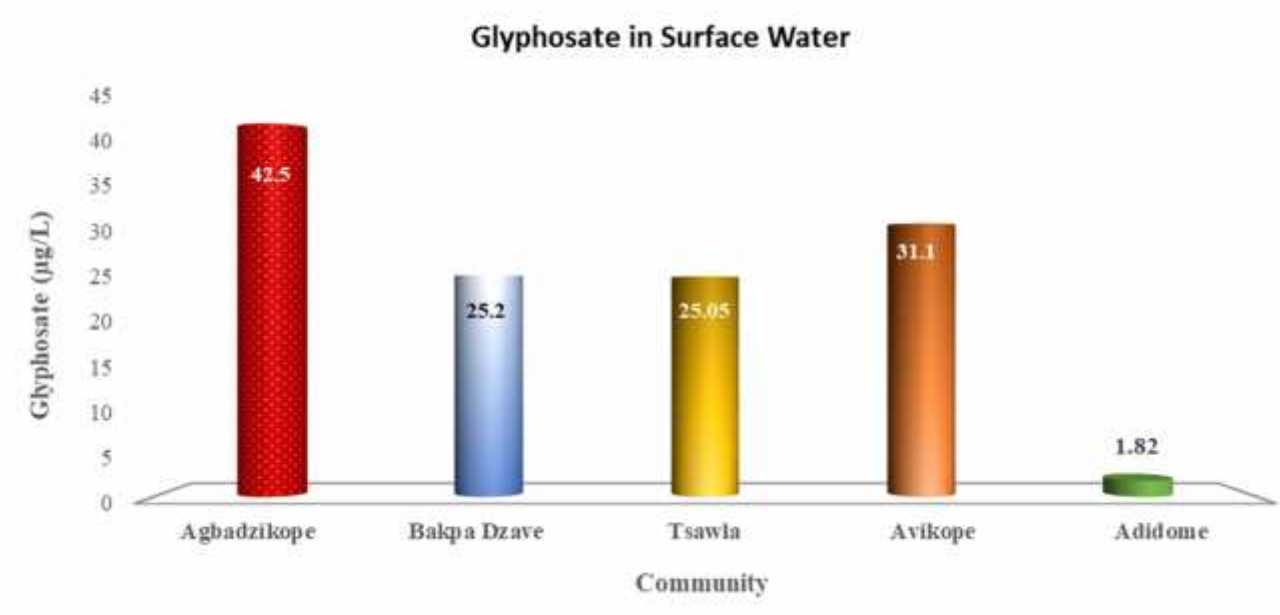

Fig. 4.6: Levels of Glyphosate Residues in Surface Water

Comparison of the levels of glyphosate residue in the surface water with the control sample (1.82 µg/L) from Adidome indicate high input of glyphosate in surface water in the farming communities. The levels of glyphosate in borehole water ranged between 2.57 and 9.21 µg/L (Fig. 4.7). The maximum glyphosate concentration in boreholes was recorded in Kanikope1 (9.21 µg/L) and the minimum in Amegakope (2.57 µg/L).

The detection/presence of glyphosate in the borehole water may be due to infiltration and leaching of Glyphosate; site conditions such as soil texture, soil permeability, soil organic matter, soil pH as well as the depth of the water table could be contributing factors for the leaching process (Perez-Lucas et al., 2018). However, the geology of the Central Tongu district is under-laid with heavy impermeable clay, the presence of glyphosate residues in groundwater/boreholes may be due to cracks and fractures in the Dahomeyan; and or the farmers may be preparing/mixing the herbicides near the wellhead of the boreholes in the district. Pesticides applied may infiltrate into and through the soil for the period of groundwater release into the aquifer (National Pesticide Telecommunication Network 2008).

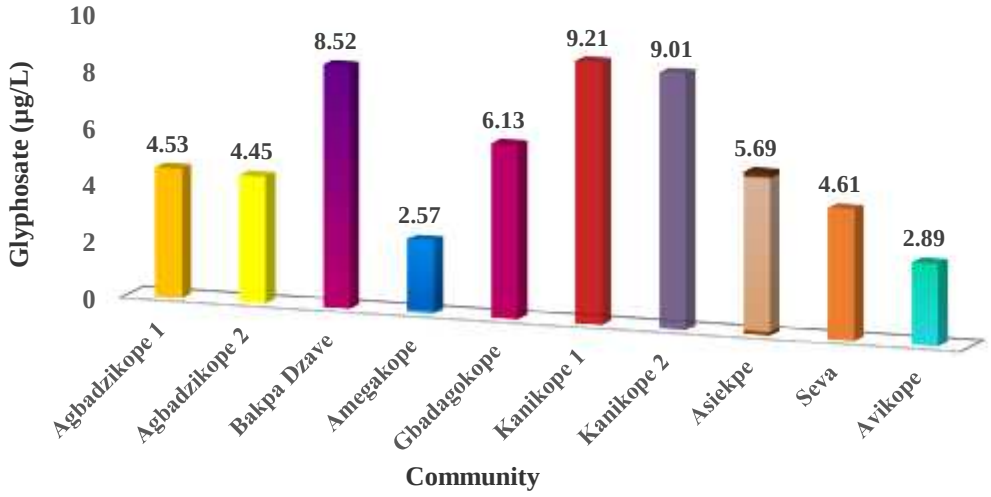

Fig. 4.7 Levels of Glyphosate Residues in Borehole Water from the studied Communities

