KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

ACCUMULATION OF COPPER FROM APPLICATION OF COPPERBASED FUNGICIDES AND ITS RELATIONSHIP WITH SOIL CHEMICAL PROPERTIES AND MICROBIAL BIOMASS IN BIBIANI-ANHWIASO-BEKWAI DISTRICT OF GHANA

BY

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A thesis submitted to the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology in partial fulfillment for the degree of

MASTER OF SCIENCE

IN ENVIRONMENTAL SCIENCE

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DECLARATION

I hereby declare that this submission is my own work towards the MSc. 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.

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Head of Dept. Name	Signature	Date

DEDICATION

This thesis is dedicated to my wife, Mrs. Stella Serwaa.

ACKNOWLEDGEMENT

I would first and foremost thank the Almighty God for blessing, protecting and strengthening me through my period of study. May his name be exalted, honored and glorified.

I express my sincere gratitude to my supervisor Dr. Osei Akoto for his time, continuous support, patience, motivation and immense knowledge towards the writing of this thesis. His matured experience and knowledge guided me all the time in the research and writing of this thesis. My special appreciation also goes to Mr. Richard

Tawiah of Ejisuman Senior High for his generous support and numerous contributions. Obviously, I owe a debt of appreciation to the staff of the Soil Research Institute, of the Council for Scientific and Industrial Research (CSIR), Kwadaso, Kumasi for the laboratory work.

ABSTRACT

Black pod disease is a major cause of yield loss in cocoa production worldwide and the disease is mostly controlled by copper-based fungicides. However, prolong usage of these fungicides might have negative impact on soil fauna and other soil chemical properties. Ghana Cocoa Board continues to assist farmers to spray their cocoa farms with copper-based fungicides. This study was conducted in selected cocoa farms and uncultivated forests close to these farms which have never been sprayed with fungicides as reference. The study was to investigate the extent of accumulation of copper from the application of copper-based fungicides and its relationship with soil chemical properties and microbial biomass in three communities namely Akaasu, Kyeikrom and Tuntum in Bibiani-Anhwiaso-Bekwai District. Soil samples were collected at two distinct depths, 0-15 cm and 15-30 cm. The fresh soil samples were divided into two. One was immediately sieved through 4 mm mesh and stored at 4 ^{0}C for the soil microbial test. The other half was air dried, sieved through a 2 mm mesh and stored in plastic bags for the chemical analysis. Results showed that Tuntum cocoa plantation recorded the highest concentration of total copper (286.54 mgkg^{-1}) while Akaasu cocoa plantation recorded the least total copper concentration of (215.63 mgkg^{-1}). Extractable and total copper vary significantly ($P < 0.05$) in both top and sub soils of the cocoa plantations from their respective reference values. However, soil pH, nitrogen and organic matter from soils of the cocoa plantations did not vary significantly ($P > 0.05$) from their reference soils. Results from correlation analysis revealed that extractable and total copper in both the top and sub soils correlated negatively with the levels of organic matter. The relationship between extractable and total copper with microbial biomass were also negatively correlated but not significant ($P > 0.05$) from the regression analysis in all the study locations.

The findings have also shown that the concentrations of copper in the soils of cocoa plantations have not reached their critical levels.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Black pod disease is a major cause of yield loss in cocoa production worldwide and about 450,000 tonnes losses per year have been estimated (Bowers *et al.*, 2001). *Phytophthora palmivora* and *Phytophthora megakarya* are the most prevalent fungal diseases of cocoa in Ghana and are differentially spread in all the six cocoa-growing regions (Western, Brong Ahafo, Eastern, Ashanti, Central, and Volta) of the country (Opoku *et al.,* 1999). The spread and level of infection by black pod disease in these regions are influenced by the climatic conditions (Opoku *et al.,* 1999).

Several methods have been used by researchers and farmers, according to Tan and Tan (1990) to control *Phytophthora* spp. that causes black pod disease of cocoa. The most common is the use fungicides to supplement good farm management practices. Chemical biocide to control fungal diseases and insect plant pest has helped to increase crop yield and food production in conventional agricultural practices (Lee,
1985).

In Ghana, copper-based fungicides are recommended for controlling the black pod disease (Opoku *et al*., 2007). These fungicides include Ridomil Gold plus, Nordox, Funguran OH, Kocide 2000, Sidalco defender, Fungikill and Champion. Prolong usage of copper-based fungicides may however, have negative effects on soil microorganisms, health of humans, animals and non-target organisms (Bengtsson and
Rundgren 1992; Reinecke *et al*., 1997).

1.2 Problem Statement

Ghana Cocoa Board continue to assist farmers to spray their cocoa farms with copperbased fungicides but since the inception of the Cocoa Disease and Pests Control

Programme (CODAPEC), no studies have been done in Bibiani-Anhwiaso-Bekwai District to investigate the accumulation of copper from copper-based fungicides and its relationship with soil chemical properties and soil microbial biomass. High levels of copper when found in soils may be toxic to plants and soil microorganisms and can lead to lower biological activity and eventually loss of soil fertility, hence the need for this study.

1.3 Justification

The government of Ghana through Ghana Cocoa board has over the past thirteen years been assisting cocoa farmers in the country to spray their farms against capsids and black pod disease in a programme called Cocoa Diseases and Pest Control (CODAPEC) popularly known as "Cocoa Mass spraying" (Opoku *et al.,* 2006). Since the inception of the programme in 2001 to 2013, estimated farmlands of about 97,600 hectares have been sprayed against black pod disease with an estimated average of 88,467 kg of copper-based fungicides yearly in Bibiani-Anhwiaso-Bekwai District (CSSVD CU, 2013) unpublished.

Many small-scale cocoa farmers in Bibiani-Anhwiaso-Bekwai District rarely use fungicides but have benefited from the government free cocoa mass spraying programme over the past thirteen years. Prolong usage of copper-based fungicides may however, have negative impact on soil microorganisms, health of humans, animals and non-target organisms (Bengtsson and Rundgren 1992; Reinecke *et al*., 1997).

Decomposition of litters and dead roots also depend highly upon soil faunal and microbial mediated in nutrient cycling which helps to improve soil fertility (Norgrove *et al.,* 1998). There is therefore the need for a study to investigate the accumulation of

copper from the long application of copper-based fungicides and assess its effects on soil micro-organisms and soil chemical properties within the study area.

1.4 Objectives

The main objective of the study was to determine the accumulation of copper (Cu) from application of copper-based fungicides and its relationship with soil chemical properties and microbial biomass in soils of cocoa plantations.

The specific objectives were to:

- Determine the accumulation of copper from application of copper-based fungicides in soils of cocoa plantations.
- Derermine the relationship between copper contamination from application of copper-based fungicides and soil microbial biomass.
- Determine the relationship between copper contaminations from application copper-based fungicides and soil chemical properties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diseases of Cocoa in Ghana

Diseases and pests pose major challenges to cocoa production Bowers *et al*. (2001). There is an increasing demand for cocoa beans on the world market but diseases and pests could limit its supply to consumers (Taylor, 2000). Cocoa swollen shoot virus disease, black pod, capsid which damage the trees and crops, stem canker, pink disease and thread blight are some of the diseases and pests that affect cocoa in Ghana (Hughes

and Ollennu, 1994; Thresh and Owusu, 1986). However, Blackpod disease is the major disease of cocoa in Ghana.

2.2 Black-Pod Disease

Black pod disease is a major cause of yield loss word wide in cocoa production and is caused by *Phytophthora* fungi. Every part of the cocoa plant can be affected by the disease, including the stem, cushion, root and pod. 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 (Hughes and Ollennu, 1994; Thresh and Owusu, 1986).

2.2.1 Economic Impact of the Disease

It was estimated that, the loss of cocoa due to black pod worldwide was £1.54 billion in 1985 (Evans and Prior, 1987). In West Africa, *Phytophthora* spp. is also a serious pathogen. Losses up to 63% in pod rot and stem canker up to 10% death of trees annually have been reported in Karkar Island, and Papua New Guinea (Prior, 1985). Black pod disease causes between 60 -100% crop losses in severely affected areas in Ghana (Dakwa, 1987). According to Guest *et al*., (1994), the Philippines, the Pacific Islands, India, Jamaica and Indonesia are countries where pod rot disease is also of economic importance.

2.2.2 Symptoms

A small brown spot which normally starts 2-3 days after infection and enlarges to cover the whole pod. It finally becomes black within 7-14 days, and sometimes covered in a white mass of sporangia. When various isolates of *Phytophthora palmivora* are

inoculated with detached pod it shows 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). Affected pods husks would remain hard and firm. When the flower cushion is infected, pod infection may also develop from the stalk (Opoku *et al*., 2007)

Oval to round and rusty-brown discoloration of the external bark are characteristics of stem cankers. The infections of the collar are characterized by irregular, dark brown, water-soaked lesions with reddish-brown exudates; and these lesions must be accompanied by a gummy exudate before they can be observed. Die-back may results if the young shoots are attacked (Zadoks, 1997)

2.2.3 Causal Agent

The disease is caused by different *Phytophthora* spp. in different parts of the world. In Central and South America and some Caribbean countries *P. capsici*, is the most dominant. Also in Brazil *P. citrophthora*, has been reported on cocoa (Campélo and Luz, 1981). Chowdappa and Chandra (1996) have also reported *P. citrophthora* on cocoa in India. In the tropical or warm and temperate countries where rainfall is high *Phytophthora* spp. is the most common. *Phytophthora* spp. is believed to have arisen in South-East Asia where much genetic diversity occurs (Blaha *et al*., 1994; Mchau and Coffey, 1994). Equatorial Guinea has also recorded *Phytophthora* spp. from the island of Bioko (Prior, 1985).

P. Palmivora and *P. megakarya* are the most prevalent in Ghana (Dakwa, 1987; Luterbacher and Akrofi, 1993; Opoku *et al*., 1999). According to Dakwa (1985/6) *P. megakarya* was observed on cocoa in 1985. *P. palmivora* is milder and relatively less

destructive compared to *P. megakarya*. Crop losses caused by *P .megakarya* are estimated at 60-100% compared with 4.9-19% for *P. palmivora* (Dakwa, 1987).

2.2.4 Control of Black Pod Disease

Several methods have been used by researchers and farmers, according to Tan and Tan (1990), to control the fungal that causes black pod of cocoa. The most common is the use of fungicides coupled with good farm management practices. Fungicides are either chemical or biological agents used to prevent, inhibit or destroy fungi from establishing and spreading (Martinez *et al*., 2006).

To help minimize or reduce yield losses in cocoa production due to black pod disease, the Government of Ghana through Ghana Cocoa Board started a programme called Cocoa Diseases and Pest Control (CODAPEC) to assist cocoa farmers in the country to control capsid and black pod disease. An average of about 659,000 hectares made up of 543,279 farms was sprayed against black pod disease with copper-based fungicides between 2001 and 2004, (Opoku *et al*., 2006) There is at least four routine sprays per year on these farms. Pods surfaces are coated with fungicide, till run-off leading to a large volume of the spray ending up on the plantation floor.

2.3 Fungicide usage in Agriculture

The use of chemical biocide to control fungal diseases and insect plant pest has helped to increase crop yield and food production in conventional agricultural practices (Lee, 1985). According to Merry *et al*. (1986) copper fungicides sprays have been used for over 100 years in food, ornamental, orchards, vineyards and vegetable crops production. Copper fungicides by their action are able to release small quantities of copper (Cu^{2+}) ions when dissolved in water, even though they can be described as insoluble compounds. (Noyce *et al*., 2006; Mehtar *et al*., 2008).

