THIN LAYER CHROMATOGRAPHIC STUDIES ON DEPLETION OF SOME HERBICIDES IN TWO SOIL ECOSYSTEMS.

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By

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DECLARATION

It is hereby declared that this Thesis is the result of the research work undertaken by the author and it has neither wholly nor partially been presented for another degree elsewhere.

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DEDICATION

THIS THESIS IS DEDICATED TO MY BELOVED WIFE, VICTORIA AND MY DAUGHTERS JOSEPHINE, LORETTA AND LOIS FOR THEIR CONCERN FOR MY WELL BEING.

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Praise, honour and adoration be to Almighty God for keeping me in his safe hands till now. To him be the glory for the great thing he has done.

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CONTENTS

ABSTRACT

Depletion rates of three triazine herbicides, atrazine, simazine and ametryne and two urea base herbicides, diuron and metobromuron, under laboratory conditions have been investigated in soil samples collected from GAEC, a coastal savannah soil, and KNUST, a forest zone soil. Two hundred grammes of the soil samples were treated with herbicides standard solution to generate herbicide-soil concentration of 10 µg/g and incubated at room temperature for 12 weeks. TLC methodology was used to monitor the decline of the herbicides from the soil and the result showed that the decline of the chemicals was comparatively faster in the KNUST soil than the GAEC soil. After two weeks of soil treatment and incubation, atrazine, simazine and metobromuron had depleted more than half of the initial amount applied. In all, the rate of depletion of metobromuron was found to be the highest and at the end of the experiment, it declined to about 2.42 % and 4.38 % of the initial concentration in the KNUST and the GAEC soils respectively. The results obtained, indicated that the kinetics involved in the process of depletion of the herbicides to a higher degree could be described by first order reaction kinetics. The half-lives of the herbicides in the GAEC soil were in the range of 14.8 – 32.2 days and 13.3 – 31.1 days in the KNUST soil. Soil moisture and organic matter content were found to facilitate the depletion of the chemicals from the soils. Out of the various solvents systems tried for the

extraction of the herbicides, acetone, acetonitrile and acetone/hexane mixture (4:1) were found to be efficient for the recovery of the chemicals in the soil ecosystems studied. With the photosynthesis inhibition method used for the detection of the herbicides, the detectability for the unclean extracts was in the range of 0.004 – 0.008 µg/g and that of the clean-up extracts was in the range of 0.024 – 0.162 µg/g.

CHAPTER ONE

INTRODUCTION

1.1 HISTORICAL BACKGROUND

A weed is essentially any plant grown in a place where it is not wanted[1]. Weeds have been a problem since man began to till the land for food and for other useful purposes. The problem of weeds was even emphasized in the parable of Jesus Christ[2], hence 2000 years ago, they must have been a burden to man. He referred to deleterious effect of weeds in two ways. First, in the parable of the sower, they choked the crop and reduced the yield . Second, in the parable of the tares sown by the enemy, the crop was disturbed and its growth impaired in the process of removing the weed from the crop.

Although weeds have challenged man's effort to survive ever since he started tilling the soil for food, advanced weeds control methods are practiced on limited scale particularly, in the tropics, where hand weeding is still widely carried out. It has been reported by LeRoy Holm[3] that more energy is still expended on the weeding of man's crop than any other single human task.

Before the introduction of advanced methods of weeds control, four measures were adapted to eradicate or limit the spread of weeds. These were manual weeding (by the use of the hand directly or by the use of implements such as

hoes and cutlasses), crop rotation, ploughing and various methods of preventing weed seeds from being dispersed. These methods suffer from a basic weakness, in that they are aids to control but they cannot prevent weeds growing with the crops. Apart from this weakness, weeds control by manual weeding through the direct use of the hand, or the use of the farm implements, i.e hoes and cutlasses is time consuming and labour intensive.

Advanced methods of weed control or weed eradication involving the use of chemicals have been practised since time immemorial. It is even an established fact that in biblical times, armies sometimes used salts or mixture of brine and ashes to sterilise land that they had conquered, the intention being to make the land uninhabitable by future generations of the enemy. Salts and various industrial by-products such as smelter wastes have also been applied to roadsides and paths to rid them of vegetation hundreds of years ago[4].

Chemicals used to control weeds are called herbicides. Herbicides are one of the main classes of pesticides. They are often employed to kill weeds, sometimes without causing injury to desirable vegetation, for example, to eliminate broad-leaf weeds from lawns without killing the grass.

Herbicides, may be considered to have been discovered in 1896 when Bonnet, a French grape grower, observed that the Bordeaux mixture he applied to his vines as protection against downy mildew, turned the leaves of the *sinapis arvensis*

black[5]. The weedkilling properties of sulphates of ammonia, zinc, iron and other metals were soon observed[5].

In the first half of the twentieth century, several inorganic compounds were used as weed killers, principal amongst them being Sodium arsenite Na_3AsO_3, Sodium Chlorate $NaClO_3$, and Copper sulphate $CuSO_4$[6]. The latter are only two of a large number of salts formerly used as herbicide sprays whose means of operation is to kill plants by the primitive action of extracting the water from them, and leaving the land to support agriculture. Later milestone in weed control was the introduction of the first organic chemical, 2-methyl-4,6-dinitrophenol in 1932. A few years later, an important discovery was made that chemicals related structurally to plant hormones could be used as selective weedkillers.

The use of non-selective and, later selective residual chemicals such as the substituted phenylureas, triazines, and the non-residual chemicals such as paraquat and diquat may be considered as more recent milestones. Inorganic and organometallic herbicides have been phased out because of their persistence in soil.

The world consumption level of herbicides between 1896 and 1968 is presented in Table 1[7].

Table 1: Estimated world consumption of Herbicides at consumer level from 1896 to 1968

Area	Consumption(million of dollars)
North America	550
Latin America	80
Near East, South East, Oceania	80
Japan	70
Western Europe	60
Africa	40
Total	**880**

It is clear from the Table 1 that more than 50 % of the world consumption of herbicide occurred in North America and the least amount was consumed in Africa within the period being discussed.