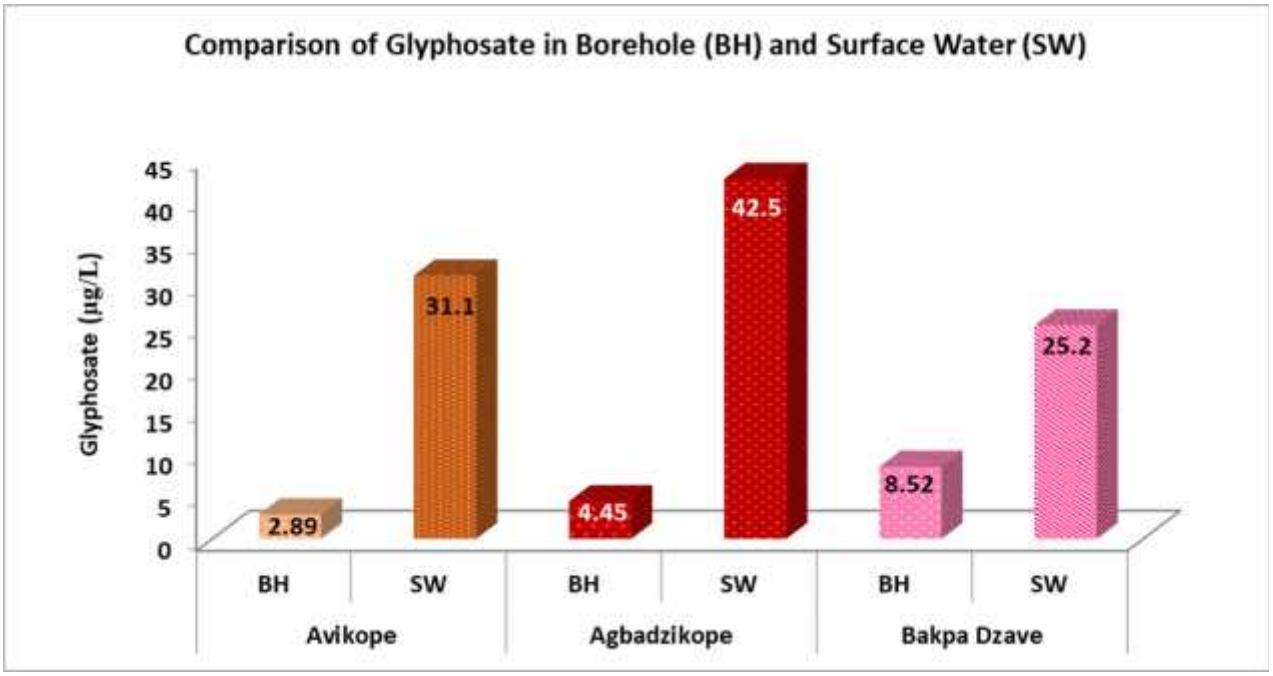

Fig. 4.8: Levels of Glyphosate Residues in Borehole Water and Surface Water from the Communities where both water types were collected

A comparison of the levels of glyphosate in borehole and surface water were in three (3) communities (Avikope, Agbadzikope and Bakpa Dzave) where both types of water were collected is presented in Fig. 4.8. The level of glyphosate in surface water from Avikope (31.1 µg/L) was about eleven (11) times higher than the borehole water (2.89 µg/L) from the same community. Glyphosate in surface water from Agbadzikope (42.5 µg/L) was ten (10) times higher than the borehole water from Agbadzikope (4.45 µg/L). For Bakpa Dzave, glyphosate in surface water was approximately three (3) times higher than that in the borehole water (8.52 µg/L).

Monsanto Company (An American Agrochemical and Agricultural Biotechnology Corporation and developer [since 1970] of the Glyphosate-based Herbicide, ***Roundup***) has stated in the past that Glyphosate is not a major problem in water because it "sometimes is detected in surface waters, but historically, Glyphosate has not been included among herbicides that cause concern in water supplies" (Mosanto, 2003). The reason given for this is that "because glyphosate binds tightly to most soils, it has a low potential to move through soil to contaminate groundwater". However, the presence of glyphosate in all water samples from the study area disputes the supposed assertion of strong sorption and comparatively fast degradation of glyphosate in soil; suggesting that leaching into groundwater is not prominent (Busse et al., 2001; Giesy et al., 2000; Vereecken, 2005).

Glyphosate residues have been detected in groundwater, even in areas/media where they are considered non-existent; a clear indication that the herbicide is transported far from the point of application (Siimes et al., 2006; Torstensson et al., 2005).

4.2.3 Comparison of Glyphosate Residues with Literature Data

The results of the glyphosate residue levels obtained in this study were compared with levels reported in scientific literature from similar studies in other countries (Table 4.3).

Table 4.3 Glyphosate Residues Obtained in this Study Compared to Literature Data

Country	Time period	Water type	Concentration range (µg/L)	Analytical Technique	Reference
Ghana	**Nov. 2019-Jan. 2020**	**Surface**	**25.02 - 42.50**	**LC-MS/MS**	**This Study**
Ghana	**Nov. 2019-Jan. 2020**	**Groundwater**	**2.57- 9.21**	**LC-MS/MS**	**This Study**
Brazil	**2013-2014**	**Tap water**	**2.3-3.3**	**HPLC-PDA**	**Delmonico et al., (2014)**
Canada	2010-2013	Groundwater	<0.010-0.663	IC-MS/MS	Van Stempvoort et al., (2016)
Argentina	2011-2012	Streams	<0.1–7.60	LC-MS/MS	Aparicio et al., (2013)
Brazil	2010-2011	Natural water	<309-1150	UV-Vis Spec.	Tzaskos et al., (2012)
Hungary	2010-2011	Surface and Ground water	<0.12 – 0.98	ELISA	Mörtl et al., (2013)
Spain	2007-2010	Groundwater	<0.009-0.939	LC-MS/MS	Sanchis et al., (2012)
USA	2001-2010	Groundwater	<0.02-2.03	LC-MS/MS	Battaglin et al., (2014)
Spain	2005-2006	Surface and Ground water	<0.05-0.85	LC-MS/MS	Ibáñez et al., (2016)
Sweden	1998-1999	Groundwater	<0.05-0.93	GC-MS	Börjesson and Torstensson (2000)
Germany	1994-1996	Surface water	<0.05-0.590	HPLC-FLD	Skark et al., (1998)

Approximately 40% of the studies in other countries (Table 4.3) used LC-MS/MS as the analytical technique (same analytical technique used in the present study). About 20% of the studies used HPLC-related analytical techniques. Roughly, 10% of the studies applied UV Visible Spectrophotometry, ELISA, IC-MS/MS and GC-MS respectively. This indicates that the analytical technique employed in this study is the predominant technique used in glyphosate detection in water samples globally.

With the exception of the studies in Germany and Sweden which were done in mid to late 1990s, all the other studies were done in early- to mid-2000s. Generally, the results obtained in this study (groundwater: 2.57-9.21 µg/L; surface water: 25.05-42.50 µg/L) were relatively lower than the results reported in similar studies in other countries, with the exception of the results obtained (<309-1150 µg/L) in the Brazilian study.

Around 36% of the studies were conducted on groundwater; only 9% was exclusively on surface water while 27% were conducted on both groundwater and surface water. Groundwater reservoirs/resources are an important source of potable drinking water in several regions. In the Central Tongu district (studied area) of Ghana; groundwater is the main water resource used by the communities as potable drinking water, crop irrigation, and animal husbandry.

