KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
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EFFECTS OF COPPER-BASED FUNGICIDE APPLICATION ON COCOA GROWING SOILS AND PLANTS IN FENASO OF THE AMANSIE CENTRAL DISTRICT, ASHANTI REGION,GHANA

By

ADJEI YEBOAH, BED (SCIENCE)

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DECLARATION

I hereby declare that this submission is my own work towards the MSc. degree award and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

ADJEI YEBOAH	________________	______________
(PG6088911)	Signature	Date

Certified by:

Dr. Patrick Addo-Fordjour	________________	______________
(Supervisor)	Signature	Date
Dr. B. Fei-Baffoe	________________	______________
(Supervisor)	Signature	Date
Dr. Isaac Tetteh	________________	______________
(**Head of Department)**	Signature	Date

ABSTRACT

Copper based fungicide remains the most effective way to control the canker that reduces the higher yield of the brown gold, cocoa for the people of Fenaso and the country at large. However excessive application of the fungicide leaves a higher percentage of residual copper in the soil and plants which affects the quality of cocoa produced in the area. 30 selected cocoa farms from Fenaso, in the Amansie Central District of Ashanti Region which had experienced repeated application of copper-based fungicide were sampled to study the total copper in two soil depth of three of spraying age categories together with cocoa parts, namely seeds, leaves and bark. The relationship between copper concentrations and the soil pH and organic matter in the soils were also studied. An unsprayed farm land at the same area was sampled for control. Mean total copper concentration for three spraying age categories-below 10 years, 10-30 years, and above 30 years of the top soil were 22.37 mg/kg, 22.46 mg/kg and 23.97 mg/kg respectively with corresponding mean of sub-soil as 18.08 mg/kg, 17.34 mg/kg and 17.91 mg/kg. Analysis of results indicated that copper concentrations of the soil samples from sprayed cocoa farms at both depths were significantly different from the corresponding copper concentrations from unsprayed farm land ($p < 0.05$). The analysis also showed a significant difference between top soil and sub soil which indicated that the mean copper concentration of top soil was always greater than corresponding sub soil. No significant difference was obtained in the three spraying age categories on total copper concentration pH values and organic matter content of soil the samples ($p > 0.05$). The regression analysis of the results indicated positive relationship between total copper concentration and soil pH as well as organic matter. Copper concentration of cocoa beans differed significantly from leaves and bark ($p < 0.05$). The mean copper concentration of the seeds, leaves and bark were 37.3 mg/kg, 16.10 mg/kg and 16.5 mg/kg respectively. Even though the cocoa growing soil of Fenaso is contaminated with copper based fungicide, the cocoa beans from the area are far below the health risk of consumption. In future, more research should be conducted on different cocoa farms nationwide to complement with the conclusions made in this study.

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LIST OF ABBREVIATIONS

A	Top soil
AAS	Atomic Absorption Spectrophotometer
ACDMTDP	Amansie Central District Medium Term Development
AC-MoFA	Amansie Central-Ministry of Food and Agriculture
AOAC	Association of Official Analytical Chemist
B	Sub soil
Bk	bark
CEC	Cation Exchange Capacity
COCOBOD	Cocoa Board
CODAPEC	Cocoa Diseases and Pest Control
COFs	Copper Oxychloride-based Fungicides
CSSVD	Cocoa Swollen Shoot Virus Diseases
EPA	Environmental Protection Agency
g	gramme
GCAP	Ghana Commercial Agriculture Project
GCSCRAR	Ghana Supply Chain Risk Assessment Report
ICCO	International Cocoa Organization
IMF	International monetary Fund
Kg	kilogramme
l	leaf
L	Liter
LC	Lethal concentration
LD	Lethal Dose
LI	Legislative Instrument

mg	milli gramme
ml	milliliter
MoFA	Ministry of Food Agriculture
MT	Metric Tones
^{O}C	Degree Celsius
OM	Organic matter
ppm	Part Per Million
S	Seed
SD	Standard Deviation
WHO	World Health Organization

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DEDICATION

This work is dedicated to the God for his guidance and protection together with my parents Mr. and Mrs. Asuming Yeboah for their financial support and encouragement.

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CHAPTER ONE

1.0. INTRODUCTION

1.1 Background

Cocoa products-Milo, chocolate, cocoa powder to name but few had been a vital delicacy for so many homes in the world as breakfast and dessert. Cocoa, scientifically known as *Theobroma cacao* L. originated from upper regions of Amazon River basin now known as Peru, Ecuador, Colombia and Brazil. Tetteh Quarshie, a native of Osu, Accra, was the first to established a cocoa farm in Ghana at Akwapim Mampong in the Eastern Region having returned with Amelonado cocoa pods in 1879 from Fernando Po as blacksmith after unsuccessful attempts by the Dutch and Basel missionaries in 1815 and 1857 respectively (Grossman-Green and Bayer, 2009). Cocoa, which is used mainly in production as chocolate, is an important agricultural export commodity to Ghana. Cocoa serves as the major source of revenue for provision of socio-economic infrastructure in the country. In terms of employment, the industry employs 60% of the national agricultural labour force (Appiah, 2004). Ghana's cocoa has been described as the backbone of Ghana's economy (Osei, 2007). It is from this light that the government of Ghana is committed to reap maximum benefit for cocoa sector to ensure that the country increases its cocoa products and also processes more of the beans into downstream product for both the local and export market (Awua, 2002). Having this goal in mind the government of Ghana, through Cocoa Board initiated a National Cocoa Diseases and Pest Control (CODAPEC), established mass cocoa spraying exercise to arrest the decline in cocoa production, popularly known as "Mass Spraying" to assist all cocoa farmers in the country to combat the Capsid/Mirid and the Black Pod disease using copper-based fungicide (CODAPEC, 2012). Consequently farmers are progressively integrating fertilizer application and spraying practices into their own cultivation of cocoa crops

(Koka *et al*., 2011). Copper fungicide like Kocide 101, Nordox, Champion, Ridomil and Caocobre Sandoz is used by farmers, leaves a higher percentage of copper residue which can bio accumulate in the cocoa trees. Even though copper in the soil is an essential trace element for proper function of many biological systems in plants, animals and humans, it is potentially toxic at an excessive level (Alloway, 1995). Copper (Cu) plays important roles in plant growth and is an important component of various enzymes involved in both photosynthesis and respiration (Demirevska-Kepova *et al*., 2004). As indicated in the study conducted by Lewis *et al*. (2001), the excessive Cu in soils is phytotoxic which leads to chlorosis and retarded growth. Cu contamination of the food chain is also injurious to human and animal health. Natural concentration of Cu in soils depends primarily on copper contamination in soil and water is on the rise worldwide due to anthropogenic input (He *et al*., 2005; Koka *al*., 2011). The application of copper-based fungicide traced back to the end of 19^{th} century. Presently a large portion of fungicides containing copper sulphate and copper (II) hydroxide are still being used. As a result, substantial amount of copper have been accumulated in the soil with total copper as high as several thousand milligrams per kilogram (wightwick *et al*., 2008). Due to this, copper has significantly accumulated in the soil, accelerating transport to the environment particularly in low pH soil (Zhang *et al*., 2003). Cu concentration at >100 mg/kg in sandy acidic soil caused phytotoxicity to plants (Alva, 1991). Studies indicate that copper exhibits strong sorption properties to many mineral surfaces as well as soil organic matter, this makes copper one of the least mobile of the heavy metals (Kabata-Pendias, 2001). Organic matter controls and dominates the adsorption of Cu in the soil (McBride *et al.*, 1997). Sanders and Bloomfield (1980) indicated that copper adsorb quite strongly to humus in the soil. Also, the removal of organic matter reduces Cu adsorption significantly even in cases of high Cation Exchange Capacity (CEC) (Elliott *et al*., 1986;

Schwer, 2010), therefore making copper one of the least mobile heavy metals. Organic matter controls and dominates the adsorption of copper in the soil (McBride *et al.,* 1997). Fenaso is a well-known cocoa producing area in Ashanti region of Ghana, have over the years indulged in repeated spraying exercise to do away black pod diseases on their farms with copper based fungicides like Kocide, Nordox , Ridomil, just to name but few. The farmers have also enjoyed the mass cocoa spraying exercise introduced by the government of Ghana since 2001.

1.2 Problem Statement

Excessive application of copper-based fungicides on cocoa farms leaves a higher percentage of copper residues in the soil, which contaminate the soil environment, ground water (Zhang *et al.*, 2003), leading to undue absorption and translocation to various vegetative parts of the tree (Aikpokpodion *et al*., 2012).

Alloway (1995) found out that the excessive levels of copper in plants pose a serious health threats to the plants. Also, excessive copper in soils is phytotoxic leading to chlorosis and retarded growth (Lewis *et al*., 2001). In the nut shell, the excessive accumulation of copper in the cocoa trees reduces the quality of cocoa beans (Adeyeye, 2006), which is produced in the world, particularly Fenaso in the Amansie Central District of Ghana. This will in turn affect the demand of cocoa beans from that area, finally leading to poverty as the people of Fenaso depends heavily on proceeds from cocoa for their livelihood. It is therefore prudent to determine the concentration of copper in the soils and cocoa trees sprayed with copper based fungicides, in order to keep the level of copper in cocoa beans within the acceptable limit set by the WHO. Therefore, a careful assessment of plantations where copper-based fungicides had been applied for years should be made to determine the level of copper residues in the soil and its accumulation in cocoa trees.