Bordeaux mixture (copper sulphate and calcium hydroxide), copper oxychloride, copper hydroxide and cuprous oxide are examples of copper-based fungicides. According to (Martinez *et al.,* 2006) they are mainly used to protect crops from fungal diseases outbreaks and to control spread of airborne spores since they are highly efficient in controlling fungal spores than established mycelia.

2.4 Mode of Action of Copper-Based Fungicides

2.4.1 Contact fungicides or protectants

When contact fungicides or protectants are applied they are not absorbed into the plant, but act on the surface to prevent infection or germination of the pathogen. According to Agrios, (2005) protectant fungicides have to be applied to the pod surface before the arrival of the pathogen or its propagules. In Ghana, most of the copper-based fungicides used to fight black pod disease of cocoa are protectants. These include Kocide 2000, Funguran-OH, and Champion, which contain copper as copper hydroxide and Nordox 75, which contain copper as cuprous oxide (Agrios, 2005). 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).

2.4.2 Systemic fungicides

When systemic fungicides are applied, they are absorbed through the leaves or roots and are translocated through the xylem. Systemic fungicides may accumulate at the leaf margins due to the upward movement in the transpiration stream but some of them e.g., fosetyl-Al, also move downward. These fungicides however are not reexported to new growth (Neumann and Jacob, 1995). When some of them are sprayed on herbaceous

plants they become translocated systemically but within the sprayed leaves most are only locally systemic. When applied in-furrow treatments or in soil drenches, or as seed treatments, and in root dips, as well as in trees injected into the trunks, many of the systemics becomes very effective. Almost all systemic fungicides are site specific; inhibiting only one or perhaps a few specific steps in the metabolism of the fungi they control (Neumann and Jacob, 1995). 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. Therefore, various strategies have been developed for preserving the usefulness of such chemicals (Dekker, 1995)

Metalm and Ridomil Gold Plus are examples of systemic fungicides approved for the prevention of *Phytophthora pod* rot of cocoa in Ghana. 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 (Dekker, 1995).

Metalaxyl is one of the best systemic fungicides against oomycetes (Schwinn and Staub, 1995). 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 (Schwinn and Staub, 1995). 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 slow (Neumann and Jacob, 1995).

2.5 Copper Accumulation in Soils

According to Merry *et al.* (1986) and Alva *et al*. (2000), significant accumulations of Cu have been recorded in surface soils with prolong use of copper fungicide application through horticultural and viticulture operations. Addo-Fordjour *et al*. (2013) have found a significant accumulation of copper in soils of cocoa plantations as a result of continues applications of copper-based fungicides to control black pod disease of cocoa. The strong interaction of copper with soil organic matter and hydrous oxides means that copper is likely to build-up in top soils for a long time (Alva *et al*., 2000) According to Georgieva *et al*. (2002), in the soil profile where there is greatest biological activity copper accumulation occurs.

Continuous applications of fungicides to prevent fungal diseases of grapes and pears have been noticed to have resulted in build-up of copper in Italian soils (Toselli *et al.,* 2009). High application of Bordeaux mixture has resulted in significant build-up of copper in surface and subsurface soils and this was revealed in a study conducted by Savithri *et al*. (2003) in India.

2.6 Impact of Copper on Soil fauna

All organisms require copper as an essential element and so deficiency may result in reduction in biological activity and possible death. However, high levels of copper when found in soils may be toxic to plants and soil microorganisms and can lead to lower biological activity and loss of soil fertility (Dumestre *et al*., 1999).

Soil microorganisms have been impacted negatively by copper residues in avocado orchards (Merrington *et al.*, 2002). Reduction in microbial biomass and increase in respiration and metabolic quotient have been noticed in copper contaminated residues,

showing that microorganisms are stressed (Merrington *et al.*, 2002). High levels of copper have been shown to impact on beneficial mycorrhizal associations (Georgieva *et al.*, 2002) as well as reduction in microbial activity and functions (Dumestre *et al.,* 1999).

According to Thrupp (1991), significant adverse effects of copper residues on fertile agricultural soils are known to range between 20 and 400 $mgkg^{-1}$. Copper residues are also known to restrict bioturbation of soil and be toxic to soil organisms, thereby resulting in accumulation of organic materials. According to Ma (1984) fewer earthworms have been observed in soils that contain significant copper residues, resulting in reduced surface activity and greater litter build-up.

Earthworms have also been observed to avoid copper contaminated soils, with little indication of breakdown of organic matter and incorporation into the sub-surface leading to a thick layer of organic matter of (10-30 cm) deep that was clearly stratified on the surface (Merrington *et al.,* 2002). Soil-copper level and the concentration of copper in earthworm tissues have been observed to have strong correlation (Morgan and Morgan 1988; Beyer *et al*., 1982). Helling *et al*. (2000) also noted that at very low concentrations of copper (9-16 mg kg-1) earthworm's exhibit sublethal toxic response. Copper contaminated soil had been observed to be actively avoided by the enchytraeid worm *Cognition sphagnetorum* (Salminen and Haimi, 2001).

According to Paoletti *et al*. (1998), earthworms have been recommended as good soil health indicators. Baker *et al.* (1994) also noted that earthworm's aid in decomposition and incorporation of organic matter, through their burrowing activity, thus improving water infiltration, aeration, root penetration, and increase microbial activity.

Total and available plant nutrients in earthworm casts and burrow walls exhibit higher concentrations than surrounding soil and plant pathogens can be reduced through digestion of fungal spores (Baker *et al.,* 1994). Therefore, reduction in soil health can result through practices that can lead to reduction in earthworm populations in soil hence causing disease or nutrients problems in soil.

2.7 Mechanism for absorption of Copper in Copper-Based Fungicides

A gradual redistribution of copper deposits controlled by environmental factors may occur after foliar application of copper fungicides. The plant cells take up some of the copper during redistribution, while most ultimately end up in litter and top soils (Mabbett, 1984). However, there is no indication of copper build-up in the soil profile at depth below 25 cm which might be due to copper's strong affinity for organic matter, thus making its interaction noticeable within the top soils (Renan, 1994).

In soils available copper is held mainly on surfaces of clay minerals or association with organic matter as a cation (Cu^{2+}). Copper are largely unavailable in a form of silicate minerals or carbonates. The predominant factors influencing copper availability are organic matter and soil pH (Schulte and Kelling, 1999). As organic matter in the soil increases, copper availability decreases. Organic matter binds copper more strongly than any other micronutrient (Schulte and Kelling, 1999). This strong interaction reduces fixation by soil mineral and leaching, as well as its availability to crops.

According to McGrath *et al*. (1988) as pH decreased, the proportion of copper present in soil solution as Cu^{2+} increased. Movement of copper along the soil profile, phytotoxicity threshold for crops and bioavailability for root uptake depend on soil pH (Chaignon *et al*., 2003), quality of organic matter, soil texture, cation exchange capacity

(Parat *et al.,* 2002; Brun *et al*., 2001). Average background levels of 20-30 $mgkg^{-1}$ have been reported in agricultural soils (Baker, 1990).

2.8 Impact of Copper on Nutrient Availability

Plants need nutrients to perform specific functions and to produce at maximum capacity. Copper binds strongly to organic matter, clay minerals and hydrated oxides of aluminium, iron, and manganese and either makes them unavailable to plants or reduces the concentration of these nutrients in soils (Schnitzer, 1969)

Savithri *et al*. (2003) found that the amount of micronutrient such as, manganese, zinc, and iron decreased as copper content in the soils of grape farms increased due to continuous application of Bordeaux mixture. Similarly, due to fungicide application available phosphorus contents of the soils decreased at both surface and sub surface layers (Caudhuri, 1964). Available phosphorus immobilization or fixation may be encouraged as a result of increasing base saturation of soils containing fungicide residues (Caudhuri, 1964). Akinnifesi *et al*. (2006) found that the amount of phosphorus available to plants reduced with increasing copper content of soils in cocoa plantations and causes nutrient imbalance.

2.9 Soil Microbial Biomass

Soil microbial biomass is both a source and sink of nutrients contained in the organic matter. It acts as the transformation agent of organic matter in soil (Jenkinson and Ladd, 1981).

The definition of soil microbial biomass according to Jenkinson and Ladd, (1981) “is the living portion of the soil organic matter, excluding plant roots and soil animals larger

than $5x10^{-3}$ um^3." The microbial biomass comprises approximately 2% of the total organic matter in soil and may be considered as of minor importance in the soil . However, the overall biological activity of the soil is controlled by soil microbial biomass as agent (Jenkinson and Ladd, 1981). According to Nannipieri *et al.* (1990) microbial biomass plays a central role in majority of the biological activity in the soil biomass. The flow of C and N in the soil, from other materials or newly deposited plant to the mineral forms of carbon dioxide and ammonium or nitrate ions clearly shows the important role of the microbial biomass.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Area

The study was carried out in Bibiani-Anhwiaso-Bekwai District in the Western Region of the Republic of Ghana. The District is located between latitude 5°54'N and 6°30'N and 2°06'W and 2°27'W longitude. It is bounded to the south by Asankragua District, to the north by Asunafo south District, to the west by Sefwi Wiawso District and to the east by Atwima Nwabiagya District (EPA, 2002).

3.1.1 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 bimodal rainfall regime, with the major one in May to July and the minor in August to September. Mean rainfall is between 1,250 mm and 1,750 mm per annum. The dry season begins in October through to early part of March (EPA, 2002).

3.1.2 Selected Cocoa Farms and Reference Forests

Cocoa farms and reference forests were selected from three communities, namely Akaasu, Kyeikrom and Tuntum in the district as shown in Figure 3.1. [Three farms each were sampled in Akaasu and Kyeikrom while four farms were sampled in Tuntum]. Uncultivated forests close to these plantations were also selected as references. These forests were labelled as reference forest F_1, F_2 and F_3 for Akaasu,
Kyeikrom and Tuntum respectively. The sampling points were plotted by the use of Geographic Positioning System (GPS) device.

