# IMPACT OF COPPER-BASED FUNGICIDE APPLICATION ON COPPER CONTAMINATION IN COCOA SOILS AND PLANTS IN THE AHAFO ANO NORTH DISTRICT, ASHANTI REGION



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## DECLARATION AND CERTIFICATION

I hereby declare that this submission is my own work towards the Master of Science (MSc.) Degree, and that, to my best of knowledge it contains no material previously published by another person or 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.



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# ABSTRACT

The control of the blackpod disease of cocoa is mainly by the application of copperbased fungicides. However, copper fungicides used might have negative impact on soil pH, available phosphorus and organic matter and may also persist in cocoa beans which can negatively impact on the health of consumers. A study was conducted in selected cocoa farms in the Ahafo-Ano North district of the Ashanti Region to evaluate the effect of copper contained in fungicides on the soil, cocoa beans and leaves. Soil analysis showed that the amount of both extractable and total copper in the soil did not vary significantly with increasing soil depth, but varied significantly ($p<0.001$) with the age of cocoa plantation. Soils of the various cocoa plantations were contaminated with regard to Contamination Factor and Geoaccumulation index distribution pattern of total copper in the soils and ranged from 0.93 and 4.08 and 0.62 to 2.72 respectively. Cocoa beans analysis revealed that, the beans were not contaminated with copper and showed no correlation with selected soil properties. This indicates that copper in the soil did not affect cocoa beans significantly. Copper content of leaves on the other hand showed positive correlation with organic matter. The amount of soil organic matter decreased with increasing soil depth from the top to the subsoil ($p< 0.04$). Whereas available phosphorous varied significantly ($p = 0.002$) with the age of farm, pH did not.

# DEDICATION

This work is entirely dedicated to the Almighty God for the gift of life and strength given me up to this time. For you also, Mr. R.K. Asante and the Gyimah Gyamfi family for the care, moral guidance, and encouragement and spiritual support up to this time of my life.

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°C degree Celsius mm

millimeters

Kg Kilogram

L Litre g

Gram ha Hectare

cm Centimeter

mL Milliliter

% Percentage

L.S.D. Least Significant Difference ANOVA

Analysis of Variance

µL Microlitres

S.E.D. Standard Error of Difference ppm

Part per million

$H_2O$ Water

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background

Cocoa (*Theobroma cacao* L.), the main source of raw material for the chocolate industry, is the most important cash crop for most of the countries in the West and Central African sub-region (mainly, Cote d'Ivoire, Ghana, Nigeria and Cameroon). In 2005/06, the sub-region produced 2,626 metric tonnes (71.4%) of the world output of 3,674 metric tonnes (ICCO, 2007). Ghana is the world's second largest producer after La Cote d'Ivoire and the crop occupies a key position in the country's economy in terms of foreign exchange generation, domestic income, and source of revenue for the provision of socio-economic infrastructure. Cocoa cultivation employs about 60% of the national agricultural labour force (Appiah, 2004). Although, there is an increasing demand for cocoa beans (Taylor, 2000), pests and diseases pose a major challenge to its production (Bowers *et al*., 2001). *Phytophthora* pod rot (black pod) is the most prevalent disease of cocoa in Ghana (Dakwa, 1987). The disease is caused by *Phytophthora palmivora* and *Phytophthora megakarya* (Dakwa, 1987; Luterbacher and Akrofi, 1993; Opoku *et al*., 1999). The control of black pod disease is a major challenge for world cocoa cultivation. According to Tan and Tan (1990), several methods have been adopted by researchers and farmers to control *Phytophthora* pod rot of cocoa. The most common is the routine use of fungicides to supplement good farm management practices. In Ghana, all the fungicides recommended for the control of black pod disease of cocoa are copper-based (Opoku *et al*., 2007). Repetitive applications (6-9 sprays/year) of high doses of these copper-based

fungicides bring serious risks to human health and an adverse effect on the sustainability of the agro-ecosystem.

### 1.2 Aims and objectives

The main aim of the study was to determine the effects of spraying of copper based fungicides on copper residues in/on cocoa beans, leaves and in soils of cocoa plantations.

The specific objectives were to:

- determine the presence and concentration of copper in the soil of cocoa farms and forest in the Ahafo Ano district of Ghana.
- determine the presence and concentration of copper on/in cocoa beans and leaves from fungicide treated pods
- determine pH levels, organic matter content of soils in the cocoa farms.

### 1.3 Justification

The continuous application of copper in agriculture is being questioned because of human health and environmental concerns (Baker, 1990). As part of efforts to address the decline in cocoa production, the Government of Ghana through Ghana Cocoa Board has over the years instituted several programmes to control black pod disease. These included free spraying of copper fungicides in *Phytophthora megakarya* outbreak areas, selective spraying of infected farms in the famous *si anonom ano kwan prɛko* and *yɛ wo afuo yie* programmes and recently, the National Cocoa Diseases and Pest Control (CODAPEC) programme popularly known as "Mass Spraying" in which cocoa farmers in the country are assisted to combat the mirid and the black Pod disease. Under the 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 copperbased fungicides. Five hundred and twenty one thousand nine hundred and fifty six farmers were involved in the spraying exercise (Opoku *et al.*, 2006).

The Ahafo Ano North is a district of Ghana in the Ashanti Region, and the vegetation is mainly rain forest. About 85% of the working populations are farmers and it is one of the most important cocoa growing regions of Ghana. The district produces the bulk of cocoa beans from the region (AANDA, 2006). Due to the favourable environmental conditions for both cocoa and *Phytophthora spp.* that causes black pod disease of cocoa, there is high incidence of the disease in the district. This has triggered the increase in the application of fungicides to control the disease. Between 2001 and 2009, an estimated average hectarage of about 33,845.54 was sprayed against black pod disease with an estimated average of 514,604.33 sachets of copper-based fungicides (Champion WP, Nordox 75WG, Funguran OH WP and Kocide 101 WP and Ridomil Gold 66 WP) in the Ahafo Ano district in the Ashanti Region (CSSVDCU, 2010). With the introduction of cocoa "Mass Spraying" exercise nationwide the rate at which pesticides are applied on cocoa farms has increased. However, there is no information on the impact of spraying copper-based fungicides on the copper level in the soil and in cocoa leaves and beans in Ghana. There is therefore the need for a study to investigate the possible effect of copper-based fungicides on the levels of copper in soil, cocoa beans and leaves in Ghana.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Origin of the Cocoa Plant

Cocoa (*Theobroma cacao* L.) is one of 22 species that constitutes the genus *Theobroma*, a member of the family Sterculiaceae. Botanically, the term 'cacao' refers to the tree and its fruits whilst 'cocoa' describe the bulk commercial dried fermented beans, as well as the powder produced from the beans. Cacao in its natural environment occurs as an understorey plant. The centre of greatest diversity and origin of the tree is the dense tropical forests of the upper Amazon and Orinoco River basins of South America, where it occurs as a sub-canopy plant. *Theobroma cacao* is assumed to have spread naturally westward and northward to Guyana, Mexico and Central America at the time of European contact in the fifteenth century. Currently, extensive production occurs in the tropics of the old world, owing to the high disease prevalence in the Native American tropics of the cacao tree. Cacao production systems vary greatly within and between countries, and planting materials are still largely made up of unselected seedling populations (Eskes, 2004).

### 2.2 Economic Importance of Cocoa

Cocoa, the source of raw material for the chocolate industry, is the economic engine for the impoverished West and Central African sub-region (mainly Cote d'Ivoire, Ghana, and Cameroon). In 2005/06, the sub-region produced 2,626 metric tons (71.4%) of the world output of 3,674 metric tons (ICCO, 2007). Ghana is the world's second largest producer after La Cote d'Ivoire and the crop occupies a key position in the country's economy in

terms of foreign exchange generation, domestic income, and source of revenue for the provision of socio-economic infrastructure. The COCOBOD News of April 2007 described cocoa as the "cash crop whose foot print is seen in every aspect of life in Ghana." Cocoa has been the backbone of the Ghanaian economy for more than sixty years and it employs about 60% of the national agricultural labour force (Appiah, 2004).