1.3 Justification

Many individuals are exceeding the WHO limit of copper intake, 10–12 mg/day for adults due to the continuous ingestion of contaminated food and water (WHO, 1996). Anthropogenic activities like spraying with copper-based fungicide contribute immensely to the concentration of Cu in the environment. The continuous use of copper-based fungicide over the years to control black pod disease contributes to heavy-metal accumulation in cocoa soils due to its non-biodegradable nature. Aikpokpodion *et al*. (2012) reported that the accumulation of copper in the soil can result in an undue absorption and translocation to various vegetative parts of trees including the beans, which is the economic part of the crop. The most common, incidental contamination of horticultural soils with copper is due to the use of copper-bearing fungicides that reach soil surfaces directly or indirectly with leaf litter (Richardson, 1997). Continual use of copper fungicide appears to impair the food quality of cocoa beans (Adeyeye *et al*, 2006). Over the years, Fenaso cocoa farmers together with other staunch farmers in the country have been spraying their cocoa farms with copper-based fungicides against black pod diseases. Fenaso cocoa farmers have also enjoyed the mass cocoa spraying exercise organized by CODAPEC programme for Ghanaian cocoa farmers. Under CODAPEC programme and between 2001 and 2004, an average of about 659,000 hectares made up of 543,279 farms was sprayed against black pod disease with copper-based fungicides. Five hundred and twenty one thousand nine hundred and fifty six farmers were involved in the spraying exercise (Opoku *et al*., 2006). The repeated application of copper based fungicide to curb the canker which reduces crop yield leaves a higher percentage of residual copper in the soil and other part of the plants. Fenaso-a small cocoa growing community in the Amansie Central District of Ashanti Region depends so greatly on cocoa for their livelihood and survival. However, they have been tormented by diseases and pest of cocoa (Bowers *et al*., 20010). For several decades they have indulged in repeated application of copper based fungicide on their farms.

1.4 General Objective

To determine the concentration of copper in cocoa growing soils and cocoa trees as affected by repeated fungicide application at selected cocoa farms in Fenaso.

1.5 Specific Objectives

1. To determine the total copper concentration in cocoa growing soils at different depths.
2. To determine the relationship between soil organic matter content and the concentration of copper in the soil.
3. To determine the relationship between soil pH and copper concentration in the soil.
4. To determine the level of copper concentration in the seeds, leaves and bark of cocoa trees.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Major Challenges Associated with Cocoa Production

Diseases and pests pose a major limitation to cocoa production (Bowers *et al*., 2001). Crop diseases and insect pests pose by far the greatest risk to the cocoa supply chain in Ghana. Events such as drought and forest fires are only significant in exceptional years and have a negligible long-term impact on national cocoa production (GCSCRAR, 2011).

2.2 Major Fungal Disease of Cocoa in Ghana

Although, there is an increasing demands for cocoa beans, diseases and pests pose a major limitation to its production (Bowers *et al*., 2001). Losses could be extremely high, if left uncontrolled. Amongst cocoa diseases and pests that affect Ghanaian cocoa farmers are the black pod, cocoa swollen shoot virus disease and capsid which damage the trees and crops (Thresh and Owusu, 1986; Hughes and Ollennu, 1994; COCOBOD News, 2007).

2.2.1 Black Pod Rot

Many of the world's most devastating crop diseases are caused by fungi in the genus: *Phytophthora*. In cocoa, three species of *Phytophthora* cause diseases commonly referred to as black pod rot, and account for approximately 450,000 MT of losses worldwide(Hebbar, 2007). *Phytophthora* species are found in all three growing regions, but the most aggressive and fast moving is *P.megakarya* which is found in West Africa (Hebbar, 2007). The first sign of the disease is small spots on the cocoa pod. Farmers may miss this initial sign, particularly in areas where other diseases are prevalent. But the farmer will notice when the spots grow and darken and the pod turns black and rotten.

Although infection of the pod is the most common, the pathogen can also infect the trunk and branches with cankers (known as trunk or stem cancer) and cause root rot (Hebbar, 2007). *Phytophthora* megakarya is unique to Central and West Africa. *Phytophthora palmivora* has a worldwide distribution and is found in tropical and subtropical regions. It infects cocoa and over 200 other plant species (GCSCRAR, 2011).

2.2.2 Swollen Shoot Virus Disease

Cocoa swollen shoot virus disease (CSSVD) is a plant pathogenic virus spread primarily by the mealy bug. The virus is retained when the insect moults, but it does not replicate in the insect. The disease can also spread via roots and interlocking branches, thus necessitating the cutting of neighboring cocoa trees for effective control. Many strains of the virus exist, each varying in symptoms and virulence. Infected trees are infectious before they show symptoms, but severe CSSVD will kill traditional West African tree varieties in 2 to 3 years (GCSCRAR, 2011).

2.3 Pest Attack on Cocoa

Cocoa production in the world, particularly Ghana is affected by a number of pest-related threats. They include the following, in approximate order of importance:

2.3.1 Mistletoe

Mistletoes (family Loranthaceae) parasitize a wide range of trees and shrubs in Ghana. On cocoa (*Theobroma cacao* L.), mistletoe infestation is of considerable economic importance because of the direct effect of reducing yield and indirect effect of contributing to the general degeneration of trees on farms. Mistletoe is especially interesting botanically because it is a partial parasite ("hemiparasite") (Redmond, 2008). One species (*Tapinanthus bangwensis*) accounts for about 70% of infestations in Ghana and is recognized by its red flowers and berries. It flowers twice a year and can live for up to 18 years. If Mistletoe growth is not checked, it can impact yield so greatly. Regular removal of mistletoe is essential for good crop management and large populations can be considered a sign of farm neglect. Mistletoe may also provide a suitable habitat for ants that cultivate CSSVD vectors (GCSCRAR, 2011).

2.3.2 Cocoa Mirids/Capsids

Cocoa mirids, also known as 'capsids', have been important pests of West African cocoa for over a century. Crops losses in cocoa due to capsids and mealy bugs have been estimated at 25% - 30% per annum (Anon, 1951; Anikwe, 2010). Mirids damage cocoa trees by feeding on tree sap. They cause characteristic lesions on the surface of cocoa pods, and often introduce pathogenic fungi. Nonetheless, the greatest damage from mirids is to the tree itself. Mirid infestation typically leads to the destruction of growing shoots and, in severe cases, the loss of the tree. Practical control measures include good tree maintenance (i.e., prevention of gaps in the canopy and removal of chupons) and applications of insecticides (GCSCRAR, 2011).

2.3.3 Cocoa Stem-Borers

Cocoa stem-borers are widespread across West Africa. They are also known to attack other crops such as coffee and cola. Regular outbreaks occur in cocoa producing countries in West Africa. In Ghana, the Ashanti, Brong Ahafo, and Western regions are most affected (GCSCRAR, 2011). Stem borers have been reported in the past as minor insect pests of cocoa and those found occurring on cocoa include *Phosphorus virescens, Phosphorus gabonator, Tragocephala castnia* and *Apate monachus* (Anikwe, 2010).

2.4 Fungicide Application

Farmers worldwide apply chemicals on crops to curb the canker that reduces the yield and quality of farm produce. The application of copper-based fungicide traced back to the end of 19th century. The most common, incidental contamination of horticultural soils with copper is due to the use of copper-bearing fungicides that reach soil surfaces directly or indirectly with leaf litter (Tiller and Merry, 1981; Richardson, 1997).

2.4.1 Categories of Fungicides

There are two general types of fungicides. Contact fungicides, sometimes called protectant fungicides, remain on plant surfaces after application and do not penetrate the plant tissue. Systemic fungicides are those that are absorbed into the plant. Some systemic fungicides move within the plant only a short distance from the site of penetration; these fungicides are called locally systemic. The dicarboximide fungicides are one example of this group. Some locally systemic fungicides simply cross the leaf blade from one leaf surface to the other but do not redistribute within the plant. In that case, they are called translaminar fungicides; trifloxystrobin is an example. Some systemic fungicides move within the water-conducting tissue (xylem), which takes them upward in the transpiration stream; downward mobility within the plant is very limited. These fungicides are called xylem-mobilesystemics. Within this group, some fungicides are moderately mobile within plants, such as certain DMI fungicides. Others are highly mobile and move readily through the xylem. Examples of highly xylem-mobile systemics include thiophanate-methyl and mefanoxam. A third type of systemic fungicide is the phloem-mobilesystemic, which moves bidirectional (from leaves to roots and vice versa). Systemic fungicides sometimes can suppress the fungus after it has infected the plant, whereas contact fungicides must be present on the plant's surfaces before infection begins in order to be effective (Paul and David, 2011).

2.4.2 Copper Based Fungicide

Copper is known to be a useful micronutrient but it is a broad-spectrum biocide at higher concentrations (Flemming and Trevors, 1989). Copper fungicides can be described as insoluble compounds, yet their action as fungicides and bactericides is due to the release of small quantities of copper ($Cu^{2+)}$ ions when in contact with water (Noyce *et al*., 2006; Mehtar *et al*., 2008).

2.4.3 Effect of Copper Based Fungicides

Although the application of copper based fungicide remains the most effective way of curbing pest and disease, however the fungicide application result in serious impacts on non- target organisms.

