DETERMINATION OF CHLOROTHALONIL AND COPPER FUNGICIDE RESIDUES IN SOIL, COFFEE, WATER AND SOME ANNUAL FOOD INTERCROP FROM KIAMBU DISTRICT IN KENYA

EGERTON UNIVERSITY LIBRARY
RECEIVED
18 MAY 2022
P. O. BOX 536,
EGERTON
ACQUISITION SECTION

BY

ATONIA PAUL IKMAT

The thesis Submitted to the Graduate School in partial Fulfillment for the Requirements of the Master of Science degree in Chemistry of Egerton University

Egerton University

August 2006

EULIB
083014

2023/119850

EGERTON UNIVERSITY
LIBRARY

DECLARATION AND RECOMMENDATION

DECLARATION

I hereby declare that this is my original work, and that it has not been presented for examination in any academic institution that I know of.

Signature: .....

Name: Atonia Paul Ikmat. Date: 1/09/2006

RECOMMENDATION

This thesis has been presented with my approval as supervisor:

Signature: .....

Name: Dr. C. M. Nguta. Date: 1/09/06

20231198

DEDICATION

To dad Michael A. Ikmat,

And mum Veronica A. Lomojong.

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATION II

RECOMMENDATION II

DEDICATION III

TABLE OF CONTENTS IV

LIST OF TABLES VII

LIST OF FIGURES VIII

ACKNOWLEDGEMENT X

ABSTRACT XI

CHAPTER ONE 1

INTRODUCTION 1

1.1 Fungicides 1

1.2 Coffee diseases 1

1.3 Statement of the problem. 2

1.4 Objectives of the study. 2

1.5.1 Hypotheses 3

1.5.2 Justification of study 3

1.5.3 Limitations of study 3

CHAPTER TWO 5

LITERATURE REVIEW 5

2.1 Chlorothalonil sources and uses 5

2.1.1 Chemical structure and properties 5

2.1.2 Dissipation and degradation metabolites 6

2.1.3 Extraction methods 9

2.1.4 Chlorothalonil residue levels 9

2.2 Copper sources and uses 10

2.2.1 Sources of copper 10
2.2.2 Fate/ bioaccumulation of copper pesticide residue 11
2.2.3 Extraction methods 12
2.3 Soil pH and hp determination. 13
2.4 Coffee processing 14
CHAPTER THREE 16
RESEARCH METHODS 16
3.1 Area of study 16
3.2 Sampling 19
3.3 Plant analysis 20
3.4.1 pH determination 22
3.4.2 Hp determination 22
3.4.3 Extraction of chlorothalonil pesticide residues from soil and sediment samples 22
3.4.4 Total copper in soil and sediment 22
3.4.5 EDTA extractable copper in soil 23
3.5 Extraction and analysis of chlorothalonil in water samples 23
3.6.1 Roasting and grinding of coffee beans 23
3.6.2 Total copper in plant tissue and marketable coffee 24
3.7 Copper and chlorothalonil standards. 24
3.8 Separation of different forms of copper in the soil. 24
3.9 Florisil clean-up 25
3.10 Eluent Selection. 25
3.11 Chlorothalonil recovery studies. 26
3.12 Instrumentation for GC-ECD and AAS. 26

CHAPTER FOUR 28

RESULTS 28

4.1 Plant Tissue 34

4.2 Water 38

CHAPTER FIVE 43

DISCUSSION 43

5.1 Copper. 43

5.2 Chlorothalonil 47

CHAPTER SIX 51

SUMMARY, CONCLUSION AND RECOMMENDATIONS 51

REFERENCE. 53

APPENDICES 60

1. GC-ECD chromatogarphs: 60
2. Questionnaires to Farm/Estate Manager 64
3. Farm Landscape feature 65
4. Kiambu Soil Chemical characteristics 66
5. Paired samples t test 67

5 a: Paired samples t test for levels of chlorothalonil in the two seasons 67

5 b: Paired samples t test for chlorothalonil levels of soils in large and small-scale. 67

6. Data for Correlation analysis 68
7. Correlation for EDTA-extractable Copper and Total Copper in the soil in the 1st season 68
8. Correlations for 1ST and 2nd season Total Copper in the soil 68
9. Rivers network and sampling points in Kiambu and Thika district 70
10. Pearson correlation data for Copper, Boron and Phosphorus in soil and plant tissue 71
11. Mechanism of copper tolerance in plants 72

12a: Soil analysis results (May - July Season). n = 24 73

12b: Soil analysis result (September – October Season) n = 24 74

LIST OF TABLES

Table 2.1: Decline of chlorothalonil residues 10

Table 2.2: Comparative water consumption totals for Kiambu District in 1994. 14

Table 3.1: Estates' name, Fungicide spray regime and the agronomic practices done. 16

Table 3.2 Intensity and frequency of spray of copper and chlorothalonil fungicides 17

Table 3.3: Climatic conditions for coffee growing regions 18

Table 3.4: Procedures for the sequential extraction of copper in soil 25

Table 3.5 Recovery Studies. 26

Table 4.1: Comparison of chlorothalonil levels in soils in large and small-scale farms 28

Table 4.2 Comparison between chlorothalonil concentrations in soils at different farm landscapes n = 24 29

Table 4.3: Total / EDTA -Extractable copper in Soil (May- July season) n = 24 32

Table 4.4 a: Forms of Copper in soil (May - July season) using $MgCl_2$ as extractant n = 8 33

Table 4.4 b: Forms of Copper in soil (May - July season) using NaOCl as extractant n = 8 34

Table 4.5 a: Levels of copper and Chlorothalonil in Potato intercrop n = 4 34

Table 4.5 b: Levels of copper and Chlorothalonil in Beans intercrop n = 4 35

Table 4.6: Levels of copper and chlorothalonil in coffee leaves n = 10 35

Table 4.7: Levels of copper and chlorothalonil in Coffee Husks n = 10 36

Table 4.8: Levels of Copper and Chlorothalonil in Coffee beans n = 8 36

Table 4.9: Copper and Chlorothalonil levels in roasted and ground coffee beans n = 4 37

Table 4.10: Copper and Chlorothalonil Levels in Kenyan marketable Coffee n = 3 37

Table 4.11: Copper and chlorothalonil level (May - July) 38

Table 4.12: Copper and Chlorothalonil levels in pulping water n = 3 39

Table 4.13: Copper and Chlorothalonil levels in Laboratory pulping water n = 3 39

LIST OF FIGURES

Figure 1: 2,3,4,6 tetrachloro - 1,3 - benzenedicarbonitrile (Chlorothalonil). 5

Figure 2: Suggested pathway for the degradation of chlorothalonil 8

Figure 3: Degradation of chlorothalonil in peat loam soil. 8

Figure 4: Outline scheme for analysis of Copper and Chlorothalonil in plant tissue 21

Figure 5: Outline scheme for analysis of Copper and Chlorothalonil in soil 21

Figure 6: Decline of Chlorothalonil in Yadini sub soil with time. 30

Figure 7: Decline of chlorothalonil in Tassia sub-soil with time 31

Figure 8: Copper levels in soil sediment 40

Figure 9: Chlorothalonil levels in sediment 41

Figure 10: Copper levels in water along Ruiru River. 41

Figure 11: Chlorothalonil levels in water along Ruiru River 42

LIST OF SYMBOLS AND ACRONYMS

CRF	- Coffee Research Foundation
CBD	- Coffee berry disease
CEC	- Cation Exchange Capacity
EDTA	- Ethlynediamine tetracetic acid
GLC	- Gas Liquid Chromatography
Hp	- Exchangeable acidity
R11	- Ruiru 11 coffee breed
WP	- Wettable powder
WTO	- World Trade Organization

ACKNOWLEDGEMENT

I wish to register my appreciation to all those, in one way or another, enabled me to successfully complete this work. Particular recognition goes to the management, staff, and friends at Coffee Research Foundation (CRF), Ruiru. To Dr. C. M. Nguta (supervisor) and Mr. J.N. Mburu of CRF, my field advisor: your input was invaluable. To my family and friends, for the patience and understanding, I say *ejok'o noi*.

ABSTRACT

In the process of application to agricultural crops, fungicides spread into the surrounding environment, such as the soil surface, on food crops growing near the area of application and the water bodies. Copper bordeaux mixtures and chlorothalonil, an organochlorine fungicide, are most commonly used to fight fungal attack in coffee. If used repeatedly on coffee farms copper-based fungicides and chlorothalonil get deposited on, or are absorbed by the coffee plant, and on being processed get to the consumer. Chlorothalonil and copper pesticide residue levels in coffee beans and leaves, food intercrop (beans and potatoes), market coffee beverages, soil and water samples were determined. A gas liquid chromatographic method was used for determining the chlorothalonil residue levels while atomic absorption spectrometry method was used for copper determination. Crop and soil samples for chlorothalonil analysis were extracted using phosphoric acid and recovered in hexane. Recoveries of chlorothalonil from soil fortified with 1.00ppm chlorothalonil were carried out and mean recovery of 74.1% recorded. Analysis of soil samples for determination of total and extractable copper by nitric – perchloric acid and EDTA methods were done. The results of these analyses on soil showed levels of 0.037 – 3.186 ppm for chlorothalonil and 2.0 – 172.2 ppm and 0.30 – 50.61 ppm for Total and Available copper in soil respectively. An experiment involving sequential extraction of soil samples for copper cations using sodium oxychloride (NaOCl) and magnesium chloride ($MgCl_2$) was carried out. Statistical analysis of data obtained from this experiment showed a positive correlation between Cu (I) in soil and total copper in plant tissue. This was an indication that application of Cu (I) sprays (Cu_2O) unlike Cu (II) (CuO, $Cu(OH)_2$) into coffee plantations could lead to high levels of copper in plant tissue and the possible build up to toxic levels. Market coffee beverages were found to have copper residue mean levels of 0.833 ppm. Mean chlorothalonil residue levels in different media were determined as follows: coffee beans 0.044 ppm, coffee leaves 0.803 ppm, leaves of potato intercrop 0.723 ppm, leaves of beans intercrop 0.677 ppm market coffee beverage 0.031 ppm, soil samples 0.542 ppm and water samples 0.457 ppm. The mean levels for both copper and chlorothalonil in marketable coffee were found to fall below the maximum recommended levels by the WHO and FAO and therefore posed no threat to health on consumption. SPSS was used for data analysis.

CHAPTER ONE

INTRODUCTION

1.1 Fungicides

Fungicides are important in the management of coffee diseases especially on susceptible varieties and in environments favorable for the fungus. In deciding when and what to spray, any given fungicide application has to be considered as a long-term investment, with effects not only in the current season but also in future seasons as well. Copper-containing fungicides are very effective in controlling coffee rust and copper has a "tonic effect" on coffee plants, that is, it increases yields independent of its effect in rust control. One disadvantage of using copper-containing fungicides is that they must be present on the leaves before infection occurs. Another disadvantage, aside from cost, is that copper with time and repeated application accumulates in the soil, particularly in the organic matter, and it can reach levels toxic to plants and to other organisms in the environment (Technical circular no.56, 1984). The timing of the applications and the coverage are important. Generally, the intervals between sprays should be less than 21 days to be sure to keep new growth covered. The timing of fungicide applications depends on the epidemiology of the pathogen and the persistence of the chemical being used. Weather conditions especially rainfall is important in determining when pathogens spread and infect crop so that in general, protection of the crop is only needed during the rainy season.

1.2 Coffee diseases

Coffee leaf rust caused by *hemilleia vastatrix* Berk, is the most serious leaf disease of coffee in the low and medium altitude districts of Kenya. Copper formulations, applied at 4-6 weeks interval using 3-5 kg/ha as the standard rate has been observed to be effective against leaf rust (Masaba, 1987). Coffee berry disease (CBD), caused by *colletotricum kahawae* Noack, has been a major disease of *coffee arabica* ever since it was reported in Kenya in 1921 (McDonald, 1926). It attacks young expanding berries (5-20 weeks old) producing young sunken anthracnose lesions that cause fruit rotting and shedding. The disease is favored by wet conditions, as rainfall is required for spore production, dispersal and infection. Fungicidal control of coffee berry disease in Kenya was the subject of a detailed study between 1956 and 1960 (Bock, 1963; Nutman and Roberts, 1960 a, b). The most effective fungicides were found to be 50% copper formulations

and phenyl mercuric acetate, although the latter was abandoned at an early stage due to phytotoxic effects. Field trials comparing the efficiency of nine fungicides for the control of CBD in Kiambu district indicated that tank mixtures of Daconil (chlorothalonil) 75% WP and copper Nordox 50% WP, applied at 3 kg/ha on a monthly basis gave the best control of CBD as well as the highest yields (Mwang'ombe and Javed, 1984). Either chlorothalonil or copper can be used alone especially during the hot season when infestation by the pest is reduced. This serves to cut down on the high pesticide cost. It is worth noting that for along time, there has been no readily available organic substitute to chlorothalonil. Lately, however, there has been field trials going on at CRF to measure the efficacy of pyrethrins as a possible substitute to chlorothalonil.

1.3 Statement of the problem.

In the process of application to agricultural crops, fungicides are known to spread in the surrounding environment, such as the soil surface, on food crops growing near the area of application and the water bodies in the surrounding. If used repeatedly on coffee farms copper-based fungicides and chlorothalonil get deposited on, or are absorbed by the coffee plant, and on being processed get to the market ready for consumer use. The amount of residue in the coffee bean is due to the frequency of application, time of application and the amount of fungicide used during each spray. If the residue values are too high in the coffee beans they might pose a danger to the consumer.

1.4 Objectives of the study.

The major objective of this study was to determine the levels of copper and chlorothalonil fungicides in Kenya's marketable coffee beans from Kiambu and Thika districts.

The specific objectives of the study are to:

1. Ascertain the levels of chlorothalonil and copper in;
   - Process water effluents, pulp, and husks.
   - Food inter-cropped with coffee (Potatoes and beans).
   - Adjacent water bodies (Rivers and dams).
2. Determine the form in which copper in soil is available to the coffee plant.
3. Determine the amount of copper and chlorothalonil in Kenyan marketable coffee beverages.

1.5.1 Hypotheses

1. During spraying of coffee, chlorothalonil and copper are not dissipated into coffee pulp, coffee husks, food inter-cropped with coffee, soil and adjacent water bodies
2. The levels of copper and chlorothalonil fungicide residues in Kenya's marketable coffee are not high.
3. There does not exist a reduction of the copper and chlorothalonil fungicide residues as the coffee berries undergo the various processing operations to yield the final product.

1.5.2 Justification of study

All agricultural products are subject to international competition, which means that they have to meet international quality standards, involving careful selection of plants, seeds and fruit trees, processing procedures, fertilizers and of course fungicide pesticide residues. Quality of food products has gained great significance in liberalized trade environment under the WTO regime. In this area quality has two distinct meanings viz., quality in terms of food value or taste and quality in terms of safety to human health. The Sanitary and Phytosanitary (SPS) Agreement under the WTO primarily addresses the quality issues concerning health aspects such as macro cleanliness, mycotoxins, pesticide residues, and trace metal contamination (FAO/WHO, 1991). The trade especially in the export food crops like coffee and tea has been subjected to these conditions in the recent years. Few studies have been carried out to investigate the fungicide residue levels of marketable coffee from Kenya. This study is aimed at investigating the levels of the copper-based (inorganic) fungicide and chlorothalonil (organic) residues in coffee (as they are the most commonly used fungicide), and in so doing lay a basis for further study into possibility of the investigation of other fungicide residues.

1.5.3 Limitations of study

While undertaking this study the following limitations were noted:

1. The sampling area was too large as it included two expansive districts, Kiambu and Thika. In this regard sampling was only done for a total of 24 coffee growers consisting of 12 large and 12 small scale farms.

2. This work entirely relied on the response of the farm managers to the questionnaires sent to them. Therefore the stated spray programs are held true to the extent of how much genuine and reliable these responses were the farm managers in the selected farms were.
3. This study intended to further use other analytical technique (HPLC and polarography) to obtain more information regarding pesticide residues in plant tissue, soil and water. However, due to higher cost implication in running this instruments this was not carried out.

CHAPTER TWO

LITERATURE REVIEW

2.1 Chlorothalonil sources and uses

2.1.1 Chemical structure and properties

Chlorothalonil (2,3,4,6 tetrachloro - 1,3 - benzenedicarbonitrile), **Figure 1** below, is a colourless, odourless, crystalline solid with a melting point of 250°C and a vapour pressure of 7.63×10^{-5} Pa (5.72×10^{-7} mmHg) at 25°C.

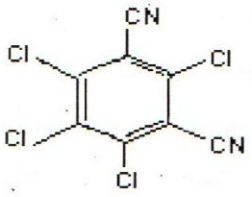
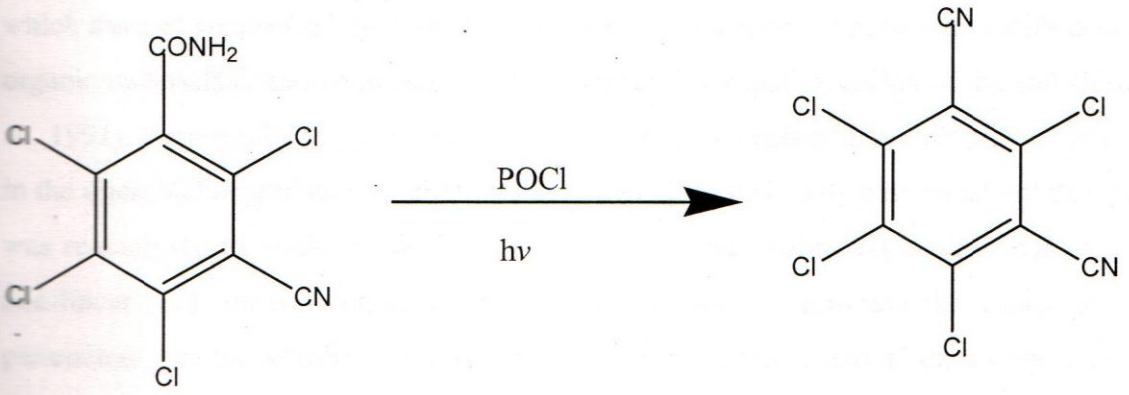


Figure 1: 2,3,4,6 tetrachloro - 1,3 - benzenedicarbonitrile (Chlorothalonil).

It has low water solubility (0.6-1.2 mg/litre at 25°C) and an octanol/water partition coefficient (log K_{ow}) of 2.882. It is hydrolysed in water slowly at pH 9 but is stable at pH 7 or below (at 25°C) (Wild, 1996). Chlorothalonil has been produced commercially since 1969 by chlorination of isophthalonitrile or by treatment of tetrachloroisophthaloyl amide with phosphorus oxychloride as shown in the reaction below.



EGERTON UNIVERSITY
LIBRARY

Several organic pesticides are available in the market for the control of pests and diseases. The organic based fungicides include triadimefon, chloropyrifos, diazinon, captan, dimetheote among others. No data on production are available but it has been estimated at 5000 tonnes annually (IARC, 1983). The annual production in Japan has been estimated to be 3000 tonnes (IARC, 1983). It is a fungicide with a broad spectrum of activity used mainly in agriculture but also on turf, lawns and ornamental plants. The three main formulations are as suspension concentrate, a water dispersible granule and a wettable powder. They are readily diluted with water and applied by ground spray systems or by air. Typical active ingredient rates are 1.2-2.5 kg/ha for crops such as beans, celery and coffee. The main sources of human exposure are during preparation and application of the products and from ingestion of crop residues in foodstuff. Considering the toxicological characteristics of chlorothalonil, both qualitatively and quantitatively, it was concluded that 0.03 mg/kg body weight per day would probably not cause adverse effects in humans by any route of exposure (IARC, 1987). The routine spraying of irrigated field crops such as potatoes and barley gave rise to low concentrations of chlorothalonil (0.04-3.6 µg/litre) in water on a small number of sampling occasions. Crop residues are composed mainly of chlorothalonil itself (IARC, 1983).