On education, the Ghana Cocoa Board scholarship scheme is notable in all the cocoa growing areas in Ghana. From 1951 when the scholarship scheme was put in place, a number of prominent Ghanaians have benefitted from the scheme (COCOBOD News, 2007). On foreign earnings, the cocoa crop generates about $1 billion annually (COCOBOD Mini Diary, 2009) and is a major contributor to Government Revenue and GDP (COCOBOD Executive Diary, 2009). In the 1964/65 cocoa season a total of 580,000 tonnes of cocoa were produced (COCOBOD News, 2007). This was about 33% of global market share then, which made Ghana the biggest producer of cocoa in that year (COCOBOD News, 2007). The industry went into decline for almost twenty years. Production figures dropped to an all-time low of 158,956 tonnes in 1983/84. This was just about 9% of global cocoa production (COCOBOD News, 2007).

### 2.3 Major fungal disease of cocoa in Ghana

Although, there is an increasing demand for cocoa beans (Taylor, 2000), diseases and pests pose a major limitation to its production (Bowers *et al*., 2001). When left uncontrolled, losses could be extremely high. Some of the 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 (COCOBOD News, 2007; Hughes and Ollennu, 1994; Thresh and Owusu, 1986).

### 2.3.1 Black-pod Disease

Of all cocoa diseases world-wide, black pod or *Phytophthora* pod rot causes the largest loss of cocoa production. The disease can affect every part of the cocoa plant, including the stem, cushion, root and pod. However, the most important aspect is pod infection, which affect pods at all stages of development. When young pods are affected, they fail to mature, and subsequently die. When mature pods are infected two months prior to ripening, the beans inside the pod may also rot.

***Economic Impact of the disease***

In 1985, a worldwide loss of cocoa due to black pod was estimated at £1.54 billion (Evans and Prior, 1987). *Phytophthora* spp. is a serious pathogen in West Africa. Pod rot and stem canker caused cocoa pod losses of up to 63%, and the death of up to 10% of trees annually, on Kar kar Island, Papua New Guinea (Guest *et al*., 1994). Black pod disease causes between 60 -100% crop losses in Ghana (Dakwa, 1987). Pod rot disease is also of economic importance in other countries such as Indonesia, the Philippines, India, the Pacific Islands and Jamaica.

***Symptoms***

The disease begins with a circular brown lesion that enlarges to cover the whole pod, which eventually becomes black and mummified, and sometimes covered in a white mass of sporangia. Inoculation of detached pods with various isolates of *Phytophthora palmivora* showed differences in colour, outline and rate of growth of the lesion, either

discrete or confluent masses of sporangia, and varying amounts of aerial mycelium Turner (1960). Pod infection may also develop from the stalk *via* an infected flower cushion. Stem cankers are characterized by oval to round, rusty-brown discoloration of the external bark. The symptoms of collar infections are dark brown, irregular, watersoaked lesions with reddish-brown exudates; these lesions are not noticeable unless accompanied by a gummy exudate. Attack of young shoots results in die-back (Zadoks, 1997).

***Causal Agent***

The disease is caused by different *Phytophthora* spp. in different parts of the world. These include *P. capsici*, which is dominant in Central and South America and some Caribbean countries. There is also *P. citrophthora*, which has been reported on cocoa in Brazil (Campélo and Luz, 1981) and India (Chowdappa and Chandra, 1996).

*Phytophthora* spp. is typically found in tropical or warm, temperate countries with high rainfall. It is believed to have arisen in South-East Asia where much genetic diversity of the *Phytophthora* spp. occurs (Blaha *et al.*, 1994; Mchau and Coffey, 1994). The record of *Phytophthora* spp. for Equitorial Guinea is from the island of Bioko (Prior, 1985).

In Ghana, the disease is caused by *P. palmivora* and *P. megakarya* (Dakwa, 1987; Luterbacher and Akrofi, 1993; Opoku *et al*., 1999). Black pod disease of cocoa caused by *P. megakarya* in Ghana started in 1985 (Dakwa, 1985/6). Comparatively, *P. palmivora* is milder and relatively less destructive. Crop losses caused by *P.megakarya* are estimated at 60-100% compared with 4.9-19% for *P. palmivora* (Dakwa, 1987).

***Control of Black pod Disease***

The control of black pod disease is a major challenge for world cocoa cultivation. According to Tan and Tan (1990), several methods have been adopted by researchers and farmers to control diseases caused by *Phytophthora* spp. in cocoa of which the most common is the use of fungicides coupled with good farm management practices. Fungicides are known in the art as either chemical or biological agents used to mitigate, inhibit or destroy fungi (Martinez *et al.*, 2006).

As part of efforts to address the decline in cocoa production due to black pod disease, the Government of Ghana through Ghana Cocoa Board initiated a National Cocoa Diseases and Pest Control (CODAPEC) programme, popularly known as "Mass Spraying" to assist cocoa farmers in the country to combat the mirid and the black pod disease in 2001. 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). On these farms, there are at least four routine sprays per farm per year. During fungicide application, pods are sprayed till runoff leading to a large volume of the spray ending up on the plantation floor.

**2.4 Heavy metal pollution**

Heavy metals are common trace constituents in the earth crust that have densities above 5 g/cm$^3$. The most frequently reported heavy metals with potential hazards in soils are cadmium, chromium, lead, and zinc and copper (Alloway, 1995). The concentration of these toxic elements in soils may increase from various sources including anthropogenic

pollution, weathering of natural high background rocks and metal deposits (Senesi *et al.*, 1999). Most heavy-metal contamination stems from high-temperature combustion sources, such as coal-fired power plants and solid waste incinerators. Local metal sources may include metal-plating industries and other metal industries. The use of leaded gasoline has led to global lead pollution even in the most pristine environments, from arctic ice fields to alpine glaciers. The primary anthropogenic sources of heavy metals are point sources such as mines, foundries, smelters, and coal-burning power plants, as well as diffuse sources such as combustion by-products and vehicle emissions. Humans also affect the natural geological and biological redistribution of heavy metals by altering the chemical form of heavy metals released to the environment. Such alterations often affect a heavy metal's toxicity by allowing it to bio-accumulate in plants and animals, bio-concentrate in the food chain, or attack specific organs of the body (http://www.osha-slc.gov/SLTC/metalsheavy).

Heavy metals are not biodegradable and hence persist in the environment (MacFarlene and Burchett, 2001). Plants show several response patterns in heavy metals uptake (Kabata-Pendias and Pendias, 1997). While most are sensitive to very low concentrations others have developed resistance, and there is a small number of plants that are hyperaccumulators of toxic metals (Chapin, 1983; Mingorance *et al.*, 2007).

### 2.4.1 Effect of heavy metals on the environment

Pollution of natural environment by heavy metals is a worldwide problem as these metals are indestructible and most of them have toxic effects on living organisms (Dalman *et al.*, 2006). Heavy metals are of high ecological significance since they are not removed from

the soil via self-purification, but rather accumulate in reservoirs and enter the food chain (Loska and Wiechula, 2003). From the environmental point of view, heavy metals are largely immobile in the soil system, explaining why they tend to accumulate and persist in agricultural soils for a long time. The concentration and distribution of heavy metals in the soil often differ from metal to metal, probably as a result of their differential accumulation rates. For instance, a study conducted on Nigerian agricultural soils, revealed that heavy metal contamination by lead, zinc and cadmium was minimal whereas copper contamination was very high (Aikpokpodion *et al*., 2010). According to Toselli *et al.*, (2009), accumulation of copper in Italian soils is due to repeated application of fungicides to control fungal diseases of pear and grapes. A study conducted by Savithri *et al*. (2003) in India also revealed that high levels of Bordeaux mixture application has resulted in significant accumulation of copper in surface and subsurface soils. These further show that horticultural operations with long history of copper fungicide application often have significant accumulation of copper in surface horizons (Merry *et al.*, 1986; Alva *et al*., 2000). There is increased awareness that heavy metals present in soil may have negative consequences on human health and on the environment (Abrahams, 2002; Selinus *et al*., 2005). This situation is gradually taking cocoa soils in the studied area to a condition of deterioration because of the contamination load and the adverse effect on soil biodiversity (Aikpokpodion *et al.*, 2010).

### 2.5 Copper as Element

Copper (Cu) occurs naturally in most soils and in fruits and vegetables. Both humans and animals need some copper in their diet. In humans, it helps in the production of blood haemoglobin (Bonham *et al*., 2002). Copper is a pliable, malleable metal, having a bright

reddish metallic lustre and is an excellent conductor of both electricity and heat. Copper occurs naturally in a wide range of mineral deposits. It is used in making textiles, marine paints, electrical conductors and wires, plumbing fixtures and pipes, as well as coins and cooking utensils (Chambers and Chambers, 1884).