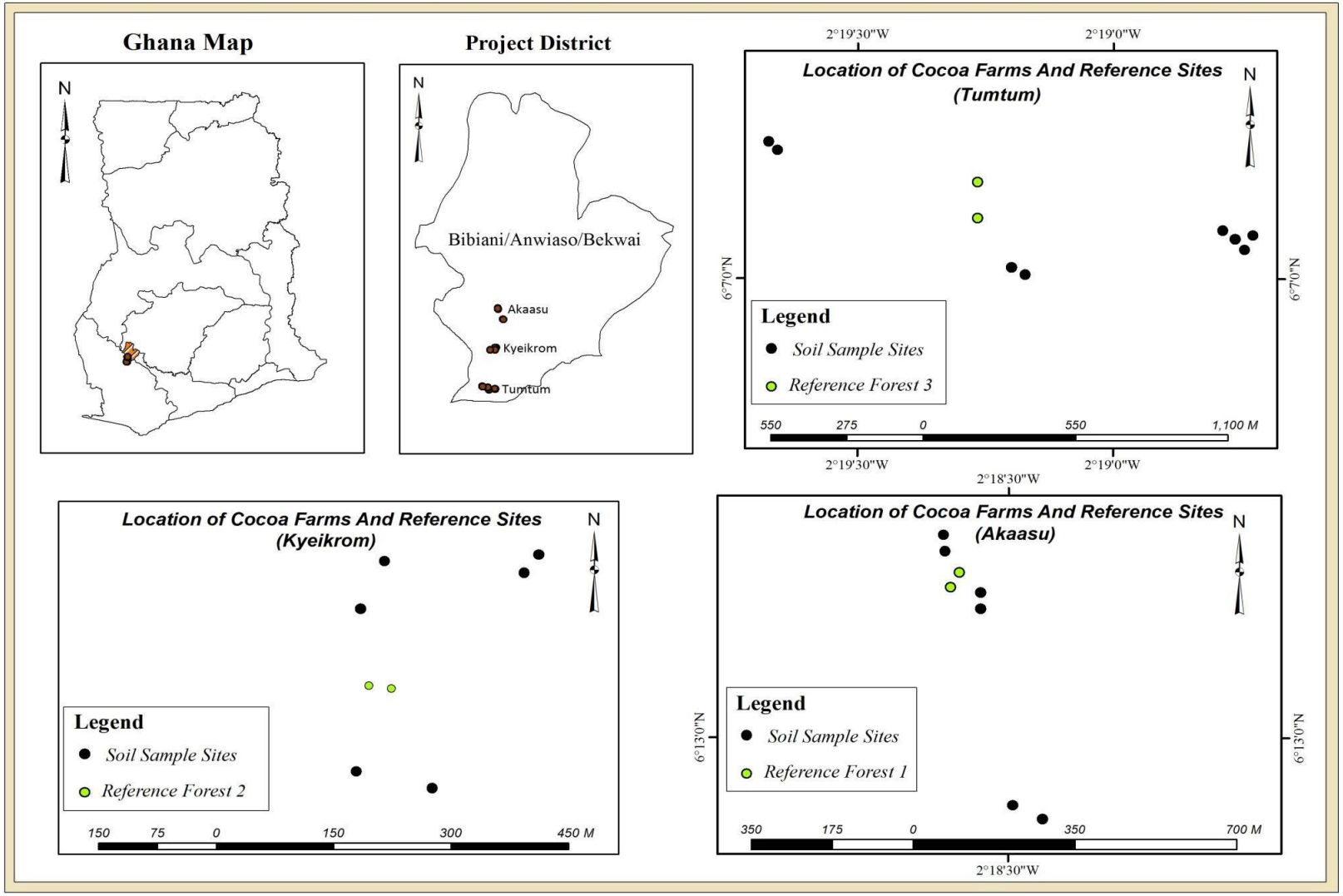

Figure 1: Map showing the study area

3.2 Soil Sampling

Soil samples were taken from ten (10) selected cocoa farms which had been sprayed with copper-based fungicide seasonally for 13 years. Soils from secondary forests close to the farms which have never been sprayed with these fungicides were selected as control. A soil auger was used for the sampling. Soil samples were collected at two distinct depths thus, 0-15 cm and 15-30 cm. For each depth, six cores were taken and mixed thoroughly in a plastic bowl into a composite sample. Two composite samples were taken from each of the 10 selected farms and labelled, giving a total of 20 samples at each depth. Labeling was done by assigning unique names and numbers to each sampling bags.

The fresh soil samples were divided into two. One was immediately sieved through 4 mm mesh and stored at 4 ^{0}C for the soil microbial test. The other half was air dried, sieved through a 2 mm mesh and stored in plastic bags for the chemical analysis. All soil samples were sent to Kwadaso, Kumasi for analysis at the Soil Research Institute of the Council for Scientific and Industrial Research (CSIR).

3.3 Chemical Analysis

3.3.1 Soil pH

Measurement of soil pH was done in a 1:1 soil-water (w/v) ratio using a pH meter (H19017). Twenty five (25 g) of soil was weighed into a 50 mL polythene beaker and 25 mL of distilled water was added and the solution was stirred thoroughly and allowed to stand for 30 minutes. The pH was read by immersing the electrode into the upper part of the soil solution and the pH value recorded after calibrating the pH meter with buffers of pH 4.01 and 7.00

3.3.2 Soil Organic Carbon

Determination of soil organic carbon was done by the modified Walkley-Black method as described by Nelson and Sommers (1982). It involves an oxidation of the organic matter with potassium dichromate. The excess dichromate was titrated against ferrous sulphate after the reaction. Air-dried sample of 1.0 g was weighed into a clean and dry 250 mL Erlenmeyer flask. Using the custom laboratory dispenser, ten (10) mL 0.1667M potassium dichromate ($K_2Cr_2O_7$) solution was accurately dispensed into the flask. The flask was swirled gently so that the sample was made wet. Afterwards 100 mL of distilled water was added and mixed well. Then 20 mL of concentrated sulphuric acid (H_2SO_4) was dispensed rapidly into the soil suspension using an automatic pipette and swirled vigorously for 1 min and allowed to stand on a porcelain sheet for about 30 min. Addition of ten (10) mL of ortho-phosphoric acid and 1 mL of diphenylamine indicator was done and titrated by adding 1.0M ferrous sulphate from a burette until the solution turned dark green at end-point from an initial purple colour. About 0.5 mL 0.1667M $K_2Cr_2O_7$ was added to restore excess $K_2Cr_2O_7$ and by adding $FeSO_4$ drop-wise to attain a stable end-point. The volume of $FeSO_4$ solution used was recorded.

The soil organic carbon content was calculated as:

$$\%\ \mathrm{O.C} = \frac{\mathrm{M} \times 0.39 \times \mathrm{mcf} \times (\mathrm{V}_1 - \mathrm{V}_2)}{\mathrm{s}}$$

Where

M = molarity of ferrous sulphate solution.

V_1 = mL of ferrous sulphate solution required for blank. V_2 = mL of ferrous sulphate solution required for sample. s = weight of dried soil samples in grams.

$$\mathrm{mcf} = \text{moisture correcting factor} = \frac{(100 + \%\ \text{moisture})}{100}.$$

$0.39 = 3 \times 0.001 \times 100\ \% \times 1.3$ (3=equivalent weight of carbon)

1.3 = a compensation factor for the incomplete combustion of the organic carbon.

Using the formula, % organic matter was calculated as;

% Organic matter = % organic C × 1.724

3.3.3 Total Nitrogen

Determination of total nitrogen was done by the Kjeldahl digestion and distillation method. Two grams (2.0 g) soil was weighed into a Kjeldahl digestion flask and 5 mL distilled water added. The flask was placed on a Kjeldahl digestion apparatus and heated initially gently and later vigorously for at least 3 hr. Selenium tablet and 5 mL of concentrated H_2SO_4 were added to the soil sample. After a clear mixture was obtained, the flask was removed and then allowed to cool. About 40 mL of distilled water was added to the digested material and transferred into 100 mL distillation tube. Twenty (20) mL of 40% NaOH was also added to the solution and then distilled using the Tecator Kjeltec distiller. The digested material was distilled for 4 min and the distillate received into a flask containing 20 mL of 4% boric acid (H_3BO_3) prepared with PT5 (bromocresol green) indicator producing approximately 75 mL of the distillate. The colour change was from pink to green after distillation, after which the content of the flask was titrated with 0.02M HCl from a burette. The volume of 0.02M HCl used was recorded and % N calculated at the end-point when the solution changed from weak green to pink A blank distillation and titration was also carried out to take care of traces of nitrogen in the reagents as well as the water used.

The percentage nitrogen in the sample was expressed as:

$$\%\,\mathrm{N} = \frac{(\mathrm{M} \times (\mathrm{a} - \mathrm{b}) \times 1.4 \times \mathrm{mcf})}{\mathrm{s}}$$

where

M = concentration of hydrochloric acid used in titration. a

= volume of hydrochloric acid used in sample titration b

= volume of hydrochloric acid used in blank titration.

s = weight of dried soil sample in grams.

mcf = moisture correcting factor

3.3.4 Total and Extractable Copper & Cadmium Determination

3.3.5 Digestion of Soil Samples for Total Copper and Cadmium Content

Two grams (2 g) of each soil sample was placed in a beaker and the metal contents extracted by adding 15 mL of 50% HNO_3 and placed on a hot plate with a watch glass cover, heated at 95 ^{0}C for 15 min. The heating was later continued with partial covering without boiling till the solution got reduced to about 5 mL, and then cooled. Two millilitres (2 mL) 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 min., The solution was filtered after cooling and the filtrate quantitatively transferred into a 50 mL volumetric flask and topped up with distilled water (USEPA, 1992). A blank sample was also treated in the same way. Each of them was filtered using a Whatman filter paper (Cat No 1001 110).

3.3.6 Digestion of soil sample for Extractable Copper and Cadmium content

Ten grams (10) g of soil sample was weighed into shaking bottle. Thirty (30) mL of ammonium acetate and ethylenediaminetetraacetic acid (EDTA) were added to the sample and then shaken for 2 hr on a reciprocating shaker. Using Whatman filter paper

No.42. the samples were then filtered into a flask. Five millilitres (5) mL of the filtrate was pipetted into a test tube and then ten (10) mL of lanthanum chloride ($LaCl_3$) solution added. The metal concentrations were determined using the Atomic Absorption Spectrophotometer (AAS) (Motsara and Roy, 2008).

3.3.7 Analysis of Total and Extractable Metal Contents

After digestion the solutions obtained were analyzed for total and extractable metals using atomic absorption spectroscopy (Buck Scientific AAS, Model 210 VGP). Separate calibration curves were prepared 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 air-acetylene flame. All samples were run in triplicates and average in $mgkg^{-1}$ values taken for each determination (Motsara and Roy, 2008).

3.4 Soil Microbial Biomass Carbon and Nitrogen

Microbial biomass was determined by the chloroform fumigation method and extraction (FE) as described by Ladd and Amato (1989). Ten grams (10) g of sieved field moist soil sample was put in a crucible and placed in a desiccator. A shallow dish containing 30 mL of alcohol free chloroform was placed by it. Ten grams (10 g) sample was also placed in a separate desiccator without chloroform as a control. The desiccators were covered and allowed to stand at room temperature for 5 days
(Anderson and Ingram, 1998).

After fumigation, 50 mL of 0.5M K_2SO_4 solution was added immediately to the soil samples to extract microbial carbon and nitrogen from the lysed microorganism. Total nitrogen in the extract was then determined by the Kjeldahl method. The amount of

microbial carbon in the extract was determined using the colorimetric method. An aliquot (5 mL) of the extract was pipetted into 250 mL Erlemeyer flask. To this were added 5 mL of (0.17 M) potassium dichromate and 10 mL concentrated sulphuric acid. The resulting solution was allowed to cool for 30 min after which 10 mL of distilled water was added.

A standard series was developed concurrently with carbon concentrations ranging from 0, 2.5, 5.0, 7.5, 10.0 mg/mL C. These concentrations were obtained when volumes of 0, 5, 10, and 20 ml of a 50 mg/mL C stock were pipetted into labeled 100 mL volumetric flasks and made up to the mark with distilled water. The absorbances of the standard and sample solutions were read on a spectronic 2ID spectrophotometer at a wavelength of 600 nm.

A standard curve was obtained by plotting absorbance values of the standard solution against their corresponding concentration. Extracted carbon concentration of the samples was determined from the standard curve. For biomass C and N calculations, k-factors of 0.35 (Sparling *et al.,* 1990) and 0.45 (Jenkinson, 1988; Ross and Tate, 1993) were used, respectively.

The following equations (Sparling and West, 1988) were used to estimate the microbial C and N from the extracted C and N, respectively:

Microbial C (mg) = Ec/k

Microbial N (mg) = E/k where

E = the extracted nitrogen produced following fumigation,

Ec = the extracted carbon produced following fumigation;
k = the fraction of the killed biomass extracted as carbon or nitrogen under standardized conditions.

3.5 Soil Microbial Biomass Phosphorus

Microbial biomass P analysis was determined using five grams (5 g) of field-moist soil weighed into a crucible and fumigated in a dessicator with 30 mL of alcohol-free chloroform for 5 days.

Another crucible containing five grams (5 g) sample was placed in a separate desiccator without fumigation as control. Both unfumigated and fumigated samples were shaken with 35 mL Bray's No.1 extracting solution (0.03M NHF + 0.025M HCI) for 10 min and filtered. Correction for adsorption of P during fumigation was made by simultaneously equilibrating unfumigated soil with a series of P containing standard solutions followed by extraction with the Bray-1 solution. The amount of P was determined according to the relationship between P added (from standard solution or microbial lysis) and P extracted by the Bray-1 solution (Oberson *et al*.,
1997).