2.1.2 Dissipation and degradation metabolites

Chlorothalonil dissipation was attributed to microbial activity, a biological contribution verified by comparing changes in concentration with time in samples of non sterile and autoclaved soil which showed suppressed dissipation in the later. Amendment of soils with easily decomposable organic materials is known to recover the suppressed dissipation ability of the soil (Katayama, et al., 1991). Such results suggest that easily decomposable organic materials play an important role in the microbial degradation of chlorothalonil in soil. In this study chlorothalonil dissipation data was re-analysed to obtain half-life estimates for this metabolite. Assuming first order kinetics, non-linear least squares regression modelling was used to estimate the values of the model parameters. For the 4-hydroxy metabolite, half-lives between 6 and 43 days were determined for the various non-sterile soils. This large range is attributable to a size of sampling area, variability in rates, frequency and duration of spraying. An alternative method of data analysis, utilizing a transformation and a linearizing approximation, was also used by Jacobson and Schollenberger (1992) which gave a similar range of half-lives.

Information about the toxicology of resultant metabolite products from chlorothalonil degradation is still scanty. The effect of mulching, liming and other agronomic practices on the degradation pathway of chlorothaloni is not well defined. Acidic conditions and moderate temperatures are thought to favour the formation the 4-hydroxy metabolite **Figure 2** presents a suggested pathway and lists the structure of the five soil metabolites that have been identified in aerobic soil studies involving ^{14}C-chlorothalonil in the laboratory (Frazier, 1993). It should be noted that the scheme is a suggested pathway (Frazier, 1993) and that there is no direct evidence that any of the five soil metabolites are converted directly to "bound" residue, but serve to illustrate the expected possibilities. The percent composition of the "bound" metabolites in the soil is not defined. **Figure 2** presents a graphical representation of the degradation of chlorothalonil in peat loam soil (Stallard & Szalkowski, 1976).

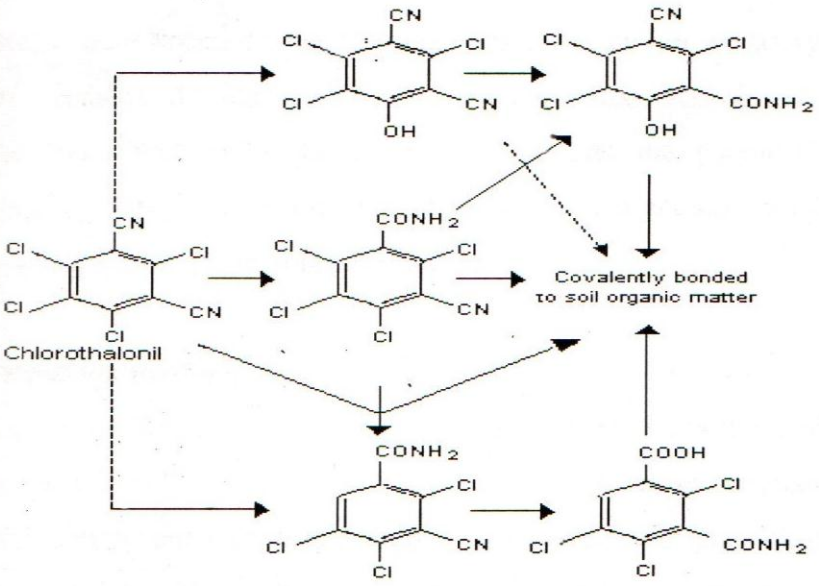


Figure 2: Suggested pathway for the degradation of chlorothalonil

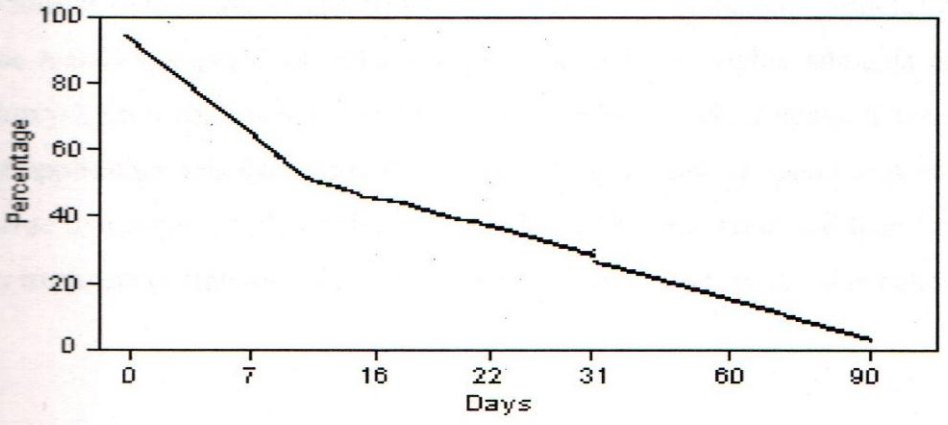


Figure 3: Degradation of chlorothalonil in peat loam soil.

Many genera of heterotrophs *(Agrobacterium, Bacillus sand Clostridium)* have been known to metabolize chlorothalonil. It is however rarely possible to predict with precision the species

responsible for the particular transformation in nature (Sato and Tanako, 1987). In the metabolism of such toxicants, heterotrophs may cause one or a variety of reactions yielding a variety of products. Products such as the chlorinated benzoic acids and the 4-hydroxy metabolites have been found to be more toxic than the parent chlorothalonil compound (Katayama *et al.*, 1991). Chlorothalonil is commonly used in crops that are susceptible to fungus attack including apples, pears, tomatoes and coffee.

2.1.3 Extraction methods

There are many multiresidue extraction methods for chlorothalonil in soils (Wild, 1996). The use of phosphoric acid helps release bound residues without causing degradation of the compounds. Partitioning with hexane will recover most of the non-polar organochlorine pesticides. The most prevalent analytical method, after sample extraction and clean-up, is gas-liquid chromatography using an electron-capture detector GC-ECD. HPLC/MS is also a predominant analytical method. However cost is the major factor inhibiting its use.

2.1.4 Chlorothalonil residue levels

The amount of chlorothalonil pesticide residue at harvest depends upon factors such as the application rate, time interval between the last application and harvest, and the type of crop. In a previous study by Stallard and Szalkowski, (1976) chlorothalonil pesticide residues were found to be mainly composed of chlorothalonil, and only negligible amounts of the metabolite 4-hydroxy-2,5,6-trichloroisophthalonitrile. The decline of chlorothalonil residues on food crops after application was demonstrated by the field treatment of apples and pears against *Botryris cynerea* by spraying with a chlorothalonil flowable formulation and then harvesting at intervals after treatment (Camoni *et al.*, 1998). The results are shown in the table below.

Table 2.1: Decline of chlorothalonil residues

Days after treatment	Pears (mg/kg)	Apples (mg/kg)
0	3.55	2.43
7	3.48	2.07
14	2.08	0.65
21	1.85	0.55

Source: Camoni et al. (1998).

Studies on the problem of chlorothalonil residues and its metabolites have been conducted for coffee in Brazil and South Africa (FAO/WHO, 1990a) and in India (Hassall, 1990). Chlorothalonil was found to translocate from the site of application to other parts of a plant. In this study it was found to metabolise only to a limited extent on plants and the 4-hydroxy metabolite is usually < 5% of the residue. Chlorothalonil has been suspected to be carcinogenic (FAO/WHO, 1989).

2.2 Copper sources and uses

2.2.1 Sources of copper

Natural sources of copper exposure include windblown dust, volcanoes, decaying vegetation, forest fires and sea spray. Anthropogenic emissions include smelters, iron foundries, power stations and combustion sources such as municipal incinerators. Its agronomic uses include use as fertilizers, bactericides, fungicides, algicides and antifouling paints. It is also used in animal feed additives and growth promoters, as well as for disease control in livestock and poultry. In coffee, copper is applied as copper (I)- based or copper (II)- based fungicide for the control of leaf rust and CBD. Agricultural use of copper products accounts for 2% of copper released to soil (Shuman, 1979). Hodgson (1963) reported that more than 98% of the copper in the soil solution was in an organically complexed form while Ellis and Knezek (1972) reviewed literature that demonstrated the existence of stable Cu complexes with organic matter. Broadbent and Bradford (1952) showed that carboxylic and phenolic functional groups are important in Cu-Organic matter bonding while Demumbrum and Jackson, (1956) suggested that copper might be bound to peat through hydroxyl group. An important aspect of copper and organic matter

complexes involves binding with fulvic and humic acids. These fulvic acid compounds may be important as the soluble forms of organic copper reported by Hodgson (1963). Humic acid is an important fraction for binding copper to organic matter in largely insoluble forms in the soil (Stevenson, 1976).

2.2.2 Fate/ bioaccumulation of copper pesticide residue

Studies on copper pesticide residues in Kenyan have concentrated in the soils and plant tissues in coffee growing areas, partly because copper based fungicides are widely used in the Kenyan coffee industry (Aduayi, 1973, Maroko, 1989, Wanjohi, 1991, Kariuki 1992). Maroko, 1989 reported a range of 10.0 – 147.0 ppm and 2.6 - 24.7 ppm of copper in the soil and coffee leaves respectively. Wanjohi (1991) observed that coffee leaves accumulated higher levels of copper showing levels of up to 5.0 – 20.0 ppm, and the coffee beans accumulate the least portraying levels of less than 3.0 ppm in Kiambu area. A study in Brazil on copper concentration in coffee plant tissue (Harris 1966) showed ranges from 5.0 – 26.1 ppm and copper deficiency occurring at less than 4.0 ppm. Copper toxicity was observed to be appearing in mature coffee leaves when levels exceed 27.0 ppm. Indian childhood cirrhosis (ICC) and idiopathic copper toxicosis (ICT) are conditions related to excess copper ingestion (FAO/WHO, 1990b).

Several processes influence the fate of copper in the aqueous environment. These include complex formation, sorption to hydrous metal oxides, clays and organic materials, and bioaccumulation. Information on the physicochemical forms of copper (speciation) is more informative than total copper concentrations as this reveal the particular copper form in different soil matrices (Aduayi, 1973). This study proposed that sufficient levels of copper are estimated at between 30 – 150 ppm. Comparing the levels of both EDTA extractable and total copper of top and sub-soil, it is clear that copper concentrations in the topsoil is higher than in the sub soil (Wanjohi, 1991; Ongeri, 1998). Much of the copper discharged to water is in particulate form and tends to settle out, precipitate out or be adsorbed by organic matter. Most copper deposited in soil is strongly adsorbed and remains in the upper few centimeters of soil (Kariuki, 1992). For various reasons there is increasing concern about copper toxicity and copper tolerance in agriculture (Willson, 1985). These reasons include the high copper content in soil caused by the long-term use of copper containing fungicides (in the coffee sector, cereals, vegetables, fruits,

and trees), industrial and urban activities and the application of pig and poultry slurry high in copper. Mechanisms of copper tolerance in plants **(Appendix 10)** are therefore of interest for crop production on copper polluted soils. High copper tolerance of high plants (including coffee trees) is as a result of its capability of restricting copper uptake and thereby preventing damage to the root cell plasma membrane (De Vos, 1992), i.e. by a combination of mechanisms 1, 2, and 5 **(Appendix 10)**. Although immobilization in the cell wall plays a role in copper tolerance, compartmentation (either as soluble or insoluble complexes) and chelation within the cytoplasm and in the vacuoles, remain the major mechanism of copper tolerance in higher plants. This holds true in particular for low molecular weight polypeptides such as phytochelatins, plastocyanins, and other polypeptides. From a copper tolerant *Agrostis* species, a phytochelatin has been isolated which has 20.8% cysteine residue and binds 54 mg copper per gram protein (Marschner, 1995). In an exploratory study to access the possibility of using husks as animal feed supplement Kiptoo (2000) labeled several micronutrients, including copper and zinc as being antinutritive agents in the pulp. This study determined levels of upto 0.64 ppm copper in the coffee husks, and went further to suggest ways on how to make coffee pulp attractive as an animal feed additive.

2.2.3 Extraction methods

Copper displays four oxidation states: metallic copper Cu^0, cuprous ion Cu^+, cupric Cu^{2+} and trivalent copper ion Cu^{3+}, the latter being highly unstable. The sequential extraction procedure which consist of subjecting a soil sample to successive attacks with two reagents of different chemical properties (acidity, redox potential, complexing properties), is most commonly used to separate between these forms (Westerman, 1990). Other researchers in the field of speciation go a step further and alter the exchangeable cations (Maroko, 1989, Ongeri, 1992), to improve on the yield of copper ions extracted. Each reagent extracted part of the copper associated with the sample:

- Mg^{2+} exchangeable, **(Cu^{2+}).**
- Related to organic matter, **(Cu^+).**
- Residual copper shall be the copper forms that are covalently bound to the soil matrix. This is total copper concentration less the total copper concentration of the above 2 steps of extractions.

Colorimetric and gravimetric methods for the measurement of copper are simple to use and are inexpensive (Shuman, 1979); however, their usefulness is limited to situations where extreme sensitivity is not essential. These colorimetric methods suffer the disadvantage that the colored complexes are decomposed by strong light. For measurement of ultra-low to low concentrations of copper in studies like pesticide residue and metal contamination in various matrices, atomic absorption spectrophotometry (AAS) and atomic emission spectrophotometry (AES) are the methods commonly used, due to their high accuracy and sensitivity.

2.3 Soil pH and hp determination.

Various factors influence the availability of copper to the coffee plant. These include organic matter as observed by Masaba, (1987), soil moisture and soil texture (Maroko 1989) and pH and CEC (Aduayi, 1973). Obviously the effect of one factor on Cu (or other micronutrients) availability cannot be treated in isolation. The inconsistencies in results obtained by studies to ascertain the availability of copper micronutrients due to a particular factor for example pH (Stevenson, 1976 and Smith, 1996) reveal the difficulty of endeavoring to relate the availability of copper to any one factor. These studies however underlined the importance of stating the pH and the Hp regime under consideration, hence the need to carry out these measurements in this study.

Soil pH can be measured colorimetrically by use of an organic acid whose undissociated form has a different colour than the anion in the acid. The disadvantages in this method are that one must remove all soil to ascertain the colour of the solution. Soil pH can also be measured electrometrically (Mitchell, 1964; Smith, 1996). In this method the potential difference between the H^+ electrode and the reference electrode is measured. The apparent pH of the soil will depend on the amount of water added to the soil to make the suspension or the dispersion. The pH increases in wet weather due to amount of water percolating the soil. It also increases in soils under crop due to the rate of nitrification. Soil pH in the field is rarely uniform; variation of up to 1.0-1.5 units may easily be found in similar soil samples taken on the same day. These variations of soil pH can be reduced by making measurements in a salt solution that is sufficiently strong to swamp the effects/changes that occur naturally, with a low or as little cation exchange as possible. Smith (1996) proposed the use of 0.01M Calcium Chloride solution for temperate soils.

Cation exchange capacity (CEC) of a soil colloid is directly related to the soil organic matter and the clay content of the soil. The negatively charged surface of the soil colloid is normally neutralized by cations (H^+, Ca^{2+}, Cu^{2+}) that are readily replaced through simple exchange by other cations in the soil solution. These are the exchangeable cations whose proportion is directly related to the concentration of the replacing cations in the soil. Exchangeable acidity (Hp) of any soil is measured where the targeted exchangeable cation is H^+. The capacity of the soil to retain and exchange H^+ cation is an important soil property and is a measure of the ability of the soil to store available nutrients for gradual release to the crops. This is normally measured using NH_4Ac at pH 7.0 (Cheng and Bray, 1953). $MgCl_2$ and NaOCl and $BaCl_2$ have also been used to measure this parameter (Shuman, 1979).

2.4 Coffee processing

Kiambu District alone produces more than 129,000 metric tonnes per annum (Anon, 1998). The wet method is predominantly used for processing arabica coffee which is the main variety grown in Kenya. The operations of this process involve the use of enormous amount of water, which sometimes overloads the disposal pits occasionally ending up in watercourses (Mwaura, 1995). The table below shows the water consumption demand of the wet method of coffee processing, as compared to agriculture, industrial, livestock and domestic water demand.

Table 2.2: Comparative water consumption totals for Kiambu District in 1994.

Sector.	**Annual water consumption (MCM).**	**%.**
Coffee processing	0.65	2.3
Agriculture	14.50	51.7
Livestock use	8.80	31.4
Domestic use	2.50	8.9
Industrial use	1.60	5.7
Total	**28.05**	**100.0**

Source: ODA, 1996. **MCM. – Metric Cubic Meters.**

Although wet processing produces high quality coffee, the waste water discharged during the operations constitute high organic matter either dissolved in effluent or associated with suspended material. This results in objectionable colour, turbidity, smell of the water, and sometimes death of aquatic organisms, implying a reduction in the rivers' resource value (Githunguri, 1991). This study revealed levels of up to 76.0 ppm copper in river sediment along the Athi River. Despite the dilution effect from increased flow in rivers during the processing season, significant impact on surface water quality from coffee processing occurs particularly where seepage pits (generally located within 30–50 m of surface water) overflow into surface waters, untreated. Mwaura (1995) in a study on Nairobi River reported that the pollution potential of effluent discharged from the production of one tonne of clean coffee is equivalent to 273 m^3 of crude domestic sewage. He reported levels of up to 46 ppm copper and 68 ppm zinc in Ruiru river water. Frazier, 1993 in study on the fate of chlorothalonil sprays in coffee found levels of up to 37.0 ppm in ground water and 101ppm in sediments in Painesville, Ohio USA.

The ultimate in coffee beverages is soluble or instant coffee manufactured industrially from Arabica coffee. It has been suggested that the process of roasting coffee and cooking beverages reduces its residue levels (Onianwa, 1998). In this study on Nigerian Robusta coffee the highest loss of chlorothalonil fungicide (over 60%) was attributed to roasting and processes involved in the preparation of fine coffee. Spray drying and freeze drying, the commercially used methods of drying coffee extracts to produce soluble coffee beverages, are long, exacting and carefully controlled processes (Clifford, 1991). Freeze drying, used since 1965, is the more common of these processes. The water in the extract is first frozen and the ice gradually sublimed off, under a high vacuum, with the controlled application of heat. It is at this stage that most coffee beverages loose a lot of pesticides residues as proposed in the studies by Frank, (1984) and Demeke (1993).

CHAPTER THREE
RESEARCH METHODS

3.1 Area of study

A questionnaire **(Appendix 2)** was designed to ascertain farms that did spray copper and chlorothalonil against those that did not. The farms so selected represented an area of 1028 ha of coffee trees, consisting of Ruiru 11 (R11) and SL 37 varieties of Arabia coffee. Other agronomic practices in each farm selected are as shown in **Table 3.1**.

Table 3.1: Estates' name, Fungicide spray regime and the agronomic practices done.

Estates/ farms	Fungicides spray regime and other agronomic practices.
Rukera, Azania estates	No copper and chlorothalonil sprayed for the past 3 years on Ruiru 11 and SL 37.
Mbucho's and Kahowai's farms (Two small holdings in Kigaa factory)	Ruiru 11 variety of Arabica coffee planted. This variety is not susceptible to CBD and leaf rust infection and hence no copper and chlorothalonil sprays necessary. Organic manure, NPK, and nitrogenous fertilizers are regularly applied. CuO was applied for food intercrop.
Kibubuti, Tassia, Yadini, Ibonia, Cianda Estates, Njoroge and Njungua's farms.	Regular copper and chlorothalonil sprays and fertilizer application. 50% Cu_2O and Bordeaux mixtures applied. Foliar spray supplements and mulching done regularly. These farms plant SL 37 and French mission variety.

Research in pesticide chemistry has been focused in the relationship between the pesticide and the microorganism, evaluation of potential pollutants to the environment and pesticide residue levels in the soil, water and plant tissue. This work starts with small plots observations, then progress to large scale sampling and testing on Coffee Research foundation farms, and then to a multi-location observation of the fields of co-operating farmers, as a prelude to a recommendation for the farmers of an area. The rates of application of the pesticides are

determined and recommended by the Coffee Research Foundation. The experimental controls are the experimental plots and estates under routine care of the Coffee Research Foundation, and for the farms, the controls are farms that are known not to have used copper and chlorothalonil pesticides sprays for the past five years. While comparing the results obtained the following differences were taken into consideration:

1. **Intensity / frequency of spray**.

Table 3.2 Intensity and frequency of spray of copper and chlorothalonil fungicides

Fungicide	Low sprayers	High sprayers
Chlorothalonil	> 2.2 kg / Ha / spray.	< 2.2 kg / Ha / spray.
Copper	**R 11.** ≥3.3kg / Ha / spray. **SL Arabicas.** ≥5.5kg/ Ha × 5 spray for leafrust control, and ≥7.7kg/ Ha × CBD months. The copper fungicides contain 50% copper.	**R 11.** <3.3kg / Ha / spray. **SL Arabicas.** <5.5kg/ Ha × 5 spray for leafrust control, and <7.7kg/ Ha × CBD months.