### 2.5 1 Sources of copper

Copper is a common trace constituent in the earth crust. Its concentration in the ambient environment has increased dramatically since the Industrial Revolution, as have lead and copper since Roman times through human activities. However, the major source of copper in agricultural soils is through the continuous application of copper-based fungicides to control diseases of crops. This situation is gradually taking cocoa soils to a condition of deterioration because of the contamination load and the adverse effect on soil biodiversity (Aikpokpodion *et al.*, 2010).

### 2.5.2 Copper as fungicides

Although copper is required as a micronutrient, it is a broad-spectrum biocide at higher concentrations (Fleming 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). Formulations of inorganic Cu, most commonly as copper hydroxide and copper sulphate, are used as agricultural pesticides to control fungi, bacteria, and in some instances, invertebrates and algae. As a result, water insoluble copper compounds are used as fungicides (Martinez *et al.,* 2006). Copper hydroxide is more water soluble at low pH (high acidity) and it is applied in spray solution such as water at a pH above 6 to

avoid phytotoxicity (Gant *et. al.*, 2007). Concentrations that are reported as toxic vary; critical factors include the organism, whether acute or chronic toxicity was determined, the extraction method, and soil characteristics such as pH and organic matter and clay content. Phytotoxic effect of copper has been known since the 19th century from spraying of Bordeaux mixture in French vineyards (Diiszel, 1991). More than 100 years ago, it was discovered in Bordeaux, France, that the downy mildew disease of grapes could be controlled by copper. Bacteria, fungi, and mollusks are generally the most sensitive to Cu compared with flowering plants and vertebrate animals (Domsch, 1989; Giller *et al.*, 1998).

Following absorption into the fungus or bacterium, the copper ions link to various chemical groups (imidazoles, phosphates, sulfhydryls, and hydroxyls) present in many proteins and disrupt the function of these proteins. Thus, the mode-of-action of copper hydroxide (or any other copper fungicide) is the nonspecific denaturation (disruption) of cellular proteins. The toxic copper ion is absorbed by the germinating fungal spore and thus for best results copper must be reapplied as plants grow to maintain coverage and prevent disease establishment (Agrios, 2005).

### 2.5.3 Copper fungicides for the control of black pod

The recommended fungicides for the control of black pod disease in Ghana are all copper-based (Opoku *et al.*, 2007). These include Ridomil Plus 66 WP (12% MetalaxylM and 60% Copper (i) oxide), Champion WP (77% copper hydroxide and 23% inert ingredient: 50% metallic copper equivalent), Nordox 75WG (86% Cuprous oxide and 14% inert ingredients: 75% metallic copper equivalent), Funguran OH WP (77% copper hydroxide

and 23% inert ingredient: 50% metallic copper equivalent), Kocide 101 WP (77% copper hydroxide and 23% inert ingredient: 50% metallic copper equivalent) and Metalm 72 WP (12% Metalaxyl-M and 60% Copper (i) oxide).



### 2.5.4 Groups of copper fungicides

Copper fungicides for the control of black pod disease of cocoa in Ghana can be grouped into two, based on their mode of action (i) contacts fungicides or protectants and (ii) systemic fungicides.

***Contact fungicides or protectants***

Contact fungicides or protectants when applied are not absorbed into the plant, but act on the surface to prevent infection or germination of the infective propagules of the pathogen. Thus, protectant fungicides have to be applied to the pod surface before the arrival of the pathogen or its propagules. Most of the copper-based fungicides used to control black pod disease of cocoa in Ghana are protectants. These include FunguranOH, Kocide 101 and Champion, which contain copper as copper hydroxide and Nordox
75, which contain copper as coprous oxide. The use of contact fungicides do not result in the development of pathogen strains resistant to the fungicides. This is because they affect several vital processes of the pathogen and many gene changes would be necessary to produce a resistant strain (Agrios, 2005).

***Systemic fungicides***

Systemic fungicides are absorbed through the foliage or roots and are translocated within the plant through the xylem. Systemic fungicides generally move upward in the transpiration stream and may accumulate at the leaf margins. A few of them, e.g., fosetyl-

Al, also move downward. These fungicides are not re-exported to new growth. Some of them become translocated systemically when sprayed on herbaceous plants, but most are only locally systemic within the sprayed leaves. Many systemics are effective when applied as seed treatments, root dips, in-furrow treatments or soil drenches, and in trees when injected into the trunks. Almost all systemic fungicides are site specific, inhibiting only one or perhaps a few specific steps in the metabolism of the fungi they control. As a result, many target fungi through simple mutation become resistant to each frequently used systemic fungicide within a few years of introduction of the compound. For this reason, various strategies have been developed for preserving the usefulness of such chemicals. To avoid abandonment of a systemic fungicide after appearance of a pathogen strain resistant to it, the fungicide must be used in combination with another broad-spectrum contact fungicide under various schemes of application (Agrios, 2005).

Systemic fungicides for the control of *Phytophthora* pod rot of cocoa in Ghana include Metalm and Ridomil Plus. Both fungicides can also be grouped as Acylalanine and have been formulated to include both metalaxyl and copper as the active ingredients to reduce the possibility of development of resistant strains of the pathogens (Agrios, 2005).

Metalaxyl is one of the best systemic fungicides against oomycetes. It is widely used as a soil or seed treatment for the control of *Pythium* and *Phytophthora* seed rot and damping-off and as soil treatment for the control of *Phytophthora* stem rots and cankers in annuals and perennials and of certain downy mildews (e.g., of tobacco). It is also effective as a curative treatment if it has to be applied after infection has begun. Metalaxyl is quite water soluble and is translocated readily from roots to the aerial parts of most plants, but its lateral translocation is slight.

### 2.5.5 The fate of copper in copper-based fungicides

After foliar application of copper fungicides, a gradual redistribution of copper deposits by the weathering effect (rainfall and dew) may occur. Some of the copper are taken up by plant cells, while most redistribution occurs in downward direction and ultimately end up in litter and soils (Mabbett, 1984). This in turn redistributes itself within the soil profiles. However, there is no evidence of copper accumulation at depth below about 25 cm of the soil profile which might be due to copper's strong affinity for organic matter, thus tending to dominate its interaction with surface soils, litter and vegetation (Renan, 1994). Some of the difference in exchangeable Cu between cocoa and forest can be attributed to pH differences, as Cu is more available at higher pH (Sauve *et al.*, 1997).

Available copper in soils is held mainly as a cation ($Cu^{2+}$) on surfaces of clay minerals or in association with organic matter. Copper present as an impurity in silicate minerals or carbonates is largely unavailable. Organic matter and soil pH are the predominant factors influencing copper availability. Copper availability decreases as organic matter in the soil increases. Organic matter binds copper more tightly than any other micronutrient. This does not only reduce fixation by soil mineral and leaching, but also reduce availability to crops. Increasing the soil pH by liming increases the amount of copper held by clay and organic matter, thereby decreasing copper availability to plants (Schulte and Kelling, 1999). McGrath *et al*. (1988) found that total Cu content was more dependent on the organic matter status, as soil organic matter forms complex with copper to prevent it from leaching. The proportion of copper present in soil solution as $Cu^{2+}$ increased and as pH decreased. In soil, Cu is restricted mainly to the top layer because of its ability to tightly

bind with carbonates, clay minerals, hydrous oxides of Al, Fe, Mn and organic matter (Mengel and Kirkby, 2001). Copper mobility along the soil profile, bioavailability for root uptake and consequently phytotoxicity threshold for crops depend on soil pH (Chaignon *et al.*, 2003), cation exchange capacity (CEC), quality of organic matter, soil texture etc. (Brun *et al*., 2001; Parat *et al*., 2002). Copper is always present at a background level, but can be of concern in situations of heavy agronomic use of copper compounds. Agricultural soils are reported to have average background levels of 20-30 ppm (Baker, 1990), with average overall US level found to be 15.5 ppm (Holmgren, 1993). Some vineyard soils in Europe, which have seen intensive use of copper sulphate containing Bordeaux mixtures for 100 years, have soil Cu concentrations ranging from 100 - 1500 ppm (Besnard *et al.*, 2001).