2.4.3.1 Effects of Copper Based Fungicide on Birds

Copper sulphate is practically nontoxic to birds. It poses less of a threat to birds than to other animals. The lowest lethal dose (LD_{50}) is 1000 mg/kg in pigeons and 600 mg/kg in ducks. The oral LD_{50} for Bordeaux mixture in young mallards is 2000 mg/kg (Extoxnet, 1996).

2.4.3.2 Effects Copper Based Fungicide on Aquatic Organisms

Copper sulphate is highly toxic to fish. Even at recommended rates of application, this material may be poisonous to trout and other fish, especially in soft or acid waters. Its toxicity to fish generally decreases as water hardness increases. Fish eggs are more resistant than young fish to the toxic effects of copper sulfate. Copper sulphate is toxic to aquatic invertebrates, such as crab, shrimp, and oysters. It is used as a pesticide to control tadpole shrimp in rice production. The 96-hour LC_{50} of copper sulphate to pond snails is 0.39 mg/L at 20 ^{o}C. Higher concentrations of the material caused some behavioral changes, such as secretion of mucous, and discharge of eggs and embryos (Extoxnet, 1996).

2.4.3.3 Effects Copper-Based Fungicide on Other Organisms

Bees are endangered by Bordeaux mixture. Copper sulphate may be poisonous to sheep and chickens at normal application rates. In some orchards, most animal life in soil, including large earthworms, has been eliminated by the past extensive use of copper containing fungicides (Extoxnet, 1996). Copper suppress rates of nitrogen fixation by the bacteria Rhizobium under some situations at relatively high copper levels of 235 ppm (Addo-Fordjour *et al*., 2013).

2.4.3.4 The Effect of Copper on Human Health

Copper (Cu) is an essential micronutrient important to plants, animals, and humans as a constituent of several enzymes and as a redox catalyst in a variety of metabolic pathway (Devez *et al.*, 2005; Yan *et al.*, 2006). The deficiency of Cu may result in reduction of biological function and potentially death (Juang *et al.*, 2008). The biological benefits of exposure to copper reflect this balance between essentiality and toxicity. Chronic toxicity of copper in humans is associated principally with Wilson disease, with the occurrence of infantile cirrhosis in areas of India (Indian childhood cirrhosis), as well as with isolated clusters of cases in other countries related to excessive copper intake (Horslen *et al.,* 1994). The oral LD_{50} of copper sulphate is 472 mg/kg in rats. Toxic response in humans has been observed at 11 mg/kg. Ingestion of copper sulphate is often not toxic because vomiting is automatically triggered by its irritating effect on the gastrointestinal tract. Symptoms are severe, however, if copper sulphate is retained in the stomach, as in the unconscious victim. Injury to the brain, liver, kidneys, and stomach and intestinal linings may occur in copper sulphate poisoning. Copper sulphate can be corrosive to the skin and eyes. It is readily absorbed through the skin and can produce a burning pain, as well as the other symptoms of poisoning resulting from ingestion. Skin contact may result in itching or eczema. It is a skin sensitizer and can cause allergic reactions in some individuals. Eye contact with this material can cause conjunctivitis, inflammation of the eyelid lining, cornea tissue deterioration, and clouding of the cornea (Extoxnet, 1996).

2.4.3.5 Effect of Copper on Nutrient Availability

Nutrients are needed by plants to produce at maximum capability and to perform specific functions within the plant (Addo-Fordjour *et al.*, 2013). When copper gets into the soil, it binds strongly to organic matter, clay minerals and hydrated oxides of Iron (Fe), Aluminium (Al) and Manganese (Mn) (Schnitzer, 1969), and either reduces the concentration of these nutrients in the soil or makes them unavailable to plants. Continuous application of Bordeaux mixture increases Copper content in the soils of grape farms (Savithri *et al*., 2003). Akinnifesi *et al*. (2006) found that increasing copper content of soils in cocoa plantations reduced the amount of phosphorus available to plants and causes nutrient imbalance.

2.4.3.6 Effect of Copper on Soil Fauna

Copper (Cu) is an essential micronutrient important to plants, animals, and humans as a constituent of several enzymes and as a redox catalyst in a variety of metabolic pathway (Devez *et al.*, 2005; Yan *et al.*, 2006). It is well recognized, however, that elevated concentrations of Cu are toxic to organisms. Van Zwieten *et al.* (2004) stated that copper disturbs the biochemical and physiological processes such as photosynthesis, enzyme activity, pigment and protein synthesis, and cell division.

2.4.4 Environmental Fate

Whilst a farmer's intention is to apply fungicides to the agricultural crop/plant, inevitably, a large proportion of the chemical spray will miss its target. Much of the lost chemical will enter our water bodies where it will persist for a period of time and potentially migrate off-site.. In addition, some of the agrochemicals applied on farm will migrate off-site due to aerial drift. Agrochemicals after off-site migration will potentially enter nearby waterways and groundwater resources where they can cause adverse effects to aquatic organisms (Wightwick and Allinson, 2007). The fate and behavior of agrochemicals, including fungicides, in the environment is influenced by the properties of the chemical (ability to bind to soil, susceptibility to degradation) and environmental factors (e.g. soil type, rainfall, topography, agricultural management practices). These environmental factors, in particular soil type, are widely varied, thus there are many scenarios to consider when assessing the potential for off-site migration and the persistence of agrochemicals in soil (Wightwick and Allinson, 2007; Arias-Estevez *et al*, 2008; Komarek *et al*., 2010).

2.5 Copper as an Element

Zheng *et al.* (2007) stated that copper is widespread in natural and agricultural environment as a consequence of various agricultural, mining and industrial activities as well as resulting from the geochemical processes.

2.5.1 Sources of Copper in the Environment

Copper from natural source is considered a trace elements, generally at a concentration of <0.1%. The source of Copper, natural or anthropogenic, can provide information on concentrations and mobility in the environment. Copper is obtained from weathering of soil parent material. In natural systems, the levels of copper is generally low and in most cases are necessary for the proper function of the ecosystem, because the background concentrations provide essential nutrients (Cu) for the resident flora and fauna (Schwer, 2010). On the contrary, anthropogenic activities, and under some natural circumstances, the trace metal-copper concentrations in soils can become highly elevated with respect to background concentrations. On the face of these circumstances, copper nutrient can pose a serious threat to the local environment, in addition to potential threat to humans through the ingestion of food and water containing elevated metal concentrations.

2.5.1.1 Natural Sources

Kabata-Pendias (2001) indicated in his studies that the highest copper concentrations occur in mafic rocks (60-120 mg/kg) and in the lowest concentration in limestone rocks (2-10 mg/kg). On average, the concentration of copper in the earth's crust is 25-50 mg/kg and is 17-30 mg/kg in the soil (Sparks, 2003; Sposito, 2008; Schwer, 2010). As indicated in the studies of Schwer (2010), copper is a component in many primary minerals, the most common being sulphides such as chalcocite (Cu_2S), covellite (CuS), and villamaninite (CuS_2). The sulphide minerals are quite soluble and provide a continuous release of Cu ions to solution where they can then interact with a variety of soil constituents.

2.5.1.2 Anthropogenic Sources

Copper can be highly elevated in surface soils due to the widespread use of copper in many anthropogenic activities particularly fungicide spraying. Addo-Fordjour *et al.* (2013) indicated that the use of copper based fungicide in agricultural sector elevates the concentration of soil Cu. The anthropogenic mobilization factor of copper is 632, meaning human activities are greatly influencing the metal cycling of copper (Sposito, 2008; Schwer, 2010). The most recognized Cu containing fungicide is the Bordeaux mixture ($Ca(OH)_2+CuSO_4$) which has been applied to control downy mildew on grapes since the end of the 19th century (Schwer, 2010). According to Brun *et al*. (2001), the extensive use of copper based fungicide leads to accumulation of copper up to 200-500 mg/kg compared to 5-30 mg/kg in soils without fungicide addition. Mining activities is a major anthropogenic source of copper in the soil environment. Other sources like waste emissions, and the application of sewage sludge, fertilizers, and fungicides in agricultural applications contributes greatly to copper concentration in the soil. (Flemming and Trevors, 1989; Schwer, 2010).

2.6 Copper Chemistry

Copper is a transition metal with atomic number and atomic mass of 29 and 63.546 respectively. It is in the period 4 and group 11 of the periodic table along with silver and gold. In its ground state copper's electronic structure is $[Ar]3d^{10}4s^1$(Whitten *et al.*, 2000). However the Cu^{2+} is the most common form. Most copper deposits exist in the form of sulphide minerals. Copper occurs in the environment as a solid metal with no charge (Cu), as the cuprous ion, Cu^+($[Ar]3d^{10}$), or as the cupric ion, Cu^{2+}($[Ar]3d^9$) (Schwer, 2010). The Cu^{2+} is a Lewis acid, which means it is highly reactive with both hard and soft Lewis bases, whereas Cu^+ is a soft Lewis acid which reacts with soft Lewis bases (Sparks, 2003). Cu is partitioned into the aqueous phase, including soluble species and free ions, the solid phase, including sorption to soil constituents and precipitates, and the biological phase, including sorption and incorporation in organic matter in the environment. $Cu^{2+}$is the most abundant copper oxidation state in the environment and is classically thought to exist as the free cupric cation $[Cu(H_2O)_6{}^{2+}]$ in solution (Schwer, 2010).