Conversion: 1.0kg/ha/spray 100 g/L

Copper in the farms selected was sprayed at the above rates twice a year during the preceding 5 years and in May and November of the current year for Ruiru 11 variety of coffee. For the SL Arabica the above rates were applied during the CBD months of May, June, July, October and November of all the 5 years. Chlorothalonil is sprayed during the CBD months (May, June, July, October and November) at the rates mentioned above. This information is also obtained from the questionnaires **(Appendix 2)**.

2. **Ecological zone**. Agro–ecological zonation of coffee growing areas in Kenya is separated into 4 zones that differ in altitude, rainfall and temperature. Kiambu district mainly being UM1 and the UM2 zones and Thika district as UM3, and the UM4 as shown in the table

below. This zonation (**Table 3.3**) takes care of the different climatic zones necessary for comparison of results caused by the difference in climatic conditions.

Table 3.3: Climatic conditions for coffee growing regions

Zone	Region	Altitude (m ASL)	Annual Rainfall (mm)	Annual Mean Temp. (°C)	Soil Description	Farms/ estates in region
UM1	Kiambu.	1500-3000	>2000	13-22	Moderate to high fertility clays	-Kibubuti. -Chianda -Ibonia
UM2	Kiambu.	1200-2000	1200-1500	18-25	Moderate to high fertility clays	-Rukera -Yadini. -Most small scale holdings.
UM3	Thika.	900-1800	600-1200	18-28	Low fertility and low drainage sands	- Tassia.
UM4	Thika	< 900	< 600	< 24	Low fertility and low drainage sands	-Azania.

3. **Scale.** Small-scale farmer (< 5 Ha) and large-scale farmers (> 5 Ha)

determined and recommended by the Coffee Research Foundation. The experimental controls are the experimental plots and estates under routine care of the Coffee Research Foundation, and for the farms, the controls are farms that are known not to have used copper and chlorothalonil pesticides sprays for the past five years. While comparing the results obtained the following differences were taken into consideration:

1. **Intensity / frequency of spray**.

Table 3.2 Intensity and frequency of spray of copper and chlorothalonil fungicides

Fungicide	Low sprayers	High sprayers
Chlorothalonil	> 2.2 kg / Ha / spray.	< 2.2 kg / Ha / spray.
Copper	**R 11.** ≥3.3kg / Ha / spray. **SL Arabicas.** ≥5.5kg/ Ha × 5 spray for leafrust control, and ≥7.7kg/ Ha × CBD months. The copper fungicides contain 50% copper.	**R 11.** <3.3kg / Ha / spray. **SL Arabicas.** <5.5kg/ Ha × 5 spray for leafrust control, and <7.7kg/ Ha × CBD months.

Conversion: 1.0kg/ha/spray 100 g/L

Copper in the farms selected was sprayed at the above rates twice a year during the preceding 5 years and in May and November of the current year for Ruiru 11 variety of coffee. For the SL Arabica the above rates were applied during the CBD months of May, June, July, October and November of all the 5 years. Chlorothalonil is sprayed during the CBD months (May, June, July, October and November) at the rates mentioned above. This information is also obtained from the questionnaires **(Appendix 2)**.

2. **Ecological zone**. Agro–ecological zonation of coffee growing areas in Kenya is separated into 4 zones that differ in altitude, rainfall and temperature. Kiambu district mainly being UM1 and the UM2 zones and Thika district as UM3, and the UM4 as shown in the table

below. This zonation (**Table 3.3**) takes care of the different climatic zones necessary for comparison of results caused by the difference in climatic conditions.

Table 3.3: Climatic conditions for coffee growing regions

Zone	Region	Altitude (m ASL)	Annual Rainfall (mm)	Annual Mean Temp. (°C)	Soil Description	Farms/ estates in region
UM1	Kiambu.	1500-3000	>2000	13-22	Moderate to high fertility clays	-Kibubuti. -Chianda -Ibonia
UM2	Kiambu.	1200-2000	1200-1500	18-25	Moderate to high fertility clays	-Rukera -Yadini. -Most small scale holdings.
UM3	Thika.	900-1800	600-1200	18-28	Low fertility and low drainage sands	- Tassia.
UM4	Thika	< 900	< 600	< 24	Low fertility and low drainage sands	-Azania.

3. **Scale.** Small-scale farmer (< 5 Ha) and large-scale farmers (> 5 Ha)

3.2 Sampling

a) River water and sediment sample.

Sampling for the analysis of river / dam water, soil and plant tissue for pesticide residue was intended to cover both the early (May–July) and the late crop (October–December) picking periods. Rivers and streams have been used as location factors for coffee factories and farms/estates in Kenya and as such, their existence very close to rivers is a common phenomenon (**Appendix 9**). This poses a threat to the ecosystem since the effluent discharged from the coffee factory's run-offs, soil-leaching can easily reach the rivers when there is a tendency of overloading of effluent seepage pits during the peak season or run off during the heavy rains. Ruiru River flows through all coffee growing zones in the district (UM 1, 2, 3 and 4). Sampling points (**R1, R2, R3, R4, R5, and R6**) were established on this river according to Zones (**Appendix 9**). Sampling point (**R1**) was placed in this region as a control as coffee is not grown in this region. Sampling points **R2, R3, R4, R5** were located in the region with the most coffee factories along the river. Sampling point **R6** was selected to help in assessing the capability of the river to do self-purification. This sampling point has completely no coffee activity but other food crops are grown. The region is completely deforested and soil erosion could be seen to have caused considerable land degradation. This sampling point was located in this region to serve as a control. The sampling points for water samples were placed sufficiently downstream of effluent entry point (100 – 300m) to ensure dispersion of the effluent over a wide cross-section of the rivers. Water and river sediment was scooped using airtight plastic bottles that were first rinsed with the river water.

b) Pulping water samples

Pulping water was sampled from 4 factories randomly selected, to observe the effect of 1st and 2nd washing on fungicide spray losses. The observations were also compared to the concentration of laboratory pulping water. They were then carefully labelled and placed in an icebox and taken to the laboratory for analysis.

c) Soil and plant tissue samples

Coffee Estates and small-scale farms located along the rivers mentioned here were selected randomly (sampling space of 24 farms) and from them, soils and plant tissue samples collected during both picking seasons. Samples of freshly harvested coffee leaf and soil samples from the selected field farms were transported to the Coffee Research Station immediately after sampling. Leaves were taken from the 4th pair position counting from the fully open leaves at the tip of a primary branch (Bould *et al.*, 1971). For purposes of analysis of copper and chlorothalonil in intercrop potato and beans leaves were sampled alongside the coffee plant tissue, soil and water samples.

d) Market coffee

Three coffee beverages found in Nairobi's Uchumi supermarket were sampled to analyze for pesticide residues in market coffee. All samples were analysed within 4 days after sampling.

3.3 Plant analysis

The analytical methods used in this study are routine procedures employed for chemical determination in soil and plant tissue (APPHA - AWWA, 1975). These methods are adopted (and sometimes slightly modified to suit convenience and cost) from the works of various investigators in the field of plant and soil chemistry. Leaf samples were first washed in deionized water, then in soap solution and finally in 0.1N HCl, and rinsed in running deionized water, in order to reduce major and trace element contamination. All the leaf samples were then dried overnight in an oven set at 65 C. After drying the leaves samples were ground separately in a Wiley micro-hammer stainless steel mill using a 1-mm sieve. The ground samples were stored in sealed plastic containers. Before chemical analysis, 10.0g of each leaf sample were re-dried at 105 C for 1 hour. At this temperature all the moisture in the sample is expected to have vapourised and therefore samples are not expected to have moisture interference, as they are completely dry. This eliminates the need for converting calculations to an oven–dry basis (Suzuki S and M. Oda, 1987). Concentrations of copper were determined in Nitric-Perchloric acid digest solution (Aduayi, 1973) using Atomic Absorption Spectroscopy. Chlorothalonil levels in leaves, husks, pulp and berries were determined using acetone as the extractant, n-hexane for partitioning and florisil clean up done (Suzuki S and M. Oda, 1987) before running on

EGERTON UNIVERSITY
LIBRARY

a GC-ECD system. The outline of procedures used for the copper and chlorothalonil analysis of plant tissue (**Figure 4)** and soil **(Figure 5)** are shown below.

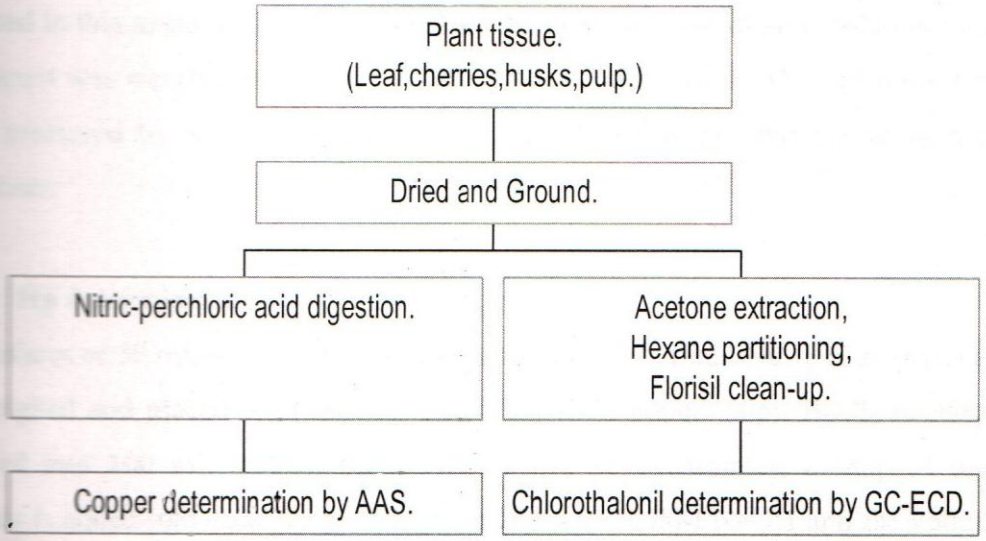


Figure 4: Outline scheme for analysis of Copper and Chlorothalonil in plant tissue

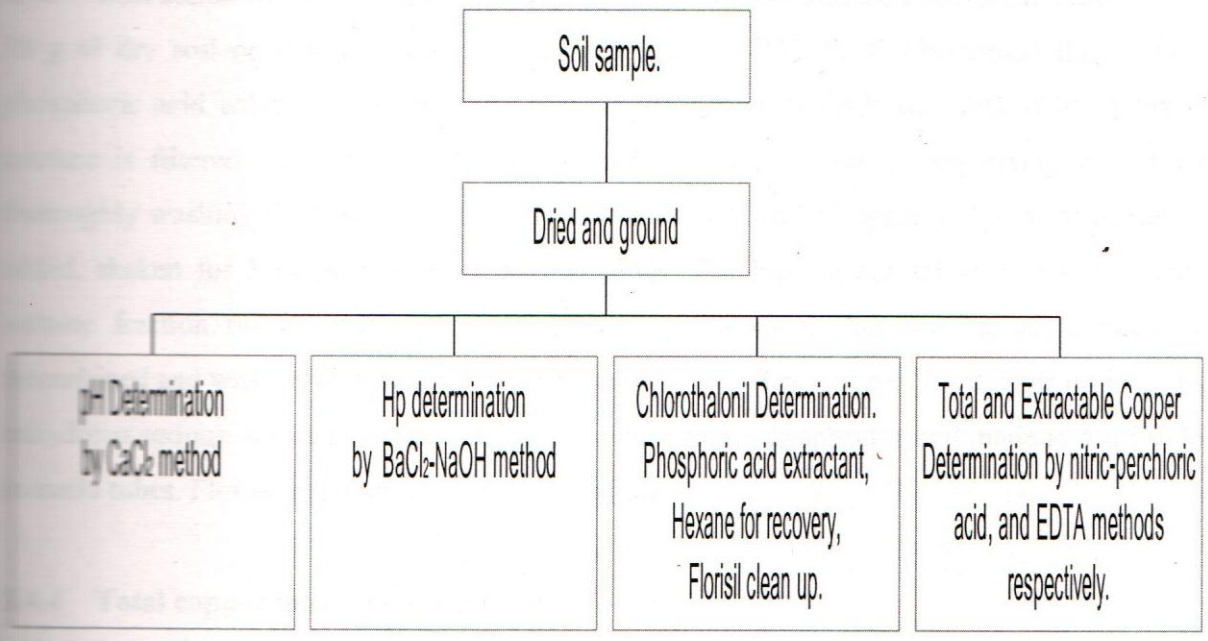


Figure 5: Outline scheme for analysis of Copper and Chlorothalonil in soil

3.4.1 pH determination

Smith (1996) proposed the use of 0.01M Calcium Chloride solution for temperate soils. The pH measured in this manner is more constant and are independent of soil- solution ratio. A 10 g dry soil aliquot was weighed into 50 ml plastic bottles and 25 ml 0.01M $CaCl_2$ solution added. The pH is measured by immersing into a pH glass meter as per the operating instruction after calibration.

3.4.2 Hp determination

The surfaces of 50 ml sintered funnels are covered by Whatman No. 1 filter paper and 5 g of dry soil weighed and placed onto the surface of the filter paper. 50ml $BaCl_2$ is added and filtrate collected into 100 ml flat-bottomed flask. Using an eyedropper, a drop of phenolphthalein indicator is added into each flask. This is titrated with 0.05M NaOH and the volume required to change the solution to pink noted. This is proportional to exchangeable acidity, Hp in m.e. / 100g soil.

3.4.3 Extraction of chlorothalonil pesticide residues from soil and sediment samples

10 g of dry soil or sediment sample was weighed into 250 ml flat-bottomed flask. 10ml of phosphoric acid solution and 60 ml of acetone are added to flask and shaken for 4 hrs. The mixture is filtered by a suction pump and collected into a 500 ml separating funnel while thoroughly washing the flask and soil residue with 2 x 10 ml of acetone. 60 ml of hexane was added, shaken for 3 minutes and allowed to settle. The hexane extract was collected and the acetone fraction re-extracted with another 60 ml of hexane. The two hexane extracts were recombined and washed with two 50 ml volumes of water. The extract was then dried in 10.0g of anhydrous sodium sulphate, evaporated in a vacuum rotary evaporator and made to 5 ml volume in evelo tubes. Florisil clean up was then carried out.

3.4.4 Total copper in soil and sediment

A pre-dried soil or sediment portion (3.0 g) is weighed into plastic sample container in duplicates, 4 mls of a digestion solution consisting of 10% concentrated sulphuric acid in an acid mixture of 3:1 nitric acid : perchloric acid is added in the container and shaken in a multiple

shaker for 1 hour. 10 mls of 0.5N HCl and 1.0 mls 0.5% $NaNO_2$ buffer is added and warmed in a hotplate, cooled, filtered, into exelotube and made to 20 cm^3 volume with distilled water and transferred to plastic specimen containers. This aliquot is used for copper determination with the AAS.

3.4.5 EDTA extractable copper in soil

A pre-dried soil portion (3.0 g) is weighed into plastic bottle. 50ml 0.1M Ethylenediamine tetraacetic acid (EDTA) added and the bottle shaken for 1 hour. The extract is then filtered into exelo tube using1.0g of charcoal to decolorize the extract. This aliquot is used for copper determination with the AAS.

3.5 Extraction and analysis of chlorothalonil in water samples

A water sample (100 ml) was placed into the separating funnel. And shaken vigorously with 60ml of acetone for 30 minutes. This was allowed to settle and the lower aqueous layer re-extracted with another 60ml of acetone. The two acetone extracts were mixed and then washed in 500ml of distilled water. The extracts are partitioned in 100 ml hexane, which separates out from the aqueous acetone layer. Florisil clean up is done followed by drying of extracts by passing through approximately 10.0 g of anhydrous Na_2SO_4. The hexane layer is then concentrated in a rotary evaporator to 5ml volume ready for injection in the GC-ECD system for quantification of the chlorothalonil pesticide residue.

3.6.1 Roasting and grinding of coffee beans

Coffee cherry was washed 3 times in distilled water after dehusking. This is then sun dried over a period of 24 hours, and then transferred to a roasting machine (model Exeter 115 Roaster) in the CRF laboratory. This, with a capacity of 10kgs fresh beans, is closed and roasted airtight at 150 C for two hours. This produces golden brown beans with an aromatic smell. The same machine has a grinding compartment into which the cooled roasted beans are placed ground to a relatively fine coffee blend ready for packaging. Samples for chemical analysis are taken at this stage, placed in sample bottles and stoppered.

3.6.2 Total copper in plant tissue and marketable coffee

A pinch of plant tissue (10 g) predried at 105°C for 1 hour in an oven, is weighed into conical flasks in duplicate and ashed overnight at 450°C. 2 ml of a digestion solution consisting of 10% concentrated sulphuric acid in an acid mixture of 3:1 Nitric acid: Perchloric acid is added into the container, heated to dryness in a hotplate, removed and let to cool. 10ml of 0.5N HCl and 1.0ml 0.5% $NaNO_2$ is added and warmed in a hotplate, cooled, filtered into exelo tube with 1.0g charcoal and made-up to $20cm^3$ volume with distilled water and transferred to plastic specimen containers. Marketable coffee beverages in form of powder or granules underwent the same procedure as plant tissue. This aliquot is used for copper determination with the AAS.

3.7 Copper and chlorothalonil standards.

Chlorothalonil standards were obtained from:

- The EPA Research Triangle Park, North Carolina, USA.
- Caffaro Societa, Milano, Italy.

A stock of 1000µg/gram (1000 ppm) chlorothalonil was prepared by accurately weighing 1.00g of the standard into a 1000ml volumetric flask and dissolving it thoroughly in distilled water before making it to the mark with distilled water. To make the 1-ppm solution, 1ml of the 1000ppm stock solution was taken into a 1000ml volumetric flask and diluted to the mark with distilled water.

3.8 Separation of different forms of copper in the soil.

Table 3.4 summarizes the sequential procedure used to separate soil copper into different forms, reagents, exchangeable cations used and references.

Table 3.4: Procedures for the sequential extraction of copper in soil

3.0g of soil from the < 2mm fraction.	Form of copper targeted.	Reference.
+ 15 ml of 1 M $MgCl_2$, pH = 4.9 (2 hrs).	Mg^{2+} Exchangeable (Cu^{2+})	Tessier *et al.*, 1979
+ 10 ml of 0.47 M NaOCl, pH = 3.3 (5 hrs)	Copper bound to organic matrix (Cu^{+})	Shuman, 1979
+ 10ml 10% concentrated H_2SO_4 in 3:1 HNO_3: POCl.	Total copper	Mckeague & Day, 1966.

Source: Westerman, 1990.

3.9 Florisil clean-up

Florisil initially deactivated by heating at 600° C in an oven and tested with lauric acid, to confirm that it is completely dry is placed in a desiccator to cool. A glass column (i.d. 22mm, length 25cm) was fitted with 1-cm thick cotton wool and then filled with 15g of florisil under a 2-cm layer of anhydrous sodium sulphate. The sample is then quantitatively transferred into the column and eluted with hexane then reduced to 5.0cm^3 using rotary vacuum evaporator at 64 C, transferred to exelotubes ready for GC-ECD analysis.

3.10 Eluent Selection.

In a study to apply the systematic identification and determination of pesticides to multiresidue analysis, Suzuki and Oda (1977), observed that of the fifteen pesticides investigated, 14 eluted in 50 % diethyl-ether and hexane elution mixtures posting recoveries of more than 90%, except for chlorothalonil which gave as low as 43% recovery. They presumed this to have resulted from adsorption of the chlorothalonil on florisil particles having the high adsorptive activity derived from some lots of florisil. From the results obtained it is evident that the elution solution to be used should be hexane because it was evident from the recovery studies below that lowering the polarity of the eluent from the 50% diethyl-ether led to higher chlorothalonil recoveries.

3.11 Chlorothalonil recovery studies.

In order to ascertain the effectiveness of the extraction method adopted and the eluent selected it was necessary to carry out chlorothalonil recovery studies. Soil samples from 3 sampling sites were processed and analyzed for recovery of the spiked chlorothalonil standard. The results are presented in the table below.

Table 3.5 Recovery Studies.