### 2.5.6 Effect of copper on nutrient availability

Nutrients are needed by plants to produce at maximum capability and to perform specific functions within the plant. 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. Savithri *et al*. (2003) found that as the copper content in the soils of grape farms increased as a result of continuous application of Bordeaux mixture, the amount of micronutrient such as zinc, manganese and iron decreased. Similarly, the available phosphorus contents of the soils decreased with fungicide application at both surface and subsurface layers. This might be due to increasing base saturation of the soils with lime-containing fungicide residues, probably encouraging fixation or immobilization of available phosphorus (Caudhuri, 1964; William, 1951).

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.

### 2.5.7 Effect of copper on soil fauna

The build-up of copper is more in the surface soil (0 – 15 cm) (Savithri *et al.*, 2003; Alva *et al*., 2000). Detrimental effects of elevated copper concentration on mycorrhizal associations (Georgieva *et al.*, 2002), microbial population and functions (Dumestre *et al.*, 1999) have been documented. It has been suggested by Potter *et al*. 1990 that to circumvent toxic effects, earth worms may avoid surface litter and soil layers contaminated by certain pesticides. Earthworm aids decomposition and incorporation of organic matter, increase water soluble aggregates, improve water infiltration, aeration, drainage, root penetration, and increase microbial activity in soil (Baker *et al*., 1994; Yeardley, 1996). Earthworm casts and burrow walls exhibit higher concentrations of total and plant-available nutrients than surrounding soil and it has been recognized that surface feeding species horizontally and vertically transport microorganisms, spores, pollen and seeds (Makeschin, 1997) and can reduce plant pathogens through digestion of fungal spores (Hirts *et al*., 1955). In some orchards, most animal life in soil, including large earthworms, has been eliminated by the extensive use of copper containing fungicides (Extoxnet, 1996; Norgrove, 2007). Long term use of Cu fungicides might have negative impacts on soil fauna and other non-target organisms. In temperate zones, it has been demonstrated that earthworms are more susceptible to heavy metals (such as Cu) than most other groups of soil invertebrates (Bengtsson and Rundgren, 1992; Reinecke *et al*., 1997), exhibiting chronic toxic responses at Cu concentrations of less than 16 mg/kg (Helling *et al.*, 2000). Cu was shown to be the primary factor influencing earthworms in

fruit plantation soils in temperate Australia (Van Zwieten *et al*., 2004). Earthworms are highly mobile in soil and hence their ability to avoid areas of contamination would have significant ecological implications (Yeardley *et al*., 1996). Akinnifesi *et al*. (2006) reported that increase in organic matter content with fungicide treatment is apparently due to the fungicide causing a decrease in the microbial population of the soil, consequently decreasing the degree of decomposition of the organic matter.

Copper is a relatively non-specific bactericide and fungicide and can kill naturally occurring microorganisms on leaves as well as those that have been applied as biocontrols including *Bacillus spp*., *Trichoderma spp*. and others. Copper has been found to suppress rates of nitrogen fixation by the bacteria *Rhizobium* under some situations, at relatively high copper levels of 235 ppm (OMRI, 2001).

### 2.5.8 Effects of copper 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 (LDLo) is 1000 mg/kg in pigeons and 600 mg/kg in ducks. The oral LD50 for Bordeaux mixture in young mallards is 2000 mg/kg (Extoxnet, 1996).

### 2.5.9 Effects of copper 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 sulphate. 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 LC50 of copper sulphate to pond snails is 0.39 mg/L at 20 C. Higher concentrations of the material cause some behavioural changes, such as secretion of mucous, and discharge of eggs and embryos (Extoxnet, 1996).

### 2.5.10 Effect of copper on human health

Long-term exposure to copper can cause irritation of the nose, mouth and eyes and it causes headaches, stomachaches, dizziness, vomiting and diarrhoea. Intentionally, high uptakes of copper may cause liver and kidney damage and even death (www.lenntech.com/periodic/elements/cu.htm). Wilson's disease is an inherited genetic disorder characterized by the body's inability to properly excrete copper, leading to accumulation of copper in the tissues which can cause liver disease and mental retardation (www.copperinfo.co.uk/health). Copper react with other elements to form compounds such as copper sulphate that is harmful to human. 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). Copper hydroxide is less acutely toxic, with an oral LD50 in rats of 833 mg/kg. It is also not readily absorbed through the skin, with a dermal LD50 of over 5000 mg/kg in rats (Nufarm Americas Inc., 2004). Absorption of copper sulphate into the blood occurs primarily under the acidic conditions of the stomach. After ingestion, more than 99% of copper is excreted in the faeces. However, residual copper is an essential trace element that is strongly bio-accumulated. Vineyard sprayers experienced liver disease after 3 to 15 years of exposure to copper sulphate solution in Bordeaux mixture. Long-term effects are more likely in individuals with Wilson's disease, a condition that causes excessive absorption and storage of copper. Chronic exposure to low levels of copper can lead to anaemia. The growth of rats was retarded when given dietary doses of 25 mg/kg/day of copper sulphate. Dietary doses of 200 mg/kg/day caused starvation and death. Copper sulphate has been shown to cause reproductive effects in test animals. Testicular atrophy increased in birds as they were fed larger amounts of copper sulphate. Sperm production was also interrupted to varying degrees. Reproduction and fertility was affected in pregnant rats given this material on day 3 of pregnancy. Copper sulphate at 10 mg/kg/day caused endocrine tumours in chickens given the material outside of the gastrointestinal tract through an intravenous or intramuscular injection. Long-term animal studies indicate that the testes and endocrine glands have been affected Heart disease occurred in the surviving offspring of pregnant hamsters given intravenous copper salts on day 8 of gestation (Extoxnet, 1996).

# CHAPTER THREE

## 3.0 METHODOLOGY

The study was broadly classified in two forms: Field survey and land laboratory analysis.

## 3.1 Study Site

### *3.1.1 Geographic area*

The study was carried out in the Ahafo Ano North district in the Ashanti region. The Ahafo Ano North District is located in the wet equatorial zone in the north-western part of the Ashanti Region, between latitude 6˚ 47'N and 7 02'N and longitude 2˚ 26'W and 2˚ 04'W. It is bounded to the south by Atwima District, to the east by Ahafo Ano South District, to the north by Tano South District and west, by Asutifi District (EPA, 2002).

### *3.1.2 Relief and Drainage*

The district is situated on an arm of the Southern Voltaian Plateau. The topography is undulating and rises up to about 500 meters above sea level. The soil is of deepweathered, well-drained and suitable for the cultivation of tree crops such as cocoa (EPA, 2002).



### *3.1.3 Climate*

The area experiences the wet semi-equatorial type of climate with a mean annual temperature of about 26 °C. The area is marked by double rainfall regime, with the major one in May to July and the minor in August to September. Mean rainfall is between 1250 mm and 1750 mm per annum. The dry season begins in October through to early part of March (EPA, 2002).

### *3.1.4 Vegetation*

The Ahafo Ano North district is found in the high forest zone of the country. The type of vegetation found is the moist semi-deciduous forest. Due to vibrant cocoa industry in the area, coupled with intensive activities of timber merchants, the original forest has almost been replaced by secondary forest. However, in most parts, vegetation similar to the interior wooded savanna has taken over, especially around Tepa, notably TepaAnyinasuso, Tepa-Akwasiase. The district is found in the agro-ecological zone of the country where the livelihood of the people depends on agriculture as the main income generating activity for a number of households. (EPA, 2002).

### 3.2 Field survey

The simplicity of administration and the fact that it takes relatively less of the respondent's time were considered in choosing questionnaires as a collection instrument to cocoa farmers in the villages and towns to determine the number of cocoa farms with history of copper fungicide application. Interviews were conducted in an informal manner and respondents expressed themselves freely, irrespective of their literate background. The questionnaire was made of both open and closed ended questions. It was pre-tested with six cocoa farmers in Tepa, a farming community in Ahafo Ano district of the Ashanti Region of Ghana to determine the clarity of the questions. Some of the questions were modified based on answers obtained during the pre-testing.

#### *3.2.1 Fieldwork and administration of questionnaires*

The sampling method used was a simple random sampling technique. To ensure representative sampling, the district was divided into five communities. Cocoa farmers were randomly sampled in towns and villages within the selected communities in Ahafo Ano North cocoa district. A sample size of 101 cocoa farmers were selected and interviewed from the districts. A total of 120 questionnaires were sent out into the field in case of any replacements. At the end of the survey period, a total of 101 questionnaires were successfully administered and collected.