2.6.1. Retention of copper in the soil

According Kabata-Pendias (2001), Copper exhibits strong sorption properties to many mineral surfaces as well as soil organic matter. As results of this, copper is one of the least mobile of the heavy metals. Also, organic matter controls and dominates the adsorption of Cu in the soil (McBride *et al.*, 1997). The amount of copper retention depends on many factors in the soil, most mainly, pH, organic matter content and clay content (Schwer, 2010). Copper adsorb quite strongly to humus horizons in the soil and the removal of organic matter reduces copper adsorption significantly even in cases of high CEC (Elliott *et al.*, 1986; Schwer, 2010).

2.6.2 Precipitation

Copper (II) hydroxide or copper (II) carbonate, usually occur on many soil constituents at higher pH surface precipitation (Schwer, 2010). Alvarez-Puebla *et al.* (2004) found out that $CuOH^+$ causes surface precipitation of $Cu(OH)_2$ on illite soil under increasing soil pH. At pH 4 Cu begins to precipitate, forming amorphous precipitates, which are the main mode of retention at pH 8. In the presence of carbonates and organic matter, copper possibly precipitated as malachite (Cavallaro and McBride, 1980; Schwer, 2010).

2.6.3 Effect of Organic Matter on Copper

The sorption behaviour of heavy metals like Cadmium, Copper, Nickel, and Zinc in soils varies from soil to soil and is influenced by soil properties, such as pH, organic matter, Cation exchange capacity(CEC), and clay contents (McBride, 1982). Soil organic matter has been of particular interest in studies of heavy metal sorption by soils, because of its significant impact on CEC, and more importantly, the tendency of transition metal cations to form stable complexes with organic ligands (Elliott *et al.*, 1986, Karaca, 2004). The amount of organic matter in soils affects the binding of heavy metals in soil and speciation in soil solution (Lo *et al.*, 1992). Vega *et al*. (2007) found out that organic matter is the main component affecting sorption of copper, especially at neutral pH, oxides control the adsorption at lower pH. Chaignon *et al.* (2003) reported of a linear positive relationship between organic carbon and soil copper concentrations.

2.6.4 Effects pH on Copper

Major plant parameters (tissue capacity, cation content, acidic ionization of biopolymers etc.) depend on the complex influence of factors in the nutrient medium, including soil. Recently, many reports take notice of heavy metals' behaviour as their agrochemical mobilization in soils and uptake by plants in toxic concentrations depends strongly on soil acidity (Merry *et al.*, 1986; Alloway and Ayres, 1994; Ross, 1994). Sorption and fixation of copper rapidly increases at a pH greater than 4 with most adsorption being non exchangeable on clays (Cavallaro and McBride, 1984). The maximum adsorption of copper occurs at a pH greater than 5 on Fe oxides, Mn oxides, organic matter and clay minerals (McLaren and Crawford, 1973; Schwer, 2010). Also, in soils the maximum copper sorption has been found at a pH greater than 5.5-6.5 (Agbenin and Olojo, 2004; Schwer, 2010). Increasing pH led to higher retention of $CuOH^+$ on illite, eventually causing surface precipitation of $Cu(OH)_2$, much of the sorption was precipitation at pH greater than 6 (Alvarez-Puebla *et al.*, 2004). Also, the desorption of copper from humic acid is decreased with increasing pH, however, this is only a small portion of the total copper adsorbed (Arias *et al.*, 2005).

2.6.5 Toxicity of Copper

Copper is a micronutrients essential for proper human nutrition. However, copper becomes very toxic at higher elevations. Continual use of copper fungicide impairs the food quality of cocoa beans (Adeyeye *et al*., 2006). Copper toxicity threshold is established at 200 mg/kg above which, a reduction of shoot growth and leaf chlorosis appeared after 2 years of copper exposure (Toselli *et al*., 2008). Giller *et al*. (1998) stated that high concentrations of heavy metals have also effected the utilization of carbon substrates, decreasing the rate of mineralization and in some cases increasing the leaf litter on the forest surface. Soil enriched in heavy metals through application of sewage sludge has also led to a decrease in microbial symbiotic nitrogen fixation (Giller *et al.*, 1998).

2.7 Mobility of Copper

The mobility of heavy metals depends on their chemical speciation, which in turn is related to the chemical properties of soil (Dudley *et al*., 1991). Much of the copper that enters environmental waters will be associated with particulate matter. Copper is largely retained in the soil as reported by Borg and Johansson (1989). They noticed that the mobility increased with the decrease of pH. They also, suggested that the mobility of copper was associated with the transport of organic material since the copper was highly adsorbed them. The mobility of most heavy metals in the soil and subsoil depends on the physico-chemical properties of the solid and liquid phases (Hossam, 2001). Many chemical changes may occur during the movement of water through the soil including dissolution/precipitation, adsorption/desorption, degradation, filtration and a variety of transport processes (Fic and Schroter, 1989). Vertical distribution of the copper in the soil profile of the Danish agricultural soils could be influenced by the mobility of clay particles (Bibak *et al.*, 1994). Bioavailability and mobility of copper in soils largely depend on its chemical forms and, consequently, on the nature of the “carrier” constituents (Miller and McFee, 1983; Tessier and Campbell, 1988). The mobility of most heavy metals in the soil and subsoil depends on the physico-chemical properties of the solid and liquid phases (Fic and Schroter, 1989). Trace metals can be found in particulate, colloidal and truly dissolved phases. The fate and transport of metals in the environment depends on these phases and the interaction between them. Soil parameters, such as pH, organic carbon content, iron and manganese oxide content and total metal content affect the distribution of copper and cadmium between different soil fractions (Wilhelmy *et al.*, 1996). Copper oxychloride-based fungicides (COFs) are widely used as foliar sprays to do away fungal diseases in a number of crops (Paradelo *et al*., 2009). Thus, there is a great likelihood that they may be released into the natural environment, especially soils.

2.8 Accumulation of Copper in Plants

Farmers all over the world apply Copper fungicides to control plant pathogens from each of the three classes of fungi (Phycomycetes, Ascomycetes and Basidiomycetes). After foliar application of copper fungicides, a gradual redistribution of deposits by the weathering effect (rainfall and dew) may occur. Some become absorbed by plant cells, while most redistribution occurs in downward direction (Alloway, 1994; Adeyeye *et al*., 2006). Heavy metals present in the soil enter the plants through their root system, as well as by diffusion through the peel (Violina *et al*., 2010). Copper being a transition metal is not biodegradable by soil microorganisms. Hence, it accumulates in soils. The build-up of copper in soil can lead to its undue absorption and translocation to various vegetative parts of the tree including the beans, which is the economic part of the crop (Aikpokpodion *et al*., 2012).

2.9 Governmental Regulation on the Use of Copper Based Fungicides

Application of Copper based fungicide is the most effective way of controlling diseases and pest on cocoa in Ghana. However, the production, management and application of all pesticides including fungicides and bactericides in the country are regulated by Environmental Protection Agency Act, 1994, Act 490. This section of Act 490 provides the rules for registration, pesticides classification, approval, clearance, using, disposing of and non-disclosure of confidential information, the granting of license, labeling and pesticides inspections. Plant Protection and Regulation Services Directorate of MoFA also regulates pesticides use in the country based Prevention and Control of Pests and Diseases of Plants Act of 1965, Act 307 now replaced by "Plants and Fertilizer Act, 2010 (GCAP, 2011). The EPA is responsible for determining the "risk" associated with pesticides and for establishing limits or "tolerances" on the amount of pesticide residues that may remain on food marketed in the Ghana to meet safety standards with "reasonable certainty of no harm" to the general population.

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3.1 The Scope of the Project

The area selected for the study is Fenaso, a cocoa growing community in Amansie central district of Ashanti region. Due to the lack of logistics and funds, the study was limited to 30 selected cocoa farms in and around Fenaso Township. Three main forms of data were collected from the study area. The first data was on the number of cocoa farms and the age of application of each cocoa farm. The second was on the quantity and concentration of copper in the soil, seeds, leafs and barks of sampled cocoa farms. The third data was on the physico-chemical properties of the soil. The physico-chemical properties comprised: the total organic matter content and pH of the soil.

3.2 Study Site

The study was conducted at Fenaso, in the Amansie Central district (Figure 1), previously part of the Amansie East district. 30 cocoa farms sprayed with copper based fungicide were sourced for all samples. A referenced unsprayed plantation was sourced for control samples.

3.2.1 Geographic Area

The Amansie Central District Assembly is one of the twenty seven (27) administrative districts in the Ashanti Region of Ghana. It was carved out of the erstwhile Amansie East District Assembly in 2004 by Legislative Instrument (L1) 1774, 2004. It has about 206 settlements with Jacobu as the Administrative Capital. The District shares common boundaries with Amansie East to the north east, Amansie West to the west, Obuasi Municipal Assembly to the south east, Adansi North to the east, Adansi South to the south and Upper Denkyira in the Central Region to the south (Figure 2). The Amansie Central District can be found within Latitude 6^{0}00N and 6^{0}30N and Longitudes 1^{0}00W and 2^{0}00W. It covers a total surface area of about 710 square kilometers (275.4 sq miles) and forms about 2.5 percent of the total area of the Ashanti Region (ACDA, 2006).