SAMPLE	SAMPLE CONCENTRATION (ppm)	% RECOVERY
Blank	0.012	
Unspiked samples		
Kibubuti estate	0.367	
Tassia estate	0.676	
Mbucho's farm	0.126	
Spiked samples		
Kibubuti estate	1.293	92.40%
Tassia estate	1.209	62.10%
Mbucho's farm	0.743	60.50%

The extraction solution was reduced to near dryness and recovered in 1ml hexane. 2.0 µL is then injected into Gas chromatogram. The recovery of the chlorothalonil spike in the above samples ranged between 60.50 - 92.40 % (average of 71.7 %).

3.12 Instrumentation for GC-ECD and AAS.

A gas chromatograph Perkin-Elmer, sigma 300 (Perkin-Elmer corporation, USA) coupled with an Electron Capture detector was used in the analysis of pesticides in this study. The chromatographic specifications were:

- Column: 3.0m, 2mm id coiled glass packed with 3% w/w, OV-1 ON 80/100 mesh chromosorb.
- Working temperatures (^{o}C): Oven 190. Injector 240. Detector 300.
- Gases: Carrier gas, N_2. Make-up gas, N_2.
- Gas pressure: Inlet 360 kPa. Inlet aux. 320 kPa.

- Flow rate: 50ml min^{-1}.
- Injection volume: 2μL.
- Detection limit: 0.002 ppm concentration of Chlorothalonil
- Detector: Electron capture, Tritium (^{3}H) source.
- Integrator: Perkin-Elmer, LCL-100.

For copper metal analysis Atomic Spectrophotometer model SP9, (Perkin - Elmer corporation) was used with an air - acetylene flame.

CHAPTER FOUR

RESULTS

Appendixes 12a and **12b** are tables of results for the analysis of soil samples for the May – July and the September – November seasons, and shall be used to compare the levels of chlorothalonil and copper in the two seasons.

Table 4.1: Comparison of chlorothalonil levels in soils in large and small-scale farms

Name of estate	Top(T) sub (S)	Chlorothalonil Conc. (ppm)
Large scale farms n=14		
Kalimi	T	0.037 ± 0.008
	S	0.239 ± 0.051
Kamu	T	0.277 ± 0.032
	S	1.448 ± 0.223
Tassia	T	0.675 ± 0.180
	S	0.308 ± 0.111
Gamba	T	0.127 ± 0.017
	S	0.234 ± 0.012
Bomia	T	0.226 ± 0.052
	S	0.328 ± 0.062
Kuiera	T	0.590 ± 0.143
	S	0.166 ± 0.003
Gituluti	T	0.585 ± 0.151
	S	3.186 ± 0.712
Range		**0. 037 – 3.186 ppm**
Small scale farms n=10		
Mbucho	T	0.126 ± 0.007
	S	0.018 ± 0.009
Njenge	T	0.092 ± 0.061
	S	0.049 ± 0.072
Kahuwai	T	0.013 ± 0.005
	S	1.479 ± 0.532
Njungua	T	0.290 ± 0.023
	S	1.543 ± 0.421
Kiarie Mukuria	T	0.095 ± 0.034
	S	0.220 ± 0.012
Range		**0.013 – 1.543 ppm**

Table 4.2 Comparison between chlorothalonil concentrations in soils at different farm landscapes n = 24

Lab number	Name of farmer	Farm landscape	Top / Sub Soil	Chlorothalonil conc. (ppm)
p1	Mbucho	ss	T	0.126 ± 0.007
p2	Mbucho	ss	S	0.018 ± 0.009
p3	Njoroge	ss	T	0.092 ± 0.061
p4	Njoroge	ss	S	0.049 ± 0.072
p9	Kiarie	ss	T	0.095 ± 0.034
p10	Kiarie	ss	S	0.220 ± 0.012
		Range		**0.018 – 0.220**
p5	Kahowai	gsu	T	0.013 ± 0.005
p6	Kahowai	gsu	S	1.479 ± 0.532
p11	Yadini	gsu	T	0.037 ± 0.008
p12	Yadini	gsu	S	0.239 ± 0.051
p13	Azania	gsu	T	0.277 ± 0.032
p14	Azania	gsu	S	1.448 ± 0.223
p23	Kibubuti	gsu	T	0.585 ± 0.151
p24	Kibubuti	gsu	S	2.140 ± 0.712
p15	Tassia	gsu	T	0.675 ± 0.180
p16	Tassia	gsu	S	0.308 ± 0.111
		Range		**0.037 – 2.140**
p7	Njungua	gsb	T	0.290 ± 0.023
p8	Njungua	gsb	S	1.543 ± 0.421
p17	Cianda	gsb	T	0.127 ± 0.017
p18	Cianda	gsb	S	0.234 ± 0.012
p19	Ibonia	gsb	T	0.226 ± 0.052
p20	Ibonia	gsb	S	0.328 ± 0.062
p21	Rukera	gsb	T	0.590 ± 0.143
p22	Rukera	gsb	S	0.166 ± 0.003
		Range		**0.166 – 1.553**

An experiment to determine the levels of chlorothalonil after every 7 days spraying in two farms was done for up to 28. The results are presented in the graphs below.

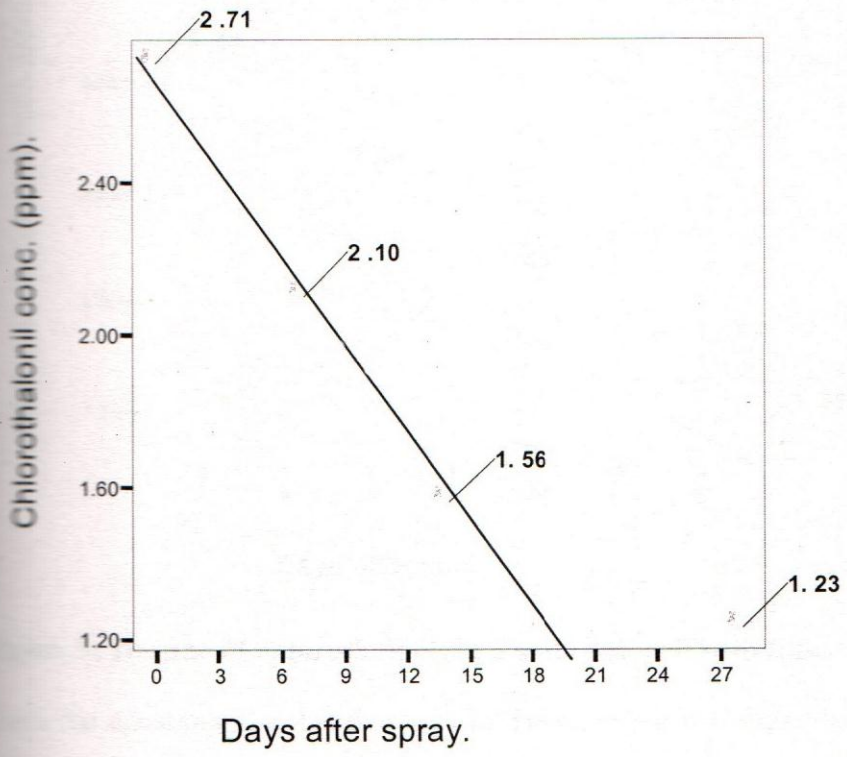


Figure 6: Decline of Chlorothalonil in Yadini sub soil with time.

Yadini estate is a light chlorothalonil sprayer. From this line graphs it is possible to estimate the chlorothalonil level of any soil sample for light sprayers of the fungicide for a period of 4 weeks after the last chlorothalonil spray. All a farmer needs to state is the last day that he sprayed his farm with the fungicide and the rate of spray. From this estimation, we can advice the farmer on whether his spraying rate and frequency is a factor contributing to the build up of chlorothalonil residue in the soil.

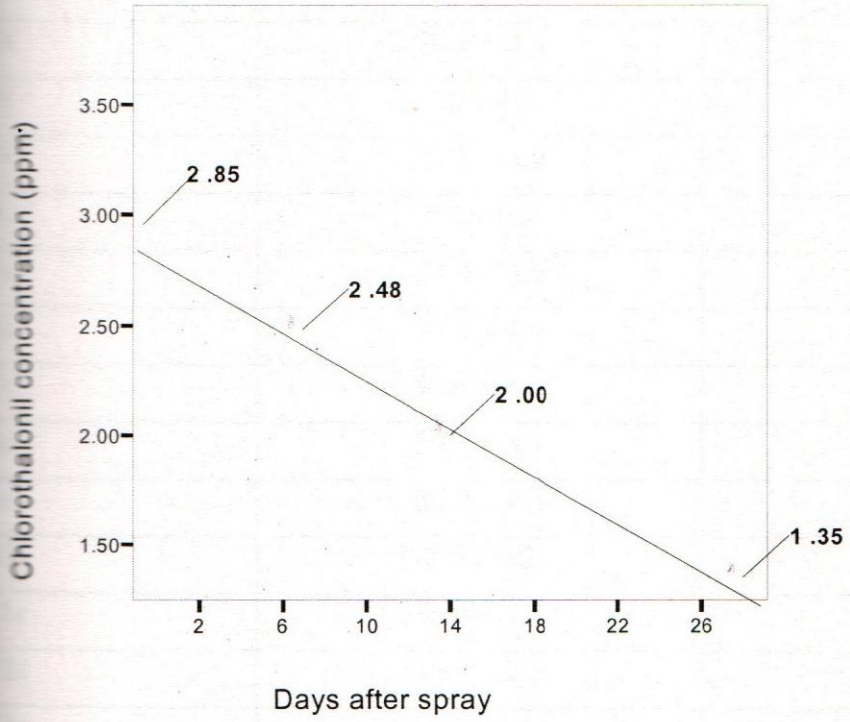


Figure 7: Decline of chlorothalonil in Tassia sub-soil with time.

From the questionnaire that was sent to Tassia estate it is clear that the farm is a high intensity sprayer of chlorothalonil (**appendix 2**). This will thus help us estimate the levels of chlorothalonil in soils of those farmers that spray at rates more than that 3.8kg A.I/Ha of chlorothalonil (heavy sprayers). This line graphs show that chlorothalonil residues in soil (at field conditions) reduce gradually at a rate of 2.301% per day for Tassia Estate and 5.29% per day in Yadini Estate, giving a general averaging of 3.78% reduction per day. This is not so much way above the figure of 2.71% reduction per day reported by Stallard and Szalkowski, (1976) for pit loam soil. The equation derived from these estates can be used to estimate the concentration of chlorothalonil residues in soil (at field conditions) from other light spraying farms. From Yadini estate a general equation **y = - 5.5E-2x + 2.71** can be forwarded as the general equation for estimating levels of chlorothalonil in farms spraying chlorothalonil at rates greater than 2.2 kg/ha /spray. For lighter sprayers (Tassia farm) the equation **y = – 7.81E-2x + 2.95** is forwarded.

Table 4.3: Total / EDTA -Extractable copper in Soil (May- July season) n = 24

Soil Sample no.	EDTA Cu (ppm)	Total copper (ppm).
1s	10.3 ± 2.0	48.5 ± 9.8
2s	7.0 ± 1.3	42.8 ± 3.6
3s	17.8 ± 3.9	133.1 ± 13.9
4s	4.4 ± 0.7	69.7 ± 8.8
5s	24.4 ± 4.9	129.3 ± 14.5
6s	17.1 ± 1.6	86.1 ± 12.3
7s	50.6 ± 8 .7	155.8 ± 18.0
8s	23.9 ± 3.7	103.2 ± 14.3
9s	30.0 ± 4.5	91.0 ± 11.7
10s	2.0 ± 0.3	12.5 ± 2.7
11s	35.0 ± 3.8	69.3 ± 7.8
12s	10.8 ± 3.3	29.6 ± 4.7
13s	6.5 ± 1.6	172.2 ± 12.8
14s	11.7 ± 3.2	38 ± 5.6
15s	19.4 ± 2.3	70.9 ± 12.6
16s	10.6 ± 2.0	27.5 ± 3.5
17s	9.2 ± 3.0	33.7 ± 4.8
18s	1.2 ± 0.6	4.9 ± 0.9
19s	8.1 ± 0.9	27 ± 4.7
20s	1.0 ± 0.2	4.1 ± 0.3
21s	0.5 ± 0.3	3.4 ± 1.4
22s	0.3 ± 0.1	2.4 ± 1.0
23s	43.0 ± 5.8	48.7 ± 2.7
24s	17.1 ± 2.0	29.8 ± 4.8
Range	**0.3 –50.6**	**2.0 –172.2**

Key:

S	- Soil	L	- Leaf sample
B	- Beans	H	- Husk samples
Sp	- Soil speciation	M	- Marketable coffee

There exists very large variability between the different farms selected. This section therefore presents some tables with only ranges as it will not be justifiable to present mean values in tables with so high standard deviations.

Table 4.4 a: Forms of Copper in soil (May - July season) using $MgCl_2$ as extractant n = 8

Sample NO	$MgCl_2$ Cu Conc. (ppm)	Total Cu (ppm)	EDTA extractable (ppm)	$MgCl_2$ Cu as % Of Total Cu	$MgCl_2$ as % of EDTA extractable Cu.
Sp 1	0.34 ± 0.12	48.5 ± 9.8	10.3 ± 2.0	0.26	3.30
Sp 2	0.37 ± 0.08	42.8 ± 3.6	7.0 ± 1.3	0.43	5.29
Sp 7	0.22 ± 0.10	155.8 ± 18.0	50.6 ± 8 .7	0.14	0.43
Sp 8	0.24 ± 0.06	103.2 ± 14.3	23.9 ± 3.7	0.23	1.04
Sp15	0.29 ± 0.14	70.9 ± 12.6	19.4 ± 2.3	0.41	1.49
Sp16	0.19 ± 0.05	27.5 ± 3.5	10.6 ± 2.0	0.69	1.79
Sp23	0.13 ± 0.02	48.7 ± 2.7	43.0 ± 5.8	0.27	0.30
Sp24	0.09 ± 0.04	29.8 ± 4.8	17.1 ± 2.0	0.30	0.53
Mean	**0.234 ± 0.098**	**65.9 ± 43.76**	**22.63 ± 15.94**	**0.34 ± 0.17 %**	**1.77 ± 1.73%**

Table 4.4 b: Forms of Copper in soil (May - July season) using NaOCl as extractant n = 8

Sample number	NaOCl Cu Conc. (ppm)	Total Cu (ppm)	EDTA extractable (ppm)	NaOCl Cu as % Of Total Cu	NaOCl Cu as % Of EDTA extractable Cu.
Sp 1	0.97 ± 0.21	48.5 ± 9.8	10.3 ± 2.0	0.75	9.42
Sp 2	2.43 ± 051	42.8 ± 3.6	7.0 ± 1.3	2.82	34.23
Sp 7	1.51 ± 0.05	155.8 ± 18.0	50.6 ± 8 .7	0.97	2.98
Sp 8	4.07 ± 0.61	103.2 ± 14.3	23.9 ± 3.7	3.94	17.70
Sp15	4.05 ± 0.07	70.9 ± 12.6	19.4 ± 2.3	5.71	20.88
Sp16	0.98 ± 0.03	27.5 ± 3.5	10.6 ± 2.0	3.56	9.25
Sp23	1.58 ± 0.29	48.7 ± 2.7	43.0 ± 5.8	3.24	3.67
Sp24	1.74 ± 0.53	29.8 ± 4.8	17.1 ± 2.0	5.84	10.18
Mean	**2.166 ± 0.655**	**65.9 ± 43.76**	**22.63 ± 15.93**	**3.36 ± 1.88%**	**13.54 ± 10 .38 %**

4.1 Plant Tissue

Tables 4.5 - 4.9 show the levels of copper and chlorothalonil in different plant tissues. It worth noting that leaves from potato and beans were sampled from mature only a few months due for harvesting.

Table 4.5 a: Levels of copper and Chlorothalonil in Potato intercrop n = 4

Potato intercrop			
Small scale farmers			
Sample number	**Name of farmer**	**Cu (ppm)**	**Chlorothalonil (ppm)**
L6	Njoroge	10.27 ± 4.54	0.646 ± 0.321
L14	Kiarie	6.85 ± 1.05	0.211 ± 0.070
L15	Kahowai	6.33 ± 1.32	0.341 ± 0.002
L3	Mbucho	6.69 ± 0.71	0.410 ± 0.038
	Range	**6.85 -10.27**	**0.211 – 0.646**
	Mean	**7.535 ± 2.137**	**0.432 ± 0.182**

Table 4.5 b: Levels of copper and Chlorothalonil in Beans intercrop n = 4

Beans intercrop			
Sample number	**Name of farmer**	**Cu (ppm)**	**Chlorothalonil (ppm)**
L11	Njoroge	4.57 ± 1.00	0.741 ± 0.10
L16	Njungua	0.25 ± 0.03	0.612 ± 0.07
L18	Mbucho	2.64 ± 0.11	0.662 ± 0.13
L21	Kahowai	2.46 ± 0.54	0.687 ± 0.24
Range		**0.25 - 4.57**	**0.612– 0.741**
Mean		**2.41 ± 1.05**	**0.677 ± 0.015**

Table 4.6: Levels of copper and chlorothalonil in coffee leaves n = 10

Small Scale Farmers			
Sample no.	**Name of farmer**	**Cu (ppm)**	**Chlorothalonil (ppm)**
L1	Mbucho Coffee	1.12 ± 0.08	0.312 ± 0.123
L7	Kahowai coffee	1.30 ± 0.11	0.216 ± 0.162
L8	Njungua coffee	1.02 ± 0.07	0.310 ± 0.114
L10	Kiarie coffee	3.37 ± 1.01	0.423 ± 0.069
L12	Njoroge coffee	7.45 ± 2.06	0.804 ± 0.098
Range		**1.02 - 7.45**	**0.216 – 0.804**
Large Scale Farmers			
L2	Azania Coffee	3.40 ± 0.76	1.203 ± 0.486
L4	Ibonia coffee	8.15 ± 2.41	1.411 ± 0.432
L5	Cianda coffee	5.33 ± 0.98	1.121 ± 0.621
L9	Yadini coffee	4.28 ± 0.41	0.984 ± 0.430
L13	Tassia coffee	6.53 ± 1.44	1.216 ± 0.508
Range		**3.40 - 8.15**	**0.984 – 1.411**

Table 4.7: Levels of copper and chlorothalonil in Coffee Husks n = 10

Sample no	Name of farmer	Cu levels (ppm)	Chlorothalonil levels (ppm)
H1	Kiarie Mukuria	0.62 ± 0.18	0.048 ± 0.004
H2	Ibonia	0.56 ± 0.14	0.064 ± 0.032
H3	Tassia	0.40 ± 0.09	0.072 ± 0.003
H4	Njungua	0.60 ± 0.11	0.096 ± 0.014
H5	Cianda	0.48 ± 0.07	0.092 ± 0.019
H6	Kahowai	0.41 ± 0.01	0.062 ± 0.009
H7	Mbucho	0.31 ± 0.10	0.041 ± 0.004
H8	Njoroge	0.42 ± 0.13	0.081 ± 0.031
H9	Yadini	0.43 ± 0.12	0.077 ± 0.039
H10	Azania	0.36 ± 0.13	0.006 ± 0.002
	Range	**0.31 – 0.62**	**0.006 – 0.096**
	Mean	**0.459 ± 0.104**	**0.063 ± 0.017**

Table 4.8: Levels of Copper and Chlorothalonil in Coffee beans n = 8

Sample no	Name of farmer	Cu levels (ppm)	Chlorothalonil levels (ppm)
B1	Kiarie Mukuria	0.72 ± 0.20	0.033 ± 0.012
B2	Ibonia	0.73 ± 0.08	0.046 ± 0.019
B3	Tassia	1.03 ± 0.31	0.052 ± 0.010
B4	Njungua	0.89 ± 0.11	0.071 ± 0.009
B5	Cianda	0.90 ± 0.21	0.066 ± 0.003
B6	Kahowai	0.64 ± 0.08	0.034 ± 0.006
B7	Mbucho	0.72 ± 0.12	0.009 ± 0.001
B8	Njoroge	0.85 ± 0.13	Nd
Range		**0.64 – 1.03**	**0.009 – 0.071**
Mean		**0.81 ± 0.12**	**0.044 ± 0.021**

Table 4.9: Copper and Chlorothalonil levels in roasted and ground coffee beans n = 4

Sample no	Name of farmer	Cu levels (ppm)	Chlorothalonil levels (ppm)
B2	Ibonia	0.26 ± 0.03	0.025 ± 0.006
B4	Njungua	0.29 ± 0.07	0.043 ± 0.003
B5	Cianda	0.53 ± 0.09	0.009 ± 0.001
B7	Mbucho	0.37 ± 0.01	Nd
	Range	**0.26 – 0.53**	**0.009 – 0.043**
	Mean	**0.42 ± 0.205**	**0.043 ± 0.009**

Table 4.10: Copper and Chlorothalonil Levels in Kenyan marketable Coffee n = 3

Sample no	Trade Name	Cu levels (ppm)	Chlorothalonil levels (ppm)
M1	Doorman's	0.21 ± 0.18	Nd
M2	Kenya coffee blend	0.43 ± 0.08	0.037 ± 0.07
M3	Kahawa Kawaida	0.62 ± 0.15	0.024 ± 0.008
	Range	**0.21 - 0.62**	**0.024 - 0.037**
	Mean	**0.42 ± 0.062**	**0.031 ± 0.009**

Key:

Nd - not detected. nd - not done. N - number of replications.