At equilibrium phosphorus adsorption is described by the following equation according to Barrow and Shaw (1975) and adapted by Morel *et al*. (1997):

$Ext_p = Ext_0 + b_1 Pad b_2$

Where

Ext_p = Pi concentration (mg/L) extracted after equilibration with different amount of P added; Ext_0 = Pi concentration extracted without P addition, b_1, b_2 = coefficients estimated by non-linear regression of mean values of Ext_p against Pad,

Pad= amount of P added (0-20 $mgkg^{-1}$).

Chloroform released is calculated from the equation, P corresponds to a P addition and

$P_{chl} = [(Ext_{chl} – Ext_0)/(b_1)]b_2$

Where

P_{chl} = chloroform released P ($mgkg^{-1}$)

Ext_{chl} = Pi concentration in extracts of fumigated samples.

The amount of microbial P is estimated by assuming a kp factor of 0.4 (Brookes *et al.,* 1982;

McLaughlin and Alston, 1986).

3.6 Statistical analysis

Statistical analyses in this thesis were executed using analysis of variance (ANOVA) techniques and correlation model. Analysis of variance (ANOVA) was conducted to compare concentrations and associations between some selected soil chemical properties in the study area. Concentrations of soil chemical properties were compared between top soils and sub soils with t-statistics analysis. Correlation analysis was conducted to determine the relationships between (1) Soil pH and extractable copper (2) Soil pH and total copper (3) organic matter and extractable copper (4) organic matter and total copper (5) extractable copper and microbial biomass carbon (6) extractable copper and microbial biomass nitrogen and (7) extractable copper and microbial biomass phosphorus. These statistical procedures were computationally implemented using the STATA (version 12) software package

(2011) at a significance level of 5%.

CHAPTER FOUR

4.0 RESULTS

4.1 Soil Chemical Properties recorded at soil depth 0-15 cm

Mean values of soil pH, nitrogen, organic matter, extractable cadmium, extractable copper, total cadmium and total copper recorded in the top soil (0-15 cm) from Akaasu, Kyeikrom and Tuntum cocoa plantations and the three reference forests are presented in table 1.

Generally, the concentrations of extractable and total copper recorded from the top soils of all the selected cocoa farms were extremely high, compared to the reference forests samples. Results from ANOVA showed that, there were high significant differences ($P < 0.05$) between the cocoa farms soils samples and the reference forests soils samples in relation to extractable and total copper in the top soils of all the three study locations.

Analysis of variance (ANOVA) showed that pH of cocoa farms soil samples selected from Akaasu, Kyeikrom and Tuntum did not differ significantly ($P > 0.05$) from soils of the reference forests. Mean values of nitrogen in the top soils of cocoa farms selected from Akaasu, Kyeikrom and Tuntum with their reference forests did not show significant differences ($P > 0.05$) from the analysis of variance. Similarly, organic carbon and organic matter showed no significant differences ($P > 0.05$) with their reference forests soils in all the three study locations.

Analysis of variance also showed no significant differences ($P > 0.05$) in the concentrations of extractable cadmium in the top soils of cocoa farms selected from the three study locations with their reference forests soils samples. However total cadmium showed significant differences ($P < 0.05$) in the top soils of cocoa farms selected from Akaasu, Kyeikrom and Tuntum from their reference forests soils samples.

Table 1: Mean and Standard deviation of Chemical Properties of Top Soils (0-15 cm) from Cocoa farms and Reference forests

Farm Location	pH (p-value)	Nitrogen (%) (p-value)	Organic Carbon (%) (p-value)	Organic Matter (%) (p-value)	Extractable Cd, $mgkg^{-1}$ (p-value)	Extractable Cu, $mgkg^{-1}$ (p-value)	Total Cd, $mgkg^{-1}$ (p-value)	Total Cu, $mgkg^{-1}$ (p-value)
Akaasu	5.57±0.29	0.14±0.01	1.64±0.13	2.83±0.23	0.11±0.05	10.75±2.64	0.67±0.02	215.63±46.34
F_1	5.20±0.33	0.18±0.01	2.15±0.37	3.70±0.29	0.05±0.01	3.64±1.01	0.23±0.07	37.80±7.32
	(0.386)	(0.111)	(0.077)	(0.079)	(0.382)	(0.029)	(0.030)	(0.044)
Kyeikrom	5.80±0.17	0.10±0.05	1.14±0.57	1.97±0.99	0.14±0.02	9.29±1.76	0.75±0.15	257.31± 2.10
F_2	5.40±0.11	0.12±0.01	1.37±0.08	2.36±031	0.04±0.01	4.02±1.55	0.21±0.01	39.90±5.57
	(0.184)	(0.762)	(0.761)	(0.767)	(0.057)	(0.038)	(0.020)	(0.046)
Tuntum	5.60±0.36	0.12±0.02	1.36±0.27	2.34±0.47	0.14±0.09	10.51± 1.80	0.72±0.08	286.54±69.26
F_3	5.50±0.21	0.13±0.01	1.38±0.22	2.37±0.26	0.05±0.01	4.03±1.73	0.22±0.03	39.91±7.99
	(0.818)	(0.707)	(0.952)	(0.962)	(0.433)	(0.031)	(0.011)	(0.049)

Values inside parenthesis represent p-values at 5% level of significance, n=52

4.2. Relationship between soil chemical properties and extractable & total copper in the top soils of Akaasu cocoa farms

Table 2 shows correlation coefficients relating soil chemical properties and extractable copper in the top soil from cocoa farms at Akaasu. Soil pH showed negative relationship with extractable copper but did not correlate significantly ($P > 0.05$). However, organic matter correlated negatively but insignificantly ($P < 0.05$) with extractable copper. Additionally, with multiple coefficient of determination (Rsquare) value of 0.559, pH and organic matter relate more than half the proportion of the total variability in the content of extractable copper in the top soils of cocoa farms located at Akaasu.

Table 2: Correlation coefficient relating selected soil chemical properties and extractable copper in the top soils of Akaasu cocoa farms (2013)

	Coefficient	t –statistic	p-value
Constant	10.179	1.16	0.329
pH	-2.083	-1.22	0.311
Organic Matter	-2.706	-2.88	0.047
F-statistic	4.17		0.049
R-square	0.559		

Correlation coefficients relating soil chemical properties and total copper in the top soil from cocoa farms at Akaasu are presented in table 3. Soil pH and organic matter showed negative insignificant correlation with total copper at 5% level.

Table 3: Correlation coefficient relating selected soil chemical properties and total copper in the top soils of Akaasu cocoa farms (2013)

	Coefficient	t _statistic	p-value

Constant	294.831	1.07	0.365
pH	-5.699	-0.29	0.790
Organic Matter	-3.009	-0.10	0.296
F-statistic	0.07		0.936
R-square	0.143		

4.3 Relationship between soil chemical properties and extractable & total copper in the top soils of Kyeikrom cocoa farms

Table 4 shows correlation coefficients relating soil chemical properties and extractable copper in the top soil from cocoa farms Kyeikrom. Soil pH and organic matter showed negative insignificant ($P > 0.05$) correlation with extractable copper.

Table 4: Correlation coefficient relating selected soil chemical properties and extractable copper in the top soils of Kyeikrom cocoa farms (2013)

	Coefficient	**t –statistic**	**p-value**
Constant	6.463	0.32	0.770
pH	-0.899	0.25	0.820
Organic matter	-2.116	-1.12	0.343
F-statistic	0.63		0.589
R-square	0.297		

Correlation coefficients relating soil chemical properties and total copper in the top soil from cocoa farms at Kyeikrom are presented in table 5. Soil pH and organic matter correlated significantly ($P < 0.05$) and negatively with total copper. In addition, with a

multiple coefficient of determination (R-square) value of 0.887, pH and organic matter relate more than half the proportion of variability in total copper concentration in the top soils of cocoa farms at Kyeikrom.

Table 5: Correlation coefficient relating selected soil chemical properties and total copper in the top soils of Kyeikrom cocoa farms (2013)

	Coefficient	t –statistic	p-value
Constant	201.645	7.41	0.005
pH	-32.371	-3.40	0.042
Organic Matter	-20.785	4.22	0.024
F-statistic	11.75		0.038
R-square	0.887		

4.4 Relationship between soil chemical properties and extractable & total copper in the top soils of Tuntum cocoa farms

Table 6 shows correlation coefficients relating soil chemical properties and extractable copper in the top soil from cocoa farms Tuntum. Soil pH and organic matter showed negative insignificant ($P > 0.05$) correlation with extractable copper.

Table 6: Correlation coefficient relating selected soil chemical properties and extractable copper in the top soils of Tuntum cocoa farms (2013)

	Coefficient	t –statistic	p-value
Constant	5.321	0.56	0.602
pH	-0.119	-0.07	0.944
Organic Matter	-0.623	-0.92	0.399
F-statistic	0.64		0.564

R-square	0.205

Correlation coefficients relating soil chemical properties and total copper in the top soil from cocoa farms at Tuntum are presented in table 7. Soil pH and organic matter showed negative insignificant ($P > 0.05$) correlation with total copper in the top soils of cocoa farms located at Tuntum.

Table 7: Correlation coefficient relating selected soil chemical properties and total copper in the top soils of Tuntum cocoa farms (2013)

	Coefficient	t –statistic	p-value
Constant	187.007	1.59	0.172
pH	-0.398	-0.95	0.387
Organic Matter	-0.664	-0.25	0.815
F-statistic	0.49		0.638
R-square	0.169		

4.5 Soil Microbial Biomass Carbon, Nitrogen and Phosphorus recorded in the top soils from cocoa farms at Akaasu, Kyeikrom, and Tumtum

Mean values of soil microbial biomass carbon, nitrogen and phosphorus recorded in top soil (0-15 cm) from Akaasu, Kyeikrom and Tuntum cocoa plantations and their reference forests soil samples are presented in table 8. Results from analysis of variance showed that soil microbial biomass carbon values recorded in soil samples from Akaasu cocoa farms did not differ significantly ($P > 0.05$) from the reference forest soil samples.

Microbial biomass carbon values recorded in soil samples from Kyeikrom were also not significantly different ($P > 0.05$) from the reference forest soil samples. Similarly Microbial biomass carbon values from Tuntum cocoa farms showed no significant difference ($P > 0.05$) from the reference forest soil samples.

From table 8, the ANOVA results showed no significant difference ($P > 0.05$) between microbial biomass N_2 values and reference forest soil samples from cocoa farms at Akaasu. The microbial biomass N_2 recorded at Kyeikrom cocoa farms was also not significant ($P > 0.05$) compared with the reference forest soil samples. Microbial biomass N_2 values recorded from cocoa farms at Tuntum and the reference forest soil samples did not differ significantly ($P > 0.05$).

Microbial biomass phosphorus recorded in soil samples from Akaasu cocoa farms and the reference forest soil samples were not significantly different ($P > 0.05$). Microbial biomass phosphorus recorded in soil samples from Kyeikrom cocoa farms did not differ significantly when compared with soil samples from reference forest ($P > 0.05$). Again, microbial biomass phosphorus in the top soils of Tuntum cocoa farms did not differ significantly ($P > 0.05$) from that of reference forest soil samples.

Table 8: Mean and standard deviation of microbial biomass carbon, nitrogen, and phosphorus in the top soils from selected cocoa farms and reference forests.