4.2.4 Comparison of Glyphosate Residues Obtained in the Present Study with Globally Recommended Drinking Water Standards

The levels of glyphosate residues obtained in groundwater and surface water (present study) were compared with Drinking Water Quality Standards set by the EU, USA and Canada. The ranges (Groundwater: 2.57-9.21 µg/L; Surface water: 25.05-42.50 µg/L) [Tables 4.2 and 4.3] obtained in this study were below the Canadian Drinking Water Quality Recommended Guideline Value of

280 µg/L glyphosate residue; as well as the USEPA maximum allowed concentration of 700 µg/L glyphosate residue (Guidelines for Canadian Drinking Water Quality, 2014; USEPA 2002). However, the levels of glyphosate residue obtained in this study were higher than the EU Recommended Glyphosate Residue Limit of 0.1 µg/L for Drinking Water (Council Directive 91/414/EEC, European Union, Brussels 1991; Horth, 2010).

4.3 Statistical Analysis of Data Obtained in the Study

4.3.1 Effect of Type of Water, pH and Temperature on Glyphosate Levels Using ANOVA

Type of Water

In this study, Analysis of Variance (ANOVA) was applied to ascertain the effect of the type of water on glyphosate residue levels (Appendix 1-5). ANOVA shows that the type of water affects levels of glyphosate. This is because there are significant differences ($p<0.05$) in the source of water and glyphosate concentration. The study recorded a Pearson's Correlation Coefficient (R) of 0.876. This means the type of water accounted for about 87.6% of the concentration of glyphosate.

pH

The effect of pH on levels of Glyphosate in the water samples was assessed using ANOVA. Analysis of Variance (ANOVA) shows that there are significant differences ($p<0.05$) between pH and glyphosate concentrations. The R-value of 0.548 obtained signify that glyphosate concentration increases with increasing pH.

Temperature

The effect of temperature on glyphosate concentration was evaluated using Analysis of Variance (ANOVA) showed that there were no significant differences ($p>0.05$) in temperature changes on glyphosate concentrations in the water samples.

4.3.2 Effect of Conductivity, Salinity and TDS on Glyphosate concentration

Conductivity

The effect of conductivity on glyphosate levels in the water samples assessed using analysis of variance (ANOVA) shows that there are significant differences ($p<0.05$) between conductivity and glyphosate concentration. R-value of -0.578 confirms that conductivity decreases with increasing glyphosate concentration and vice versa.

Salinity

The effect of salinity on glyphosate levels in the water samples assessed using analysis of variance (ANOVA) shows that there are significant differences ($p<0.05$) between salinity and levels of glyphosate. R-value of -0.548 showed that salinity decreases with increasing glyphosate concentration and vice versa.

Total Dissolved Solids (TDS)

The effect of total dissolved solids (TDS) on Glyphosate levels in the water samples assessed using analysis of variance (ANOVA) shows that there are significant differences ($p<0.05$) between total dissolved solids (TDS) and glyphosate concentration. R-value of -0.586 confirms that total dissolved solids (TDS) decreases with increasing glyphosate concentration and vice versa.

4.3.3 Pearson Correlation Analysis of Data Obtained

The Pearson correlation method is the most common method to use for numerical variables; it assigns a value between -1 and 1 (where 0 is no correlation). A correlation of 1 is total positive correlation, and − 1 is total negative correlation. The interpretation is as follows: a correlation value of 0.7 between two variables would indicate that a significant and positive relationship exists between the two. A positive correlation signifies that if variable 'A' goes up, then 'B' will also go up, whereas if the value of the correlation is negative, then if A increases, B decreases (Nettleton, 2014). Pearson correlation coefficient, also known as Pearson R statistical test, measures strength between the different variables and their relationships (CFAI, 2020).

Pearson correlation was conducted for the various parameters. The study showed varying relations among the indexes measured. The study found a direct relationship between pH and the glyphosate content. Statistically, when the Glyphosate content is increased the pH content also increases and vice versa. This accounted for 27.5% of the correlation matrix. The study however found an inverse relationship between glyphosate and temperature, conductivity, salinity and TDS. Increasing glyphosate level was accompanied by a decrease in temperature (-22.6%), conductivity (-42.1%), salinity (-41.7%) and TDS (-43.2%) and vice versa (Appendix 4).

4.3.4 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a powerful pattern recognition technique that attempts to explain the variance of a data set of inter-correlated variables with a smaller set of independent variables (principal component) **(**Frimpong et al., 2013**)**. PCA in rotation mode was performed on the mean glyphosate residues data. Varimax rotation was used to maximize the sum of the variance of the factor coefficients.

From the analysis, the variables were reduced to two principal components from the data set with Eigenvalues > 1 (Appendix 6). Components with Eigenvalues < l accounted for less variance than the original variable (Var 1) and so are of little use. Approximately, 86.5% data variance was explained from the data set (Appendix 6).