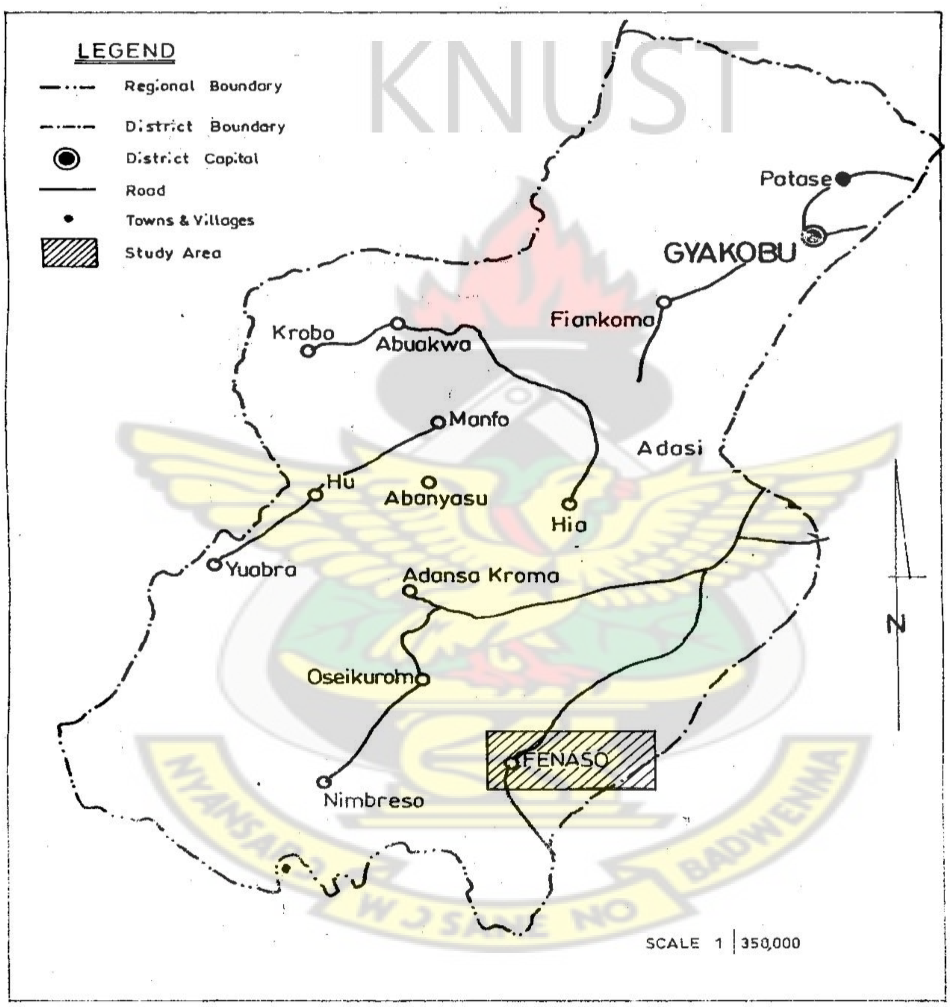

Figure 1: Map of Amansie Central District Showing the Study area.

3.2.2 Relief and Drainage

The district is located within the forest plateau region with an average height of about 150m above sea level. Topographically, the area is relatively flat with occasional undulating upland of 240 m to 300 m above sea level around areas such as Numereso, Apitisu, Tweapease and Abuakwaa. There are two main rivers in the District. These are Oda and Offin which are perennial. There are other minor rivers, some of which flow throughout the year (AC-MoFA, 2013*)*.

3.2.3 Climatic Conditions

The district experiences bi-modal rainfall distribution with the major season falling between March and July, whilst the minor season is between September and November. This is separated by short cool dry season in August and a relatively long dry season from November to March. The annual amount of rainfall ranges between 1500 mm and 1800 mm with mean relative humidity of about 70 percent (AC-MoFA, 2013*)*. Temperature ranges between 20°C and 32°C with mean at 28°C. Incidence of high rainfall pattern increases the prevalence of black pods diseases (Addo-fordjour *et al*., 2013).

3.2.4 Vegetation

The vegetation in the district is semi-equatorial forest. Some of the tree species found in the area includes Odum, Wawa, Edinam, Mahogany and Sapele. There are two main forest reserves in the district and these are Oda and Subin (AC-MoFA, 2013*)*.

3.3 Field Survey/ Fieldwork

Having identified the high illiteracy rate of the people of Fenaso cocoa growing community, a structured interview was used as a collection instrument to collect reliable information from the farmers (Appendix A). The respondents expressed themselves freely, by providing the location, age of cocoa farms, duration and types of fungicide application. In all 72 cocoa farmers were interviewed. Based on the information derived from the farmers on location, age and period of fumigation, 30 farms were selected for sampling (Appendix B-Table B.1 and B.2).

3.4. Sampling Techniques

Regular and random sampling was used for soil and plant materials sampling respectively.

3.4.1 Sampling for Chemical Analysis

Information derived from farmers, especially the period of spraying were used to categorize the sampled cocoa farms into three; farms sprayed for less than 10 years, farms sprayed for a period of 10-30 years and farms sprayed beyond 30 years (Appendix B-Table B.1). Through the interview, it was realized that farmers spray their cocoa farms at least once every year with copper based fungicides to do away with pest and diseases in order to improve yield and maximize profit (Appendix B-Table B.2). 30 cocoa farms were selected (10 farms for each category). Both soil and plant samples were collected from these farms except for the control where only soil which has not been sprayed with copper bearing fungicide.

3.4.2 Soil Sampling

30 cocoa farms were sampled for the analysis with unsprayed plantation as control. Soil samples were collected from an undisturbed area of soil profile at two different depths, between 0-10 cm and 10 cm-30 cm. Five sampling spots of 20 m apart from each other were mapped out for each soil sample collection within each farm. Earth chisel together with scoop were used to dig and collect soil samples from two depths having used meter rule to measure the required depths. Top soil samples collected at 0-10 cm depth from 5 mapped spots were mixed up to make composite with each other in a plastic bowl (Plate

1). A soil sample of about 500 g were taken, bagged and labeled as A (Plate 1 and 2). Soil samples collected at 10-30 cm depth (sub soil) were also treated as A but labeled as B (Plate 2). This procedure was carried out throughout the 30 cocoa farms as well as the unsprayed plantation.

3.4.3 Sampling of Cocoa Bark, Leaves and Beans

Plant materials-barks, leaves and cocoa pods (seeds) were collected (randomly) from 10 different cocoa plants in each plantation. A stainless steel machete was used to collect cocoa bark from 10 randomly selected cocoa trees, mixed to make composite with each other. It was then kept in a transparent polythene bag and labeled as Bk (Plate 2). Ten fresh but matured leaves were collected from each of the ten selected cocoa tree, mixed and a composite of each other were bagged in a transparent polythene bag and labeled as L (Plate 2). A pod of cocoa was collected from ten different cocoa trees already sampled for bark and leaves. The seeds were removed and mixed thoroughly with each other. A composite of each other were taken, bagged in a polythene bag and labeled as S (Plate 2). The same activities were carried out in all the 30 cocoa farms. The samples collected were prepared and digested at faculty of Renewable Natural Resources Department of KNUST whiles the total copper was determined at Environmental quality laboratory, AngloGold Ashanti.

Plate 1: Sourcing for soil samples

Plate 2: Bagging of samples

3.5 Laboratory Analysis

Visible pieces of debris and gravels were removed from the soil samples by hand picking. The barks and leaves were washed thoroughly with distilled water prior to the analysis. The samples-soil, bark, leaves and seeds were all air dried (Adeyeye, 2006). The dried soil samples were ground and sieved through 2 mm nylon mesh sieve. Prior to the analysis of Cu concentration, the soil samples were analyzed for pH value, and Organic matter. The dried samples-bark leaves and seeds were then milled at Department of Soil and Crop Science, KNUST.

Plate 3: Milling of plant sample at soil and crop science laboratory, KNUST.

3.6 Soil pH Determination

Soil pH was determined in a 1:5 of soil water ratio in accordance with standard analytical method. The pH meter was calibrated with three buffer solution of pH 4, 7 and 9. 10 g of the soil samples was weighed into a beaker and 50 ml of distilled water was added to it. The solution was stirred continuously with a magnetic stirrer for 10 minutes and allowed to stand for 30 minutes. The solution was then stirred again for 5 minutes. The electrode of the pH meter was placed in the slurry, swirled carefully, and the pH read and recorded (Motsara and Roy, 2008).

3.7 Organic Matter Determination

The organic matter content was determined by ignition method. 10 g of the soil sample was weighed into a crucible. The soil was then put in an oven at a temperature of 105 ^{o}C for 4 hours. The content of the crucible was then weighed and recorded as M_o. The crucible was thereafter put in a furnace at 400 ^{o}C for 4 hours. The crucible was removed from the furnace and cooled. The content of the crucible from the furnace was reweighed and recorded as M_f (Motsara and Roy, 2008).

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The organic matter content was determined as follows:

$\%OM = \frac{Mo-Mf}{Mo}$ 100 %, Equation 1.

OM represents organic matter.

Mo represents mass of soil sample from oven.

Mf represents mass of soil sample from furnace.