The situation in Africa today however, shows an improvement with respect to the use of herbicides. Wandiga[8] in the review of pesticide use in Africa reported that between 1986 to 1990 a total of 6830 tonnes of herbicides were imported into Kenya for use. In Ghana the situation is not different, and available information indicates that 21 different kinds of herbicides were imported into the country for agricultural purposes between 1995 and 2000[9]. Notably among

them were paraquat, atrazine, bromacil, diuron, glyphosate, propanil, ametryne, oxadiazon and alachlor.

Table 2 below shows the total amount of herbicides imported into Ghana from 1995 to 2000.

Table 2: Quantity of herbicides imported into Ghana from 1995 – 2000[9].

Year	Amount imported (in kilogrammes)
1995	88,587
1996	55,414
1997	132,292
1998	224,816
1999	97,584
2000	52,030

1.2 BACKGROUND OF STUDY

In recent years, there has been a considerable increase in the use of herbicides in Ghana. This has come about as a result of the intensification of agricultural activities to meet the Country's food needs and the emphasis on the promotion of non-traditional agriculture products.

The use of herbicides makes food production convenient and to some extent easy. This is because a number of them selectively kill the target weeds and leave the cultivated crops/plants intact, thus saving the farmer the problem of

having to use farm implements to clear the unwanted weeds. In Ghana, such herbicides as atrazine, diuron, ametryne, glyphosphate,bromacil, paraquat and simazine[10] are now being used extensively for the weed control in commercial cultivation of food crops such as rice, maize, pineapple, banana and some vegetables.

Despite the immense advantages with the use of herbicides, the associated environmental problem that arises is a matter of concern. After application of the pesticide product on the target pest, the chemical is gradually lost as a result of breakdown, evaporation etc, and the ***residue*** is the amount that remains after application. Residues of pesticides in food in general have been a major problem particularly, in the developing countries. When it rains the residues in the field are washed away by flood, run-off and seepage into ground and surface water supplies.

As a result of the environmental problem associated with the use of herbicide, it is imperative to embark on a study to have a better understanding of the depletion rate of these chemicals in our environment. In assessing the depletion rates of pesticides in the environment, their behaviour in the soil becomes very important since on application the soil is one of the first points of contact.

Depletion of pesticides from treated soil comprises leaching, evaporation and degradation [11]. The first two are purely physical phenomena and are governed

by the physico-chemical properties of the compound and soil characteristics (i.e. solubility, adsorption capacity, water content etc). The series of chemical conversions, which finally lead to the breakdown of the pesticide, is called degradation [11]. Such conversions may proceed on biotic pathway due to light, temperature and pH or chemical composition of the soil. The microorganisms of the soil carry out some of the conversions, either by metabolising the compounds or by catalytic effect of the free enzymes originating from the living organisms. These factors have a complicated interrelation and make it difficult to predict the fate of pesticides in the soil.

1.2.1 STATEMENT OF PROBLEM

Depletion of herbicides in the soil has been investigated in other parts of the world. These studies have been advanced to determine the fate of the chemicals in the environment[12]. Although some progress has been made in this field of research, there had been no work done in Ghana on the depletion rates and the lifetime of these herbicides in the Ghanaian soils. Knowledge of depletion of these chemicals in Ghanaian soils would offer a better understanding of their fate and duration in our environment. This would ultimately guide the agronomists and the environmentalists in predicting the amount and nature of residues remaining in the soil after application.

1.2.2 PURPOSE AND SCOPE OF THE WORK

In this study, three commonly used triazine herbicides, atrazine. simazine and ametryn, and two substituted urea herbicides, diuron and metobromuron will be studied in a coastal savannah and a forest zone soils with the objectives of

- determining the depletion rates of the herbicides in the two soil ecosystems for comparative purposes.
- studying the kinetics involved in the process of depletion of the herbicides for the purpose of assessing their half-lives in the chosen soil ecosystems.
- determining the physico-chemical soil properties such as moisture content, particle size, soil pH, organic matter etc for the purpose of establishing the relation between these soil properties and herbicide depletion rate.

CHAPTER TWO

LITERATURE REVIEW

2.1 HERBICIDES DEPLETION IN SOILS

Work done on pesticides in Ghanaian soils focussed mainly on insecticides such as lindane, endosulfan and propoxur. Very little work has been done on herbicides depletion in Ghanaian soils, particularly, in the area of kinetics of their depletion.

Appoh *et. al.*[13] studied persistence of lindane in Ghanaian coastal savannah top soil using a radiotracer technique. They reported that dissipation pattern favours a second order kinetics. They further indicated that dissipation from all the soils exhibited a biphase nature with a more rapid dissipation occurring within the first 2 – 6 days followed by relatively, slower phase. Persistence was also observed to be dependent on the organic matter content of the soil.

Antwi-Boakye *et. al.*[14] also studied persistence of lindane and endosulfan in two soil ecosystems under laboratory conditions, and established that the degradation pattern of chemicals in both ecosystems was similar. However, after six weeks of incubation, endosulfan had degraded more than lindane. He further indicated that soil moisture facilitated the degradation process. Lowor[15] on the other hand, investigated the fate of propoxur, in cocoa ecosystem using TLC and GC methodology. The investigation established that residues levels of propoxur

determined by the two methods were not significantly different. He further indicated that the residue levels of the chemical in the soil decreased rapidly and, by the twenty-first day after application none was detected in the top soil (0 – 6 ins).

Lowor *et. al.*[16] investigated persistency of atrazine in tropical soils, and concluded that the herbicide is lost from the top soil within five weeks of application. The loss of atrazine was attributed to either degradation or leaching. They further reported that TLC determination of atrazine gave comparable residue data as that of GC.

Suess *et. al.*[17] studied the degradation of herbicides and herbicides metabolite in different soils and observed that 3.6 to 10.2 % of the applied amount of 1 ppm of atrazine disappeared during an incubation time of 16 weeks, and 77.6 to 77.9 % was adsorbed into the soil complex. No correlation was observed between the degraded amount of atrazine and the amount adsorbed to the soil complex. Espinosa-Gonzalez[18], investigated the fate and effects of pesticides under tropical field conditions, and showed that atrazine and simazine used for the production of maize, dissipated slowly. He further indicated that the half-life of atrazine in clay soil was of the order of 50 – 60 days. In the study of persistence of some horticultural herbicides in soils at 26 sites throughout United Kingdom, Davison and Clay[19] reported no significant relationship between persistence of simazine and any soil factor, although there was a trend toward lower soil pH.