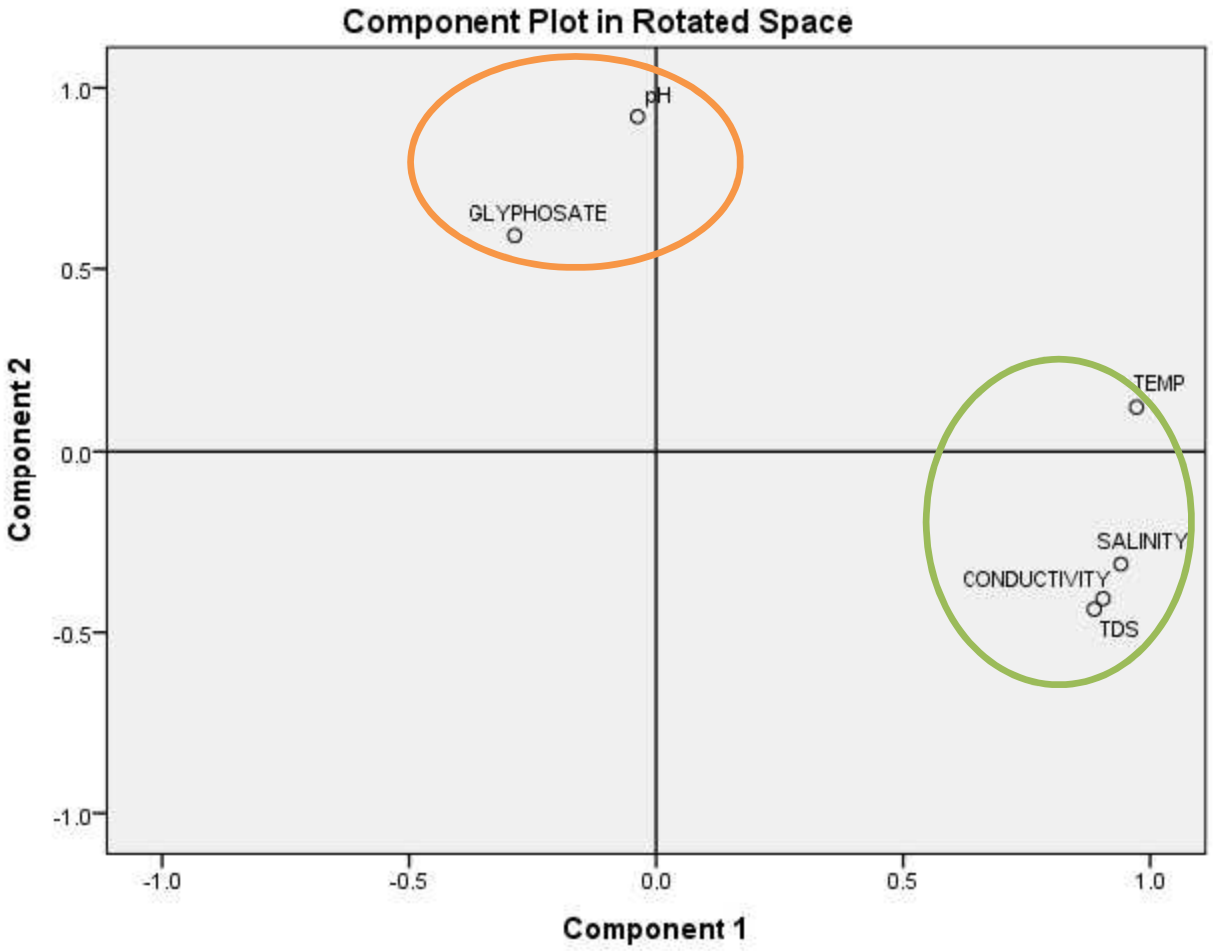

Fig. 4.9 PCA Loading plot showing how strong each Variable influences a Principal Component

The variables have reduced to two Principal Components: PCA 1 and PCA 2. Conductivity, salinity and total dissolved solids formed PCA 1 whiles PCA 2 represents pH and glyphosate. From Fig. 4.9, pH correlated highly and positively to glyphosate; thus from the study, glyphosate levels in both groundwater and surface water is pH-dependent. This result (Fig. 4.9) is in agreement with similar observations by De Jonge et al. (2001) and Al-Rajab et al. (2008).

From Fig. 4.9, conductivity, salinity and total dissolved solids (TDS) clustered strongly together; and decreases with increasing pH and glyphosate levels. This implies that salinity, conductivity, and TDS correlates inversely with pH and levels of glyphosate. Again, from Fig. 4.9, there is a positive but weak correlation between glyphosate levels and water temperature.

4.3.5 Hierarchical Cluster Analysis

Hierarchical Clustering Analysis (HCA) is an algorithm that is used to group the data points having similar properties, these groups are termed as clusters, and as a result of hierarchical clustering; a set of clusters are obtained where these clusters are different from each other (EDUCBA, 2020). Hierarchical Cluster Analysis (HCA) was used to assess the association between glyphosate levels and physicochemical parameters; as well as the association between the glyphosate levels and the water resources from the studied communities.

Association between Glyphosate Levels and Physical Parameters

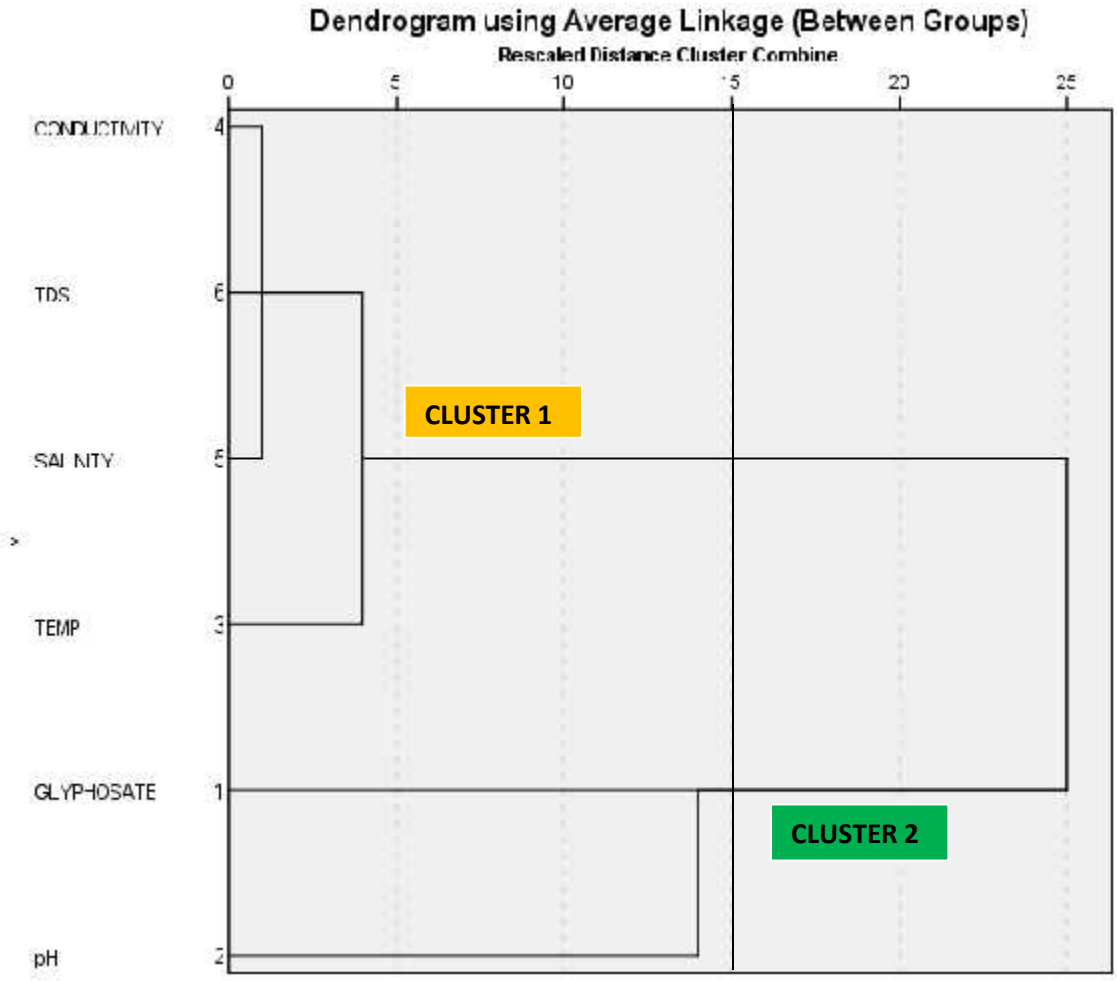

Fig. 4.10 HCA dendrogram for Physical parameters and Glyphosate analyzed: A demonstration of successful discrimination of two clusters