4.2 Water

Table 4.11: Copper and chlorothalonil level (May - July)

River water analysis results sheet.					
Lab number	District	Water Source/ sampling **point**	**Adjacent Estate/Factory/Farm**	**Copper** (ppm)	**Chlorothalonil** (ppm)
1	Thika	Ruiru river/**R5**	Tassia	15.43 ± 2.11	0.685 ± 0.18
2	Kiambu	Cianda dam	Cianda Estate	35.02 ± 6.42	0.198 ± 0.06
3	Thika	Ruiru river/**R3**	Rukera Farm	10.54 ± 3.26	0.792 ± 0.12
4	Thika	Azania dam	Azania Farm	4.08 ± 2.11	0.115 ± 0.09
5	Kiambu	Gathara-ini river	Ibonia Estate	14.87 ± 2.94	0.075 ± 0.02
6	Thika	Kandakari river/**R1**	Small Scale Farmer - Njung'ua	4.43 ± 0.41	0.004 ± 0.00
8	Thika	Ruiru river/**R4**	Yadini Estate	10.90 ± 1.01	0.397 ± 0.16
9	Thika	Kimaratio river	Small Scale Farmer - Kiarie Mukuria	2.81 ± 0.64	1.013 ± 0.14
10	Thika	Magotha river	Small Scale Farmer - Peter Mbucho	12.12 ± 0.66	0.015 ± 0.01
11	Kiambu	Ruiru river/**R2**	Kagwanja Factory	6.62 ± 1.03	0.653 ± 0.16
12	Thika	Kigaa river	Kigaa Factory	4.42 ± 0.73	0.073 ± 0.19
14	Thika	Ruiru river/**R6**	-	6.46 ± 0.66	0.211 ± 0.17
		Range		**2.81 - 35.02**	**0.004 - 1.013**

Ruiru River, which cuts across Thika and Kiambu Districts and empties its waters into the Athi River is an important water source for people living along the river. The water is used for consumption, domestic uses like washing and other economic activities including farming. The tributaries of Ruiru River include Komothai and Gathara-ini Rivers in Kiambu District and Magotha, Kimaratio and Kigaa Rivers in Thika District.

Table 4.12: Copper and Chlorothalonil levels in pulping water n = 3

Lab no.	District	Name of factory	Estate	Copper (ppm)	Chlorothalonil (ppm)
15	Kiambu	Ibonia 1st Washing	Ibonia Estate	44.32 ± 12.10	1.275 ± 0.312
19	Thika	Kigaa 1st Washing	Kigaa	36.03 ± 13.02	1.526 ± 0.721
18	Thika	Tassia 1st Washing	Tassia	32.35 ± 12.11	0.342 ± 0.051
21	Kiambu	kibubuti 1st Washing	Kibubuti	40.76 ± 14.10	0.372 ± 0.008
Mean				**40.17 ± 4.19**	**0.891 ± 0.201**
17	Kiambu	Ibonia Factory 2nd washing	Ibonia Estate	13.21 ± 5.97	0.569 ± 0.061
16	Thika	Kigaa Factory 2nd Washing	Kigaa	19.49 ± 1.90	0.464 ± 0.013
Mean				**16.35 ± 3.13**	**0.515 ±0.043**

Table 4.13: Copper and Chlorothalonil levels in Laboratory pulping water n = 3

Lab no	District	Estate / farm	Copper (ppm)	Chlorothalonil (ppm)
22	Thika	Tassia	13.23 ± 1.21	0.433 ± 0.08
23	Thika	Yadini	42.76 ± 4.32	0.163 ± 0.04
24	Thika	Kahowai	12.23 ± 2.16	1.333 ± 0.03
25	Kiambu	Cianda	nd	0.120 ± 0.06
26	Kiambu	Ibonia	23.87 ± 3.68	0.120 ± 0.02
27	Thika	Rukera	nd	0.011 ± 0.03
28	Thika	Azania	9.98 ± 2.96	0.029 ± 0.06
29	Kiambu	Njungua	28.74 ± 3.84	0.054 ± 0.07
31	Thika	Njoroge	10.03 ± 1.36	0.038 ± 0.12
32	Thika	Kiarie	12.65 ± 0.88	0.939 ± 0.17
33	Kiambu	Kibubuti	37.45 ± 3.21	0.734 ± 0.23
Range			**9.98 – 42.76**	**0.011 – 1.333**

Ibonia Estate and Kigaa cooperative society factories are two factories that perform 1st and 2nd washing of the parchment coffee after pulping (**Table 4.12**). 2nd washing further washes as much as 20 - 30 % of the 1st washing making it an important step in reducing pesticide both copper and

chlorothalonil residues in processing. Factories are thus encouraged to perform 2nd washing of the parchment coffee during pulping.

Performing a similar factory pulping process in the laboratory, and analyzing the resulting pulping water for copper and chlorothalonil pesticide residues, it was observed that the copper concentration of the pulping water ranged from 9.98-42.76 ppm (Mean 21.12) while the chlorothalonil concentration ranged between 0.011–1.333 ppm (Mean 0.361). Copper and chlorothalonil concentrations of pulping water from factories selected are not far away from these figures and therefore the laboratory pulping is a good indication of the field pulping conditions.

Copper and chlorothalonil levels in sediment and water along Ruiru River are presented in **Figures 8** to **11** below.

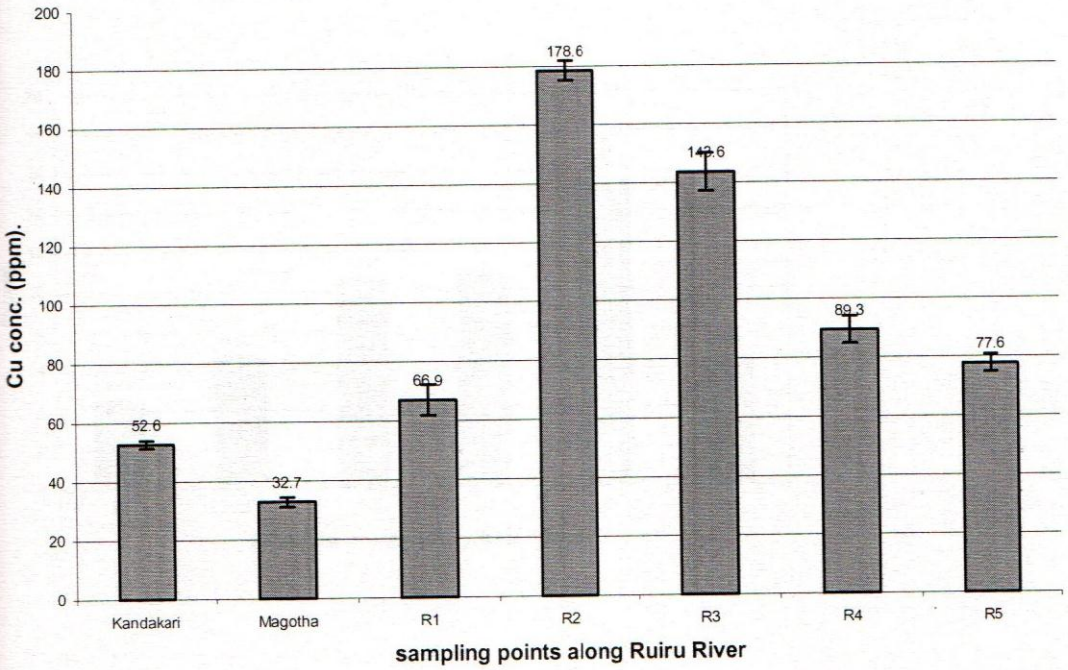


Figure 8: Copper levels in soil sediment

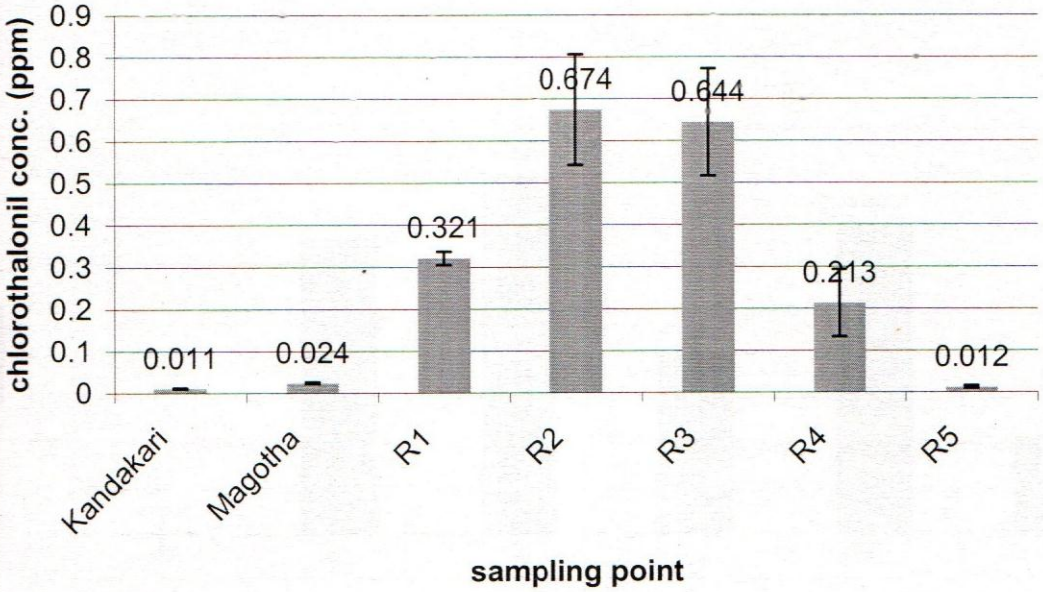


Figure 9: Chlorothalonil levels in sediment

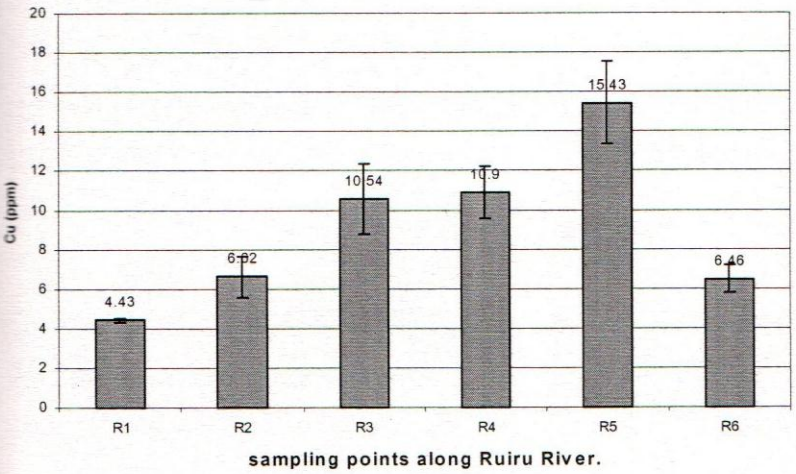


Figure 10: Copper levels in water along Ruiru River.

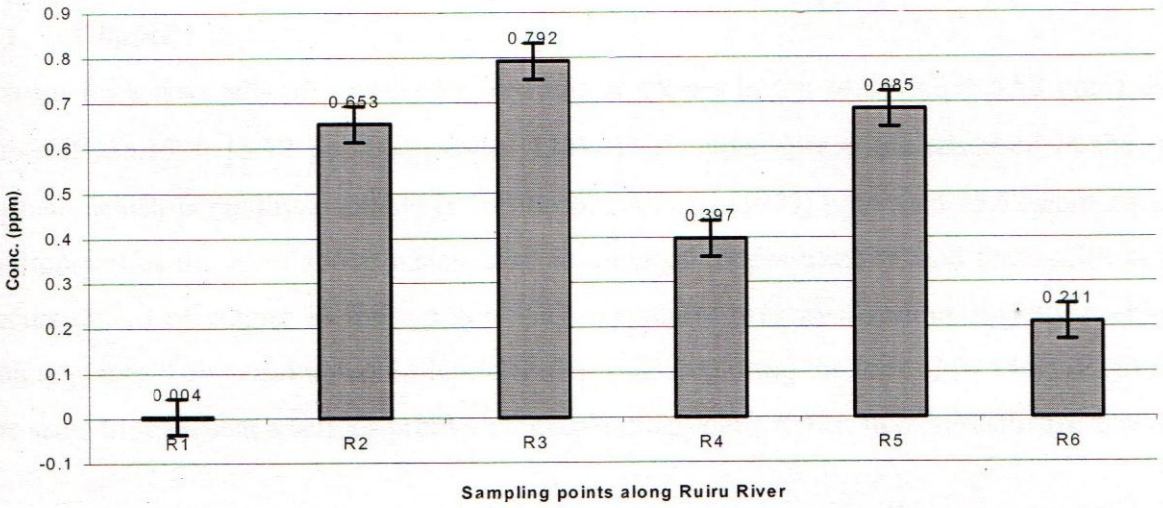


Figure 11: Chlorothalonil levels in water along Ruiru River

CHAPTER FIVE
DISCUSSION

5.1 Copper.

Except for a few, all soil samples showed higher copper in top-soil (99.2 ± 5.88 ppm) than in sub-soil (55.15 ± 15.12 ppm, **appendix 12b)**. The copper sprays are adsorbed in the humic surface, which is readily available in the topsoil. Aduayi (1973) presented 15.52 ppm copper in the top-soil as the level above which there is onset of copper toxicity, and that such excessive accumulation of copper in the top soil may eventually present a serious toxicity problem to young plants. This build up could injure feeder roots exploring the topsoil (< 15cm deep) and at the same time present a serious problem for replanting young coffee in old established farms.

Total-copper in soil ranged between 2.0-172.2 ppm and EDTA-extractable copper ranged between 0.3-50.6 ppm (**Table 4.3**). The implication of this revelation is that approximately 27.39 % of copper in the soil is what constitutes the amount of copper available for absorption by the plant roots as this is the proportion that can be brought into solution by complexing with EDTA (Cheng and Bray, 1953). Data on total and EDTA extractable copper showed a positive Pearson correlation **(appendix 6 and 7)** of 0.538 at the 0.01 significant level. Analysis of copper for the 2 seasons of study also showed significant correlation of 0.964 at the 0.01 significance level **(appendix 8).**

Some small-scale farmers although light-users/non-users of copper still exhibited high levels of copper in soil (> 50 ppm), coffee and the intercrops leaf samples (>5.0 ppm), and coffee bean samples (>0.5 ppm) . From the questionnaires sent to and received from farmers, it was observed that there existed a system of intercropping comprising of beans, potatoes, and tomatoes with coffee. The source of copper was thus not only from the spraying of coffee, but also from the spraying of intercrops (tomatoes, potatoes and beans). There was observed elevated copper levels in the soils of farms where there was intercrop between tomatoes and coffee. High total soil-copper resulted in higher plant-tissue copper uptake and accumulation in the leaves and beans of coffee and intercrops

An observation from the data acquired is that acidic soils with pH levels at near neutrality (4.0 – 5.5 pH) and relatively high phosphorus levels (> 50 ppm, **Appendix 4**) showed high total copper in soil (> 100 ppm Cu; **appendix 12a and 12b**) yet the coffee plant did not show any toxicity effects. Phosphorus and the near neutral soil pH, as earlier speculated by Masaba (1987) and Aduayi (1973), are observed to provide a buffer against such toxicity. Aduayi, (1973) stated that heavy use of copper sprays (over 2.2 kg/Ha copper) would lead to high levels of copper in the soil and plant tissue. It was however observed that although the soil had high copper concentrations, plant tissue samples from these farms did not have elevated copper levels. This could be attributed to the levels of phosphorus in the analyzed soil samples of 34-142 ppm. (Mean 88.0 ppm, **Appendix 4**). The coffee trees in these farms were observed to have sufficiently good morphological growth attributable to the NPK (which supplies the soil with phosphorus) fertilizer application.

Copper levels in the leaves of potato and bean intercrops were mostly determined in the small-scale farms as inter-cropping is not common in the large-scale farms. Copper levels were found to lie between 0.85 -19.27 ppm (mean 7.53 ppm) for potatoes and 0.25 - 4.57 ppm (mean 2.41 ppm) for beans (**Table 4.5 a and 4.5 b**). From these results it can be said that potato leaves accumulate about three times more copper (mean 7.53 ppm) than both bean leaves (mean 2.41 ppm) and coffee leaves (mean 2.85 ppm, **Table 4.6**) in the small-scale farms. Various plausible explanations can be presented for this observation. First, potatoes/tomatoes are highly susceptible to fungi attack, (Bacterial Spot, and Bacterial Canker) and are thus heavily sprayed with red-copper for its control. Copper which accumulates in the topsoil is translocated to the leaves of the potatoes, hence the elevated copper values detected. Secondly, in bean leaves, high copper supply enhances the synthesis of proteins that are almost identical to plastocyanins (Marschner, 1995). The enhanced synthesis of these phytochelatins (in beans unlike in the potatoes; Marschner, 1995), in response to the high copper supply is suspected to have lead to compartmentation and chelation of the copper in the bean cytoplasm showing lower levels in its leaves. The copper content of 32 bean samples ranged between 4.0 – 12.2 ppm (Marschner, 1995). Maroko (1989), observed elevated levels as high as 18 ppm copper in potato leaves. The maximum residue level acceptable to FAO/WHO (1991) is 5.00 ppm for leaves of most annual

food crops. However, there is need for further investigation to determine the levels of copper not only in the leaves but also in the tubers, roots and stems of food crops intercropped with coffee.

The values of $MgCl_2$ extractable copper are distinctly lower than those obtained by the NaOCl extractant as recorded in **Table 4.4a and 4.4b**. This difference in copper extraction shows that copper ionic species are held on the soil exchange sites with correspondingly different bonding energies, as earlier proposed by Aduayi (1973). Cu^+ (being larger than Cu^{++}) will have lower ionic charge per unit area than Cu^{++}. It would thus be rational to accord Cu^{++} the higher bonding energy pool and the high radius Cu^+ the lower bond energy pool. Thus $MgCl_2$ must be primarily extracting the lower radius Cu^{++} ionic species from the soil exchange matrix and the higher radius Na^+ should be viewed as the Cu^+ ion extractant. This extraction process is believed to be through displacement reaction by ions of near similar size (Tessier *et. al.*, 1979) and through cation exchange reactions involving the copper cations and the extracting cation (Shuman, 1979). The values of copper extracted by $MgCl_2$ and NaOCl from the soil were low, just as was observed from these earlier studies. This experiment need to be explored further using other potential cation exchange extractants with larger or smaller ionic radii than Mg^{++} or Na^+ (Lithium, Calcium and Potassium as the cation exchangers). This step will help us see if we can get even better yields of the copper extracted.

Correlation analysis between Total soil-copper, EDTA-extractable copper, and the form of copper extracted by $MgCl_2$ and NaOCl, and the copper in coffee plant tissues are shown in **Appendix 11**. Though not significant, a linear negative correlation between the exchangeable copper extracted by $MgCl_2$ (most likely the Cu^{++} ion), and the copper residue levels in coffee leaves ($r^2 = -0.184$), coffee husks ($r^2 = -0.985$) and coffee beans ($r^2 = -0.180$) at 0.05 significance level is observed. A direct positive correlation between the copper ions extracted by the NaOCl from the soil (which is mostly Cu^+ ions), and the amount of copper in the coffee leaves ($r^2 = 0.862$) and coffee bean ($r^2 = 0.418$), is shown in **Appendix 11**.