#### *3.2.2 Sampling for chemical analysis*

Information gathered from the interviews were used to group the farms into three different age categories; farms less than 10 years, farms between 10 and 30 years and farms more than 30 years old. Due to the high amount of rainfall in the study area the incidence of

black pod disease is very high. Consequently, all farmers apply fungicides in order to protect their yield and maximise profit. Due to this, there were no available cocoa farms without fungicide spraying which could be used as control of the study. Some secondary forests close to the farms were selected to act as control. All the questionnaires were numbered and assigned codes and random selection was done based on the age category. A total of 15 farms were selected (5 farms for each category). Both soil and plant samples were collected from these farms except for the control where only soil was sampled.

### *3.2.3 Soil sampling*

Two composite samples were collected per field for the 15 plantations, giving a total of 30 soil samples. The factors considered were slope, drainage and erosion. In each plantation two quadrats of 80 x 80 metres were marked. In each quadrat, 15 cores were made and composited as one sample. Labelling was done by assigning unique names and numbers to each demarcated area as well as sampling bags. A soil auger was used for sampling. The auger was calibrated from zero to twenty-five centimetres. Sampling was done at 0-5cm and 5-15cm depths. For each depth, cores were mixed thoroughly in a clean plastic bowl and a sub sample of 500 g was taken and labelled. All soil samples were sent to the laboratory at the Soil Research Institute of the Council for Scientific and Industrial Research (CSIR), Kwadaso, Kumasi for analysis.

### *3.2.4 Sampling of leaves and beans*

For each quadrat of the fifteen plantations, ten cocoa trees were randomly selected and 50 fresh and matured cocoa leaves taken and a composite of each was bagged in a labelled envelope. Ten cocoa pods were taken randomly on the same ten cocoa trees and kept in

labelled bags. Both leaves and pods were sent to the laboratory at Soil Research Institute of CSIR, Kwadaso in Kumasi.

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Plate 1: Coring of soil in a cocoa plantation with an auger at Achina, Tepa.

Plate 2: Sampled soil in an auger.

Plate 3: Soil samples in sampling bowls.

Plate 4: Labelling and storing of soil samples.

### 3.3 Laboratory analysis

Soil samples were air dried ground and passed through a 2 mm sieve. The pods were separated into husk and beans. The beans and leaves were washed with distilled water and air dried to constant weight and ground.



#### *3.3.1 Chemical analysis of soil sample*

The soil samples were analyzed for pH (1:1), total copper, extractable copper, organic matter percentage and available phosphorus.

#### *Soil pH*

Soil pH was measured with a pH meter with a glass electrode using a 1:1 soil: water ratio (Mclean, 1982). The pH meter was calibrated using standard buffer solutions (pH 4.01 and pH 7.01). Twenty five grams of each soil sample was weighed into a beaker. Twenty five millilitres of distilled water was added and the solution stirred vigorously for 15 seconds. This was left to stand for 30 minutes and intermittent stirred for 10 minutes. The electrodes of the pH meter were placed in the slurry, swirled carefully, and the pH read and recorded.

#### *Organic matter determination*

Organic carbon was determined by the wet combustion method (Walkey and Black, 1934). One gramme of soil sample was weighed out into a 500 ml Erlenmeyer flask and exactly 10 ml of 1.0 N Potassium dichromate ($K_2Cr_2O_7$) solution added from a burette (Potassium dichromate oxidizes Carbon in the organic matter, itself being reduced in the process). This was followed by the addition of 20 ml conc. $H_2SO_4$ to generate heat to facilitate the

reaction between C and $Cr_2O_7$. The mixture was swirled for one minute to ensure that the solution was in contact with all the particles of the soil. The flask and the content were allowed to cool on an asbestos sheet for 30 minutes. Two hundred millilitres of distilled water was added, followed by 10 ml of orthophosphoric acid (to sharpen the colour change at the end point of titration). One milliliter of diphenylamine indicator was added and the solution titrated with 1.0M normal ferrous sulphate solution until the colour changed to blue, and then finally to a green end-point. The titre value was recorded and the blank solution corrected (≥ 10.5).

Organic carbon was calculated using the formula below;

$$\%\ organic\ C\ in\ soil = \frac{([m.e.K_2Cr_2O_7 - m.e.FeSO_4] \times 0.003 \times f \times 100)}{weight\ of\ soil}$$

where

m.e. = milli equivalent = normality of solution × ml of solution used

0.003 = m.e. weight of C f = correction factor = 1.33

% Organic matter was calculated using the formula;

% organic matter = % organic C × 1.724

***Available Phosphorous***

Available Phosphorous was determined by extraction with Bray $P_1$ (Bray and Kurtz, 1945). Five grams of soil was weighed into a 100 ml shaking bottle. 35 ml of extracting solution was added to the sample, and shaken at 200 rpm for 10 minutes at room temperature of 27°C. The sample was filtered into a receiving flask using Whatman filter paper No. 42. Five millilitres

of the filtrate was pipetted into 50 ml test tube and 10 ml of the colour reagent (Aluminium molybdate, bismuth of carbonate and sulphuric acid) added to it. A pinch of ascorbic acid was added to the sample and left for 15 minutes for blue colour to fully develop. The absorbances of the samples were then read on the spectrophotometer at a wavelength of 660 nm after calibrating the spectrophotometer with standard solutions.

Available Phosphorous was calculated using the formula below:

$$\text{Available Phosphorous}(mg/kg) = 7 \times \left(\frac{\text{Absorbance(Abs)}}{0.0878}\right)$$

Absorbance (Abs) is the reading on spectrophotometer, the constant 7 is Dilution factor and 0.0878 as the equation on graph.

### 3.3.1.1 Total and extractable copper determination

***Digestion of Soil Samples for Total Copper Content***

Two grams of each soil sample was placed in a beaker and the copper content extracted by adding 15 ml of 50 % $HNO_3$ and placed on a hot plate with a watch glass cover, heated at 95 $^oC$ for 15 minutes. The heating was later continued with partial covering without boiling till the solution got reduced to about 5 ml, and then cooled. Two millilitres of distilled water and 3 ml of 30 % $H_2O_2$ were then added and heated gently to start the peroxide reaction. This was followed by the addition of 5 ml concentrated HCl and 10 ml distilled water, and refluxed again for 15 minutes without boiling. After cooling, the solution was filtered and the filtrate quantitatively transferred into a 50 ml volumetric flask

and topped up with distilled water (U.S. EPA, 1986). A blank sample was also treated in the same way. Each of them was filtered using a Whatman filter paper (Cat No 1001 110).

***Digestion of soil sample for extractable Copper content***

Ten grams of soil sample was weighed into shaking bottle. Thirty millilitres of ammonium acetate and ethylenediamine tetraacetic acid (EDTA) added to the sample and then shaken for two hours on a reciprocating shaker after which the samples were removed and filtered into a receiving flask using Whatman filter paper No.42. Five millilitres of the filtrate was pipetted into a test tube and then ten millilitres of lanthanum chloride ($LaCl_3$) solution added. The concentration of the extractable copper was determined using the Atomic Absorption Spectrophotometer (AAS) Buck Scientific, Model 210 VGP (Motsara and Roy, 2008).

Extractable Copper was calculated as:

$$Extractable\ Copper\left(\frac{mg}{kg}\right) = AAS\ reading \times Extraction\ factor$$

***Analysis of total and extractable copper content***

Filtrates obtained after the digestion were analyzed for total and extractable Cu using atomic absorption spectroscopy (Buck Scientific AAS, Model 210 VGP). Calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. The digested solutions were aspirated individually and atomized in an airacetylene flame. All samples were run in triplicates and average in mg/kg values taken for each determination (Motsara and Roy, 2008).

***Ashing and Digestion of sampled cocoa leaves and beans for analysis***

A composite sample of dried leaves and beans were milled and homogenised. Half gram of the sample was then weighed into crucibles and placed in a muffled furnace at a temperature of 450$^{o}$C for a period of 3 hours. The ash samples were then removed after cooling. An ash solution was prepared using 10 ml of 1:2 $HNO_3$ solutions which had been heated on a hot plate at 100 °C for 15 minutes to destroy easily oxidizable materials and carbonates. This was filtered using a Whatman filter paper (Cat No 1001 110) into a receiving flask of 100 ml volume. The filtrate was then topped up to the 10 ml mark with distilled water. The concentration of copper was determined using the AAS after calibrating with the standard solution. All samples were run in triplicates and average in mg/kg values taken for each determination (Motsara and Roy, 2008).