Similar to PCA, HCA has correctly classified the variables into two clusters; glyphosate and pH in one cluster; with conductivity, salinity and total dissolved solids in another cluster (Fig. 4.10). This also confirms a strong relationship between glyphosate concentration and pH (De Jonge et al., 2001; Al-Rajab et al., 2008).

Association between Glyphosate Levels and Water Resources from the Communities

Sampling locations were coded as follows: abbreviated community name followed by the type of water (indicated by G [groundwater] or S [surface water]). For instance, AVG is groundwater sample from Avikope, and so on. From Fig. 4.11, the water samples grouped into two clusters (according to the water type). Cluster 1 consists of all the groundwater samples except that of Asiekpe, Amegakope and Avikope. With Cluster 2, all the surface water samples grouped together. However, the groundwater samples from Asiekpe, 'Amegakope' and Avikope were found in the surface water group (Cluster 2) due the comparable nature of the levels of glyphosate residues found in groundwater from these communities with the general levels in surface water (Table 4.2).

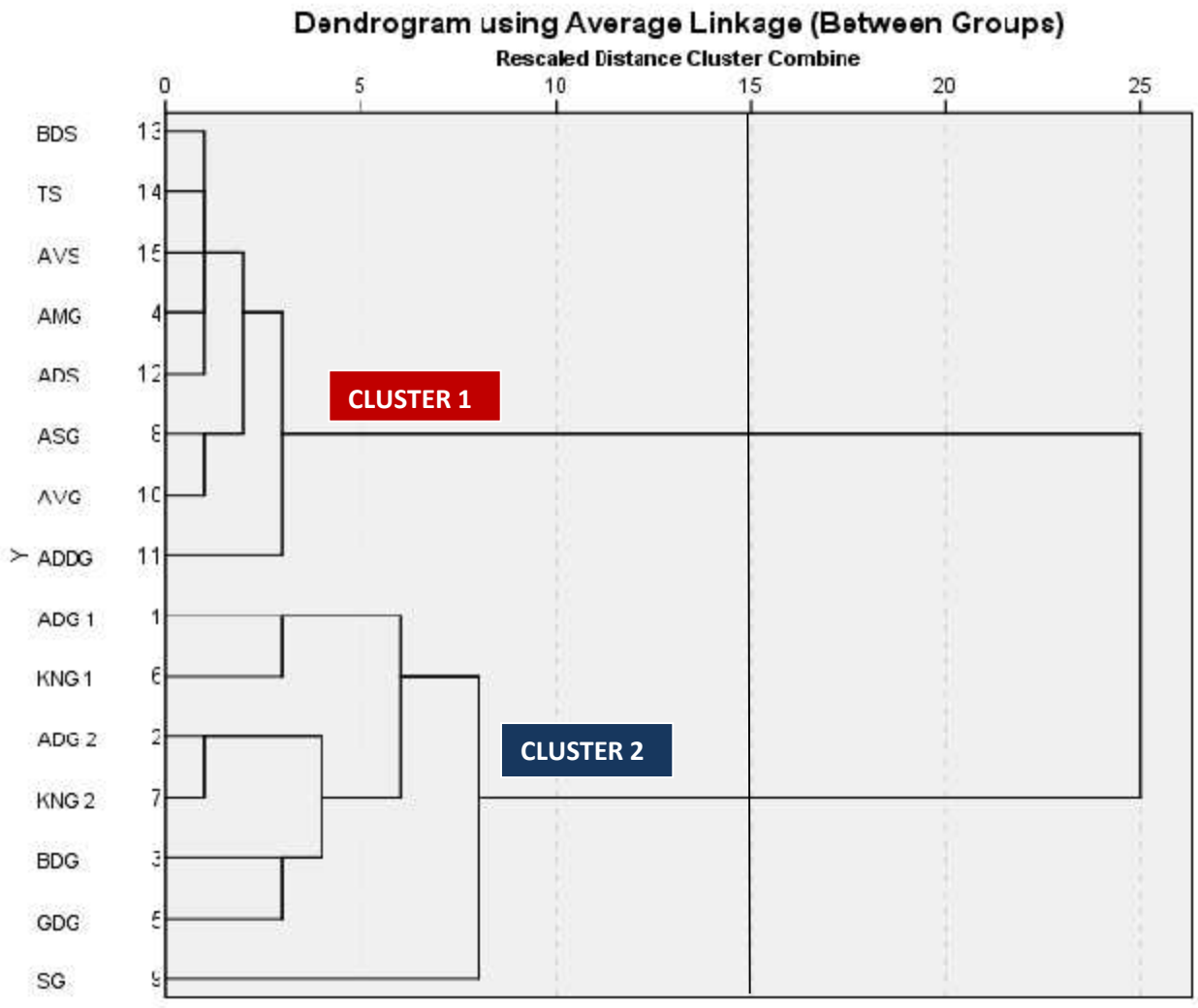

Fig. 4.11 HCA dendrogram demonstrating successful separation of cases into two clusters

4.4 Human Health Risk Assessment of Glyphosate in Groundwater and Surface Water

The non-carcinogenic risk associated with drinking groundwater (borehole) and surface water from the studied communities was assessed using hazard quotient indices. The hazard quotient (HQ) is "the ratio of the average daily intake (ADI) and the reference dose (R_fD)." This value indicates the degree to which exposure is greater or less than the R_fD. When the ratio is equal to or greater than 1, exposure exceeds the R_fD and the exposed population may be at risk of detrimental health effects occurring (IRIS, 1994).