The rate of loss of diuron from soil have also been assessed in different soils in both laboratory test and field trials[20]. No relationship was established between any soil property and the rate of loss. In the laboratory, only 27 % of the variations in the rate of loss could be accounted for by a multiple linear regression with soil properties. The fate and activity of some herbicides in soils was studied by Ogle and Warren[21] who concluded that monuron and diuron decreased progressively from light sandy soil through a silt loam to an organic soil. In the same study, simazine degraded more rapidly in soils with higher organic content. Green *et al*[22], also investigated the fate of linuron, monolinuron and metobromuron in water and soil. They observed that metobromuron was soluble in water and is less persistent in soil.

In the study of dissipation of herbicides from soil, Kearny *et. al.*[23] concluded that depletion of the herbicides follows a first order reaction. This implied that at any time, the rate of loss would be proportional to the concentration in the soil. This type of loss is typical of majority of pesticides. Kaufman[24] also investigated the dissipation of certain biodegradable herbicides from soil and reported that a lag phase might occur after initial application in which relatively little pesticide is lost. This is followed by rapid disappearance as a result of microbial metabolism.

2.1.1 Kinetics of Depletion of Herbicides

Depletion of some pesticides is characterized by initial lag-period during which little or no change in concentration occurs. This is followed by rapid degradation which appear linear with time, but detailed analysis may show it to follow first order kinetics[25]. Herbicides, with this degradation patterns, include 2,4-dichlorophenoxyacetic acid, dalapam, chloridazon, propham and all compounds of short persistence in soil[26]. Because of the lag-phase, presentation of half-lives for such compounds is not very meaningful.

With other compounds there is no lag-phase and the rate of degradation is proportional to concentration so that the result can often be interpreted using first order kinetics, $C=C_oe^{-kt}$

Where C is the concentration after time t, C_O is the initial concentration, k is the rate constant, Thus, a plot of the logarithm of concentration against time gives a straight line with slope proportional to the rate constant.

Writing $t_{1/2}$ as the time taken for 50 % depletion, the half-life is

$$T_{1/2}=0.693/k$$

Which is independent of the initial concentration. The half-life concept is a valuable tool in comparing rates of herbicides depletion.

Burschel[27] and Freed[28] commented that since the quantity of herbicides in soil is very small in relation to other components, the herbicide concentration is expected to be the rate limiting step, so that first order reaction kinetics should

apply. However, since soil is a complex biological and chemical medium, the kinetics of adsorption and desorption might affect rates of loss by controlling the amount of herbicide available for degradation, while the activities of soil microorganisms may vary with time depending on the availability of nutrients and other energy sources. It is therefore not surprising that deviations from simple first order kinetics are observed.

Hance and Mckone[29] showed that neither zero-order, half-order, first order nor Michaelis-Menten kinetics described precisely the breakdown of atrazine and linuron in the laboratory.

Empirical curve fitting has also been suggested and Hamaker[30] proposed a power-rate equation

$$C = [\ C_o^{(1-n)} + (n-1)kt\ ]^{1/(1-n)}$$

Where C is the concentration after time t, Co is the initial concentration, n the apparent order of the reaction and k the rate constant. This equation was used by Kempson-Jones and Hance[31] in the study of kinetics of linuron and metribuzin degradation. In only 10 to 40 separate experiments did the value 1 fall within the 95 % confidence limits of the determined value of n. In the remaining experiments the apparent reaction order was greater than 1, and in eight of these, it was greater than 4. Hamaker[30] reported similar deviations from first-order reaction kinetics when he used the equation above to calculate apparent order of reaction from previously published degradation data.

When degradation is followed for extended periods, many compounds show rates of depletion that are disproportionately slow at lower residual concentrations. Hamaker and Goring[32] suggested a "two compartment" model to explain this. The pesticide is considered divided between available and unavailable fractions, with only the available subject to depletion. Appropriate rate constants control movement into and out of the unavailable pool. Freshly added chemicals are mainly in the available state and the initial rate of degradation is rapid. However, the rate of degradation falls as the pesticide is transferred to the unavailable state and eventually, rate of release from the unavailable pool controls the rate of degradation. Hamaker[33] and Hamaker Goring[32] demonstrated that this model could describe the kinetics of degradation of a number of soil-applied pesticides.

There is little doubt that rate equations are valuable for describing pesticide degradation, but it seems unlikely that a universal equation will be found which applies to all compounds and soils. When rate equations are used, their approximate nature must always be kept in mind.

2.1.2 Factors Influencing Rate of Loss

2.1.2.1 Concentration

With first order kinetics, rate constant should be independent of initial concentration, but for atrazine, Armstrong *et. al.*[34] and for simazine, Walker[35] indicated that the rate of loss decreased as initial concentration increased. With

some compounds, a lag-phase was demonstrated at increased initial concentrations. Picloram[36] and tri-allate[37] were found to belong to this group. Hance and Mckone[38] suggested that reduced degradation rates at higher initial concentration might result from a limitation in the number of reaction sites in the soil. Hurle[39] suggested that toxic effects on microorganisms or enzymes inhibition might be involved.

2.1.2.2 Herbicide Adsorption

Adsorbed herbicides may be degraded more rapidly since the density of microorganisms near colloidal surfaces is greater in the soil[41]

Hurle[39] reported increased persistence of atrazine and 2-methyl-4,6-dinitrophenol in soil when straw ash was added. In unamended soils, increasing half-lives with increased adsorption have been reported for simazine, atrazine, ametryne and propazine[42]. Moyer *et. al.*[43] found lower degradation rates for atrazine but not linuron in soil amended with activated charcoal although increased adsorption of the compound was observed.

Clay minerals used as carriers may catalyse degradation of pesticide in dust formulation[44]. Chloro-s-triazine has been observed to hydrolyse when adsorbed on clay minerals[45]. Armstrong *et. al.*[46] suggested that adsorption catalysed hydrolysis, however, later studies by Armstrong and Chesters[47] indicated that adsorption does not necessarily lead to catalyse hydrolysis but specific adsorption bonds are required for this to occur.