These direct interactions suggest that there is a relationship between the form of copper biologically extracted by the coffee plant from the soil and translocated to the leaves, beans and the husks and the amount that was chemically extracted by $MgCl_2$ and NaOCl. The direct positive correlation observed between the copper extracted by NaOCl and the Cu^+ species in the soil proposes that copper is transported into the plant tissue in this form. It can therefore be said that copper in the soil is easily transported into the coffee leaves, coffee husks and coffee berry as Cu^+. From this observation, it can be said that spraying coffee with copper in the Cu^+ species will result in high copper residue levels in the leaves, husks and coffee bean. Therefore, frequent application of copper (I)-based fungicides can lead to build-up of the heavy metal in the coffee plant-tissue, unlike spraying the coffee plant with copper (II)-based fungicides, which according to this study does not translocate and therefore has a low possibility of build-up in the plant tissue. It is hereby recommended that stake holders in the agrochemicals industry be cautious in the use of Cu^{2+}-based fungicides (the copper hydroxide, $Cu(OH)_2$ and Di-copper oxychloride fungicide), and the Cu^+-(the Cuprous oxide, Cu_2O) based fungicides for the control of leaf rust and CBD diseases of coffee. In terms of efficacy (but not possibility of build up) using either of these fungicide has not been found to be different (Technical circular no. 63, 1990). Much more work has to be done to ascertain this proposition.

Boron enhances copper uptake by the coffee plant from the soil (Shuman, 1979). Phosphorus has been found to suppress copper absorption by the plant roots and it translocation to plant parts, hence the negative correlation between the soil phosphorus and leaf copper observed. Phosphorus is also known to react with soil copper to form the insoluble copper phosphate, which gets adsorbed in the soil matrix (Rendina and Iorio, 1996).

$$Cu^{2+}(aq) \quad + \quad PO_4^{2-}(aq) \quad \longrightarrow \quad CuPO_4(s)$$

Copper levels along Ruiru River water seems to increase downstream from **(R1 to R5; Figure 8 and 10)**, as the coffee growing activities increases. Effluent is discharged directly to water bodies or seeps from the seepage pits into watercourses resulting in greater amount of copper residue loading in the rivers and soils. The results obtained from sampling point **R1** reveal that Ruiru River at the upland stretch has low levels of copper and chlorothalonil and therefore least

polluted (**Figure 10 to 11**). This can be associated with high flow rates and good dilution of the river water by tributaries. There is a gradual increase in the pesticide residue levels from **R2 - R5**. These sampling points are within the co-operative sector and show that control measures undertaken to protect the water course from the coffee industry's pesticide application and the resulting coffee processing effluents are not effective in controlling water pollution by the pesticide residues. The residue levels of the river downstream **(R6)** decrease, as a result of dilution by self purification of river water, hence the lower levels (as compared to **R2** to **R5**). This is a region within the estate sector, and one can also attribute the lower level to good handling and discharge of the coffee effluent (dilution tanks, seepage pits, channelling of effluent into coffee farms as manure) by factories/Estates located within this zone.

The high copper levels **(Table 4.13)** point to pulping as a main source of copper in the river water. It is observed that the river sections showing high levels of copper also have high levels of copper deposited in the sediment **(Figures 8 and 10)**. This trend is also visible in chlorothalonil levels in river water and river sediments **(Figures 9 and 11)**. These broad and indicative trends confirm results from other studies carried out on heavy metal loading of rivers, that localized pollution from pesticides use and coffee processing does occur (Mwaura, 1995; Githunguri, 1991).

5.2 Chlorothalonil

The levels of chlorothalonil in soils, plant tissues and water are presented in **Tables 4.1 – 4.13**. This fungicide is only next to the copper-based fungicide in affordability and for this reason it is very popular with farmers. It is therefore no surprise that the fungicide was detected in all the samples analyzed. Concentrations in the range of 0.007-3.186 ppm (Mean 0.513 ppm) chlorothalonil in soil were detected for the 1st season (May – July) whereas the 2nd season (September – October) values ranged between 0.042 – 2.410 ppm (mean 0.573 ppm). These results are comparable to levels found by Jacobsen (1992), who obtained levels of 0.41ppm chlorothalonil in Painville, Ohio. Katayama (1991) observed levels of between 0.64 ppm and 2.86 ppm chlorothalonil in soils from Congo. In **Table 4.1,** it is observed that soils in the well-managed coffee plantations in the UM2 zone showed higher levels of chlorothalonil (mean 0.602

ppm) than from the small-scale farms (mean 0.39 ppm). These large-scale commercial farms have the capital and the resources to adequately spray chlorothalonil above the average rate of 2.2-kg chlorothalonil active ingredient (A.I) per hectare as recommended by the Coffee Research Foundation.

The farm landscape (**Appendix 3)** and the exact location of the sampling points are factors that affect the concentration of the nutrients in the soil. Chlorothalonil levels in the soil of farms located in different farm topographies are shown in **Table 4.2**. Farms in steep slopes (ss) are observed to have relatively lower concentrations (0.02 – 0.22ppm, Mean 0.10 ppm) of chlorothalonil relative to the Gentle Slope Upper (gsu) with concentrations ranging between 0.01 – 3.19 ppm with a mean of 0.59 ppm. The concentrations of chlorothalonil in the Gentle Slope Base (gsb) are highest with Concentration ranging between 0.13 – 1.54 ppm (Mean 0.69 ppm). This is evidence of transport of chlorothalonil by rainwater from the gentle slope upper (gsu) and steep slope (ss), and its accumulation at the gentle slope base (gsb). Thus chlorothalonil when applied to steep coffee terrain will easily seep into the watercourses. When it rains after a chlorothalonil spray the chemical accumulates in the subsoil very rapidly. Within 28 days chlorothalonil reduces at the area of accumulation (sub-soil) to less than 50% of the initial level. The disappearance can either be physical leaching / horizontal dissipation via drainage forces or biochemical degradation. If the latter is dominant pathway then this seems to give a half live of about 18-21 days. This is in contradiction to the proposition by Jacobsen (1992) and Hassall, (1990) that chlorothalonil is immobile in soils to some extent. Results from this study however indicate that chlorothalonil can actually be translocated, leached or transported by spill-off water and runoffs to regions of lower topographic location in the farms, elevating its concentration in the soil. This proposition has however been supported in some work done by Sato and Tanako (1987) on the degradation and metabolism of chlorothalonil in Japanese soil.

It was observed that chlorothalonil concentrations were higher in sub-soil as compared to its concentration in the topsoil for both seasons and in both Kiambu and Thika districts. This can be attributed to the leaching of chlorothalonil through the topsoil into the sub-soil. Chlorothalonil is reportedly persistent and as such can accumulate in soils to high levels posing an environmental pollution risk. Reduction trends of chlorothalonil in sub-soil showed that chlorothalonil reduced

following 1st order kinetics, with an estimated half-life of approximately 18 - 21 days (**Figures 6 and 7**). This is consistent with findings by Stallard and Szalkowski (1976) and Sato and Tanako (1987).

The tropical biotic conditions of the kikuyu loams growing coffee and the attendant field activities including the frequency of chlorothalonil application in each farm investigated could be influencing factors to the rate of chlorothalonil degradation. Due to the observed high levels of chlorothalonil there is risk for actual accumulation of the metabolite under severe CBD months (May, June, July and November) when the spray interval is sometimes reduced to 14 days. These preliminary results show that the threshold spray interval should be drawn at 21 days if chlorothalonil is the fungicide of choice. This applies to the upper Kiambu CBD prone high altitude coffee farming area. In order to alleviate from this problem pyrethrins have been forwarded as a good substitute to chlorothalonil (Technical Circular no. 63, 1990).

Bean leaves are seen to build-up approximately one and a half times more chlorothalonil than potatoes leaves (**Table 4.5a**), while bean leaves had about twice as much chlorothalonil as that taken into the coffee leaves in the small holdings (**Table 4.6**). It would therefore appear that beans posses the potential to transfer substantial amounts of chlorothalonil or its derivatives into the human beans-based dietary menu. This trend was also observed by Bowen, (1987) in a study to examine the physiology of genotypic differences in zinc and copper uptake in rice and tomato.

Chlorothalonil residues in Ruiru River water increases from **RI** through **R6.** The farms along this river seem to let-off more chlorothalonil into Ruiru River probably through dumping of irrigation water, seepages and rain run-offs. This high load of chlorothalonil (and copper) in pulping water after 1st and 2nd washing show that offloading these effluents into the river will only help to contaminate the same water for factories pulping downstream and domestic users.

It has been suggested that the process of roasting and cooking beverages reduces its residue levels (Onianwa, 1998). In this study on Nigerian Robusta coffee, Onianwa observed that the highest loss of the fungicide (over 60%) was attributed to roasting and preparation of fine coffee. Results obtained in the current study confirm this proposition and is seen that the process of

roasting coffee beans reduces copper and chlorothalonil residues by 44.7 % and 52.32 % respectively (**Table 4.8 and 4.9**). Depulping and dehusking, which essentially involves the physical removal of the coffee pulp and husks so as to remain with the coffee been are important steps leading to losses of up to 32.0 and 28.7 % copper and chlorothalonil, respectively. The levels of copper and chlorothalonil in coffee collected from supermarkets in Nairobi were determined (**Table 4.10),** and were observed to be much lower as compared to the coffee beverages roasted and prepared in the laboratory during study. This is because of the thorough processing procedures fine coffee undergoes, hence loosing alot of the residues along the process.

The Paired-Samples t- Test procedure compares the means of two variables that represent the same group at different times (soil concentrations for May and for October seasons). The mean values for the two variables are displayed in the Paired Samples Statistics table (**Appendix 5a, and b**). A low significance value for the t test (typically less than 0.05) indicates that there is a significant difference between the two variables. If the confidence interval for the mean difference does not contain zero, this also indicates that the difference is significant. From **appendix 5a** the significance difference between the two variables is 0.277 and the confidence interval contains zero. Thus there is no significant difference between the means of chlorothalonil in the two seasons. This statistical data propose that data for chlorothalonil obtained from studying either small or large scale farms in either the May - July or October - December seasons will give equivalent results.

A significant difference in the levels of copper in the soil between the two seasons is observed. This implies that in order to study copper, data for both dry and wet seasons are required as the two give significantly different sets of data. This is because of the dynamics of copper, as proposed in a study by Aduayi (1973), and the inherent climatic conditions, pH, CEC, and the organic content of the soil. The view is equally shared by Maroko (1985), Kariuki (1992) and Thuo (1994) who proposed that in order to study most micronutrients in higher plants there is need to obtain data from different regions and climatic conditions.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

High levels of total soil-copper due to copper spray application resulted in high plant-tissue copper, uptake and accumulation in the leaves and beans of coffee and intercrops. Similarly, elevated levels of chlorothalonil were observed in these tissues. However, the mean levels of copper and chlorothalonil fungicide residues in Kenyan market coffee obtained in this study were found to fall below the maximum residue limit (MRL) recommended by Food and Agriculture Organization and World Health Organization of 1.00 ppm for copper and 1.00 ppm for most organochlorine pesticides, including chlorothalonil (FAO/WHO, 1988) and therefore deemed to be safe for human consumption.

The heavy rains averaging 16.32 mm/day during this period resulted in run-offs, which led to overloading of the disposal pits causing raw effluents to enter into the watercourses untreated. This caused the river water to get more polluted with the copper and chlorothalonil residues resulting from coffee washings, particularly in the 1st season during which heavier rains were received in the region. During the course of this study it was observed that not all factories had stabilization pits. Those that had, the pits were poorly maintained and not well protected from run-off water. Wastewater drainage channels in some cases were blocked thus diverting the effluents to rivers instead of disposing it into the seepage pits. It was observed that treatment of coffee effluent in the major coffee growing areas (UM and UM3) was not taken seriously as a management practice.

This study has shown that bean leaves contain about one and a half times more chlorothalonil than potatoes leaves while bean leaves also had about twice as much chlorothalonil as that taken into the coffee leaves in the small holdings as a result of spraying with chlorothalonil. It is inferred therefore that that beans posses the potential to transfer substantial amounts of chlorothalonil or its derivatives into the human beans-based dietary menu. Although the coffee research foundation (CRF) discourages intercropping in coffee farms, smallscale farmers are known to favour this practice as it is a good source of livelihood subsistence.

Recirculation systems were either partially operational or broken down. It was observed that pollution in coffee growing areas is rampant and hence the need to initiate an environmental impact assessment. This is to ensure continuous water quality monitoring in this region since the vast number of coffee factories did very little environmental impact analysis during their planning and construction phases. It is clear that the enforcement of the Water Act in this region is suspect or absent and that water pollution appears to go unabated putting the general community at a risk of using contaminated water for domestic purposes. It is hereby suggested that seepage pits be taken care of sufficiently and appropriate management practices be put in place by the factory managers in order to alleviate water pollution by factory effluents, and that community participation be encouraged while undertaking water management programmes.

For purposes of assessing the toxicological potential of each biodegradation product, it is suggested that the products of chlorothalonil degradation (metabolites) be analyzed closely, and that the micro flora associated with the various biodegradation products be bioassayed and data on their optimum multiplication conditions established through literature or investigation. This would form the basis for positively interfering with the decay pathway to encourage production of least toxic chemicals from the parent metabolite (chlorothalonil).

The following recommendations are forwarded:

a) That there is need to develop a database of the recommended spray programs for all food crops that can be intercropped with coffee (beans, Irish potatoes, tomatoes, onions, capsicums, dhania, managu, and amaranthus) with optimized spray frequencies that allows intercropping with no or reduced harmful effect to the consumer.

b) That a study on copper oxidation state (species) preference on coffee root uptake. This study will serve to investigate the observation that Cu (I) was more mobile in coffee than Cu (II).

REFERENCE.

Aduayi, E. E., 1973. Effect of increasing levels of copper on the mineral nutrition and growth of *coffee Arabica* in Kenya. Ph.D. Thesis, University of Nairobi.

Anon, 1998. Recirculation of water in a coffee factory. Kenya coffee, **26** (304): 119 – 126.

APPHA-AWWA, 1975. Standard methods for the examination of water and waste water, 14th Edition. McGraw Hill Inc. USA. Pp. 493

Bernrd D., Knezek and Boyd G. E., 1976. 'Essential Micronutrients IV: Copper, Iron Manganese and Zinc'. In applied soil trace elements. Eds. B.E. Davies. John Wiley and Sons Ltd. Pp. 259 – 286

Bock, K.R., 1963. The Control of CBD in Kenya. *Empire Journal of Experimental Agriculture*. **63**: 1198 – 1201

Bould, C., E.A. Aduayi and B.S. Kimeu, 1971. A leaf analysis service for coffee growing. Kenya coffee. Pp. 37 – 39.

Bowen, J.E., 1987. Physiology of genotypes differences in zinc and copper uptake inrice and tomato. *Plant and soil science.* **99**:115 – 125

Broadbent, F.E. and G.R. Bradford, 1952. *Soil Sci.* **74**:447-457.

Camoni I, A. DiMuccio, D. Pontecorvo, M. Rubbiani, L. Vergori, and C. Lugaresi, 1998. Residue levels in apples and pears field-treated with two experimental chlorothalonil formulations. *Bull. Environ. Contam. Toxicol,* ***46***: 361-367

Cheng, K.L, and R.H. Bray, 1953. Two specific methods of determining copper in soil and plant materials. *Anal. Chem.* **25**: 655 -659

Clifford M.N., 1991. Phenols and caffeine in wet processed beans and coffee pulp. Food chemistry **40,** 35 – 42

Demeke S., 1993. Nutritive value of forage ensiled coffee pulp suplemented with *sesbania sesban.* International foundation for science IFS workshop. Kampala, Uganda. 1993

Demumbrum, L.E. and M. L Jackson, 1956. *Soil Sci.* **8**1:353-357

De Vos C.H.R, 1992. Increased resistance to copper-induced damage of the root cell plasmalema in copper tolerant silane cucubalus. *Phsiol plant.* **82**: 523 - 528

Ellis. B. G. and B. D. Knezek, 1972. 'Adsorption reactions of micronutrients in soils' In micronutrients in agriculture. (Eds). J.J. Mortredt, P.M. Gordano. Soil Sci. Soc. Am., Inc., Madison, WI. Pp. 331-338.

FAO/WHO, 1988. Pesticide residues in food. 1987. Evaluations: Part II - Toxicology. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper 86/2).

FAO/WHO, 1989. Guide to Codex recommendations concerning pesticide residues. Part 8: Recommendations for methods of analysis of pesticide residues, 3rd ed. Rome, Codex Committee on Pesticide Residues, Food and Agriculture Organization of the United Nations (CAC/PR8-1989).

FAO/WHO, 1990a. Pesticide residues in food - 1990. Evaluations: Part I - Residues. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper 103/1).

FAO/WHO, 1990b. Pesticide residues in food - 1990. Evaluations: Part II - Toxicology. Rome, Food and Agriculture Organization of the United Nations (FAO Plant Production and Protection Paper 103/2).

FAO/WHO, 1991. Pesticide residues in food - 1990. Evaluations: Part II - Toxicology. Geneva, World Health Organization (WHO/PCS/91.47).

Frazier H.W., 1993. Chlorothalonil: A response to Environmental Fate and Ground Water Branch (EFGWG) review. Painesville, Ohio, Ricerca, Inc. (Document No. 3254-93-0280-EF-001, submitted to WHO by ISK Biosciences Corporation, Mentor, USA).

Githunguri E.W., 1991. Ecological impact of urban sewage and wastewater on the Nairobi and Athi rivers. M.Sc. Thesis, University of Nairobi.

Greenland, D.J. and Hayes M.H.B., 1978. Soils and Soils chemistry, in D.J. Greenland and M.H.B. Hayes (eds), the chemistry of soil components, Wiley – Interscience, Chichester and New York. Pp. 1-27

Harris, C. R., 1966. Exploratory studies on the occurrence of organochlorine insecticide in agricultural soils in Brazil. *J. Agric. Fd. Chem.* **14:** 398-403

Hassall A.K., 1990. The biochemistry and uses of pesticides 2nd edition. Macmillan publ. Delhi, India. p.p. 27-29

Hodgson, J. F., 1963. *Advance Agron.* **15**:119 – 159

IARC, 1983. Chlorothalonil. In: Miscellaneous pesticides. Lyon, International Agency for Research on Cancer, pp 319-328 (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Volume 30).

IARC, 1987. Overall evaluations of carcinogenicity: An updating of IARC monographs, volumes 1 to 42. Lyon, International Agency for Research on Cancer, p 60 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7).

Jacobson, B.M., and J.M. Schollenberger, 1992. Dissipation of 4-hydroxy-2,4,5-trichloroisophthalonitrile (SOS-3701) in soil: A re-analysis of chlorothalonil soil dissipation data. Painesville, Ohio, Ricerca, Inc. (Proprietary report No. 1874-T, submitted to WHO by ISK-Biotech, Mentor, USA).

Kiptoo, J., 2000. Investigation into the use of coffee pulp as animal feed. An exploratory study. Work in progress. Coffee Research Foundation, 1999. (Unpublished report.)

Katayama A, H. Isemura, and S. Kuwatsuka, 1991. Suppression of chlorothalonil dissipation in soil by repeated applications. *J Pestic Sci,* **16**: 233-238.

Kariuki J.K, 1992. Distribution of fungicide copper in a coffee farm in Kiambu. M.Sc. Thesis Kenyatta University, Kenya.

Marschner H., 1995. Mineral nutrition of higher plants. Academics press, London. Pp. 268

Masaba, D. M., 1987. Efficiency of reduced rates of copper formulations against coffee leaf rust in Kenya. Kenya coffee, volume **65:** 112-132

Maroko J.B., 1989. Copper levels in soil and plant materials in Kenya. Kenya coffee. Vol. **54:** 13-17

McDonald, J. A., 1926. Preliminary account of green coffee berries in Kenya colony. *Trans Britain Mycological Society*. **4**: 113 - 114

Mckeague, J. A. and J. H. Day, 1966. Dithionate- and oxalate-extractable Fe and Al as aids in differentiating various classes of soils. C*anadian Journal of Soil Science.* **46:** 313 - 321

Mitchell, R.L., 1964. Trace elements in soils. In Bear F.E. (ed). Chemistry of the soil. Reinhold, New York. Pp. 421

Mwan'gombe, A. W. and Z. U. R. Javed, 1984. Field evaluation of fungicides for the control of CBD in 1981/1982 period in Kenya . Kenya coffee, volume **64:** 24-27.

Mwaura, P.K., 1995. Environmental impact of waste from coffee processing of coffee on river water quality. Unpurblished report. Diploma, Kenya Polytechnic, Kenya.