According to the USEPA (2005), direct ingestion of water, dermal absorption of water adhered to the human skin and inhalation through showering are the common pathways by which humans could be at risk or exposed to potential health hazards in utilizing glyphosate-contaminated groundwater and surface water for domestic purposes. For most domestic conditions, the contribution of inhalation pathway is insignificant compared the ingestion and dermal pathways to risks associated with contaminants in water (Lee et al., 2002). Therefore, the present study considered only ingestion pathway in evaluating the health risks associated with the consumption of groundwater and surface water.

4.4.1 Acute Exposure and Risk Assessment

Glyphosate residue levels in borehole water and surface water were used to assess human exposure through oral intake/ingestion. The population groups considered in this study was adults and children. The average daily intake/dose of glyphosate was determined using Eq. (4.1), following recommendations from the U.S. Environmental Protection Agency (USEPA, 1999) and Jaipieam et al. (2009).

Potential Dose

$$Dpot = C \times IngR \quad (4.1)$$

Where:

Dpot = Potential Dose

C (µg/L) = Pesticide concentration,

IngR (L/day) = Intake/Ingestion Rate of water

Assumption:

- Intake/ingestion rate of water was 2.3 L/day (adult) and 1.4 L/day (children) [Avigliano, 2016].

Average Daily Intake

The potential dose was converted to an 'Average Daily Intake/ Dose' by dividing it by the assumed bodyweight (Eq. (4.2).

$$ADI = \frac{Dpot}{BW} \quad (4.2)$$

Where:

BW = Body Weight (average bodyweight of an individual for each group)

Assumption:

-Average body weights used: 65 kg for adult and 20 kg for children (Del Pino et al., 2011).

Hazard Quotient (HQ)

The Hazard Quotient was estimated based on Eq. 4.3:

$$HQ = \frac{ADI}{RfD} \quad (4.3)$$

Table 4.4: Hazard Quotient Indices of Glyphosate Residues in Water samples due to Acute Exposure

Sampling Location (Mafi)	Hazard Quotients (HQ) for Acute Exposure			
	Borehole Water		Surface Water	
	Adult	Children	Adult	Children
Agbadzikope **1**	0.32	0.63	**3.01**	**5.95**
Agbadzikope **2**	0.32	0.62		
Bakpa Dzave	0.60	**1.19**	**1.78**	**3.53**
Gbadagokope	0.43	0.86		
Kanikope **1**	0.65	**1.29**		
Kanikope **2**	0.64	**1.26**		
Amegakope	0.18	0.36		
Asiekpe	0.40	0.80		
Seva	0.31	0.65		
Avikope	0.20	0.40	**2.20**	**4.35**
Tsawla			**1.77**	**3.51**
*Tsawla			0.13	0.25

* Stand pipe water collected at Tsawla with Adidome as origin; HQ > 1 is in bold

For Acute Exposure, the hazard quotient for groundwater ranged from 0.18 to 0.65 for adults; 0.36 - 0.80 and 1.19 - 1.29 (for two communities; Bakpa Dzave and Kanikope) for children. For surface water, hazard quotient (HQ) ranged from 1.77 - 3.01 (Adults) and 3.51 - 5.95 (children) [Table 4.4]. Accordingly, there is generally no health risk associated with drinking groundwater by adults and children through acute exposure. However, children from two communities (Bakpa Dzave and

Kanikope) are potentially at risk; due to acute exposure to glyphosate in groundwater. Consumption of surface water poses a potential health risk to both children and adults.

4.4.2 Chronic Exposure and Risk Assessment

Assessment of glyphosate residue intake from chronic exposure was estimated as Chronic Daily Intake (CDI). The CDI value indicates the amount of chemical substance ingested (USEPA, 1996); and calculated using equation 4.3.

$$CDI = \frac{C \times IngR \times EF \times ED}{(BW \times AT)} \qquad 4.4$$

Where:

CDI= Chronic Daily Intake

C= Concentration of Chemical (µg/L)

IngR= Ingestion Rate (2.3 L/day for adult and 1.4 L/day for children) [Avigliano, 2016]

EF= Exposure Frequency (365 days/year [USEPA, 1989])

ED= Exposure Duration (70 years for adults and 6 years for children [USEPA, 2002])

BW= Body Weight (65 kg for adult and 20 kg for children [Del Pino et al., 2011])

AT= Average Time (ED×365 [USEPA, 1989])

For Chronic Exposure, the Hazard Quotient (HQ) was estimated using Eq. 4.4 (USEPA, 1999):

$$HQ = \frac{CDI}{RfD} \qquad 4.5$$

Table 4.5: Hazard Quotient Values of Glyphosate Residues in Water Samples due to Chronic Exposure

Sampling Location (Mafi)	Hazard Quotient (HQ) for Chronic Exposure			
	Borehole Water		Surface Water	
	Adult	Children	Adult	Children
Agbadzikope **1**	0.09	0.18	0.86	**1.70**
Agbadzikope **2**	0.09	0.17		
Bakpa Dzave	0.17	0.34	0.51	**1.00**
Gbadagokope	0.12	0.25		
Kanikope **1**	0.19	0.37		
Kanikope **2**	0.18	0.36		
Amegakope	0.05	0.10		
Asiekpe	0.11	0.23		
Seva	0.09	0.18		
Avikope	0.06	0.12	0.51	**1.24**
Tsawla			0.51	**1.00**
*Tsawla			0.04	0.07

* Stand pipe water collected at Tsawla with Adidome as origin; HQ > 1 is in bold

For chronic exposure, the hazard quotient for groundwater ranged from 0.05 - 0.19 (Adults) and 0.10 - 0.37 (Children); whiles the hazard quotient for surface water ranged from 0.51 - 0.86 and 1.00 - 1.70 for adults and children respectively (Table 4.5).

For groundwater, chronic exposure does not pose potential health risk to both children and adults (HQ<1). For surface water, chronic exposure does not pose potential health risk to adults. It however, poses a potential health risk to Children (HQ>1).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

Overall, the study investigated the occurrence/presence of glyphosate residues in groundwater and surface water from selected farming communities within the Central Tongu district; and evaluated the health risk posed to children and adults. This was achieved through the following:

(i) assessment of the levels of glyphosate residues in groundwater (boreholes) and surface water within farming communities in Central Tongu;

(ii) appraisal of the physical parameters of the waters, and assess their influence on the levels of glyphosate residues;

(iii) investigation of the relationship between glyphosate levels and water resources within Central Tongu district; and,

(iv) estimation of the Human Health Risk associated with consumption of groundwater (borehole) and surface water by inhabitants of the farming communities in Central Tongu.