3.8 Digestion of Soil, Bark, Leaves and Seeds Samples for Total Copper Content

Wet digestion method was used for the analysis of Cu in soil, bark, leaves, and seeds. 1 g of each of the sample was weighed into a 300 ml beaker. 10 ml of a diacid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a ratio of 9:4 was added to the sample in the beaker and content were mixed by swirling. The mixture was then heated on hot plate in a fume hood to drive off $NO_{2\ (g)}$ fumes, started at 80-90 ^{O}C and rose to 150 ^{O}C until red $NO_{2\ (g)}$ seized. The content was then cooled and about 20 ml of distilled water was added. The solution was filtered into 100 ml volumetric flask using a filter paper. The beaker was rinsed several times into the volumetric flask until it reached the calibrated mark. The Total copper estimation was made with aid of Atomic Absorption Spectrophotometer model 220. A blank sample was also treated in the same way. (Motsara and Roy, 2008).

3.9 Analysis of Total Copper Content

Filtrates obtained after the digestion were analyzed for total Cu using Atomic Absorption Spectrophotometry (AAS) analysis. AAS 220 model was used in determining the total dissolved copper concentration in the previously digested samples. The acetylene gas and compressor were fixed and compressor turned on and the liquid trap blown to rid of any liquid trapped. The Extractor was turned on and the AAS 220 power turned on (AOAC, 2002). The capillary tube and nebulizer block were cleaned with cleansing wire and opening of the burner cleaned with an alignment card. The worksheet of the AAS software on the attached computer was opened and the hollow cathode lamp inserted in the lamp holder. The lamp was turned on; ray from cathode aligned to hit target area of the alignment card for optimal light throughput, then the machine was ignited. The capillary was placed in a 10 ml graduated cylinder containing deionized water and aspiration rate measured, and set to 6 ml per minute. The analytical blank was prepared, and a series of calibration solutions of known amounts of analyte were made. The blank and standards were atomized in turn and their responses measured. A calibration graph was plotted for each of the solutions, after which the sample solutions were atomized and measured. Copper concentration from the sample solution was determined from the calibration, based on the absorbance obtained for the unknown (AOAC, 2002).

3.10 Statistical Analysis

The mean soil copper concentration and soil pH and organic matter content were compared between different aged sprayed farms using analysis of variance (ANOVA). Likewise copper concentrations in various plant parts were compared with ANOVA. Significant difference in soil copper concentrations between the top and sub soil for different farms ages, and sprayed and non-sprayed farms were tested using t-test. The relationship between soil copper concentrations, and soil pH and organic matter were determined by linear regression analysis.

3.11 Quality Assurance

All chemicals used were of reagent grade and pure distilled water was used throughout the experimentation. All plastics were soaked in 10% $HN0_3$ Procedural blanks preparation of standard solutions under clean laboratory environment (Aikpokpodion *et al.,* 2012). The pH meter was calibrated with three buffer solution of pH 4, 7 and 9 (Motsara and Roy, 2008).

CHAPTER FOUR

4.0 RESULTS

4.1 Copper Concentration of Sprayed Cocoa Growing Soil and Unsprayed Plantation Soil

The profile of plantations indicated that mean copper concentration of sprayed soils was higher than unsprayed soils. A significant difference ($p < 0.05$) was obtained between the mean copper concentration of sprayed cocoa growing soils and unsprayed plantation soil at both soil depths (Table 1).

Table 1:Mean values(±SD) of Copper Concentration of Sprayed Soil and Unsprayed Soil

	Copper conc. of farm soil (mg/kg)			
Soil Depth	Top Soil		Sub Soil	
Sprayed soil	22.93[a]	±9.70	17.78[a]	±7.67
Unsprayed soil	8.60[b]	±0.08	7.90[b]	±0.08
P-values	0.0002		0.0008	

Mean values in the same column of different letters are significantly different at p<0.05

4.2 Copper Concentration of Three Spraying Age Categories at Two Soil Depths

The mean concentrations of copper in the top soil of all spraying age categories were significantly ($p<0.05$) higher than the corresponding mean concentration of sub-soil (Table 2).

Table 2: Mean values (±SD) of Copper Concentration (mg/kg) of Spraying Age Categories at Different Depth

Soil Depth	< 10 Years	10-30 Years	>30 Years	All Farms
Top Soil	22.37[a] ± 9.43	22.46[a] ±7.40	23.97[a] ±12.56	22.93[a] ±9.70
Sub Soil	18.08[b]±7.95	17.34[b]±7.06	17.91[b]±8.72	17.78[b]±7.60
P-value	0.003	0.001	0.023	<0.001

Within the same column numbers followed by different letters are significantly different at p<0.05

4.3 Copper Concentrations of Various Spraying Age Categories at Different Depths

There was no significant difference ($p > 0.05$) in the means of copper concentration of all the spraying age categories at both depths. However, the concentrations of each the spraying age category decreases with increasing depth (Table 3).

Table 3: Mean values(±SD) of Concentration of Copper of Sprayed Plantations at Two Depths

	Copper Conc.(mg/kg)	
Sprayed Farm Lands	Top soil	Sub soil
<10 years	22.37[a] ±9.43	18.08[a] ±7.95
10-30 Years	22.46[a] ±7.40	17.34[a] ±7.06
>30 years	23.97[a]±12.56	17.91[a] ±8.72
P-value	0.9229	0.9765

Means with similar letters within the same columns are not significantly different (*p >0.05*)

4.4 Selected Physico-Chemical Properties of Sprayed and Unsprayed Soil Samples at Two depths

The mean values of each of soil pH and Organic matter of both sprayed and unsprayed soil samples decreased with increasing soil depth. However, there was no significant difference ($p > 0.05$) between the mean values of soil pH of sprayed and unsprayed plantations. On the contrary, a significant difference ($p < 0.05$) was recorded between mean organic matter content of sprayed and unsprayed soil (Table 4).

Table 4: Mean values(±SD) of Physico-Chemical Properties of Sprayed and Unsprayed soil Samples

Soil type	pH		Organic matter (%)	
	Top soil	Sub soil	Top soil	Sub soil
Sprayed soil	6.61^{a} ±0.62	5.54^{a}±0.66	3.23^{a}±1.42	1.32^{a}±0.61
Unprayed soil	6.22^{a} ±0.11	5.86^{a}±0.02	3.58^{b}±0.01	1.96^{b}±0.01
P-value	0.1307	0.2924	0.0167	<0.0001

Mean values in the same columns with similar letters are not significantly different p>0.05

4.5 Selected Physico-Chemical Properties of Sprayed Soils at Different Depths

The mean values of top soil and sub soils of each of soil pH and organic matter were not significantly different (p < 0.05; Table 5).

Table 5:Mean value (±SD) of Physico-Chemical Properties at two Different Depths

Soil Depth	Physco-Chemical Properties	
	pH	Organic matter
Top Soil	6.61±0.62	3.23±1.42
Sub soil	5.54±0.66	1.32±0.61
p-value	<0.0001	<0.0001

Mean values in the same column of different letters are significantly different at p<0.05

4.6 Physico-Chemical Properties and Copper Concentration of Spraying Age Categories.

The analysis of results in indicated that no significance difference (p > 0.05) was obtained between mean values of each of the spraying age categories of pH, top soil of organic matter and copper concentrations. On the other hand, a significant difference (p < 0.05) was obtained in the mean organic matter content of the sub soil (Table 6).

Table 6: Mean values (±SD) of Physico-chemical properties and concentration of copper

Spraying Age Categories	pH		Organic matter (%)		Copper Conc.(mg/kg)	
	Top soil	Sub soil	Top soil	Sub soil	Top soil	Sub soil
<10 Years	6.67[a]±0.87	5.95[a]±0.72	2.45[a]±1.38	0.98[a]±0.05	22.37[a]±9.43	18.08[a]±7.95
10-30 Years	6.52[a]±0.30	5.41[a]±0.50	3.41[a]±1.09	1.70[b]±0.53	22. 46[a]±7.40	17.34[a]±7.06
> 30 Years	6.65[a]±0.61	5.27[a]±0.59	3.83[a]±1.52	1.30[b]±0.61	23.97[a]±12.56	17.91[a]±8.72
P-value	0.8433	0.0448	0.0808	0.0246	0.9229	0.975

Within the columns, mean value with the same letters are not significantly different (*$p>0.05$*)

4.7 Relationship between Soil Copper Concentration and Selected Physico-Chemical Properties

A linear positive relationship was observed between copper concentration and soil pH for all soil samples ($p < 0.05$). This was observed in both top and sub soil layers (Figures 2 and 3). A similar pattern was observed between copper concentration and organic matter content of the top soil. ($p =0.01$; Figure 4). However, there was no significant correlation between sub-soil copper concentration and sub soil organic matter content ($p=0.454$: Figure 5).