## 3.4 Determination of contamination levels of the soil

### *3.4.1 Index of geoaccumulation ($I_{geo}$) of soil*

Index of geoaccumulation ($I_{geo}$) was used to evaluate the degree of metal (copper) contamination in the soil for both the top and subsoil (Müller, 1979; Singh *et al.*, 1997).

$I_{geo}$ is expressed as:

$$I_{geo} = \log_2 \frac{Cn}{1.5\ Bn}$$

Where:

$I_{geo}$ is the geoaccumulation index. C is the measured concentration of the element n in the n soil and B is the geochemical background value of n element in the background or control within the study area. The constant 1.5 is a factor which allows analysis of natural fluctuations in the content of a given substance in the environment and very small anthropogenic influence. Müller (1979) differentiated six classes of the geoaccumulation index as indicated in Table 1.

**Table 1: The classes of geoaccumulation index specifying the different levels of contamination**

| $I_{geo}$ Value | Designation of soil quality |
|---|---|
| $I_{geo} = 0$ | Practically uncontaminated |
| $0 < I_{geo} < 1$ | Uncontaminated to moderately contaminated |
| $1 < I_{geo} < 2$ | Moderately contaminated |
| $2 < I_{geo} < 3$ | Moderately to heavily contaminated |
| $3 < I_{geo} < 4$ | Heavily contaminated |
| $4 < I_{geo} < 5$ | Heavily to extremely contaminated |

### *3.4.2 Contamination Factor (CF)*

Anthropogenic Contamination Factor (CF) is a quantification of the degree of the contamination as a single-metal index. The measure is relative to either average crustal composition of the respective metal or to a measured background from uncontaminated area. The CF is estimated as:

$$CF = \frac{Cm}{Bm}$$

Cm is the measured concentration in soil; Bm is background (adjacent forest) concentration value of copper metal. Hakanson (1980) recognized four descriptive classes for degree of contamination (Table 2).

**Table 2: Contamination factor levels and their degree of contamination interpretation**

| Contamination Factor (CF) index | Degree of Contamination |
|---|---|
| CF < 1 | Low contamination |
| 1 ≤ CF ≥ 3 | Moderate contamination |
| 3≤ CF ≥6 | Considerable contamination |
| CF > 6 | Very high contamination |

### 3.5 Statistical analyses

Concentrations of soil properties were compared between topsoil and subsoil with the ttest analysis. Furthermore, concentrations of extractable and total copper were compared between topsoil and subsoil using the t-test analysis. Copper concentrations (extractable and total copper) in cocoa soils were compared with those of forest soils using the t-test analysis. Analysis of variance (ANOVA) was conducted to compare extractable and total copper contents between the different aged plantations. The ANOVA was run separately for extractable copper in the topsoil and subsoil, and for total copper in the same soil depths.

Correlation analysis was conducted to determine the relationships between the following pairs of variables: (1) soil properties and soil copper content (extractable and total copper), (2) soil properties and plant copper content (leaf and beans), (3) soil copper content (extractable and total copper) and plant copper content (leaf and beans), and (4) bean copper and leaf copper contents. Correlation analyses were conducted separately for

extractable and total copper in topsoil and subsoil. All analyses were conducted with the MINITAB 15 software at a significance level of 5 %.



# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Referenced Forest soil and cocoa farm soils at different depths

Analysis of variance showed that from Table 3, there was a high significant difference between the cocoa farm soils and the reference forest soils at the depth of 0-5 cm and 515 cm in relation to the total and extractable copper. The significant difference between the forest and cocoa farms were very high at (p=0.000).

Table 3: Mean values of forest and cocoa farm soils at different depths

| **Habitat** | **Total Copper** | | **Extractable Copper** | | **0-5cm** | **5-15cm** |
|---|---|---|---|---|---|---|
| | **0-5cm** | **5-15cm** | | | | |
| **Forest** | 6.47a | 5.97a | 3.98a | 3.52a | | |

| | | | | |
|---|---|---|---|---|
| **Cocoa Farm** | 17.53b | 13.51b | 10.65b | 8.05b |
| **P-value** | 0.000 | 0.000 | 0.000 | 0.000 |

*Within the same column numbers followed by different letters are significant at LSD (0.05)*

### 4.2 Selected topsoil and subsoil properties

Mean values of soil properties and copper content for the two depths are presented in Table 4. The amount of extractable copper in the soil decreased with increasing depth. However, analysis of variance showed no significant differences in the amount of extractable copper at 0-5 and 5-15 cm soil depths. There were no significant differences in total copper, available phosphorus and soil pH for the two soil depths; levels of these parameters however showed a decreasing trend with soil depth. There was a significant ($p = 0.04$) difference in organic matter content at the different soil depths. Organic matter at 0 – 5 cm was significantly higher than the amount at depth 5 – 15 cm.

Table 4: Mean values of soil properties and copper content for the two depths

| **Depth** | **pH 1:1 $H_2O$** | **Org. Matter (%)** | **Avail. P ($mgkg^{-1}$)** | **Extractable Cu ($mgkg^{-1}$)** | **Total Cu ($mgkg^{-1}$)** |
|---|---|---|---|---|---|
| **0-5 cm** | 6.4a | 3.7 a | 8.7a | 10.65a | 17.52a |
| **5 – 15 cm** | 6.2a | 1.9 b | 5.4a | 8.05a | 13.51a |
| **p-value** | 0.37 | 0.04 | 0.15 | 0.22 | 0.053 |

*Within the same column numbers followed by similar letters are not significant at LSD (0.05)*

### 4.3 Effect of age of Plantation and Extractable copper (0-5cm)

Soils under plantations less than 10 years old had the lowest level of extractable copper, whilst plantations aged between 10 and 30 years had the highest level (Table 5). Soil copper content of plantations less than 10 years old was significantly lower than that of plantations aged between 10 and 30 years and above 30 years ($p < 0.001$). Extractable

copper content of plantations at ages between 10 and 30 years was similar to that of plantations above 30 years.

Table 5: Mean copper content in the topsoil of different aged plantations

| **Age of plantation (years)** | **Extractable copper (mg/kg)** |
|---|---|
| **< 10** | 4.88 b |
| **10-30** | 14.02 a |
| **>30** | 13.06 a |

*Within the same column numbers followed by similar letters are not significant at LSD (0.05)*

**4.4 Effect of age of Plantation and Extractable copper (5-15cm)**

The status of subsoil extractable copper from the different plantations is shown in Table 6. Analysis of variance showed significant differences (p= 0.005) in the levels of extractable copper at the different plantations. The amount of extractable copper in plantations aged between 10 and 30 years was significantly higher than farms less than 10 years, but similar with plantations above 30 years.

Table 6: Mean copper content in the subsoil of the different plantations

| **Age of plantation (years)** | **Extractable copper (mg/kg)** |
|---|---|
| **< 10** | 3.98 b |
| **10 – 30** | 11.48 a |
| **>30** | 8.69 a |

*Within the same column numbers followed by similar letters are not significant at LSD (0.05)*

### 4.5 Soil properties under the various plantations

Table 7 shows selected soil properties for the various cocoa plantations. Organic matter content and soil pH of the soils were generally similar. Available phosphorus in soils from plantations less than 10 years was not significantly different from plantations aged between 10 and 30 years, but significantly higher ($p = 0.002$) than plantations above 30 years.

Analysis of variance revealed significant difference in the amount of extractable copper in the various plantations. Plantations less than 10 years old had mean extractable copper that was significantly lower than that of plantations aged between 10 - 30 years and above 30 years ($p = 0.003$). However, there was no significant difference between 10 - 30 years and above 30 years old plantations ($p = 0.464$). There was a significant difference in the amount of total copper between the various plantations. Total copper content from farms less than 10 years was significantly lower than that of farms between 10 and 30 years and farms above 30 years ($p = 0.020$). There was no significant difference in total copper content at farms between 10 and 30 years and farms 30 years ($p = 0.207$). Farms less than 10 years had 35% of its total amount of copper to be extractable, whilst farms between 10 and 30 years and farms with ages above 30 years had 73.5% and 63.37% respectively.