5.1 CONCLUSION

The study established the presence of glyphosate residues in groundwater and surface water within the Central Tongu district. The presence of glyphosate residues in the water samples is attributable to intense farming activities (Pepper, Cassava and Maize) within the district.

pH was identified as the sole physical parameter influencing the glyphosate residue in the water resources through the use of principal component analysis (PCA). Glyphosate residues were high in slightly basic water samples; and low in slightly acidic water samples.

The study through hierarchical cluster analysis (HCA) also established close association between levels of glyphosate and the type of water resources. The levels of glyphosate in groundwater were relatively low, compared to the high levels of glyphosate in surface water.

Assessment of non-carcinogenic risk using hazard quotient (HQ) for acute exposure and chronic exposure revealed some level of risk associated with the consumption of groundwater and surface water from the selected farming communities in the Central Tongu district.

Groundwater/borehole water did not pose any health risk to both adults and children from chronic exposure. Though surface water did not pose any health risk chronically to adults, children are at risk.

For acute exposure, borehole water did not pose a health risk to both adults and children; notwithstanding, children in Bakpa-Dzave and Kanikope were at risk. Acute exposure to surface water poses a health risk to both adults and children.

5.2 RECOMMENDATION

Based on the results obtained from the study, the following recommendations are proffered:

(i) Farmers should be educated on the use of glyphosate-based herbicides in land clearing and its impact on water resources;

(ii) The Public and Environmental Health Directorate of the Central Tongu district Assembly should constantly monitor water resources in the district to assess risk;

(iii) Further studies should be conducted to cover the entire district in order to ascertain the state of water resources in the district with respect to glyphosate contamination;

(iv) The Ministry of Food and Agriculture (MoFA), Environmental Protection Agency (EPA), Community Water and Sanitation Agency (CWSA) and Ministry of Local Government and Rural Development (MLGRD) should consider funding of such researches since data generated will help in policy revision as well as formulation.

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APPENDICES

Appendix 1a: ANOVA Glyphosate and Type of water

		Sum of squares	Df	Mean square	F	Sig.
Between groups	Combined	6368.531	2	3184.265	174.508	0.000
	Linearity	5459.787	1	5469.787	299.761	0.000
	Deviation	898.743	1	898.743	49.254	0.000
Within groups		766.380	42	18.247		
Total		7134.911	44			

Appendix 1b: Measures of Association

	R	R squared	Eta	Eta squared
Glyphosate*type of water	.876	.767	.945	.893

Appendix 2a: ANOVA Glyphosate and pH

		Sum of squares	Df	Mean square	F	Sig.
Between groups	Combined	6000.334	34	176.480	1.555	.233
	Linearity	2146.369	1	2146.369	18.918	.001
	Deviation	3853.965	33	116.787	1.029	.514
Within groups		1134.578	10	113.458		
Total		7134.911	44			

Appendix 2b: Measures of Association

	R	R Squared	Eta	Eta Squared
Glyphosate * pH	.548	.301	.917	.841

Appendix 3a: ANOVA Glyphosate and Temperature

		Sum of squares	Df	Mean square	F	Sig.
Between groups	Combined	4374.960	27	162.036	.998	.515
	Linearity	113.332	1	113.332	.698	.415
	Deviation	4261.628	26	163.909	1.010	.504
Within groups		2759.951	17	162.350		
Total		7134.911	44			

Appendix 3b: Measures of Association

	R	R Squared	Eta	Eta Squared
Glyphosate * Temperature	-.126	.016	.783	.613

Appendix 4a: ANOVA Glyphosate and Conductivity

		Sum of squares	Df	Mean square	F	Sig.
Between groups	Combined	7134.905	40	178.373	1.130E5	0.000
	Linearity	2383.299	1	2383.299	1.510E6	0.000
	Deviation	4751.606	39	121.836	7.521E4	0.000
Within groups		.006	4	.002		
Total		7134.911	44			

Appendix 4b: Measures of Association

	R	R Squared	Eta	Eta Squared
Glyphosate * Conductivity	-.578	.334	1.000	1.000

Appendix 5a: ANOVA Glyphosate and Salinity

		Sum of squares	Df	Mean square	F	Sig.
Between groups	Combined	7134.906	41	174.022	9.373E5	0.000
	Linearity	2139.324	1	2139.324	1.152E6	0.000
	Deviation	4995.582	40	124.890	6.727E4	0.000
Within groups		.006	3	.002		
Total		7134.911	44			

Appendix 5b: Measures of Association

	R	R Squared	Eta	Eta Squared
Glyphosate * Salinity	-.548	.300	1.000	1.000

Appendix 6a: ANOVA Glyphosate and TDS

		Sum of squares	Df	Mean square	F	Sig.
Between groups	Combined	7134.647	39	182.940	3.465E3	0.000
	Linearity	2450.659	1	2450.659	4.642E4	0.000
	Deviation	4683.988	38	123.263	2.335E3	0.000
Within groups		.264	5	.053		
Total		7134.911	44			

Appendix 6b: Measures of Association

	R	R Squared	Eta	Eta Squared
Glyphosate * TDS	-.586	.343	1.000	1.000

Appendix 7: Rotated Component Matrix explaining 86.49 % of the Total variance for the variables

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.061	67.685	67.685	4.061	67.685	67.685	3.522	58.692	58.692
2	1.128	18.808	86.492	1.128	18.808	86.492	1.668	27.800	86.492
3	.732	12.198	98.690						
4	.066	1.108	99.798						
5	.011	.190	99.988						
6	.001	.012	100.000						

Extraction Method: Principal Component Analysis.

Appendix 8: Parameters distribution for Exposure and Risk, Input data.