2.1.2.3 Soil Type

The influence of soil type on herbicide persistence is not well understood. The fact that soil microorganisms are usually involved in degradation means that soil organic matter might be expected to have some effect since microbial activity is often higher in more organic soils. However, adsorption of most herbicides also increases with an increase in soil organic matter and since adsorption reduces the amount of herbicides available in the soil solution, it might provide protection from degradation. Hamaker[33] suggested that an increase in organic matter might increase rates of degradation in mineral soil up to a limiting value, above which the rate of loss would be retarded. Briska *et. al.*[40] reported that simazine degraded more rapidly in soil with higher organic contents.

2.1.2.4 Soil pH

Soil pH may affect depletion directly if the stability of chemical is pH dependent. With simazine, Nearpass [48] found faster depletion in two soils at pH 5.4 and 3.9 than at 6.8 and 7.0. Atrazine depletion was more rapid at pH 5.5 than in the same soil adjusted to pH 7.5[49]. This is consistent with result of Hiltbold and Buchanan [50] working with soil adjusted to pH 5.6 and 7.0.

Walker and Thompson[51] found a significant negative correlation between rates of simazine degradation and pH in 18 unamended soils.

Hance[52] working with soils adjusted to four different pH in the range of 5.0 to 8.0 reported only slightly increased decomposition rates for atrazine with decreasing pH in one soil, whereas in the other, rates of loss decreased.

Corbin and Upchurch[53], investigated the rates of degradation of several herbicides in two organic matter soils adjusted to pH levels in the range of 4.3 to 7.5. They reported maximum degradation of dicamb and 2,4-dichlorophenoxyacetic acid at pH 5.3, and aminotriazole at pH 6.5, but no effect of pH on the rate of loss of diuron or chloramben. These results show that soil pH can influence depletion rates. There is however, very little understanding of the principle involved, and more information is required before general conclusions could be made.

2.1.2.5 Soil Amendments

It is generally assumed that the rate of depletion of most herbicides is influenced by soil microbial activity. Since addition of easily degradable organic substrates and mineral nutrients results in a spontaneous increase in microbiological activity, one would expect enhanced herbicide degradation by such treatments. However, this does not always occur. McClure studied accelerated degradation of herbicides in soil and reported that accelerated degradation of minuron, atrazine, dicamba and diuron occurred when microbial nutrient broths were added to the soil[54]. Glucose enhanced disappearance of atrazine[55], ground vetch plant increased atrazine and diuron decomposition[55].

Wolf and Martin[56], reported an enhance decomposition of bromacil and tercil in the soil on addition of maize and bean straw.

Hance[57], working on two soils indicated that farm yard manure or straw accelerated atrazine degradation in one soil but not in the other, whereas mineral fertilizers (N, P,K) plus straw accelerated degradation in both soils.

2.1.2.6 Temperature and Moisture

Since increasing temperature increases the rates of both non-biological reactions and biological processes, rate of herbicide depletion should be expected to also increase with temperature. Hamaker[30], reviewed the literature for herbicides and other soil-applied pesticides and found that this is generally the case. The dependence of the rate constant, k of a chemical on temperature can be expressed by the Arrhenius equation

$$k = A_o e^{-(Ea/RT)}$$

Where Ao is a constant, R the gas constant, T the absolute temperature and Ea the activated energy. With first-order reactions, the dependence of half-life on temperature can be expressed by

$$\log H_1 - \log H_2 = Ea/[4.575(1/T_1 - 1/T_2)]$$

where H_1 and H_2 are half-lives at absolute temperatures T_1 and T_2 respectively. This equation has been satisfactorily applied to degradation data for a number of soil applied herbicides[58].

Adequate water as well as high temperature is essential for microbiological activity, but in addition, water acts as a solvent and transport agent, a reaction medium for both biological and non-biological processes and a reagent in hydrolytic reactions. There is evidence that herbicides degradation rates are increased under moist soil conditions, which, in most instances probably reflects increased biological activity.

In recent years, reports of studies with wide range of compounds have been published[59], and these have shown the expected effect of increase degradation rates with increasing soil moisture up to field capacity.

2.2 VARIABILITY IN MEASUREMENT OF HERBICIDES RESIDUES

In laboratory experiments, a well sieved and therefore relatively uniform particle soil samples can be used, the herbicides can be distributed uniformly through the soil and incubation condition can be controlled. In the field, none of these factors can be controlled with precision. Much of the variation in the field compared with laboratory experiment will result from differences in the initial distribution of the herbicides on the soil surface. Some examples of these errors can be taken from data of Fryer and Kirkland[60]. Over six years of repeated treated of plots with four different herbicides, initial recoveries of the nominally applied rate varied from 42 to 100 % with 2-metyl-4-chlorophenoxyacetic acid, 37 to 90 % with tri-allate, 60 to 104 % with simazine and 32 to 144 % with linuron. Similar observation was observed for atrazine. Hence to assess quantitatively the kinetics of degradation under field conditions, detailed measurements of the amount present initially are essential.

Fryer and Kirkland[60] also measured the point to point variation in deposition of the spray application. In experiment with linuron, the mean deposition on several 12.9cm^2 filter papers placed at random on the soil surface was 94, 65, 73, 81 and 95 % of the theoretical quantity in different tests and the coefficient of variation was 16, 60, 23, 17 and 11 % respectively. Mechanical incorporation of

herbicides applied to the soil surface increases this variation[61]. Variations in residual concentrations from one point to another within a treated area tend to increase with time, presumably because of variations in soil properties which affect degradation rates[62].

2.3 ANALYTICAL PROCEDURE FOR PESTICIDES RESIDUE ANALYSIS

Regardless of the analytical methods used, the procedure for pesticide residue analysis follows the following steps.

1. Sampling and Sample Preparation
2. Sample Extraction
3. Extract Clean-up
4. Determination of Residues

2.3.1 Sampling and Sample Preparation

The importance of careful, unbiased and representative sampling in the field cannot be overemphasized. It should always be remembered that chemical residue analyses are usually time- consuming as well as expensive and therefore the minimum sampling to obtain reasonable validity is of paramount importance. Samples collected must have the following characteristics[63].

a. Sample must be accurate. The final accuracy of the residue determination is largely dependent on the original field sample. Residue data may be precisely determined but woefully inaccurate due to inadequate field sampling.

b. The sample must be valid. A valid sample is the one that is selected in a manner that ensures that each unit of material in the batch being sampled has an equal chance of being selected for the extraction and ultimate test.
c. The sample must be representative. A representative sample is not only a random sample but the proportion of each type of the sample material should be identical to that of the gross sample from which it was originally selected.