Table 7: Mean values of soil properties and copper content under the different cocoa plantations

| **Age of Plantation** | **pH** | **Org. Matter (%)** | **Avail. P ($mgkg^{-1}$)** | **Extractable Cu ($mgkg^{-1}$)** | **Total** |
|---|---|---|---|---|---|
| **> 10 yrs** | 6.4a | 2.4a | 9.2b | 4.2 a | 12.0a *(35.0 %)* |
| **10-30 yrs** | 6.1a | 2.6a | 8.7b | 12.8 b | 17.4b *(73.5% )* |
| **>30 yrs** | 6.4a | 3.4a | 3.3a | 10.9 b | 17.2b *(63.4 %)* |

*Within the same column numbers followed by similar letters are not significant at LSD (0.05).Percentage of total copper that is extractable in parenthesis*.

### 4.6 Copper content of leaves and cocoa beans

Analysis of variance showed significant differences in the levels of copper content in cocoa leaves (Table 8). The level of copper in leaves from farms between 10 and 30 years was significantly higher than farms less than 10 years and farms above 30 years of age ($p = 0.001$). However, no significant difference was observed between farms less than 10 years and above 30 years. Copper content of the cocoa beans did not differ significantly with respect to the age of the plantation ($p = 0.227$).

Table 8: Copper content in leaf and bean of cocoa

| **Age of Plantation** | **Total Cu ($mgkg^{-1}$)** | |
|---|---|---|
| | **Leaf** | **Bean** |
| **<10 yrs** | 21.8 b | 33.0a |
| **10-30 yrs** | 87.1 a | 35.6a |
| **>30 yrs** | 38.8 b | 33.5a |

*Within the same column numbers followed by similar letters are not significant at LSD (0.05)*

### 4.7 Relationship between soil properties and total copper content in cocoa beans and leaves

Table 9 shows correlation coefficients relating topsoil properties and total copper content in cocoa leaves and beans. Soil pH, organic matter content, available phosphorus and

extractable copper did not correlate significantly with total copper in cocoa beans and leaves.



Table 9: Correlation coefficient relating selected soil properties at 0-5cm and total copper in leaf and bean of cocoa

| Soil properties | Total Copper($mgkg^{-1}$) Leaf | Bean |
|---|---|---|
| **Soil pH** | 0.075 (0.96) | 0.051 (0.76) |
| **Organic Matter** | 0.016 (0.79) | 0.089 (0.86) |
| **Available phosphorus** | -0.383 (0.16) | -0.180 (0.53) |
| **Extractable copper** | 0.071 (0.81) | 0.063 (0.82) |
| **Total copper** | 0.142 (0.62) | 0.183 (0.52) |

*The values in the bracket are p-value*

Table 10: Correlation coefficient relating selected soil properties at 5-15 cm and total copper in leaf and bean

| Soil properties | Leaf | Bean |
|---|---|---|
| **Soil pH** | -0.104 (0.72) | 0.352 (0.62) |
| **Organic Matter** | 0.540 (0.04) | 0.144 (0.20) |
| **Available phosphorus** | -0.223 (0.43) | -0.073 (0.80) |
| **Extractable copper** | 0.775 (0.0004) | 0.277 (0.32) |
| **Total copper** | 0.528 (0.04) | 0.213 (0.44) |

*The values in the bracket are p-values*

Correlation coefficients relating selected subsoil soil properties and copper in leaves and beans of cocoa are presented in Table 10. Soil pH and available P showed no significant correlation with copper in cocoa leaves. None of the soil properties showed significant correlation with copper in cocoa bean. Organic matter and total copper in the soil correlated significantly and positively ($p = 0.04$) with leaf copper. Extractable copper showed very strong correlation ($p = 0.0004$) with copper in cocoa leaves.

Correlation analysis between total amount of copper and amount of extractable copper in the soil at depth of 0 – 5 cm (topsoil) showed strong positive correlation ($p = 0.001$; $r = 0.568$). Very high correlation coefficient was obtained between the amount of total copper and the amount of extractable copper in the soil at a depth of 5-15 cm (subsoil) ($p = 0.000$; $r = 0.689$). Correlation analysis between the total amount of copper and amount of extractable copper, irrespective of depth, indicated positive correlation ($r = 0.510$, $P = 0.004$) between the two variables Table 11.

Table 11: Correlation coefficient relating total copper to extractable copper

| **Soil depth (cm)** | | **Extractable copper** |
|---|---|---|
| **0 – 5** | **Total copper** | 0.568 (0.001) |
| **5 – 15** | **Total copper** | 0.689 (0.000) |

*The values in the bracket are p-values*

Correlation matrix for all sources of copper is shown in Table 12. Extractable copper very strongly ($p<0.0001$) correlated with total copper and with leaf copper ($p= 0.03$).

Copper in cocoa leaves significantly correlated ($p= 0.02$) with copper in cocoa bean.

Table 12: Correlation matrix for all the sources of copper

| | **Extractable copper** | **Total copper** | **Leaf copper** | **Bean copper** |
|---|---|---|---|---|
| **Extractable copper** | - | 0.653 (<0.0001) | 0.406 (0.03) | 0.164 (0.31) |
| **Total copper** | | - | 0.314 (0.09) | 0.189 (0.32) |
| **Leaf copper** | | | - | 0.435 (0.02) |
| **Bean copper** | | | | - |

### 4.8 Copper contamination levels of soils in the various cocoa plantations

The results of total copper contamination factor and geoaccumulation index in the soils from the various cocoa plantations are summarized in Table13. Geoaccumulation index ($I_{geo}$) of total copper in the topsoil ranged from 0.62 to 2.72 with a mean value of 1.81, while that of the subsoil ranged from 0.28 to 2.37 with a mean value of 1.00. Total copper contamination factor level at the topsoil was between 0.93 and 4.08 with a mean value of 2.71, while that of the subsoil ranged from 0.42 to 3.56 with a mean value of 1.80.

Table 13: Mean total copper concentration (±SD), contamination factor (CF) and geoaccumulation index ($I_{geo}$) of copper at different depths in the cocoa farms

| Cocoa | 0-5 cm | | | 5-15 cm | | |
|---|---|---|---|---|---|---|
| **Farms** | **Mean Conc. ± SD** | **CF** | **$I_{geo}$** | **Mean Conc. ± SD** | **CF** | **$I_{geo}$** |
| 1 | 14.80±0.100 | 2.29 | 1.52 | 4.140±0.010 | 0.69 | 0.46 |
| 2 | 19.20±0.100 | 2.97 | 1.98 | 5.880 ± 0.030 | 0.98 | 0.66 |
| 3 | 25.20±0.200 | 3.89 | 2.50 | 19.32 ± 0.000 | 3.24 | 2.16 |
| 4 | 14.00±0.380 | 2.16 | 1.44 | 18.24 ± 0.000 | 3.06 | 2.04 |
| 5 | 20.40±0.200 | 3.15 | 2.10 | 14.46 ± 0.010 | 2.42 | 1.61 6 |
| | 25.20±0.200 | 3.89 | 2.50 | 12.125±0.005 | 2.03 | 1.35 7 |
| | 14.80±0.900 | 2.29 | 1.52 | 5.520 ± 0.000 | 0.92 | 0.62 |
| 8 | 18.80±0.100 | 2.91 | 1.94 | 8.820 ± 0.030 | 1.48 | 0.98 |
| 9 | 15.60±0.100 | 2.41 | 1.61 | 3.900 ± 0.000 | 0.65 | 0.44 |
| 10 | 6.00± 0.020 | 0.93 | 0.62 | 3.720 ± 0.060 | 0.62 | 0.42 11 |
| | 13.60±0.300 | 2.10 | 1.40 | 9.060 ± 0.440 | 1.52 | 1.01 |
| 12 | 16.40±0.300 | 2.53 | 1.69 | 2.700 ± 0.080 | 0.45 | 0.30 |
| 13 | 26.40±0.580 | 4.08 | 2.72 | 21.24 ± 0.030 | 3.56 | 2.37 |
| 14 | 13.20±0.000 | 2.04 | 1.36 | 2.920 ± 0.020 | 0.49 | 0.33 |
| 15 | 19.20±0.000 | 2.97 | 1.98 | 2.520 ± 0.010 | 0.42 | 0.28 |
| **MEAN** | | **2.71** | **1.81** | | **1.50** | **1.00** |
| **MINIMUM** | | **0.93** | **0.62** | | **0.42** | **0.28** |
| **MAXIMUM** | | **4.08** | **2.72** | | **3.56** | **2.37** |

The results of extractable copper contamination factor and geoaccumulation index in the soils from the various cocoa plantations are summarized in Table 14. Geoaccumulation index ($I_{geo}$) of extractable copper in the topsoil range from 0.51 to 4.02 with a mean value of 2.02, while that of the subsoil range from 0.48 to 4.02 with a mean value of 1.60.