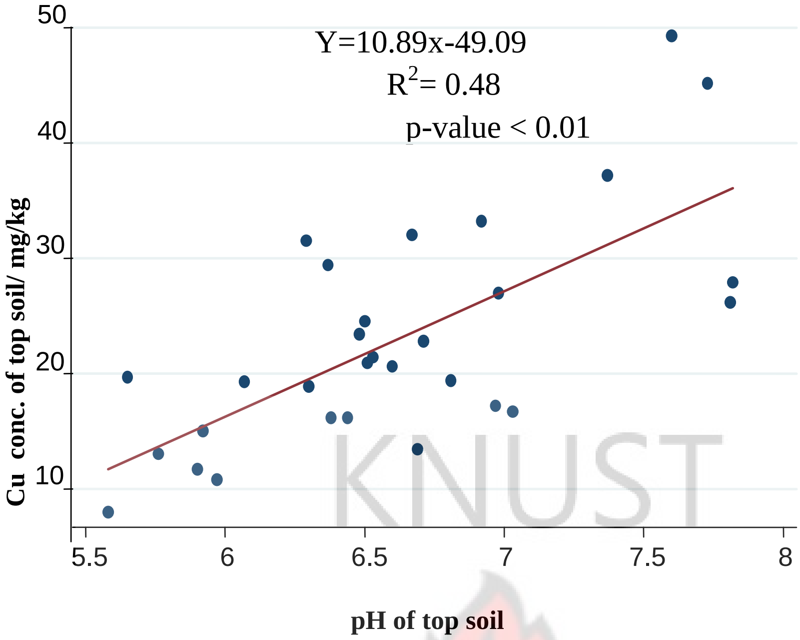

Figure 2: Copper Concentrations in the Top Soil against Corresponding Soil Ph

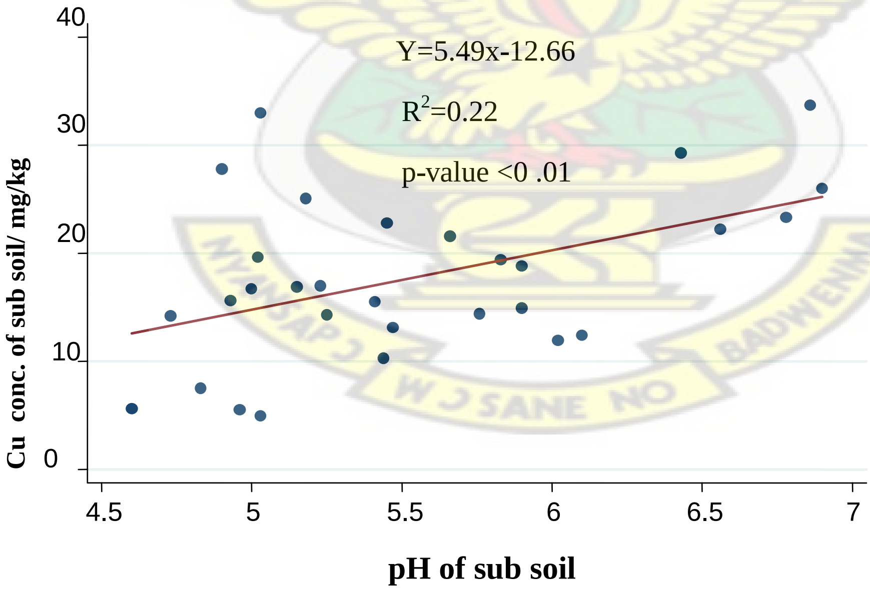

Figure 3: Copper Concentration in the Sub Soil against Corresponding Soil pH.

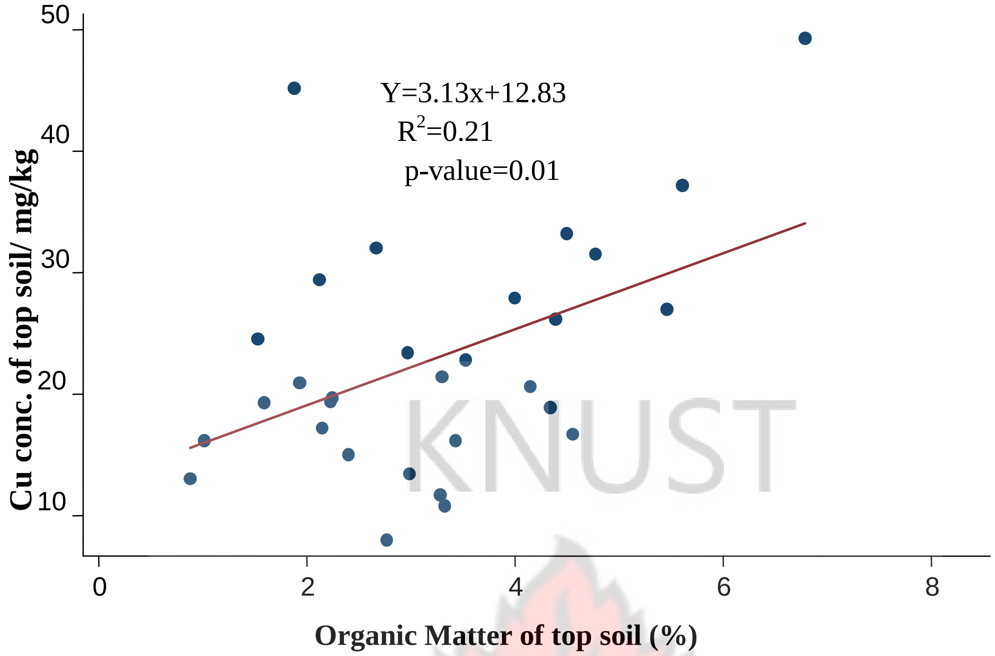

Figure 4: Relationship between copper concentration and organic matter content of the top soil

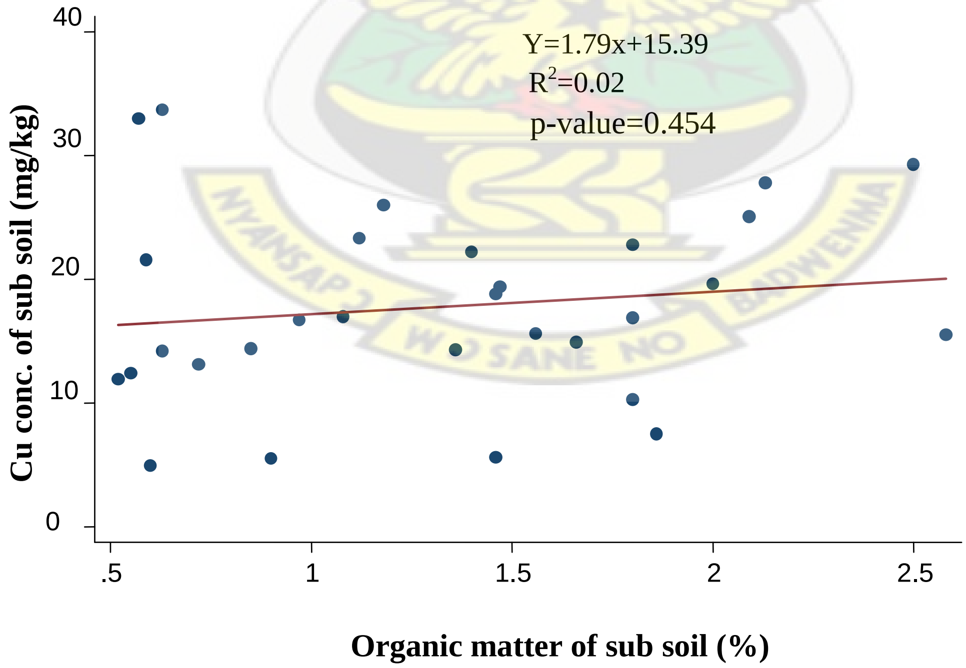

Figure 5: Relationship between copper concentration and organic matter content of the sub soil

4. 8 Concentration of Copper of Various Plants Parts

The concentration of Copper in the seed was twice higher than each of the bark and the leaf. Consequently, copper concentration in the cocoa seeds was significantly higher ($p < 0.05$) than the copper concentrations in the leaves and barks. On the contrary, the copper concentrations of the leaf and bark were not significantly different ($p > 0.05$; Table 7).

Table 7: Mean values(±SD) Copper Concentrations of Plant Parts

Plant parts	Cu Conc.(mg/kg)
Seed	32.73[a] ± 7.73
Leaf	16.10[b] ± 2.86
Bark	16.47[b] ±7. 66
p-value	<0.0001

Mean values in the columns with different letters are significantly different p>0.05

4.9 Copper Concentration of Plant Parts of the Various Sprayed Plantations at Different Age Categories

The mean copper concentrations of the various plant parts: seeds, leaves and bark from the various spraying age categories were significantly similar ($p > 0.05$) to each other.

Table 8: Mean Values(±SD) Concentration of Plant Parts: Seed, Leaf, and Bark

Spraying Age Category	Copper Conc.(mg/kg)		
	Seed	Leaf	Bark
<10 years	34.02[a]±7.65	15.91[a]±3.47	18.17[a]±7.40
10-30 years	29.73[a]±8.65	15.88[a]±3.18	18.03[a]±10.52
>30 years	34.44[a]±6.65	16.50[a]±2.00	13.20[a]±2.44
P-value	0.3323	0.8699	0.2587

Within the same column numbers followed by similar letters are not significant at p<0.05

CHAPTER 5

5.0 DISCUSSION

5.1 Concentration of Copper in the Soil Samples

Soil samples from sprayed plantations had elevated copper concentration at both depths as compared to the corresponding referenced samples from unsprayed plantations. The wide difference may be due to the continuous application of copper-bearing fungicide. This finding is in support of the work of Addo-Fordjour *et al.* (2013) who indicated that the use of copper based fungicide in agricultural sector elevates copper concentration of sprayed farm soils. The profiles of the three spraying age category (<10 years, 10-30 years and >30 Years) showed similarity with respect to their copper distributions for the specific depths (top and sub soil). This may be due to the presence of other copper retention factors in the plantation soils which were distributed equally in the three age categories other than age of spraying. This trend is at variance with the findings of Adeyeye *et al*. (2006), Aikpokpodion *et al*. (2010), Addo-Fordjour *et al*. (2013) in which the age of spraying on plantations was influential in copper accumulation of soils. The copper concentration of the top soil was on the other hand, significantly higher than the corresponding sub soil for all age category soil samples. This might have resulted from higher accumulation of humus in the top soil than the sub soil. This result is in agreement with the findings of Pakula and Kalembasa (2013) who reported that the highest copper was detected in humus horizon of Eutric Cambisol and decreases with depth. Additionally, Sanders and Bloomfield (1980) indicated that copper adsorb quite strongly to humus in the soil.