2.3.2 Sample Extraction

2.3.2.1 Pre-analysis

Once a valid, representative field and sub-samples have been selected for eventual residue analysis, the next major problem for the residue chemist is to quantitatively remove the pesticides or its metabolites from the surrounding biological environment. Extraction techniques must be adequate to yield extract which accurately reflect the toxicant residue level. Bann[64] reviewed three basic extraction procedures commonly used for pesticide residue removal. These extraction procedures were represented as follows:

a. the whole crop surface rinsed with a suitable solvent.
b. maceration of sample with crystalline anhydrous sodium sulphate and extraction with suitable solvent.
c. Maceration of the sample in the presence of a suitable solvent or solvent combination.

2.3.2.2 Solvent Extraction

The first step in the analysis of pesticide residues is usually separation of the pesticide from the environmental sample by solvent extraction. For efficiency, the solvent must remove the pesticide in a reproducible manner without removing large amount of the co-extractives from the sample. For the past several years, many specialized solvents have been developed for the extraction of pesticides from agricultural samples. The analyst must consider the method of analysis before extraction is begun. For instance to analyse for lindane in a sample using Schechter-Hornstein procedure[65], sample should not be extracted with benzene, since benzene is the material to be detected in the final step of analysis.

Unless adequate experimental data concerning the solvent purity is known the solvents should be distilled before use. This is especially important in the case of chlorinated solvents such as chloroform, methylene chloride and carbon tetrachloride. These solvents often form phosgene on standing, which not only produce negative analytical results, but may also be hazardous to the analyst. Before the more unstable pesticides were developed, samples were often dried, ground and extracted in a soxhlet or some other type of continuous extractor. However, drying was soon found to cause loss of many of the new organic pesticides.

2.3.2.3 Wet-Processing Technique

Residue analysts have found that the wet-processing extraction technique is probably most satisfactory for consistent recovery of pesticides residues[65]. Two satisfactory systems for extracting most pesticides, excluding those that are water-soluble, seem to be extraction with benzene-alcohol mixture, or extraction with chloroform. There are essentially two methods of wet-processing of raw or processed agricultural sample[66].

a. Extraction by rotating or shaking with solvent.

Removal of pesticides by shaking or rotating the sample with a single solvent has the major advantage of usually removing the pesticide without the inclusion of large amounts of co-extractives. This method can often be used for soil samples, raw fruits or vegetables crops with surface residue when the pesticide has not been absorbed by the plant tissue.

b. Extraction by blending with one or more solvents

Extraction of most pesticides can be done best by blending the substrate with the solvents. It has been shown that extraction is most efficient with two solvents. Blending is convenient in warring blender or a large mixing apparatus such as that described by Gunther and Blinn[67]. Some workers in the pesticide analysis field recommended that the sample be blended with ethyl or isopropyl alcohol before adding a water-immiscible solvent[68]. In general, the amount of immicible solvent should be 2 mL per gramme of subtrate.

2.3.2.4 Extraction of Pesticides from Vegetables, Fruits, Forage and Soil

Klein *et. al.*[69] have indicated that co-solvents extraction (extraction with a mixture of two or more solvents) is highly recommended for fresh and frozen leafy vegetables containing considerable quantities of extraneous water. Co-solvent extraction is not usually necessary or advisable for forage, dried fruits and vegetable, oily crops, nut and shells etc. Precise extraction techniques for the removal of pesticides from the soils cannot always be established because chemical changes affecting adsorptive capacity may be occurring. The use of highly polar solvent such as acetone will yield satisfactory recoveries. Extraction of pesticide with 10 % acetone in hexane has been found to be adequate for the removal of most pesticides without excessive extractive interfering substances[70].

2.3.2.5 Storage of Extracts

Patterson and Lehman[71] have shown that stripping or extracted pesticide solutions should be stored under conditions that will permit no change in the pesticide until analysis is performed. If delay is unavoidable, a recovery can be made under the same condition of extraction, and stored along with the sample extracts. Even under optimum conditions, however, prolonged storage of extracts is not advisable, Extract should be stored near $0^{O}C$ in screw-cap bottles with aluminium liners in the caps. Liners of waxed paper should be avoided, since the wax is usually dissolved by the solvent. Even at low temperatures, pesticide

materials may be lost, For example, siven is lost from chloroform extracts at very short time even at low temperatures, however, small volume of added ethanol helps preserve the siven[72]. Extracts should therefore be analysed as soon as possible after extraction unless there is considerable experimental evidence showing that the technical pesticide is stable in the solvent.

2.3.2.6 Concentration of pesticide in stripping or extracting solution

After extraction of the pesticide from the sample material, the pesticide is usually at such a low concentration that direct measurement is difficult, so the solution must be concentrated by removal of the solvent. Concentration or the removal may be achieved in several ways, but distillation or evaporation of the solvent are most practical.

2.3.2.6.1 Air evaporation

In most cases, evaporation is achieved either by blowing warm air over the sample in a beaker or blowing filtered dry air or nitrogen over a sample held in a warm-water bath. If the sample is being evaporated by stream of air from the laboratory line, it is well to filter the air just before use. A convenient air filter with replaceable cartridge is satisfactory to remove water, oil and rust particle. The temperature of the water bath usually should not be over 50^{O}C, and in some cases must be lower since at higher temperatures most of the pesticides will be lost through evaporation.

2.3.2.6.2 Concentration using vacuum

Vacuum can often be used in concentrating the solutions that are sensitive to heat. A Rinco evaporator utilizes the principle of spreading a thin film of solution over a large, rotating surface area and subjecting it to negative pressure. This is a convenient method for vacuum concentration of extracts containing pesticides.

2.3.3 Clean up or purification of extract

Usually one day prior to laboratory analysis the frozen extracts are removed from freezer and allowed to thaw at room temperature, and then purified. In other words, the pesticide of interest must be isolated from the previous environment by suitable solvent. Thus, a clean–up procedure must be devised to quantitatively separate the original applied pesticide from associated interfering materials co-extracted from the original biological environment.