Nutman, F. J. and F. M, Roberts, 1960a. Investigation on a disease of *coffee Arabica* caused by a form of *Colletotricum coffeanum* Noack. 1. Some factors affecting infection by the pathogen. Trans Britain Mycological Society. Pp123-138.

Nutman, F. J. and F. M, Roberts, 1960b. Investigation on a disease of *coffee Arabica* caused by a form of *Colletotricum coffeanum* Noack. 2. Some factors affecting germination and infection and their relation to disease infection. Trans Britain Mycological Society. Pp139 – 143.

Ongeri, D.M., 1998. Determination of pesticide residues in rivers water, sea water and fish. Unpublished M.Sc Thesis. University of Nairobi.

Onianwa, P. C., I. G. Adetola, C. M. A. Iwegbue, M. F. Ojoo and O. O. Tella, 1998. Trace heavy metals composition of some Nigerian beverages and food drinks. *Food Chemistry.* **66:** 275:279.

Rendina, A., A.F. Iorio, 1996. Determinacion absorciometrica de fosforo en plantes. *Rev. fac. Agron.* **93**:1267 - 1300.

Sato, K. and H. Tanako, 1987. Degradation and metabolism of a fungicide 2,4,5,6-TPN in soil. *Biol. fert. soils.* **3:** 205 - 209

Shuman. L.M., 1979. Zinc, Manganese and copper in soil fractions. *Soil science.* **127**:10 - 17.

Smith, K. A., 1996. Soil analysis: Modern instrumental techniques 2nd Edition. Cambridge University Press. London. Pp.273.

Stallard D.E. and M.E. Szalkowski, 1976. Effect of micro-organisms upon the soil metabolism of Daconil (i.e., chlorothalonil) and 4-hydroxy-2,5,6-trichloroisophthalonitrile. Painesville, Ohio, Ricerca, Inc. (Report No. 1000-3EF- 76-2087-001, submitted to WHO by ISK Biosciences Corporation, Mentor, USA).

Stevenson F. J., 1976. ***Soil Sci. Soc. Am. J.,*** **40**:665-672

Suzuki S and M. Oda, 1977. Simultaneous determination of chlorothalonil, captan and diazinon in apples. *Bull Agric Chem Insp Stn.*(Tokyo), **17**:43-45.

Thuo J.T., Mburu J.N., and Kamau H.M. 1994. Dynamics of accumulated copper in the soil and leves of coffee Arabica L. Kenya coffee, Vol. 59. No. 689. pp 68 -75.

Technical Circular no. 56. Control of Coffee Berry Disease and leaf rust in 1982. Kenya Coffee, January 1984.

Technical Circular no. 63. Control of Coffee Berry Disease and leaf rust in 1987. Kenya Coffee, January 1990.

Tessier, A., P.G.C. Campbell and M. Bisson, 1979. Sequential extraction procedure for the speciation of particulate trace element. *Analytical Chemistry.* **51:** 844 - 851

Wanjohi, J., 1991. Copper levels in soils collected from coffee farms along Ruiru River in Kiambu District and Rutui River in Kirinyaga. Unpublished M.Sc. Thesis, University of Nairobi.

Westerman, R. W., 1990. Soil testing and plant analysis. SSSA book series: 3 Soil Science Society of America, Inc. Madison, Wisconsin. Pp. 43 – 56.

Wild, A., 1996. Soil and Environment: An Introduction. Cambridge University Press. Pp 213 – 243.

Willson K.C., 1985. Critical levels of nutrients in plant leaves. In Coffee: botany, biochemistry and production of beans and Beverages. Clifford M.N., and Willson K.C., (eds). Croom - helm, London. Pp. 150 –154.

APPENDICES

1. GC-ECD chromatogarphs:

a) Standards

b) Soil

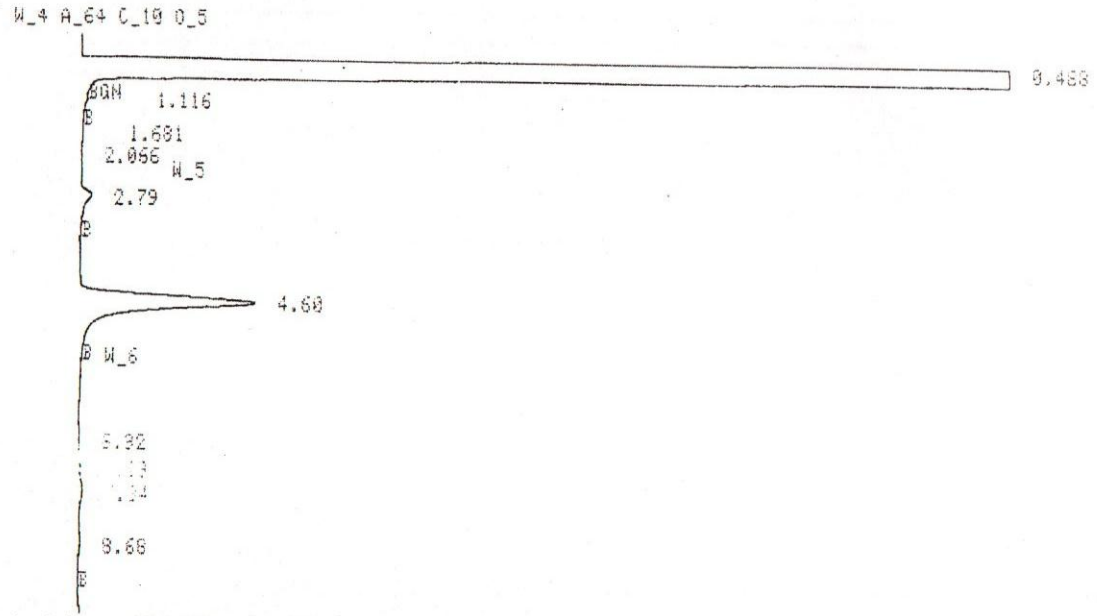
PERKIN-ELMER CORP PART NO. N625-1026 5101

FILE 21 RUN 21 STARTED 13:24.7 02/08/07 DAC. RESIDUES
% METHOD 1 PEST RESIDUES LAST EDITED 10:24.1 02/08/07

RT	AREA	HEIGHT	BC	AREA PERCENT	HEIGHT PERCENT
1.033	287840	33.1583	T	10.1330	16.1757
1.142	224744	24.5875	T	7.9118	11.9946
1.476	211957	17.6122	T	7.4617	8.5918
1.646	127449	10.2358	T	4.4867	4.9938
1.966	19135	3.2875	T	0.6736	1.6037
2.114	53134	4.6012	T	1.8705	2.2446
2.344	41091	3.6588	T	1.4465	1.7849
2.560	89199	7.0674	T	3.1401	3.4477
3.138	79736	5.2485	T	2.8070	2.5604
3.466	208985	13.9736	T	7.3570	6.8167
3.792	(673308)	49.0238		23.7029	23.9154
4.63	572425	20.9966	V	20.1515	10.2428
6.13	210782	9.1527	T	7.4203	4.4650
6.70	40827	2.3839		1.4373	1.1629

c) Water

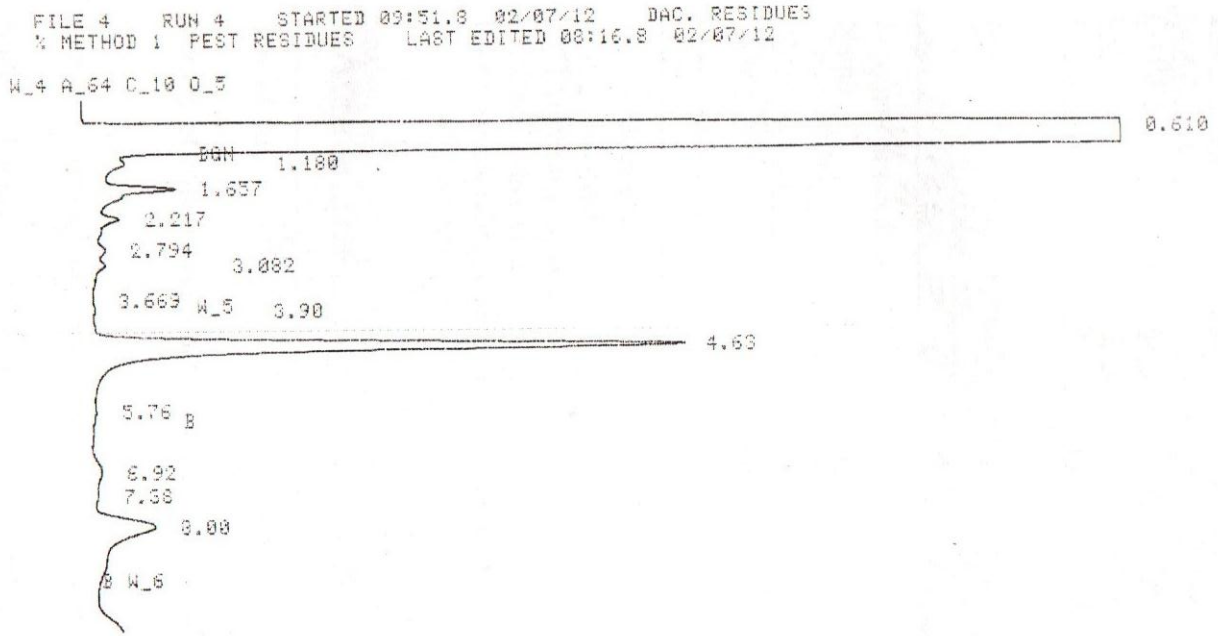
FILE 2 RUN 2 STARTED 09:27.5 02/07/12 DAC. RESIDUES
% METHOD 1 PEST RESIDUES LAST EDITED 08:16.8 02/07/12



FILE 2 RUN 2 STARTED 09:27.5 02/07/12 DAC. RESIDUES
% METHOD 1 PEST RESIDUES LAST EDITED 08:16.8 02/07/12

RT	AREA	HEIGHT	BC	AREA PERCENT	HEIGHT PERCENT
1.115	32773	3.0481		1.5845	2.3418
1.681	6746	0.4196	V	0.3261	0.3224
2.066	1608	0.2198	V	0.0777	0.1689
2.79	103113	7.0459		4.9853	5.4132
4.60	1810798	114.8794		87.5486	88.2598
6.92	5558	0.2910	T	0.2687	0.2235
7.39	13010	0.7920	T	0.6290	0.6085
7.84	[illegible]	2.4947	T	3.0036	1.9167
8.68	[illegible]	0.9700		1.2764	0.7452

9 PEAKS > AREA REJECT 2068334 TOTAL AREA



FILE 4 RUN 4 STARTED 09:51.8 02/07/12 DAC. RESIDUES
% METHOD 1 PEST RESIDUES LAST EDITED 08:16.8 02/07/12

RT	AREA	HEIGHT	BC	AREA PERCENT	HEIGHT PERCENT
1.180	891450	22.9517	T	5.3549	4.6134
1.657	759892	51.8594	T	10.3350	10.4240
2.217	304026	17.6434	T	4.1590	3.5464
2.794	140444	8.7416	T	1.9212	1.7571
3.082	125837	8.1291	T	1.7351	1.6340
3.669	1182	0.1522	V	0.0162	0.0306
3.90	1888	0.2949	V	0.0258	0.0593
4.63	4496551	345.8672	T	61.5109	69.5213
5.76	19176	1.1942		0.2623	0.2400
6.92	118510	4.9379	T	1.6090	0.9926
7.38	[illegible]	1.6751	V	0.3508	0.3367
8.00	[illegible]	34.0513		12.6299	6.8445

10 PEAKS % AREA REJECT 7310150 TOTAL AREA

2. Questionnaires to Farm/Estate Manager

Survey questionnaire.

1. Name of Farmer / Farm /Estate ---Tarnia
2. Name of Farm Manager ---George England
3. Name of pesticide chemicals applied before and the rate

Name	Rate of application
DC-TRON	150 ml / 15L of water
DIAZINON	30 ml / 15L of water
DIPEL	0.5 kg / 1000L of water
CU	3.8 kg / ha or 5.5 kg / ha (green cu)
Dacomil	2.2 kg / ha or 3.3 kg / ha (when raining)

4. Name of pesticides applied recently (current season) and the rate ----------
   (1) DIAZINON = 30ml / 15L of water
   (2) DIPEL = 0.5kg of / 1000L of water
5. Frequent application of the pesticides

Name	Frequency
DC-TRON	ONCE A YEAR
DIAZINON	TWICE / EVERY SPRAY / AFTER 14 DAYS
DIPEL	TWICE AFTER 14 DAYS

1 Coppers.
2 Dacomil

~~a)~~ Every rainy season.
b) Once every year.

6 Rivers surrounding your plantation and factory
   a. Plantation---River River
   b. Factory---River River

7. Season, day or time for harvesting (Month and Week days if possible)
   APRIL TO DECEMBER.
8. Size of farm---1/4 HA
9. Variety of coffee trees planted at the farm FRENCH MISSION
10. The intercrops planted ---N/A
11. Name of Factory that does processing for farmer/estate. TARRIA COFFEE ESTATE

A) Primary processing ---✓

B) Secondary processing ---SOCFINAF COFFEE MILLS.

Survey questionnaire.

1. Name of Farmer / Farm /Estate KENTMERE DIV / KIBUBUTI ESTATES
2. Name of Farm Manager MR. SAMUEL WEJULI
3. Name of pesticide chemicals applied before and the rate

Name	Rate of application
Copper x	2.2 Kg/ha or
Dacomic	3.3 Kg/ha as
TANK	Tank mixture.
MIXTURES	

4. Name of pesticides applied recently (current season) and the rate
Copper x Dacomil tank mixture

5. Frequent application of the pesticides

Name	Frequency
Copper x	often during
Dacomil	rainy season at
	21 day
	interval.

1 Coppers.
2 Dacomil

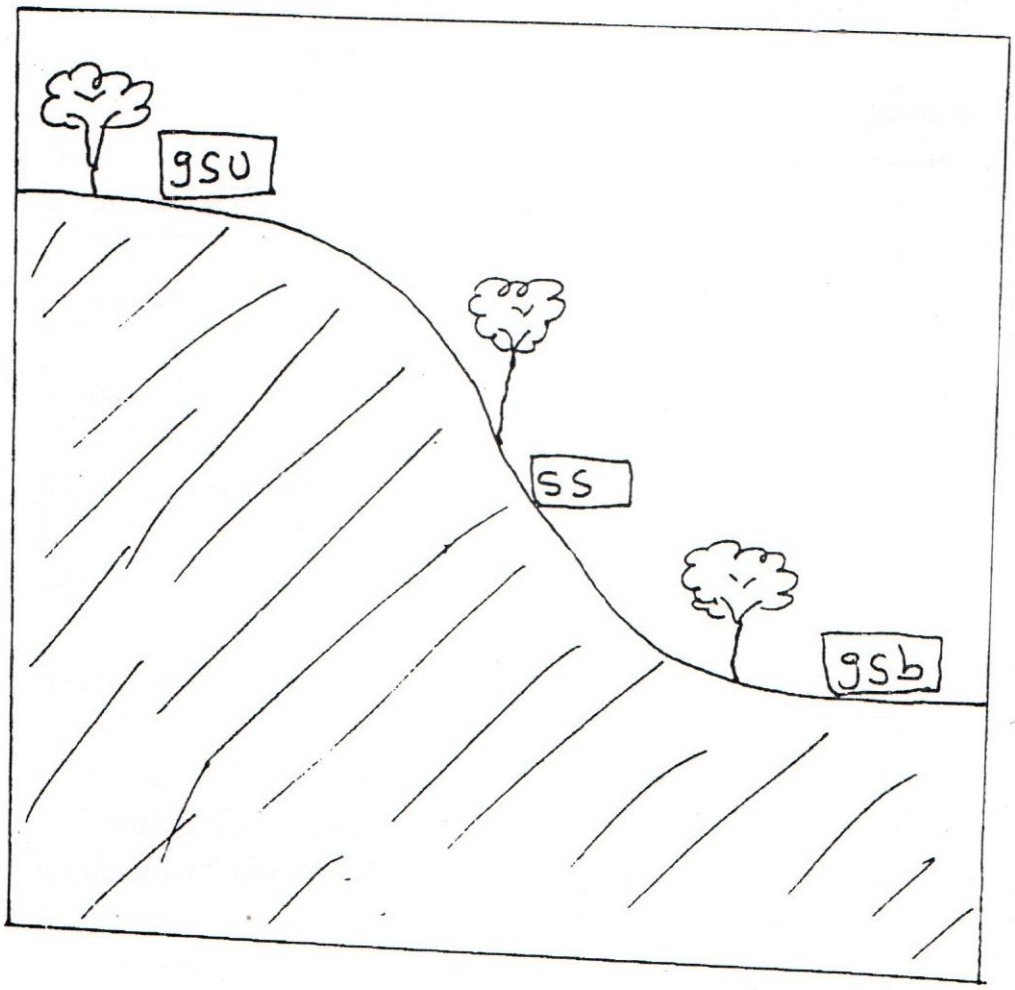
a) Every rainy season.
b) Once every year.

6. Rivers surrounding your plantation and factory
   a. Plantation---------Dam.
   b. Factory----------Dam.
7. Season, day or time for harvesting (Month and Week days if possible)
MAY – JANUARY
8. Size of farm----------1043 Acres.
9. Variety of coffee trees planted at the farm French mission, SL 28, K 47
10. The intercrops planted ---------None.
11. Name of Factory that does processing for farmer/estate.

A) Primary processing ---------✓
B) Secondary processing ---------✓

} Done in the Estate factory

3. Farm Landscape feature



KEY:

- gsu - Gentle slope upper.

ss - steep slope.

gsb - Gentle slope base.

4. Kiambu Soil Chemical characteristics

Chemical characteristics of soils in Copper sprayed (high sprayed) coffee plantation and unsprayed (low sprayed).

		Unsprayed		Sprayed	
ITEM	**T/S**	**Range**	**Mean**	**Range**	**Mean**
pH (0.01M $CaCl_2$)	T	4.1-5.0	4.5	3.9-5.5	4.5
	S	4.8-5.1	4.9	4.5-5.0	4.7
Available P (ppm)	T	25.5-72	48	34-142	88
	S	21-123	72	17-101	59
Available B (ppm)	T	0.40-0.95	0.65	nd	nd
	S	0.55-0.70	0.6	nd	nd
Extractable K (meq/100 soil)	T	0.78-1.40	1.02	0.60-1.48	1.09
	S	0.68-2.00	1.15	0.48-1.04	0.72
Extractable Ca (meq/100 soil)	T	0.40-3.60	1.7	0.80-12.0	5.5
	S	0.60-2.20	0.97	0.80-9.40	3.8
Extractable Mg (meq/100 soil)	T	1.80-2.80	2.21	1.30-3.50	2.57
	S	1.50-2.80	2.14	1.50-3.90	2.49
EDTA extractable CU (ppm) soil		0.3-24.4		1.0-50.6	15.52
Extractable CU (ppm) Leaf		1.12-3.4		3.37-8.15	4.19
Extractable CU (ppm) Bean		0.72-0.90		0.84-1.03	0.93
Extractable CU (ppm) husk		0.31-0.41		0.4-0.62	0.459

Source: Aduayi, 1973.