Locations	Microbial biomass C, $mgkg^{-1}$ (p-value)	Microbial biomass N $mgkg^{-1}$ (p-value)	Microbial biomass P $mgkg^{-1}$ (p-value)
Akaasu	170.32±19.99	6.51±3.00	15.92±5.11
F_1	144.74±11.90	5.22±0.60	17.26±4.91
	(0.589)	(0.289)	(0.757)

Kyeikrom	158.61±9.98	5.92±0.50	8.94±4.88
F_2	141.38±7.59	5.52±1.73	18.33±7.55
	(0.353)	(0.348)	(0.078)
Tuntum	174.36±13.70	6.71±0.69	10.34±4.73
F_3	143.00±13.15	5.74±0.30	15.77±2.21
	(0.336)	(0.186)	(0.293)

Values inside parenthesis represent p-values at 5% level of significance

4.6. Relationship between extractable copper and microbial biomass carbon, nitrogen phosphorus in the top soils of cocoa farms at Akaasu

The results presented in Table 9 shows correlation coefficients relating extractable copper and microbial biomass carbon in the top soils of cocoa farms located at Akaasu. Extractable copper showed negative relationship with microbial biomass carbon but insignificant at 0.05 levels.

Table 9: Correlation coefficient relating extractable copper and microbial biomass carbon in the top soils of Akaasu cocoa farms (2013)

	Coefficient	t –statistic	p-value
Constant	170.321	2.87	0.046
Extractable Copper	-5.641	-0.54	0.616
F-statistic	0.30		0.616
R-square	0.169		

Table 10 shows correlation coefficient relating extractable copper and microbial biomass nitrogen in the top soils from cocoa farms located at Akaasu. Extractable

copper showed negative insignificant (P > 0.05) correlation with microbial biomass nitrogen in the top soils of cocoa farms at Akaasu.

Table 10: Correlation coefficient relating extractable copper and microbial biomass nitrogen in the top soils of Akaasu cocoa farms (2013)

	Coefficient	t –statistic	p-value
Constant	8.334	2.31	0.082
Extractable Copper	-0.283	-0.54	0.615
F-statistic	0.30		0.615
R-square	0.169		

Correlation coefficients relating extractable copper and microbial biomass phosphorus in the top soils of cocoa farms located at Akaasu are presented in Table 11. Extractable copper negatively correlated with microbial biomass phosphorus but insignificant (P > 0.05) in the top soils of cocoa farms selected from Akaasu.

Table 11: Correlation coefficient relating extractable copper and microbial biomass phosphorus in the top soils of Akaasu cocoa farms (2013)

	Coefficient	t –statistic	p-value
Constant	13.862	2.21	0.092
Extractable Copper	-0.318	0.35	0.743
F-statistic	0.12		0.743
R-square	0.030		

4.7 Relationship between extractable copper and microbial biomass carbon, nitrogen phosphorus in the top soils of cocoa farms at Kyeikrom

Table 12 shows correlation coefficient relating extractable copper and microbial biomass carbon in the top soils from cocoa farms located at Kyeikrom. Extractable copper showed negative insignificant (P > 0.05) correlation with microbial biomass carbon the top soils of cocoa farms at Kyeikrom. R-square value of 0.234 indicates that only 23.4% of the total variability of microbial biomass carbon in the top soils of cocoa farms at Tuntum could be attributed to its relationship with extractable copper

Table 12: Correlation coefficient relating extractable copper and microbial biomass carbon in the top soils of cocoa farms at Kyeikrom (2013)

	Coefficient	t –statistic	p-value
Constant	169.352	16.14	0.000
Extractable Copper	-1.442	-1.11	0.331
F-statistic	1.22		0.331
R-square	0.234		

Table 13 shows correlation coefficient relating extractable copper and microbial biomass nitrogen in the top soils from cocoa farms located at Kyeikrom. Extractable copper showed negative insignificant (P > 0.05) correlation with microbial biomass nitrogen the top soils of cocoa farms at Kyeikrom**.**

Table 13: Correlation coefficient relating extractable copper and microbial biomass nitrogen in the top soils of cocoa farms at Kyeikrom (2013)

	Coefficient	t –statistic	p-value
Constant	6.457	12.23	0.000
Extractable Copper	-0.071	-1.09	0.337
F-statistic	1.19		0.337

R-square	0.229

Correlation coefficients relating extractable copper and microbial biomass phosphorus in the top soils of cocoa farms located at Kyeikrom are presented in Table 14. Extractable copper negatively correlated with microbial biomass phosphorus but insignificant ($P > 0.05$) in the top soils of cocoa farms selected from Kyeikrom. Additionally, with R-square value of 0.210, only 21.0% of the total variability in microbial biomass phosphorus in the top soils of Kyeikrom could be attributed to its relationship with extractable copper.

Table 14: Correlation coefficient relating extractable copper and microbial biomass phosphorus in the top soils of cocoa farms at Kyeikrom (2013)

	Coefficient	t –statistic	p-value
Constant	3.968	0.76	0.489
Extractable Copper	-0.667	-1.03	0.361
F-statistic	1.06		0.361
R-square	0.210		

4.8 Relationship between extractable copper and microbial biomass carbon, nitrogen phosphorus in the top soils of cocoa farms at Tuntum

Table 15 shows correlation coefficient relating extractable copper and microbial biomass carbon in the top soils from cocoa farms located at Tuntum. Extractable copper showed negative insignificant ($P > 0.05$) correlation with microbial biomass carbon the top soils of cocoa farms at Tuntum. R-square value of 0.105 indicates that only 10.5%

of the total variability of microbial biomass carbon in the top soils of cocoa farms at Tuntum could be attributed to its relationship with extractable copper.

Table 15: Correlation coefficient relating extractable copper and microbial biomass carbon in the top soils of cocoa farms at Tuntum (2013)

	Coefficient	t –statistic	p-value
Constant	159.405	8.62	0.000
Extractable Copper	-2.619	-0.84	0.434
F-statistic	0.70		0.434
R-square	0.105		

Table 16 shows correlation coefficient relating extractable copper and microbial biomass nitrogen in the top soils from cocoa farms located at Tuntum. Extractable copper showed negative insignificant ($P > 0.05$) correlation with microbial biomass nitrogen the top soils of cocoa farms at Kyeikrom**.** R-square value of 0.104 indicates that only 10.4% of the total variability of microbial biomass nitrogen in the top soils of cocoa farms at Tuntum could be attributed to its relationship with extractable copper

Table 16: Correlation coefficient relating extractable copper and microbial biomass nitrogen in the top soils of cocoa farms at Tuntum (2013)

	Coefficient	t –statistic	p-value
Constant	5.966	6.44	0.001
Extractable Copper	-0.131	-0.84	0.435
F-statistic	0.70		0.435
R-square	0.104		

Correlation coefficients relating extractable copper and microbial biomass phosphorus in the top soils of cocoa farms located at Tuntum are presented in Table 17. Extractable copper negatively correlated with microbial biomass phosphorus but insignificant ($P > 0.05$) in the top soils of cocoa farms selected from Tuntum. Rsquare value of 0.114 indicates that only 11.4% of the total variability in microbial biomass phosphorus in the top soils of Tuntum could be attributed to its relationship with extractable copper.

Table 17: Correlation coefficient relating extractable copper and microbial biomass phosphorus in the top soils of cocoa farms at Tuntum (2013)

	Coefficient	t -statistic	p-value
Constant	4.975	0.78	0.463
Extractable Copper	-0.940	-0.88	0.414
F-statistic	0.77		0.414
R-square	0.114		

4.9 Soil Chemical Properties recorded in the sub soil (15-30cm)

Table 18 shows the soil pH, nitrogen, organic carbon, organic matter, extractable cadmium, extractable copper, total cadmium and total copper recorded at soil depth of 15-30 cm from cocoa farms located within the study areas and the three reference forests.

Generally, the concentrations of extractable and total copper recorded from sub soils of all the selected cocoa farms were higher, compared to the reference forests soil samples. Results from ANOVA showed that, there were significant differences ($P < 0.05$) between cocoa farms soils samples and the reference forests soils samples in relation to extractable and total copper in the sub soils of all the three study locations.

Analysis of variance (ANOVA) showed that pH from selected cocoa farms soil samples at Akaasu, Kyeikrom and Tuntum did not differ significantly ($P > 0.05$) from soils of their reference forests in the sub soils. Mean values of nitrogen in the sub soils of cocoa farms selected from Akaasu, Kyeikrom and Tuntum with their reference forests also did not show significant differences ($P > 0.05$) from the analysis of variance. Similarly, organic carbon and organic matter showed no significant differences ($P > 0.05$) with their reference forests soils in all the three study locations in the sub soils.

From table 18 analyses of variances showed no significant differences ($P > 0.05$) in the concentrations of extractable cadmium in the sub soils of cocoa farms selected from the three study locations with their reference forests soils samples. Total cadmium also showed no significant differences ($P > 0.05$) in the sub soils of cocoa farms selected from Akaasu, Kyeikrom and Tuntum from their reference forests soils samples.

Table 18: Mean and standard deviations of chemical properties of sub soils (15-30 cm) from cocoa farms and reference forests

Farm Location	pH (p-value)	Nitrogen (%) (p-value)	Organic Carbon (%) (p-value)	Organic Matter (%) (p-value)	Extractable Cd $mgkg^{-1}$ (p-value)	Extractable Cu $mgkg^{-1}$ (p-value)	Total Cd $mgkg^{-1}$ (p-value)	Total Cu $mgkg^{-1}$ (p-value)
Akaasu	5.30±0.10	0.05±0.01	0.52±0.13	0.90±0.22	0.14±0.03	6.95± 1.02	0.64±0.02	150.40±42.47
F_1	5.10±0.07	0.08±0.01	0.88±0.17	1.52±0.27	0.05±0.02	1.66±0.33	0.21±0.09	33.40±2.74
	(0.225)	(0.122)	(0.131)	(0.134)	(0.096)	(0.038)	(0.133)	(0.039)
Kyeikrom	5.47±0.32	0.05±0.03	0.58±0.42	0.99±0.72	0.11±0.02	6.84± 1.25	0.60±0.24	204.51±28.27
F_2	5.10±0.27	0.06±0.01	0.62±0.20	1.07±0.37	0.03±0.01	1.43±0.30	0.17±0.05	34.25±3.93
	(0.427)	(0.874)	(0.937)	(0.935)	(0.103)	(0.035)	(0.264)	(0.035)
Tumtum	5.50±0.29	0.06±0.02	0.58±0.29	1.01±0.51	0.15±0.10	5.99± 1.56	0.58±0.18	223.10±30.29
F_3	5.40±0.18	0.05±0.01	0.26±0.08	0.43±0.25	0.06±0.01	1.19±0,37	0.21±0.10	30.04±3.74
	(0.781)	(0.863)	(0.397)	(0.380)	(0.520)	(0.0.032)	(0.173)	(0.011)

Values inside parenthesis represent p-values at 5% level of significance

CHAPTER FIVE

5.0 DISCUSSION

5.1 Variation in the concentration of soil chemical properties from the cocoa farms and the reference forests in the study areas

Results from the study indicate that, the concentrations of extractable copper in both the top and sub soils were higher in the cocoa plantations than the reference soils. Similarly, total copper concentrations were significantly higher in the soils from the cocoa plantations than the reference forest soils. This finding is consistent with the work of Addo-Fordjour *et al*. (2013) in a study of impact of copper-based fungicides application on contamination of cocoa plants and soils.

These differences might be due to the continuous application of copper-based fungicides to control fungi in the cocoa plantations. Savithri *et al.* (2003) found that the amount of micronutrient such as, manganese, zinc, and iron decreased as the copper content in the soils of grape farms increased due to continuous application of Bordeaux mixture. Similarly, due to fungicide application the available phosphorus contents of the soils decreased at both surface and sub surface layers. Immobilization of available phosphorus or fixation may be encouraged as a result of increasing base saturation of the soils with fungicide residues (Caudhuri, 1964). Akinnifesi *et al.* (2006) also found that the amount of phosphorus available to plants reduced with increasing copper content of soils in cocoa plantations and causes nutrient imbalance.