Gunther and Blinn[67], Schechter and Hornstein[65] discussed in detail clean-up and isolation of chemical residues from accompanying interfering extractants.
The extracted toxicant must be free of most accompanying extractants before precise and valid chemical analysis can be undertaken.
Most clean-up procedures are based on

a. Chromatographic separation with materials exhibiting a selective adsorption for a compound being determined.

b. Chemical removal of interference through oxidation, reduction, saponification or hydrolysis without detrimental effect on the compound itself.
c. Physical separation by solvent partition, steam distillation, freezing.

2.3.3.1 Solvent partitioning

An example of this is the preferentially solubility of chlorinated pesticides in acetonitrile. Thus, following partitioning of butter fat between petroleum ether and acetonitrile, the pesticides will be concentrated in acetonitrile while the fat is retained in the non polar solvent.

2.3.3.2 Acid clean-up

Many pesticides are stable in strong acid medium. Thus, treatment of fats and oil with fuming sulphuric acid will remove the fats while transferring the pesticides to the solvent phase. A typical example is toxaphene.

2.3.3.3 Column chromatography clean-up

This is probably the most widely used but least understood clean-up step. Adsorbents normally used are alumina, silica, charcoal, diatomaceous earth, C-18 and florisil. Solid phase extraction (SPE) is the most widely used column chromatography clean-up.

2.3.3.3.1 Solid phase extraction (SPE) clean-up method

Solid phase extraction (SPE) is relatively new technology that is gaining popularity, where low concentrations of analyte can be concentrated from a large sample volume. A typical SPE consists of four major steps:

1) conditioning the sorbent beds with solvent to improve the reproducibility of the pesticide retention and to reduce the concentration of any contaminant present.
2) Sorbing the pesticide on the bed, together with undesirable matrix constituents.
3) Rinsing the column with weak solvent to remove undesirable matrix component
4) eluting the pesticide with a sufficiently strong solvent, while leaving the undesirable components on the bed. Useful adsorbents in the extraction of the pesticides include diatomaceous earth, C-18, silica gel and silica supports bonded with ethyl, octyl, octadecyl, cyclohexyl, and cyanopropyl functionalities. SPE is mostly used off-line, the adsorbent being packed in disposable columns or cartridges.

2.3.4 Qualitative and Quantitative determination of pesticide residue

2.3.4.1 Introduction

The final step for both qualitative and quantitative determination of pesticide residue usually involved a form of chromatography. The most important chromatographic technique for both qualitative and quantitative analysis is Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC). Spectrophotometry can also be used for many pesticides, and calorimetric kits are available for cholinesterase inhibiting insecticides and some fungicides.

In this work, TLC was used for the study because it offers opportunity to undertake analysis where there is inaccessibility of instrumental facilities, lack of spare parts and lack of continuous supply of electricity.

Thin Layer Chromatography has made a strong impact on analytical chemistry. The popularity of this technique has grown rapidly in the past few years and the technique has been used for the analysis of pesticide residues in soils, plants and vegetables[73], water[74] and urine[75]. Thin layer chromatography is simple and rapid, and is more selective for a greater variety of separations than paper chromatography. The large number of adsorbents are available and the ease of changing conditions, give the chemist a considerable number of parameters which can be varied to obtain a desired separation[76].

Thin layer chromatography has genuine and general utility for pesticides residue analysis. The reasons are, it is applicable to most of the type of analytical

problems in which column chromatography, paper chromatography, gas chromatography and electrophoresis can be used, and it is much simpler and faster than these other techniques[76].

2.3.4.2 Previous work done with TLC

Stammach *et. al.*[77] chromatographed triazine herbicides, atrazine, atratone and prometryne and determined their R_f values along with those of related compounds which occur in the commercial products. Silica gel G coated plates were used in all cases. For the analysis of atrazine, ethyl acetate/ petroleum ether mixture (3:7) was used as the developing solvents, and in the case of atratone and prometryne, chloroform/absolute ethanol ethyl acetate (90:5:5), and toluene/acetic acid/water (10:10:1) were used respectively to develop the chromatograms.

Henkel and Ebing[78] analysed a group of six triazine herbicides by using a two-step development on air-dried silica gel plates, with chloroform/diisopropyl ether as the developing solvent. Henkel[79] further, reported on the separation of triazine herbicides, using a chloroform/nitromethane solvent in the ratios of 1:1 and 5:1. The most sensitive reagent for detecting these chemicals was silver nitrate spray using a 0.02-0.1M silver nitrate solution. Other reagents that were used for the detection was Dragendorff reagent and a 0.25% potassium permanganate solution.

Abott *et. al.*[80] chromatographed agroup of eight triazines herbicides in seven solvent systems on silica gel G and on kieselguhr-silica gel (1:1) in a single solvent system. Quantitatively, the compounds were determined by plotting the square root of the spot area against the logarithm of the weight of the material. For quantitative work the spot were visualized by spraying with 0.5 % brilliant green in acetone followed by exposure to bromine vapour.

Substituted urea herbicides were analysed by Henkel[81], on air-dried plates of silica gel G with chloroform/ nitromethane (1:1), using a development distance of 10 cm. He reported of the following R_f values, fenuron 0.31, monuron 0.41, diuron 0.53, monochlorlinuron 0.72, linuron 0.79 and neburon 0.77.

Golad[82] investigated the thin-layer separation of trifluralin and related compounds by two-dimentional thin-layer chromatography. Using silica gel GF coated plates, chromatograms were developed in the first direction with benezene/1,2-dichloroethylene (1:1) and in the second direction with n-hexane/methanol (98:2). Using the natural colour of some of the compounds or the blue absorbing spots under ultraviolet radiation, the sensitivity of detection was 0.5 µg.

Bache[83] analysed and detected amiben in tomatoes. Separation was achieved on silica gel G coated plate with benzene/acetic acid (5:1) as developing solvent. With this elution system, he reported R_f value of 0.44. Detection was accomplished by spraying first with 1 % sodium nitrite in 1 M hydrochloric acid

followed by a light spray of 0.2 % ethylene diamine dihydrochloride in 2 M hydrochloric acid. Using the extract from the equivalent of 2 g of tomatoes, the method was sensitive to 0.1 ppm.

2.3.4.3 TLC Technique

Chromatography can be defined as a technique for the resolution of components of a mixture as a result of differential migration[76]. Thin layer chromatography is analogous to other adsorptive techniques. An adsorbent is spread on a plate and a drop of sample applied. The plate is placed in developing chamber containing a solvent that act as a mobile phase. As the solvent migrates along the plate, it carries the components of the sample mixture along. A continuous adsorption-elution process takes place, and the most mobile compounds travel fastest, causing complex mixtures to be resolved into series of spots.