Extractable copper contamination factor ranged from 0.68 to 5.34 with a mean value of 2.68, while that of the subsoil range from 0.72 to 5.49 with a mean value of 2.29.



Table 14: Mean extractable copper concentration (±SD), contamination factor (CF) and geoaccumulation index ($I_{geo}$) of copper at different soil depths in the cocoa farms

| **Cocoa Farms** | **0-5 cm** | | | **5-15 cm** | | |
|---|---|---|---|---|---|---|
| | **Mean Conc. ± SD** | **CF** | **$I_{geo}$** | **Mean Conc. ± SD** | **CF** | **$I_{geo}$** |
| 1 | 13.32±0.000 | 3.35 | 2.52 | 4.140±0.010 | 1.18 | 0.78 |
| 2 | 18.84±0.010 | 4.73 | 3.57 | 5.880±0.030 | 1.67 | 1.11 |
| 3 | 7.800±0.100 | 1.96 | 1.48 | 19.32±0.000 | 5.49 | 3.66 |
| 4 | 8.880±0.010 | 2.23 | 1.68 | 18.24±0.000 | 5.18 | 3.45 |
| 5 | 9.600±0.100 | 2.41 | 1.82 | 14.46±0.010 | 4.11 | 2.74 |
| 6 | 16.98±0.000 | 4.27 | 3.22 | 12.13±0.005 | 3.44 | 2.20 |
| 7 | 13.98±0.000 | 3.51 | 2.65 | 5.520±0.000 | 1.57 | 1.05 |
| 8 | 15.30±0.300 | 3.84 | 2.80 | 8.820±0.030 | 2.51 | 1.67 |
| 9 | 4.500±0.100 | 1.13 | 0.85 | 3.900±0.000 | 1.11 | 0.74 |
| 10 | 5.160±0.020 | 1.20 | 0.98 | 3.720±0.060 | 1.06 | 0.70 |
| 11 | 9.060±0.440 | 2.28 | 1.72 | 9.060±0.440 | 1.65 | 1.72 |
| 12 | 2.700±0.080 | 0.68 | 0.51 | 2.700±0.080 | 1.01 | 0.51 |
| 13 | 21.24±0.030 | 5.34 | 4.02 | 21.24±0.030 | 2.70 | 4.02 |
| 14 | 2.960±0.030 | 0.74 | 0.56 | 2.920±0.020 | 0.83 | 0.55 |
| 15 | 9.420±0.010 | 2.37 | 1.78 | 2.520±0.010 | 0.72 | 0.48 |
| **MEAN** | | **2.68** | **2.02** | | **2.29** | **1.60** |
| **MINIMUM** | | **0.68** | **0.51** | | **0.72** | **0.48** |
| **MAXIMUM** | | **5.34** | **4.02** | **5.49** | **4.02** | |

# CHAPTER FIVE

## 5.0 DISCUSSION

Concentrations of total and extractable copper in the soil at both depths were significantly higher in the cocoa plantations compared to the reference forests. This difference could be attributed to cocoa spraying which introduced copper into the soil. The distribution pattern of copper in the soil according to CF and $I_{geo}$ which suggests that the cocoa plantation soil is contaminated, gives credence to the above finding. Thus, cocoa spraying activity in the plantations has impacted significantly on copper content in the soil. This finding is supported by a similar study conducted by Aikpokpodion *et al*. (2010) in which cocoa plantations which were sprayed with copper-based fungicides became contaminated with copper.

A comparison of the current study with other similar studies revealed that copper concentrations in the cocoa beans and soils recorded in this study were lower. The Cu concentration in cocoa beans and leaves in this study ranged from 27-38.8 mg/kg and 17-104.5 mg/kg respectively compared to the findings of Adeyeye *et al*. (2006) in which Cu concentration of beans and leaves ranged from 56-300 mg/kg and 122-1444 mg/kg respectively. The difference could be partly due to differences in the rate of application and concentration of fungicides solution in the two study areas. The two indicators of contamination (CF and $I_{geo}$) revealed that soils in the cocoa plantations ranged from marginally to heavily contaminated. This result is consistent with a similar study which was conducted in Nigeria (Aikpokopodin *et al*. 2010). The findings of these two studies

indicate that the use of copper-based fungicides in cocoa farms impact greatly on copper levels in the soil.

Some of the soil properties related significantly with extractable and total copper contents in the subsoil but not in the top soil. This may be an indication that soil properties influenced copper concentrations in the subsoil more than in the topsoil. Soil copper (both extractable and total) was significantly lower in the young cocoa plantations (< 10 years) compared to the older ones (10-30 and > 30 years). For instance, in the < 10 year old category, mean extractable copper was 4.2 $mgkg^{-1}$ which was about one-third that of the 10-30 year old category (12.8 $mgkg^{-1}$). This pattern is an indication of copper accumulation in the soil with time. Thus, the age of cocoa plantation was a factor that contributing to copper contamination of soils in the cocoa plantations. A similar trend was obtained for total copper concentration under different aged plantations (Lepp *et al*. 1984; Aikpokpodion *et al*., 2010).

Copper becomes more available to plants at higher pH (Sauve *et al.*, 1997). Soil pH of most of the plantations was either neutral or close to neutrality, indicating that copper was probably readily available to cocoa plants in the study area. Foliar copper concentration related significantly with copper content in the subsoil, indicating that concentration of copper in the leaves might have been a function of plant uptake in the subsoil. Besides total and extractable copper content in the soil, organic matter which determines copper solubility, and thus availability to plants was important in determining copper levels in the leaves. This finding is supported by the work of Ginocchio *et al*. (2002). On the other hand, copper concentration in the cocoa beans did not correlate with soil and leaf copper contents, demonstrating that soil and leaf might not have been major sources of copper in

the beans. Copper residues deposited on cocoa pods might have been absorbed into the beans. Alternatively, copper residues deposited on the bark of cocoa plant might have been absorbed and translocated to the beans (Hughes *et al.*, 1980). In this regard, direct spraying on the plant played an important role in the distribution of copper in the beans.

**Implication of the study for conservation**

Contamination of soils in cocoa plantations presents a serious environmental concern in view of the negative effects it could have on the ecosystem. Excess copper in the soil may cause iron chlorosis in plants (Alva and Graham, 1991) affecting productivity and biodiversity. In addition, it has the potential of suppressing the rates of nitrogen fixation by *Rhizobium* thereby reducing soil fertility. Copper contamination of soil may also limit soil biological diversity. Other works have shown that copper have diversified influence on microorganisms (Aikpokpodion, 2010). Thus, continuous accumulation of copper in the soils could affect fertility of the soil, and therefore, its ability to support plant life. Furthermore, runoff from the soil could introduce copper into nearby water bodies, thus contaminating them.

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATIONS

### 6.1 CONCLUSION

The results of the study revealed that the amount of copper in the soil of cocoa plantations were higher than that of the forest resulting from repeated application of copper-based fungicides in cocoa plantations to control blackpod disease of cocoa. This has caused the soils in cocoa plantations to be contaminated. However, the findings revealed that the cocoa beans were not contaminated by copper. Comparison of the current study with studies from other countries indicated that the amount of copper in cocoa beans was far lower. The smaller values obtained indicate that the cocoa product from the beans in the current study can be consumed without adverse health effect. The comparison also showed the copper contained in the soil of the assessed plantations was far lower. The amount of copper contained in the soil of the various cocoa plantations resulting from application of copper-based fungicides increased with increase in the age of the plantations.

The amount of organic matter in the soil of cocoa plantations varied with soil depth although this trend appears not to have influenced copper (both total and extractable) distribution in the soil. The amount of copper in leaves increased with increase in the application of copper-based fungicides.

### 6.2 RECOMMENDATIONS

On the basis of the findings of this study, it is recommended that:

1. A similar study should be conducted in other areas of the country where organic cocoa farms (farms which have not experienced fungicide application) are available. This would make it possible for copper concentrations in cocoa beans and leaves to be compared between fungicides treated farms and non-fungicide treated farms.

2. A study should be conducted to determine the amount of copper that leach or washed from the soil to ground water and streams.

3. An alternative form of fungicide devoid of copper should be explored for the control of the black pod disease.

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