The Tuntum cocoa plantations recorded the highest mean values of total copper in the top and sub soils of 286.54±69.26 $mgkg^{-1}$ and 223.10±30.29 $mgkg^{-1}$ respectively.

Though the concentrations of total copper have not exceeded the critical levels of 800 $mgkg^{-1}$ for crops receiving foliar copper-based fungicidal spray as noted by Alva, *et al*. (2000), prolong usage of copper-based fungicides will gradually increase their levels and adversely affect soil biodiversity.

Results of the study also showed that the concentration of copper was higher in the top soils in all the study locations than their respective reference forests. This could be attributed the high affinity of Cu for soil organic matter and hydrous oxides which means that Cu is likely to build-up in top soils due to its strong interaction over a long period of time (Alva *et al.,* 2000). According to Georgieva *et al.* (2002), in the soil profile where there is greatest biological activity Cu accumulation is likely to occur which corresponds to the zone in the top soils.

Results from the correlation analysis revealed that pH and organic matter correlated negatively with extractable and total copper with multiple coefficient of determination which suggests that pH and organic matter relate more than half the total proportion of variability in the content of extractable and total copper in all the three study locations.

The mean pH was generally higher in the top soils than the sub soils for all the study locations. Soil samples from cocoa plantations recorded higher values of soil pH in top and sub soils than the reference forests. Among the cocoa farms locations, Kyeikrom recorded the highest pH in the top soil, while Tuntum cocoa plantations recorded the highest pH in the sub soil.

The more acidic or lower values of pH recorded in the reference forests suggests to the fact that, as pH decreased, the proportion of copper present in soil solution as Cu^{2+}

increased. Movement of copper along the soil profile, phytotoxicity threshold for crops and bioavailability for root uptake depend on soil pH (Chaignon *et al.,* 2003), quality of organic matter, soil texture, cation exchange capacity (Parat *et al.,* 2002; Brun *et al.,* 2001).

The predominant factors influencing copper availability are Organic matter and soil pH (Schulte and Kelling, 1999). As organic matter in the soil increases, copper availability decreases. Organic matter binds copper more strongly than any other micronutrient (Schulte and Kelling, 1999). This strong interaction reduces fixation by soil mineral and leaching, as well as its availability to crops. When the soil pH is increased, the amount of copper held by clay and organic matter increases, making copper availability to plants also to decreased (Schulte and Kelling, 1999).

The levels of organic carbon in the top soils were higher than the sub soils. Also, reference samples F_1 from Akaasu cocoa farms recorded the highest levels of organic carbon in both top and sub soils. According to Jain *et al*. (1997) organic carbon correlates with organic matter in soils and that soil organic matter acts as the major sink and source of organic carbon with measured soil organic carbon content often serving as a proxy for soil organic matter.

Results from the study also showed that organic matter contents were higher in the cocoa plantations than the reference samples except F_1. This might be due to decomposition of materials from the trees (litters, residues of pod husks) from the cocoa plantations over periods under cultivation. The organic matter content also decreased with increasing soils depth. Findings from ordinarily least square regression revealed that extractable and total copper in the top correlated negatively with the levels of

organic matter. This could be attributed to the fact that organic matter has the capability of binding copper, therefore making it difficult for it to be released for plant use. These findings are in line with studies done by McGrath *et al*. (1988) to determine the effect of soil organic matter levels in extractabilities of zinc, manganese, and copper in soil solutions concentrations.

Nitrogen levels in the top soils were also higher than the sub soils for all the study locations. The levels of nitrogen in the sub soils from all the cocoa farms were not different from the reference samples. Reference samples F_1 from Akaasu recorded the highest nitrogen levels in the top soil. Total nitrogen of the soil correlates with the organic carbon content and a change in the content of the organic matter in soil whether by addition or by loss is usually accompanied by a change in the content of nitrogen according to Wild (1998). LaMotte (1998) also noted that nitrogen of the soil usually exists almost entirely in the organic matter. In this form, nitrogen is not available for use by plants directly but must first be transformed by soil bacteria (oxidation) to an available form such as nitrates which are soluble in water and which may be absorbed by plants.

Extractable and total cadmium were higher in soil samples from the cocoa plantations than the reference samples. The higher values of cadmium observed in the cocoa plantations might be due to application of cocoa fertilizers (Asaasewura, Cocofeed) by farmers of the selected cocoa farms in the past years under cultivation of the land. Zarcinas *et al.* (2004) noted in peninsular Malaysia, high levels of Cd in soils and excessive concentrations in cocoa (*Theobroma cacao*) to be the input from phosphate

fertilizers. The most likely origin of the excess cadmium in soils according to Stephen and Calder (2005) is from heavy contaminated phosphate fertilizers.

Results from the study showed that the levels of microbial biomass carbons, nitrogen and phosphorus were not significantly different from their respective reference forests in all the study locations. This suggests that soil microbial biomass were not adversely affected by the application of copper based fungicides. The soil microbial biomass is both a source and sink of the nutrients contained in the organic matter. It acts as the transformation agent of the organic matter in soil (Jenkinson and Ladd, 1981). However, the overall biological activity of the soil is been controlled by soil microbial biomass as agent. Findings from ordinarily least square regression revealed that extractable correlated negatively with concentrations of microbial biomass carbon, nitrogen and phosphorus in all the study locations.

All organisms required copper as an essential element and deficiency may results in reduction in biological function and potentially death. However, high levels of copper when found in soils may be toxic to plants and soil microorganisms and can lead to lower biological activity and eventually loss of soil fertility (Dumestre *et al.,* 1999). Georgieva *et al.* (2002) also found that high copper concentration reduces microbial activity and function in soils. Soil microorganisms have been impacted negatively by copper residues in avocado orchards according to Merrington *et al.,* (2002).

The study has shown that the concentrations of copper in the soils of cocoa plantations will gradually increase with prolong application of copper-based fungicides which will eventually affect the activities of soil microorganisms. This phenomenon may pose a

threat leading to reduced surface activity, greater litter buildup and consequent loss of soil fertility in cocoa plantations.

It was observed that the selected cocoa farms from Tuntum had the highest accumulation of total copper amongst the three study locations.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The findings presented in this thesis revealed that the levels of total copper in the soils of cocoa plantations were higher than that of the uncultivated forests.

The reference forests soils were more acidic compared to that of the cocoa plantations. It was found that the availability of extractable and total copper in the soils of cocoa plantations decreased with increasing levels of soil pH. Moreover, the cocoa plantations appeared to be richer in organic matter than that of the reference forests. The availability of extractable and total copper correlated negatively with the amount of organic matter.

The study have shown that the concentration of copper in the soils of cocoa plantations have not reached their critical levels, prolong usage will increase their levels which eventually may affect soil microorganisms. This may pose a threat leading to loss of soil fertility in cocoa plantations, if effective measures are not put in place.

Soil pH and organic matter decreased with increasing soil depths. However, the concentration of nitrogen and organic carbon in soils from the plantations did not differ significantly from the reference soils. Extractable and total cadmium were higher in both top and sub soils from the cocoa plantations than the uncultivated reference forests.

6.2 Recommendations

On the basis of the findings in this study, the following recommendations are made.

1. Periodic monitoring of copper levels in soils should be carried out.

2. This study should be repeated in other parts of the country where copper-based fungicides are used to control plant diseases to have a broader idea on its accumulations in soils.
3. Further studies should be carried out to determine the relationship between fungicides derived copper and soil physico-chemical properties such as soil texture, porosity and bulk density

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Appendix

PH Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.100833491	1	.100833491	1.21	0.3860
Within groups	.166666667	2	.083333333		
Total	.267500157	3	.089166719		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.119999962	1	.119999962	4.00	0.1835
Within groups	.060000076	2	.030000038		
Total	.180000038	3	.060000013		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.008000004	1	.008000004	0.06	0.8178
Within groups	.380000229	3	.126666743		
Total	.388000233	4	.097000058		

Nitrogen Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.001008334	1	.001008334	7.56	0.1107
Within groups	.000266667	2	.000133333		
Total	.001275001	3	.000425		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.0003	1	.0003	0.12	0.7621
Within groups	.005000001	2	.0025		
Total	.0053	3	.001766667		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.00008	1	.00008	0.17	0.7067
Within groups	.0014	3	.000466667		
Total	.00148	4	.00037		

Organic Carbon Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.195075054	1	.195075054	11.54	0.0768
Within groups	.033800007	2	.016900004		

```
------------------------------------------------------------------------------
    Total           .228875061      3   .076291687
```

Kyeikrom

```
                       Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------------
Between groups         .039675      1      .039675      0.12     0.7611
 Within groups      .655199923      2   .327599962
------------------------------------------------------------------------------
    Total           .694874923      3   .231624974
```

Organic Carbon

```
                       Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------------
Between groups      .000319999      1   .000319999      0.00     0.9516
 Within groups      .221399997      3   .073799999
------------------------------------------------------------------------------
    Total           .221719997      4   .055429999
```

Organic Matter Akaasu

```
                       Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------------
Between groups      .567675058      1   .567675058     11.11     0.0794
 Within groups      .102200053      2   .051100027
------------------------------------------------------------------------------
    Total           .669875111      3   .223291704
```

Kyeikrom

```
                       Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------------
Between groups      .112133269      1   .112133269      0.12     0.7665
 Within groups      1.94486682      2    .97243341
------------------------------------------------------------------------------
    Total           2.05700009      3   .685666696
```

Tumtum

```
                       Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------------
Between groups      .000604998      1   .000604998      0.00     0.9619
 Within groups      .673675021      3    .22455834
------------------------------------------------------------------------------
    Total           .674280019      4   .168570005
```

Cadmium Akaasu

```
                       Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------------
Between groups      .003008333      1   .003008333      1.24     0.3819
 Within groups      .004866667      2   .002433333
------------------------------------------------------------------------------
    Total              .007875      3      .002625
```

Kyeikrom

```
                       Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------------
```

```
Between groups      .007008333      1   .007008333     16.17     0.0566
Within groups      .000866667      2   .000433333
-----------------------------------------------------------------------
    Total              .007875      3      .002625 Tumtum
                      Analysis of Variance
    Source              SS         df      MS            F     Prob > F
-----------------------------------------------------------------------
Between groups         .006125      1      .006125      0.82     0.4325
 Within groups         .022475      3   .007491667
-----------------------------------------------------------------------
    Total                .0286      4       .00715
```

Cadmium Akaasu

```
                      Analysis of Variance
    Source              SS         df      MS            F     Prob > F
-----------------------------------------------------------------------
Between groups      12.6896332      1   12.6896332      1.82     0.3099
 Within groups      13.9562678      2    6.9781339
-----------------------------------------------------------------------
    Total           26.6459009      3   8.88196698
```

Kyeikrom

```
                      Analysis of Variance
    Source              SS         df      MS            F     Prob > F
-----------------------------------------------------------------------
Between groups      8.00333287      1   8.00333287      2.60     0.2485
 Within groups      6.16826457      2   3.08413228
-----------------------------------------------------------------------
    Total           14.1715974      3   4.72386581 Tumtum
                      Analysis of Variance
    Source              SS         df      MS            F     Prob > F
-----------------------------------------------------------------------
Between groups         .006125      1      .006125      0.82     0.4325
 Within groups         .022475      3   .007491667
-----------------------------------------------------------------------
    Total                .0286      4       .00715
```

Copper Akaasu

```
                    Analysis of Variance
    Source              SS         df      MS            F     Prob > F
-----------------------------------------------------------------------
Between groups      12.6896332      1   12.6896332      1.82     0.3099
 Within groups      13.9562678      2    6.9781339
-----------------------------------------------------------------------
    Total           26.6459009      3   8.88196698
```