2.3.4.3.1 Qualitative Analysis (Location and Identification) using TLC

The location of spot of a chromatogram is an index to the chemical composition and identification of the compounds separated. The migration is usually expressed as R_f value (relative factor), which is determined by the ratio of distances. The R_f is expressed as

$$\frac{\text{Distance of centre of the spot from starting point}}{\text{Distance of solvent front from starting point}}$$

Variation in temperature, adsorbent batches, moisture, layer thickness and developing chamber saturation may affect the reproducibility of the R_f values. For

these reasons it is recommended that a standard quantity of the pesticide under analysis be run concurrently. The variations may then be correlated and represented by the value of RR_f(relative retention factor). RR_f is given by

$$\frac{\text{Distance of centre of sample spot from starting point}}{\text{Distance of centre of standard spot from starting point}}$$

2.3.4.3.2 Quantitative Determination using TLC

The important quantitative methods using TLC are as follows:

1. Semi-quantitative: A number of standards are spotted on the plate with the samples. After development and visualization, the sample spots are visually compared with the standard spots. The concentration of the sample is taken as that of the standard spot for which the intensities and size are the same.
2. The diameters of standard spots can be taken and plotted against their concentrations to give a calibration curve for which the concentration of the sample can be determined.
3. Colour development on the ***tlc*** plate, followed by extraction and colorimetry of the coloured material. This involves spraying the plate with a colour developing reagent and treatment to develop the colour. An area around and including the spot is removed, extracted, and the absorbance of the resulting solution is measured at a characteristics wavelength of the compound, and the concentration is then determined from the a calibration curve of standards of known concentration measured at the same wavelength.

2.4 CLASSIFICATION OF HERBICIDES

2.4.1 Chemical Classification

There is today an extensive array of herbicides of widely different chemical type which influence the metabolism and hence the growth and behaviour of plants in a number of ways. Two closely related chemicals may also behave quite differently because physical factors such as their adsorption to plant components, volatility, acid or base dissociation differ and these factors may to varying degrees change the apparent activity of the chemical in a plant.

In chemical classification, herbicides are divided broadly into inorganic and organic. The organic chemicals are sub-divided into families such as aliphatic and aromatic acids and nitriles, amides, ureas and triazines, where a group is common to a number of herbicides. Inorganic herbicides include chemicals such as ammonium sulphate, ammonium sulphamate, sodium arsenite, sodium tetraborate, calcium cyanamide and others.

2.4.2 Classification by Phytotoxicity

In view of the complexity of the system into which herbicides are introduced, it is not surprising to find that their mode of action within plants is not very well understood. For the majority, a number of physiological and morphological effects have been observed, but there is a measure of conjecture in deciding what is direct or indirect effect in relation to herbicidal action.

Various authors have attempted to group herbicides by their important biochemical effects in plant. Van Overbeck[84] observed that just two physiological actions account for the herbicidal activity of about 70 named herbicides, and classified herbicides as the hormone weedkillers which produce growth abnormalities and the triazines and substituted ureas which inhibit photosynthesis.

King[85] in 'weed of the world' classifies herbicides as inhibitors of cell growth, inhibitor of growth and tropic responses, inhibitors of chlorophyll formation and photosynthesis.

The third example is that of Moreland[84] who used three biochemical activities, namely, modifications of respiration and mitochondrial electron transport, inhibition of photosynthesis and Hill reaction, and interference with nucleic acid metabolism and protein synthesis to classify herbicides.

Inhibition of photosynthesis is the only biochemical effect, which is mentioned in all the three classifications above. One must conclude that there exists no

general accepted classification of herbicides based on their physiology. Table 3 lists herbicides that function by photosynthesis Inhibition.

Table 3: Herbicides that act by inhibiting photosynthetic electron transport[86]

General Grouping	Specific Examples
Ureas	Diuron Linuron Monuron Metobromuron
Triazines	Ametryne Atrazine Cyanazine Prometryne Simazine Terbutryne
Uracils	Bromacil Lenacil Terbacil
Acylanilides	Pentanochlor Propanil

2.4.3 General Information on the selected Herbicides

Atrazine

Chemical Name: 2-chloro-4-ethylamino-6-isopropyamino-s-triazine

Molecular Formula: $C_8H_{14}ClN_5$

Molecular Weight: 215.69

Herbicidal activity: Atrazine is pre and post emergence herbicides suitable for general and selective use. Its actual and potential fields of selective application are corn, sorghum, rice, millet, sugar cane, pineapple and fruit trees.

Acute oral toxicity: LD_{50} for mouse 1750 mg/kg, for rat 3080 mg/kg. Rabbit 750 mg/kg.

Chronic toxicity: A series of rats fed for 2 years with daily oral amount of 2, 20, and 200 ppm of atrazine was comparable in all respect to the controls

Physical properties: Melting point :173 – 175°C.

Solubility in Water at 0°C: 0.0022 % (22 ppm)

27°C: 0.007 % (70 ppm)

Chemical properties: It sublimes at higher temperatures and stable in neutral, slightly acidic or basic media. It is hydrolysed to the herbicidally inactive 2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine in acidic or basic media, especially at higher temperatures.

Simazine

Chemical Name: 2-choro-4,6-bis-ethylamino-s-triazine

Molecular Formula: $C_7H_{12}ClN_5$

Molecular Weight: 207.66

Herbicidal activity: Simazine is a pre-emergence herbicide for general and selective use. The actual and potential fields of selective application are corn, vine, small grains, sugar cane, pineapples, tea, coffee, cocoa.

Acute oral toxicity: LD_{50} for mouse, rat, rabbit, chicken – more than 5000 mg/kg.

Chronic toxicity: A series of rats fed for 2 years with a daily oral administration of 2, 20 and 200 ppm of simazine 50W were comparable in all respect with the control.