The findings of this study showed that total copper concentration of top and sub soil range from 8.0-49.3 mg/ kg and 4.9-33.7 mg/kg respectively with corresponding mean of

22.93 mg/kg and 17.78 mg/kg. This results is consistent with the range of values reported by Addo-Fordjour *et al.* (2013) on total copper concentration of soil.

5.2 Concentration of Copper in Plant Samples

Copper concentration of the seeds was twice higher than the concentrations of other plants parts (leaf and bark). This may be due to absorption of copper into the seeds through the pods during spraying, since the pods are the main target part of cocoa during spraying exercise. The result obtained was in line with the results of Adeyeye *et al.* (2006). The concentration of copper in the seeds, leaves and bark of this study ranged from 9-48 mg/kg, 10-22 mg/kg and 19-38 mg/kg respectively with corresponding mean of 32.73 mg/kg, 16.10 mg/kg and 16.47 mg/kg respectively. This was so far lower than the concentrations reported by Adeyeye *et al*. (2006) in which the concentrations of copper in seeds, leaves and bark were 104-642 mg/kg, 129-1435 mg/kg and 56-300 mg/kg respectively. The wide variation may be due to differences in physico-chemical properties of the two plantation soils and composition of chemical used in spraying. In the spraying age categories of seeds, bark and leaf, no significant difference was obtained in their copper concentrations. This implies that other factors of copper accumulation, other than age of spraying might have distributed equally in all the soils, which finally ended up in the parts of the plant. Also, translocation factor of the spraying age categories might have been similar to one another.

5.3 Relationship of Copper Concentration with Soil pH and Organic Matter

The results of the study showed a linear positive relationship between copper concentration and pH for the two soil depths of the three spraying age categories. This finding shows that soil pH is responsible for substantial retention of copper in the plantation soils. This finding is supported by the study of McLaren and Crawford (1973), in which the specific sorption of copper onto soil constituent increases as the pH rises. Also Alvarez-Puebla *et al.* (2004) buttressed this point by stating that copper (I) hydroxide causes surface precipitation of copper (II) hydroxide on illite soil under increasing soil pH. At pH between 6 and 7 copper accumulates in the surface horizon of the soil (Reuther and Smith, 1954) leading to formation of complexes with organic matter (Stevenson and Fitch, 1981) in the form of insoluble copper (II) hydroxide (Elsayed-Ali *et al*., 2009). Thus, soil pH influenced the concentrations of total copper in the studied soils. Hence, the level of copper in the studied soil can be regulated by adjusting the soil pH.

A linear positive correlation was revealed in this study, between soil copper concentrations and organic matter of the soil samples from the top soil. This implies that the concentration of copper in plantation soils depends greatly on available organic matter. The finding was in line with the results reported by Vega *et al*. (2007) who stated that organic matter supplies organic chemicals to the soil solution, which may serve as chelates and increase metal availability to plants. Adding to this point, Juang *et al*. (2008) claimed that organic carbon is responsible for a substantial retention of copper in vineyard soils. However, there was no significant correlation between sub-soil copper concentration and sub soil organic matter content. The positive dependent of copper concentration on organic matter in the top soil shows that copper concentration of plantation soils can be controlled by managing soil organic matter content.

5.5 Ecological Implications of the Study

Copper based fungicides, even though useful in the agricultural sector poses a major threat in our environment. Excess copper in soils is however phytotoxic leading to chlorosis and retarded growth (Lewis *et al*., 2001). Toselli *et al*. (2008) buttress this by establishing copper toxicity threshold at 200 mg/kg beyond which will cause a reduction of shoot growth and leaf chlorosis after 2 years of Cu exposure. Van Zwieten (2004) indicated in his studies that copper disturbs the biochemical and physiological processes such as photosynthesis, enzyme activity, pigment and protein synthesis, and cell division. Continuous accumulation of copper in the soils could affect fertility of the soil, and therefore, its ability to support plant life (Addo-Fordjour *et al*., 2013). Copper suppress rates of nitrogen fixation by the bacteria Rhizobium under some situations at relatively high copper levels of 235 ppm (Addo-Fordjour *et al*., 2013). Agrochemicals, after off-site migration will potentially enter nearby waterways and groundwater resources where they can cause adverse effects to aquatic organisms (Wightwick and Allinson, 2007). This therefore necessitates for an alternative and environmental friendly method of controlling pest and diseases.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The findings of this study indicated that the concentration of copper in the cocoa growing soils was significantly higher than the referenced unsprayed soil. This is due to repeated application of copper based fungicide to control black pod diseases on cocoa plantations. However, no significant difference was obtained in the copper concentrations in the soils of the three age categories at both depths. This might have resulted from equal distribution of some factors of copper retention in the soil other than age of spraying.

The study also revealed significance difference between copper concentration of the top soil and sub soil. This may be due to accumulation of copper in the top soil from anthropogenic source owing to factors of retention.

A linear positive correlation was obtained between copper concentrations of cocoa growing soil with physico-chemical properties (pH and organic matter) which indicated that the physico-chemical properties are influential in the accumulation of copper in the soil. Hence, the concentration of copper in plantation soils can be controlled by regulating the physico-chemical parameters of the soil.

The analysis of the results on copper concentration of plant parts revealed that the concentration of copper in the seeds was significantly higher than that of the leaves and the barks. This may be due to absorption of copper into the seeds via the pods of cocoa during spraying, since the pods are always targeted during spraying. The findings showed that the copper concentrations of soils and cocoa plants in the study area were below the contamination level. However, the continuous applications of copper based fungicide may reduce the quality of cocoa beans. Hence, an alternative and environmental friendly method of controlling pest and diseases on cocoa must be adopted.

6.2 Recommendations

Based on the findings of study, the following recommendations have been identified:

1. In future, an extensive study could be conducted to identify possible remediation to reduce or control the excessive accumulation of copper in cocoa growing soils from anthropogenic source.
2. A future research must be carried out to investigate how soil pH and organic matter content can be regulated to control the level of copper in plantations soils.
3. A periodic assessment of the cocoa beans produced in the country must be carried out to ascertain the level of copper in the beans before processing or exporting them.

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APPENDICES

Appendix A: Sample of the structured interview used.

1. What is your name?

2. How many cocoa plantations do you have?

3. Where are they located?

4. Did you cultivate it yourself or you inherited it?

5. When did you begin spraying?

6. How often do you spray your farms within a year?

7. What chemical do you use?

8. Have you enjoyed mass spraying exercise before?

9. If yes, for how long?

Appendix B

Table B.1: Respondents response on spraying ages

Pattern of spraying	Frequency	%
Twice a year	40	55.6
Once a year	25	34.7
Irregular	7	9.7
Total	72	100

Table B.2: Respondents response on spraying exercise per year

Age of spraying	Frequency	%
Below 10 years	27	37.5
10-30 years	30	41.7
Above 30 Years	15	20.8
Total	72	100

Table B 5: Correlation between soil copper concentration and pH and organic matter

1.

BELOW 10 YEARS - TOP SOIL

Cu/pH/OM	Cu	pH	OM_A
Cu	1.0000	0.7154	0.1381
pH	0.7154	1.0000	0.6353
OM	0.1381	0.6353	1.0000

2.

BELOW 10 YEARS - SUB SOIL

Cu/pH/OM	Cu	pH	OM
Cu	1.0000000	0.7677177	-0.2159735
pH	0.7677177	1.0000000	-0.4567807
OM	-0.2159735	-0.4567807	1.0000000

3.

BETWEEN 10 &30 YEARS - TOP SOIL

Cu/pH/OM	Cu	pH	OM
Cu	1.0000000	-0.4774997	-0.1812586
pH	-0.4774997	1.0000000	0.1041186
OM	-0.1812586	0.1041186	1.0000000

4.

BETWEEN 10 &30 YEARS - SUB SOIL

Cu/pH/OM	Cu	pH	OM
Cu	1.0000000	0.2631277	0.1739904
pH	0.2631277	1.0000000	0.1516301
OM	0.1739904	0.1516301	1.0000000

5.

ABOVE 10 YEARS - TOP SOIL

Cu/pH/OM	Cu	pH	OM
Cu	1.0000000	0.8685033	0.3675005
pH	0.8685033	1.0000000	0.5442136
OM	0.3675005	0.5442136	1.0000000

6.

BELOW 10 YEARS - SUB SOIL

Cu/pH/OM	Cu	pH	OM
Cu	1.0000000	0.9031652	0.8410358
pH	0.9031652	1.0000000	0.6386746
OM	0.8410358	0.6386746	1.0000000