Kyeikrom

```
                      Analysis of Variance
    Source              SS         df      MS            F     Prob > F
-----------------------------------------------------------------------
Between groups      8.00333287      1   8.00333287      2.60     0.2485
 Within groups      6.16826457      2   3.08413228
-----------------------------------------------------------------------
    Total           14.1715974      3   4.72386581 Tumtum
```

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	4.93024436	1	4.93024436	1.52	0.3058
Within groups	9.74947725	3	3.24982575		
Total	14.6797216	4	3.6699304		

Total Cadmium Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.143008331	1	.143008331	612.89	0.0016
Within groups	.000466668	2	.000233334		
Total	.143474998	3	.047824999		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.221408336	1	.221408336	9.37	0.0922
Within groups	.047266673	2	.023633337		
Total	.268675009	3	.089558336		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.196020004	1	.196020004	31.79	0.0110
Within groups	.0185	3	.006166667		
Total	.214520004	4	.053630001		

Total Copper Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	23716.7432	1	23716.7432	11.05	0.0438
Within groups	4293.96748	2	2146.98374		
Total	28010.7107	3	9336.90355		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	35449.2433	1	35449.2433	8043.93	0.0001
Within groups	8.81391369	2	4.40695684		
Total	35458.0573	3	11819.3524		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	48663.0579	1	48663.0579	10.15	0.0491
Within groups	14389.3927	3	4796.46422		
Total	63052.4506	4	15763.1126		

Correlation

Akaasu

Copper, Total copper on PH

```
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    27.4
       Model |  .054126109     2  .027063055           Prob > F      =  0.0406
    Residual |  1.29787438     3  .432624794           R-squared     =  0.5400
-------------+------------------------------           Adj R-squared = 0.3403
       Total |  1.35200049     5  .270400098           Root MSE      =  .65774

------------------------------------------------------------------------------
          PH |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |   .0041413   .1126362     0.04   0.973    -.3543174    .3625999
 TotalCopper |  -.0023223   .0067808    -0.34   0.755     -.023902    .0192574
       _cons |   5.899116   1.326771     4.45   0.021     1.676738    10.12149
------------------------------------------------------------------------------
```

Copper, Total copper on Organic matter

```
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    8.49
       Model |  3.53258899     2  1.76629449           Prob > F      =  0.0483
    Residual |  .964694268     3  .321564756           R-squared     =  0.7855
-------------+------------------------------           Adj R-squared =  0.6425
       Total |  4.49728326     5  .899456651           Root MSE      =  .56707

------------------------------------------------------------------------------
OrganicMat~r |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |   .3185663   .0971083     3.28   0.046     .0095243    .6276083
 TotalCopper |  -.0092903    .005846    -1.59   0.210    -.0278951    .0093144
       _cons |   2.634611   1.143864     2.30   0.105    -1.005676    6.274898
------------------------------------------------------------------------------
```

Copper, TotalCopper on Microbial C

```
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    8.60
       Model |  6248.11907     2  3124.05954           Prob > F      =  0.0273
    Residual |  11743.3442     3  3914.44805           R-squared     =  0.6473
-------------+------------------------------           Adj R-squared =  0.3879
       Total |  17991.4632     5  3598.29265           Root MSE      =   2.566

------------------------------------------------------------------------------
  MicrobialC |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |  -1.406458   1.21414     -1.15   0.041    -35.50365    32.69073
 TotalCopper |  -.7298563   .6450056    -1.13   0.046    -2.782552     1.32284
       _cons |   325.8179   126.2047     2.58   0.032    -75.82188    727.4576
------------------------------------------------------------------------------
```

Copper Total Copper on microbial N

```
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    0.80
       Model |  15.6572339     2  7.82861694           Prob > F      =  0.5272
    Residual |  29.4148524     3  9.80495081           R-squared     =  0.3474
-------------+------------------------------           Adj R-squared = -0.0877
       Total |  45.0720863     5  9.01441726           Root MSE      =  3.1313

------------------------------------------------------------------------------
  MicrobialN |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |  -.0708112   .5362224    -0.13   0.903     -1.77731    1.635688
 TotalCopper |   -.036525   .0322813    -1.13   0.340    -.1392585    .0662085
       _cons |   14.29276   6.316304     2.26   0.109    -5.808542    34.39406
------------------------------------------------------------------------------
```

Copper Total and Copper on microbial P

```
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    0.05
       Model |  4.54141882     2  2.27070941           Prob > F      =  0.9484
    Residual |  126.198911     3  42.0663038           R-squared     =  0.0347
-------------+------------------------------           Adj R-squared = -0.6088
       Total |   130.74033     5   26.148066           Root MSE      =  6.4859
------------------------------------------------------------------------------
  MicrobialP |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |   .2712605   1.110681     0.24   0.823    -3.263423    3.805944
 TotalCopper |   .0080666   .0668645     0.12   0.912     -.204726    .2208592
       _cons |   12.54658     13.083     0.96   0.408    -29.08938    54.18254
------------------------------------------------------------------------------
```

PH as independent and microbial as independent

```
regress MicrobialC PH

      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  1,     4) =    0.47
       Model |  1904.19642     1  1904.19642           Prob > F      =  0.5292
    Residual |  16087.2668     4   4021.8167           R-squared     =  0.1058
-------------+------------------------------           Adj R-squared = -0.1177
Total |  17991.4632     5  3598.29265           Root MSE      =  63.418

------------------------------------------------------------------------------
  MicrobialC |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          PH |  -37.52904   54.54095    -0.69   0.529     -188.959    113.9009
       _cons |   375.2302   298.9169     1.26   0.278    -454.6962    1205.157
------------------------------------------------------------------------------


. regress MicrobialN PH

      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  1,     4) =    0.47
       Model |  4.77376728     1  4.77376728           Prob > F      =  0.5291
    Residual |   40.298319     4  10.0745798           R-squared     =  0.1059
-------------+------------------------------           Adj R-squared = -0.1176
Total |  45.0720863     5  9.01441726           Root MSE      =   3.174

------------------------------------------------------------------------------
  MicrobialN |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          PH |  -1.879067   2.729764    -0.69   0.529    -9.458106    5.699972
       _cons |   16.76804   14.96073     1.12   0.325    -24.76961    58.30569
------------------------------------------------------------------------------


. regress MicrobialP PH

      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  1,     4) =   51.35
       Model |  121.291388     1  121.291388           Prob > F      =  0.0020
    Residual |  9.44894232     4  2.36223558           R-squared     =  0.9277
-------------+------------------------------           Adj R-squared =  0.9097
Total |   130.74033     5   26.148066           Root MSE      =   1.537

------------------------------------------------------------------------------
  MicrobialP |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
```

```
          PH |   -9.47167   1.321823    -7.17   0.002    -13.14164   -5.801702
       _cons |   67.63198   7.244376     9.34   0.001     47.51837    87.7456
------------------------------------------------------------------------------
```

PH as dependent

```
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  3,     2) =   19.85
       Model |  1.30806931     3  .436023102           Prob > F      =  0.0483
    Residual |  .043931185     2  .021965592           R-squared     =  0.9675
-------------+------------------------------           Adj R-squared =  0.9188
       Total |  1.35200049     5  .270400098           Root MSE      =  .14821

------------------------------------------------------------------------------
          PH |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
  MicrobialC |   1.126785   .7549287     1.49   0.274    -2.121411    4.374982
MicrobialN |  -22.52194   15.08213    -1.49   0.274    -87.41512    42.37125
  MicrobialP |  -.0986578   .0135958    -7.26   0.018    -.1571559   -.0401597
       _cons |  -38.30538    30.3958    -1.26   0.335    -169.0879    92.47717
------------------------------------------------------------------------------
regress PH OrganicMatter
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  1,     4) =    0.63
       Model |  .182880954     1  .182880954           Prob > F      =  0.4732
    Residual |  1.16911954     4  .292279885           R-squared     =  0.1353
-------------+------------------------------           Adj R-squared = -0.0809
       Total |  1.35200049     5  .270400098           Root MSE      =  .54063
------------------------------------------------------------------------------
          PH |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
OrganicMat~r |   .2016551    .254932     0.79   0.473    -.5061496    .9094597
       _cons |   4.889652   .7540566     6.48   0.003     2.796055    6.983249
------------------------------------------------------------------------------
```

Kyeikrom

```
. regress PH Copper TotalCopper

      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    0.46
       Model |  .251810563     2  .125905282           Prob > F      =  0.6690
    Residual |  .819272996     3  .273090999           R-squared     =  0.2351
-------------+------------------------------           Adj R-squared = -0.2748
       Total |  1.07108356     5  .214216712           Root MSE      =  .52258
------------------------------------------------------------------------------
          PH |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |  -.0198351   .0718709    -0.28   0.800    -.2485604    .2088901
 TotalCopper |  -.0105669   .0110161    -0.96   0.408    -.0456252    .0244913
       _cons |   8.482622   2.908972     2.92   0.062    -.7750246    17.74027
------------------------------------------------------------------------------

. regress OrganicMatter Copper TotalCopper

      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    7.19
       Model |   2.3645932     2   1.1822966           Prob > F      =  0.0492
    Residual |  1.61953986     3   .53984662           R-squared     =  0.5935
-------------+------------------------------           Adj R-squared =  0.3225
```

```
       Total |  3.98413306     5  .796826613           Root MSE      =  .03474
------------------------------------------------------------------------------
OrganicMat~r |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |   .1042932   .1010496     2.03   0.047    -.4258781    .2172917
 TotalCopper |   -.023474   .0154885    -0.52   0.227    -.0258175    .0727655
       _cons |  -3.061936   4.089978    -0.75   0.508    -16.07807      9.9542
------------------------------------------------------------------------------
regress MicrobialC PH
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  1,     4) =    2.22
       Model |  177.740344     1  177.740344           Prob > F      =  0.2108
    Residual |  320.728724     4  80.1821809           R-squared     =  0.3566
-------------+------------------------------           Adj R-squared =  0.1957
       Total |  498.469068     5  99.6938136           Root MSE      =  8.9545
------------------------------------------------------------------------------
  MicrobialC |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          PH |  -12.88194   8.652214    -1.49   0.211    -36.90434    11.14046
       _cons |   232.3195   49.63987     4.68   0.009     94.49712    370.1419
------------------------------------------------------------------------------


regress MicrobialN PH
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  1,     4) =    2.19
       Model |  .444181161     1  .444181161           Prob > F      =  0.2126
    Residual |  .809568653     4  .202392163           R-squared     =  0.3543
-------------+------------------------------           Adj R-squared =  0.1929
Total |  1.25374981     5  .250749963           Root MSE      =  .44988
------------------------------------------------------------------------------
  MicrobialN |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          PH |  -.6439741   .4346955    -1.48   0.213    -1.850882    .5629342
       _cons |   9.609605   2.493955     3.85   0.018     2.685276    16.53393
------------------------------------------------------------------------------



regress MicrobialP PH
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  1,     4) =    3.05
       Model |  51.5127609     1  51.5127609           Prob > F      =  0.1558
    Residual |  67.6071826     4  16.9017956           R-squared     =  0.4324
-------------+------------------------------           Adj R-squared =  0.2906
       Total |  119.119943     5  23.8239887           Root MSE      =  4.1112

------------------------------------------------------------------------------
  MicrobialP |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          PH |  -6.934988   3.972416    -1.75   0.156    -17.96418    4.094207
       _cons |   48.61469   22.79073     2.13   0.100    -14.66251    111.8919
------------------------------------------------------------------------------
```