KEY:

nd	*not done*
meq	*milliequivalent*
T	*topsoil*
S	*subsoil*
ppm	parts per million

5. Paired samples t test

5 a: Paired samples t test for levels of chlorothalonil in the two seasons

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	MAY - OCTOBER	-5.061667E-02	.222599	4.54378E-02	**-.144612**	**4.33786E-02**	-1.114	23	**.277**

5 b: Paired samples t test for chlorothalonil levels of soils in large and small-scale.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	LARGE - SMALL	-2.690000E-03	.790895	.250103	**-.568462**	**.563082**	-.011	9	**.992**

6. Data for Correlation analysis

No	MgCl Cu	NaOCl Cu	Total	EDTA Cu	Leaf Cu	Husk Cu	Berry Cu	B	P
1	0.34	0.97	123	10.3	1.12	0.31	0.72	0.55	123
2	0.37	2.43	86.1	7.0	-	-	0.73	0.65	50
3	0.22	1.51	155.8	50.6	1.08	0.60	1.03	0.75	37
4	0.24	4.07	103.2	23.0	-	-	0.89	0.6	20.5
5	0.29	4.05	70.9	19.4	6.53	0.40	0.9	0.95	30
6	0.19	0.98	27.5	10.6	-	-	0.64	0.40	35
7	0.13	1.58	48.7	43.0	4.41	0.71	0.8	0.75	17
8	0.09	1.74	29.8	17.1	-	-	0.85	0.75	142

7. Correlation for EDTA-extractable Copper and Total Copper in the soil in the 1st season

		EDTA-COPPER	TOTAL COPPER
EDTA-COPPER	Pearson Correlation	1.000	.538**
	Sig. (2-tailed)	.	.007
	N	24	24
TOTAL COPPER	Pearson Correlation	.538**	1.000
	Sig. (2-tailed)	.007	.
	N	24	24

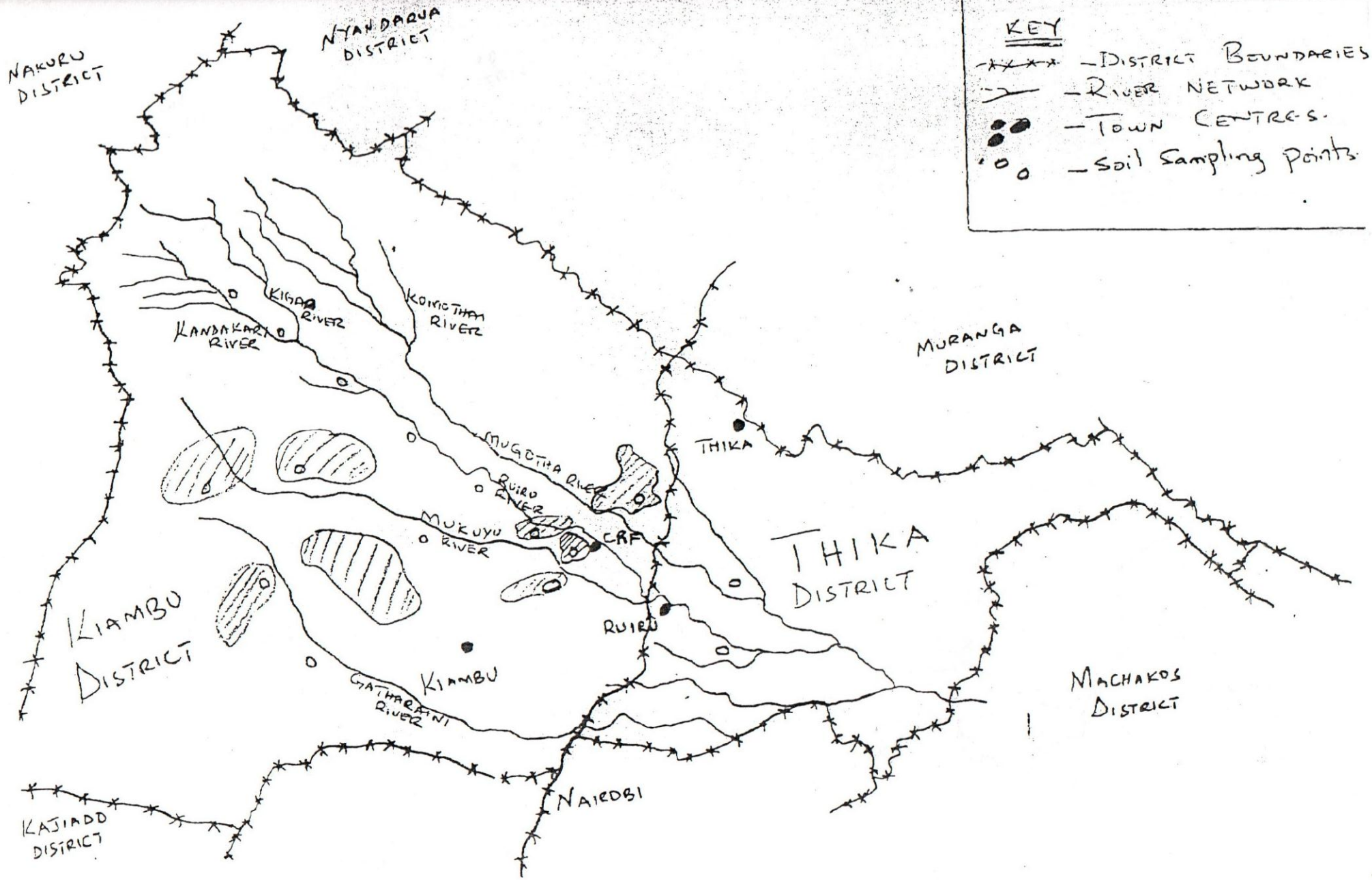
** Correlation is significant at the 0.01 level (2-tailed).

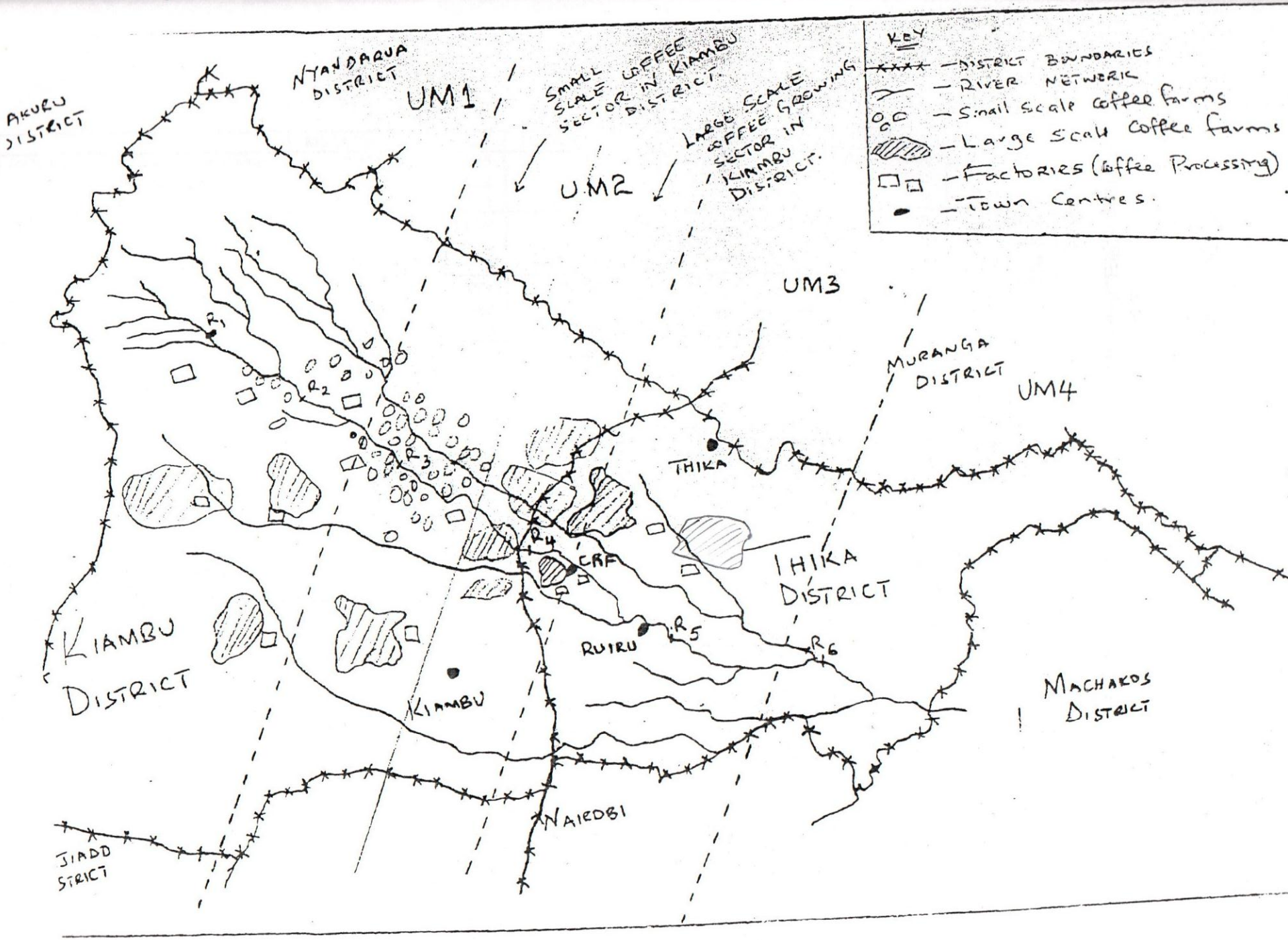
8. Correlations for 1ST and 2nd season Total Copper in the soil

		1st	2nd
1st	Pearson Correlation	1.000	.964**
	Sig. (2-tailed)	.	.000
	Sig. (2-tailed)	.	.000
	N	24	24
	N	24	24
2nd	Pearson Correlation	.964**	1.000
2nd	Pearson Correlation	.964**	1.000

	Sig. (2-tailed)	.000	.
	N	24	24

** Correlation is significant at the 0.01 level (2-tailed).

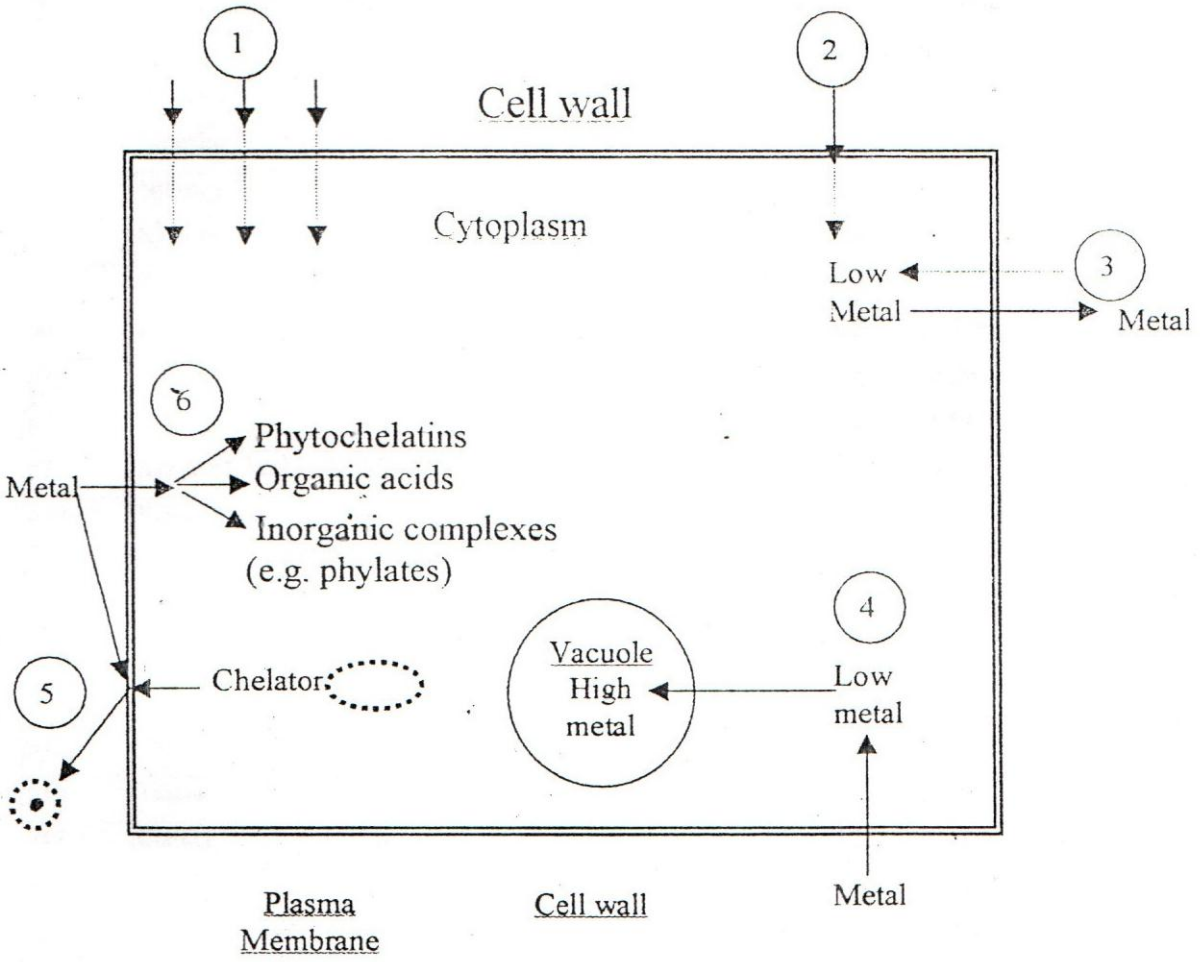

KEY
-DISTRICT BOUNDARIES
-RIVER NETWORK
-TOWN CENTRES.
-Soil Sampling Points
NAKURU DISTRICT
NYANDARUA DISTRICT
MURANGA DISTRICT
THIKA
THIKA DISTRICT
MACHAKOS DISTRICT
KIAMBU DISTRICT
KIAMBU
RUIRU
CRF
NAIROBI
KAJIADO DISTRICT
KANDAKARI RIVER
KIGAA RIVER
KOIMOTHAN RIVER
MUGOTHA RIVER
RUIRU RIVER
MUKUYU RIVER
GATHARAINI RIVER


KEY
— DISTRICT BOUNDARIES
— RIVER NETWORK
— Small scale coffee farms
— Large scale coffee farms
— Factories (coffee Processing)
— Town Centres.
AKURU DISTRICT
NYANDARUA DISTRICT
UM1
UM2
UM3
UM4
SMALL SCALE COFFEE SECTOR IN KIAMBU DISTRICT.
LARGE SCALE COFFEE GROWING SECTOR IN KIAMBU DISTRICT.
MURANGA DISTRICT
THIKA
THIKA DISTRICT
KIAMBU DISTRICT
KIAMBU
RUIRU
CRF
R1
R2
R3
R4
R5
R6
MACHAKOS DISTRICT
NAIROBI
JIADO STRICT

10. Pearson correlation data for Copper, Boron and Phosphorus in soil and plant tissue

		MGCLCU	NAOCLCU	TOTALCU	EDTACU	LEAFCU	HUSKCU	BEANSCU
MGCLCU	Pearson Correlation	1.000	.245	.539	-.441	-.184	-.985*	-.180
	Sig. (2-tailed)	.	.559	.168	.275	.816	.015	.670
	N	8	8	8	8	4	4	8
NAOCLCU	Pearson Correlation	.245	1.000	.074	-.060	.862	-.195	.418
	Sig. (2-tailed)	.559	.	.862	.888	.138	.805	.303
	N	8	8	8	8	4	4	8
TOTALCU	Pearson Correlation	.539	.074	1.000	.355	-.834	-.249	.521
	Sig. (2-tailed)	.168	.862	.	.388	.166	.751	.186
	N	8	8	8	8	4	4	8
EDTACU	Pearson Correlation	-.441	-.060	.355	1.000	-.148	.916	.715*
	Sig. (2-tailed)	.275	.888	.388	.	.852	.084	.046
	N	8	8	8	8	4	4	8
LEAFCU	Pearson Correlation	-.184	.862	-.834	-.148	1.000	.070	-.009
	Sig. (2-tailed)	.816	.138	.166	.852	.	.930	.991
	N	4	4	4	4	4	4	4
HUSKCU	Pearson Correlation	-.985*	-.195	-.249	.916	.070	1.000	.368
	Sig. (2-tailed)	.015	.805	.751	.084	.930	.	.632
	N	4	4	4	4	4	4	4
BERRYCU	Pearson Correlation	-.180	.418	.521	.715*	-.009	.368	1.000
	Sig. (2-tailed)	.670	.303	.186	.046	.991	.632	.
	N	8	8	8	8	4	4	8

11. Mechanism of copper tolerance in plants



Possible mechanism of heavy metal tolerance of plants (De Vos, 1992):

1) Binding to cell wall.
2) Restricted influx through the plasma membrane.
3) Active influx.
4) Compactmentation in vacuole.
5) Chelation at the cell wall-plasma membrane interface.
6) Chelation in the cytoplasm.

12a: Soil analysis results (May - July Season). n = 24

Lab Number	Name of Farm/farmer	Small or large scale farming	Top (T)/ Sub (S) soil.	Farm Landscape	pH	Hp	Chlorothalonil (ppm)	Copper (ppm)
p1	Mbucho	small	T	ss	5	0.3	0.126	48.5
p2	Mbucho	small	S	ss	4.9	0.3	0.018	42.8
p3	Njoroge	small	T	ss	5.3	0.4	0.092	133.1
p4	Njoroge	small	S	ss	4.9	0.4	0.049	69.7
p5	Kahowai	small	T	gsu	5	0.2	0.013	129.3
p6	Kahowai	small	S	gsu	5	0.2	1.479	86.1
p7	Njungua	small	T	gsb	4.8	0.4	0.290	155.8
p8	Njungua	small	S	gsb	4.4	1.6	1.543	103.2
p9	Kiarie Mukuria	small	T	ss	4.7	0.6	0.095	91.0
p10	Kiarie Mukuria	small	S	ss	4.3	1.1	0.220	2.0
p11	Yadini	Large estate	T	gsu	4.3	1.4	0.037	69.3
p12	Yadini	Large estate	S	gsu	4.5	1.3	0.239	29.6
p13	Azania	Large estate	T	gsu	5	0.1	0.277	172.2
p14	Azania	Large estate	S	gsu	4.3	1.5	1.448	38
p15	Tassia	Large estate	T	gsu	3.8	3.3	0.675	70.9
p16	Tassia	Large estate	S	gsu	4	2.2	0.308	27.5
p17	Cianda	Large estate	T	gsb	3.8	5.1	0.127	33.7
p18	Cianda	Large estate	S	gsb	4.6	0.9	0.234	4.9
p19	Ibonia	Large estate	T	gsb	4.6	1	0.226	27
p20	Ibonia	Large estate	S	gsb	5.3	1	0.328	4.1
p21	Rukera	Large estate	T	gsb	4.1	1.4	0.590	3.4
p22	Rukera	Large estate	S	gsb	4.8	0.1	0.166	2.4
p23	Kibubuti	Large estate	T	gsu	4.8	0.5	0.585	48.7
p24	Kibubuti	Large estate	S	gsu	4.7	0.9	3.186	29.8
Range							**0.018 –3.186**	**2.0 – 172.2**

Hp -Total exchangeable acidity.

ppm -Parts per million. **ss**,-Steep slope. **Gsu** -Gentle slope upper. **Gsb** -Gentle slope base.

EGERTON UNIVERSITY
LIBRARY

12b: Soil analysis result (September – October Season) n = 24

Lab Number	Name of farm/farmer	Top (T) / Sub (S) soil.	Farm Landscape	pH	Chlorothalonil (ppm)	Copper (ppm)
TOP SOIL						
p1	Mbucho	T	ss	4.8	0.166	161.4
p3	Njoroge	T	ss	5.0	0.103	139.7
p5	Kahowai	T	gsu	5.1	0.045	163.1
p7	Njungua	T	gsb	4.6	0.411	168.2
p9	Kiarie M.	T	ss	4.6	0.101	102.1
p11	Yadini	T	gsu	4.6	0.042	74.0
p13	Azania	T	gsu	4.7	0.300	164.8
p15	Tassia	T	gsu	4.2	0.692	88.4
p17	Cianda	T	gsb	4.0	0.186	38.4
p19	Ibonia	T	gsb	4.4	0.661	28.0
p21	Rukera	T	gsb	4.0	0.668	13.2
p23	Kibubuti	T	gsu	4.5	0.640	49.1
Range:					**0.042 – 0.692**	**13.2 – 168.2**
Mean:					**0.334 ± 0.026**	**99.2 ± 5.884**
SUB SOIL						
p2	Mbucho	S	ss	4.8	0.042	104.7
p4	Njoroge	S	ss	5.0	0.073	84.9
p6	Kahowai	S	gsu	5.0	1.274	107.3
p8	Njungua	S	gsb	4.8	1.634	143.4
p10	Kiarie M.	S	ss	4.8	0.346	13.7
p12	Yadini	S	gsu	4.3	0.244	42.0
p14	Azania	S	gsu	4.6	1.492	66.6
p16	Tassia	S	gsu	4.1	0.774	33.3
p18	Cianda	S	gsb	4.4	0.348	12.3
p20	Ibonia	S	gsb	4.8	0.541	16.4
p22	Rukera	S	gsb	4.4	0.347	13.9
p24	Kibubuti	S	gsu	4.5	2.410	23.4
Range:					**0.042 – 2.410**	**12.3 – 143.4**
Mean					**0.7935 ± 0.174**	**55.15 ± 15.12**

EULIB
083014