Parameter	**Symbol**	**Unit**	**Value**
Chemical concentration	C	µg/L	
Body weight adult	BWA	kg	65
Bodyweight children	BWC	kg	20
Averaging time	AT	Day	ED x 365
Exposure frequency	EF	Day /year	365
Exposure duration adult	ED A	Year	70
Exposure duration children	ED C	Year	6
Ingestion rate of water adults	IngR (a)	L /day	2.3
Ingestion rate of water children	IngR (c)	L /day	1.4

Appendix 9a: Hazard Quotient Due To Acute Exposure (Adults)

Sampling Location	Glyphosate (µg/L)	IngR	Dpot	BW	ADI	ARfD	HQ
Agbadzikope BH	4.53	2.3	10.419	65	0.16029	0.5	0.32058
Agbadzikope BH	4.45	2.3	10.235	65	0.15746	0.5	0.31492
Bakpa Dzave BH	8.52	2.3	19.596	65	0.30148	0.5	0.60295
Amegakope BH	2.57	2.3	5.911	65	0.09094	0.5	0.18188
Gbadagokope BH	6.13	2.3	14.099	65	0.21691	0.5	0.43382
Kanikope BH	9.21	2.3	21.183	65	0.32589	0.5	0.65178
Kanikope BH	9.01	2.3	20.723	65	0.31882	0.5	0.63763
Asiekpe BH	5.69	2.3	13.087	65	0.20134	0.5	0.40268
Seva BH	4.61	2.3	10.603	65	0.16312	0.5	0.32625
Avikope BH	2.89	2.3	6.647	65	0.10226	0.5	0.20452
*Tsawla	1.82	2.3	4.186	65	0.0644	0.5	0.1288
Agbadzikope SW	42.5	2.3	97.75	65	1.50385	0.5	**3.00769**
Bakpa Dzave SW	25.2	2.3	57.96	65	0.89169	0.5	**1.78338**
Tsawla SW	25.05	2.3	57.615	65	0.88638	0.5	**1.77277**
Avikope SW	31.1	2.3	71.53	65	1.10046	0.5	**2.20092**

Appendix 9b: Hazard Quotient Due To Acute Exposure (Children)

Sampling Location	Glyphosate (µg/L)	IngR	Dpot	BW	ADI	ARfD	HQ
Agbadzikope BH	4.53	1.4	6.342	20	0.3171	0.5	0.6342
Agbadzikope BH	4.45	1.4	6.23	20	0.3115	0.5	0.623
Bakpa Dzave BH	8.52	1.4	11.93	20	0.5964	0.5	**1.1928**
Amegakope BH	2.57	1.4	3.598	20	0.1799	0.5	0.3598
Gbadagokope BH	6.13	1.4	8.582	20	0.4291	0.5	0.8582
Kanikope BH	9.21	1.4	12.89	20	0.6447	0.5	**1.2894**
Kanikope BH	9.01	1.4	12.61	20	0.6307	0.5	**1.2614**
Asiekpe BH	5.69	1.4	7.966	20	0.3983	0.5	0.7966
Seva BH	4.61	1.4	6.454	20	0.3227	0.5	0.6454
Avikope BH	2.89	1.4	4.046	20	0.2023	0.5	0.4046
*Tsawla	1.82	1.4	2.548	20	0.1274	0.5	0.2548
Agbadzikope SW	42.5	1.4	59.5	20	2.975	0.5	**5.95**
Bakpa Dzave SW	25.2	1.4	35.28	20	1.764	0.5	**3.528**
Tsawla SW	25.05	1.4	35.07	20	1.7535	0.5	**3.507**
Avikope SW	31.1	1.4	43.54	20	2.177	0.5	**4.354**

Appendix 10a: Hazard Quotient Due To Chronic Exposure (Adults)

Sampling Location	Glyphosate (µg/L)	IngR	EF	ED	BW	AT	CDI	RfD	HQ
Agbadzikope BH	4.53	2.3	365	70	65	25550	0.1603	1.75	0.0916
Agbadzikope BH	4.45	2.3	365	70	65	25550	0.1575	1.75	0.08998
Bakpa Dzave BH	8.52	2.3	365	70	65	25550	0.3015	1.75	0.17227
Amegakope BH	2.57	2.3	365	70	65	25550	0.0909	1.75	0.05196
Gbadagokope BH	6.13	2.3	365	70	65	25550	0.2169	1.75	0.12395
Kanikope BH	9.21	2.3	365	70	65	25550	0.3259	1.75	0.18622
Kanikope BH	9.01	2.3	365	70	65	25550	0.3188	1.75	0.18218
Asiekpe BH	5.69	2.3	365	70	65	25550	0.2013	1.75	0.11505
Seva BH	4.61	2.3	365	70	65	25550	0.1631	1.75	0.09321
Avikope BH	2.89	2.3	365	70	65	25550	0.1023	1.75	0.05844
*Tsawla	1.82	2.3	365	70	65	25550	0.0644	1.75	0.0368
Agbadzikope SW	42.5	2.3	365	70	65	25550	1.5038	1.75	0.85934
Bakpa Dzave SW	25.2	2.3	365	70	65	25550	0.8917	1.75	0.50954
Tsawla SW	25.05	2.3	365	70	65	25550	0.8864	1.75	0.50651
Avikope SW	31.1	2.3	365	70	65	25550	1.1005	1.75	0.62884

Appendix 10b: Hazard Quotient Due To Chronic Exposure (Children)

Sampling Location	Glyphosate (µg/L)	IngR	EF	ED	BW	AT	CDI	RfD	HQ
Agbadzikope BH	4.53	1.4	365	6	20	2190	0.3171	1.75	0.1812
Agbadzikope BH	4.45	1.4	365	6	20	2190	0.3115	1.75	0.178
Bakpa Dzave BH	8.52	1.4	365	6	20	2190	0.5964	1.75	0.3408
Amegakope BH	2.57	1.4	365	6	20	2190	0.1799	1.75	0.1028
GbadagokopeBH	6.13	1.4	365	6	20	2190	0.4291	1.75	0.2452
Kanikope BH	9.21	1.4	365	6	20	2190	0.6447	1.75	0.3684
Kanikope BH	9.01	1.4	365	6	20	2190	0.6307	1.75	0.3604
Asiekpe BH	5.69	1.4	365	6	20	2190	0.3983	1.75	0.2276
Seva BH	4.61	1.4	365	6	20	2190	0.3227	1.75	0.1844
Avikope BH	2.89	1.4	365	6	20	2190	0.2023	1.75	0.1156
*Tsawla	1.82	1.4	365	6	20	2190	0.1274	1.75	0.0728
Agbadzikope SW	42.5	1.4	365	6	20	2190	2.975	1.75	**1.7**
Bakpa Dzave SW	25.2	1.4	365	6	20	2190	1.764	1.75	**1.008**
Tsawla SW	25.05	1.4	365	6	20	2190	1.7535	1.75	**1.002**
Avikope SW	31.1	1.4	365	6	20	2190	2.177	1.75	**1.244**