Tumtum

```
regress PH Copper TotalCopper
      Source |       SS       df       MS              Number of obs =       8
-------------+------------------------------           F(  2,     5) =    0.55
       Model |  .312254751     2  .156127376           Prob > F      =  0.6106
    Residual |  1.43134532     5  .286269064           R-squared     =  0.1791
-------------+------------------------------           Adj R-squared = -0.1493
       Total |  1.74360007     7  .249085724           Root MSE      =  .53504
------------------------------------------------------------------------------
          PH |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |  -.0480881   .1246388    -0.39   0.716    -.3684822    .2723061
```

```
 TotalCopper |   -.002611   .0031947    -0.82   0.451    -.0108233    .0056013
       _cons |   6.538005   .9470624     6.90   0.001     4.103504    8.972507
------------------------------------------------------------------------------
  regress OrganicMatter Copper
TotalCopper

      Source |       SS       df       MS              Number of obs =       8
-------------+------------------------------           F(  2,     5) =    0.64
       Model |   1.9749636     2  .987481801           Prob > F      =  0.5655
    Residual |  7.71198659     5  1.54239732           R-squared     =  0.2039
-------------+------------------------------           Adj R-squared = -0.1146
       Total |   9.6869502     7  1.38385003           Root MSE      =  1.2419
------------------------------------------------------------------------------
OrganicMat~r |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |   .3151921   .2893104     1.09   0.326    -.4285039    1.058888
 TotalCopper |   -.000168   .0074156    -0.02   0.983    -.0192304    .0188944
       _cons |  -.0728771   2.198313    -0.03   0.975    -5.723821    5.578066
------------------------------------------------------------------------------
regress MicrobialC PH
      Source |       SS       df       MS              Number of obs =       8
-------------+------------------------------           F(  1,     6) =    0.09
       Model |  18.6698953     1  18.6698953           Prob > F      =  0.7786
    Residual |  1295.63647     6  215.939411           R-squared     =  0.0142
-------------+------------------------------           Adj R-squared = -0.1501
       Total |  1314.30636     7  187.758052           Root MSE      =  14.695
------------------------------------------------------------------------------
  MicrobialC |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          PH |  -3.272258   11.12865    -0.29   0.779    -30.50309    23.95857
       _cons |   192.6154   62.31483     3.09   0.021     40.13655    345.0943
------------------------------------------------------------------------------
regress MicrobialN PH

      Source |       SS       df       MS              Number of obs =       8
-------------+------------------------------           F(  1,     6) =    0.09
       Model |  .047570669     1  .047570669           Prob > F      =  0.7768
    Residual |  3.24618002     6  .541030004           R-squared     =  0.0144
-------------+------------------------------           Adj R-squared = -0.1498
Total |  3.29375069     7  .470535813           Root MSE      =  .73555

------------------------------------------------------------------------------
  MicrobialN |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          PH |  -.1651757   .5570411    -0.30   0.777    -1.528206    1.197855
       _cons |    7.63418   3.119149     2.45   0.050     .0018975    15.26646
------------------------------------------------------------------------------
regress MicrobialP PH
      Source |       SS       df       MS              Number of obs =       8
-------------+------------------------------           F(  1,     6) =    0.08
       Model |    2.123941     1    2.123941           Prob > F      =  0.7836
    Residual |  154.467245     6  25.7445408           R-squared     =  0.0136
-------------+------------------------------           Adj R-squared = -0.1508
Total |  156.591186     7  22.3701694           Root MSE      =  5.0739

------------------------------------------------------------------------------
  MicrobialP |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
          PH |   1.103692   3.842547     0.29   0.784    -8.298681    10.50606
       _cons |    4.18515   21.51632     0.19   0.852    -48.46339    56.83369
------------------------------------------------------------------------------
.
```

15-30cm ANOVA:

PH

Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.030000038	1	.030000038	3.00	0.2254
Within groups	.020000057	2	.010000029		
Total	.050000095	3	.016666698		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.100833316	1	.100833316	0.98	0.4274
Within groups	.206666622	2	.103333311		
Total	.307499938	3	.102499979		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.008000004	1	.008000004	0.09	0.7811
Within groups	.259999886	3	.086666629		
Total	.267999889	4	.066999972		

Nitrogen

Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.000675	1	.000675	6.75	0.1217
Within groups	.0002	2	.0001		
Total	.000875	3	.000291667		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.000033333	1	.000033333	0.03	0.8740
Within groups	.002066667	2	.001033333		
Total	.0021	3	.0007		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.00002	1	.00002	0.04	0.8630
Within groups	.0017	3	.000566667		
Total	.00172	4	.00043		

Organic Carbon

Akkaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.097200008	1	.097200008	6.19	0.1306
Within groups	.0314	2	.0157		
Total	.128600007	3	.042866669		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.001408335	1	.001408335	0.01	0.9372
Within groups	.356066626	2	.178033313		
Total	.357474961	3	.11915832		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.0845	1	.0845	0.97	0.3965
Within groups	.260299988	3	.086766663		
Total	.344799987	4	.086199997		

Organic Matter

Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.291408298	1	.291408298	6.00	0.1339
Within groups	.097066666	2	.048533333		
Total	.388474964	3	.129491655		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.004408342	1	.004408342	0.01	0.9348
Within groups	1.03386661	2	.516933305		
Total	1.03827495	3	.34609165		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.271444989	1	.271444989	1.05	0.3800
Within groups	.772275027	3	.257425009		
Total	1.04372002	4	.260930004		

Cadmium

Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.005633333	1	.005633333	8.89	0.0964
Within groups	.001266667	2	.000633333		
Total	.0069	3	.0023		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.004408333	1	.004408333	8.27	0.1027
Within groups	.001066667	2	.000533333		
Total	.005475	3	.001825		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.00578	1	.00578	0.53	0.5204
Within groups	.032900003	3	.010966668		
Total	.038680003	4	.009670001		

Copper

Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	1.24807508	1	1.24807508	1.21	0.3859
Within groups	2.06179998	2	1.03089999		
Total	3.30987506	3	1.10329169		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	8.73813294	1	8.73813294	2.33	0.2661
Within groups	7.4848673	2	3.74243365		
Total	16.2230002	3	5.40766675		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	7.30840461	1	7.30840461	3.00	0.1817
Within groups	7.30827545	3	2.43609182		
Total	14.6166801	4	3.65417002		

Total Cadmium

Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.140833339	1	.140833339	325.00	0.1331
Within groups	.000866667	2	.000433334		
Total	.141700006	3	.047233335		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.138674995	1	.138674995	2.36	0.2644
Within groups	.117599994	2	.058799997		
Total	.256274989	3	.085424996		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	.108045009	1	.108045009	3.18	0.1728
Within groups	.102075008	3	.034025003		
Total	.210120017	4	.052530004		

Total Copper

Akaasu

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	10266.1649	1	10266.1649	5.69	0.0398
Within groups	3606.87021	2	1803.4351		
Total	13873.0351	3	4624.34503		

Kyeikrom

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	21741.3506	1	21741.3506	27.21	0.0348
Within groups	1598.30014	2	799.150072		
Total	23339.6507	3	7779.88358		

Tumtum

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	29816.9583	1	29816.9583	32.49	0.0107
Within groups	2753.22765	3	917.74255		
Total	32570.186	4	8142.54649		

Akaasu

```
regress PH Copper TotalCopper
```

```
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    1.85
       Model |  .098000166     2  .049000083           Prob > F      =  0.2997
    Residual |  .079483054     3  .026494351           R-squared     =  0.5522
-------------+------------------------------           Adj R-squared =  0.2536
       Total |   .17748322     5  .035496644           Root MSE      =  .16277
------------------------------------------------------------------------------
          PH |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |  -.1309788    .069676    -1.88   0.157    -.3527188    .0907613
 TotalCopper |  -.0008599   .0018714    -0.46   0.677    -.0068157    .0050958
       _cons |   6.005924   .4930456    12.18   0.001     4.436833    7.575015
------------------------------------------------------------------------------
regress OrganicMatter Copper TotalCopper
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    0.01
       Model |  .005333446     2  .002666723           Prob > F      =  0.9917
    Residual |  .952016471     3  .317338824           R-squared     =  0.0056
-------------+------------------------------           Adj R-squared = -0.6574
       Total |  .957349917     5  .191469983           Root MSE      =  .56333
------------------------------------------------------------------------------
OrganicMat~r |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |   .0128878   .2411393     0.05   0.961    -.7545251    .7803006
 TotalCopper |   .0008393   .0064768     0.13   0.905    -.0197728    .0214514
       _cons |   .7012381   1.706365     0.41   0.709    -4.729178    6.131654
------------------------------------------------------------------------------
```

Kyeikrom

```
regress PH Copper TotalCopper
      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    0.05
       Model |  .037090431     2  .018545216           Prob > F      =  0.9479
    Residual |  1.02044354     3  .340147845           R-squared     =  0.0351
-------------+------------------------------           Adj R-squared = -0.6082
       Total |  1.05753397     5  .211506793           Root MSE      =  .58322
------------------------------------------------------------------------------
          PH |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |   .0522721   .1626574     0.32   0.769    -.4653762    .5699204
 TotalCopper |   .0017935   .0079638     0.23   0.836    -.0235508    .0271378
       _cons |   4.840328   2.270503     2.13   0.123    -2.385427    12.06608
------------------------------------------------------------------------------
regress OrganicMatter Copper TotalCopper

      Source |       SS       df       MS              Number of obs =       6
-------------+------------------------------           F(  2,     3) =    6.74
       Model |  2.19983371     2  1.09991686           Prob > F      =  0.0776
    Residual |  .489299486     3  .163099829           R-squared     =  0.8180
-------------+------------------------------           Adj R-squared =  0.6967
Total |   2.6891332     5   .53782664           Root MSE      =  .40386

------------------------------------------------------------------------------
OrganicMat~r |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |  -.1227423   .1126333    -1.09   0.356    -.4811915     .235707
 TotalCopper |   .0138214   .0055146     2.51   0.087    -.0037284    .0313712
       _cons |  -1.114171   1.572226    -0.71   0.530    -6.117696    3.889355
------------------------------------------------------------------------------
```

Tumtum

```
. regress PH Copper TotalCopper
      Source |       SS       df       MS              Number of obs =       8
-------------+------------------------------           F(  2,     5) =    2.83
       Model |   .79530017     2  .397650085           Prob > F      =  0.1506
    Residual |  .702186744     5  .140437349           R-squared     =  0.5311
-------------+------------------------------           Adj R-squared =  0.3435
       Total |  1.49748691     7  .213926702           Root MSE      =  .37475

------------------------------------------------------------------------------
          PH |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |  -.1565873   .0764545    -2.05   0.096    -.3531199    .0399453
 TotalCopper |  -.0036268   .0022307    -1.63   0.165     -.009361    .0021074
       _cons |   6.904359   .6692889    10.32   0.000     5.183897    8.624821
------------------------------------------------------------------------------

regress OrganicMatter Copper TotalCopper
      Source |       SS       df       MS              Number of obs =       8
-------------+------------------------------           F(  2,     5) =    1.79
       Model |  .821231474     2  .410615737           Prob > F      =  0.2593
    Residual |  1.14735594     5  .229471188           R-squared     =  0.4172
-------------+------------------------------           Adj R-squared =  0.1840
       Total |  1.96858741     7  .281226773           Root MSE      =  .47903

------------------------------------------------------------------------------
OrganicMat~r |      Coef.   Std. Err.      t    P>|t|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
      Copper |   .1767987   .0977296     1.81   0.130    -.0744232    .4280205
 TotalCopper |   .0026555   .0028514     0.93   0.394    -.0046743    .0099854
       _cons |  -.6092941   .8555323    -0.71   0.508     -2.80851    1.589922
------------------------------------------------------------------------------
```