Physical properties: Melting point : 225 – 227^{o}C

Solubility at 0^{o}C : 0.0002 % (2 ppm)

20^{o}C: 0.0005 % (5 ppm)

85^{o}C: 1.2 % (12000 ppm)

Methanol at 20^{o}C: 0.04 % (400 ppm)

Chemical properties: It sublimates at higher temperatures, is stable in neutral, slightly acidic or basic media. It is hydrolysed to the herbicidally inactive 2-hydroxy-4,6-bis-ethylamino-s-triazine in acidic or basic media.

Ametryne

Chemical Name: 2-methythio-4-ethylamino-6-isopropylamino-s-triazine

Molecular formula: $C_9H_{17}N_5S$

Molecular weight: 227.33

Herbicidal activity: Ametryne is pre- and post-emergence herbicide for general and selective use. The potential fields for its selective application include sugar cane, small grains, peanuts and soybean

Acute oral toxicity: LD_{50} for mouse 965 mg/kg, rat 1110 mg/kg.

Subchronic toxicity: A series of rats fed for 90 days with a daily oral administration of 20 and 200 mg ametryne 50W was comparable in all behaviour with the control.

Physical properties: Melting point : 84 – 86° C

Solubility at 20°C: 0.0185 % (185 ppm), in organic acids is high.

Chemical properties: It is stable in neutral, slightly acidic or basic media, hydrolyses to the herbicidally inactive 2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine in acidic or basic media.

Diuron

Chemical Name: 3-(3,4-dichlorophenyl)-1,1-dimethylurea

Molecular Formula: $C_9H_{10}Cl_2N_2O$

Molecular Weight: 233.1

Herbicidal activity: Diuron is pre-and post- emergence herbicide used for the control of annual weeds in citrus, avocadros, bananas, pineapples, sugar cane.

Physical and Chemical Properties: The phenyl alkyl ureas are only sparingly soluble in hydrocarbons. They are stable toward oxidation and hydrolysed under

normal conditions, but at elevated temperatures, they can be hydrolysed quantitatively. Much of their analytical chemistry is based on the latter reactions.

Melting point : 158 – 159 ^{O}C, solubility in water – 42 ppm

Metobromuron

Chemical Name: 3-(4-bromophenyl)-1- methoxy-1-methylurea

Molecula Formula: $C_9H_{11}BrN_2O_2$

Molecular Weight: 259.11

Acute oral toxicity: LD_{50} for rats is 2603 mg/kg

Physical and Chemical Properties: The urea is colourless, crystalline solid, and it is more soluble in water than diuron. Solubility in water is 330 ppm at 20^{O}C. Melting Point : 95 – 96^{O}C and vapour pressure : 0.40 mPa at 0^{o}C.

CHAPTER THREE

EXPERIMENTAL METHODS

3.1 CHEMICALS AND REAGENTS

The chemicals and reagents used in the experiments were obtained from Merck, Darmstadt, Germany and Fluka, Switzerland, unless otherwise stated. The, atrazine, simazine, ametryne, diuron and metobromuron standards were obtained from Dr. Ehrenstorfer, Gmbh. They were of 98 – 99.5 % purity and were used without further purification.

Preparation of sodium hexametaphosphate (Calgon). 5 g of sodium hexametaphosphate was dissolved in 100 mL flasks with distilled water and made up to the mark (5 % solution).

Preparation of Digestion accelerator. 10 g of potassium sulphate (K_2SO_4), 1 g of hydrated copper sulphate ($CuSO_4.5H_2O$) and 0.1 g of selenium (Se) were thoroughly mixed together.

Preparation of Mixed indicator. 0.13 g of methyl red was added to 0.066 g of methyl blue and dissolved in 100 mL of 95 % ethanol.

Preparation of O-Tolidine + Potassium Iodide[OTKI] reagent. 0.5 g of O-tolidine was dissolved in 100 mL of glacial acetic acid, 2 g of KI was dissolved in10 mL of distilled water, and the two solutions were mixed and diluted to 500 mL with distilled water.

Preparation of Borax($Na_2B_4O_7$. $10H_20$) buffer solution. Used to prepare dichlorophenol-indolphenol sodium salt (DCPIP) reagent. 3.325 g of borax were dissolved in 175 mL of distilled water and the solution was added to 75 mL of 0.1M Hydrochloric acid.

Preparation of DCPIP reagent. It was prepared by dissolving 0.1 g of 2,6-dichorphenol-indolphenol sodium salt in 250 mL of the borax buffer.

Preparation of the Spraying reagent. 30 g of *Panicum maximum* leaves and 5 g of sea sand were smashed in a mortar with a pestle. 15 mL of distilled water and 3 mL glycerine were added. These were mixed thoroughly and the liquid squezzed through knapsack into 50 mL flask. 20 mL of this was added to 13 mL of DCPIP reagent to give the spraying reagent.

3.2 EQUIPMENT

Auger. This is a metallic implement with a pointed blade at the end, where it is used to dig the soil.

2mm mesh-size sieve. The sieve was used to screen the soil samples to remove stones and other debris.

Hydrometer. The hydrometer was used for particle size analysis

TOA pH meter HM-30S. The pH meter was used for the measurement of soil pH.

Kjeldahl apparatus. This apparatus was used for the determination of total nitrogen in the soil samples.

Sp 1800 Spectrophotometer (Unicam). The spectrophotometer was used to measure available phosphorus in the soil samples.

10x20cm and 20x20cm silica gel 60 tlc plates, with ***tlc*** basic set(camag). Also including were ***application guide, development tank, 5 µL*** **and** ***10 µL microsyringe*** with needle(Hamilton)

Gallenkamp Flask shaker. The flask shaker was used for the extraction of the herbicides in the soil samples.

Forevac diffusion pump. The equipment was used for the vacuum dry of the solid phase extraction (SPE) cartridges after passing the extracts through the SPE columns.

3.3. SAMPLING FIELDS

Coastal savannah and forest zone soils obtained from Ghana Atomic Energy Commission(GAEC),and Kwame Nkrumah University of Science and Technology (KNUST) respectively were used for the investigation. Sketch Maps showing the sampling sites are presented in Fig. 1 and Fig. 2 below.

At the time of sampling, the savannah field at GAEC was not under cultivation of any crop. However, around the field were pineapple and plantain farms. The field had no history of pesticide application for the past seven years.

The field at Kwame Nkrumah University of Science and Technology was under semi-deciduous forest ecological zone. The field was used for the cultivation of yams and cassava in 1998 main farming season. The yams had been harvested

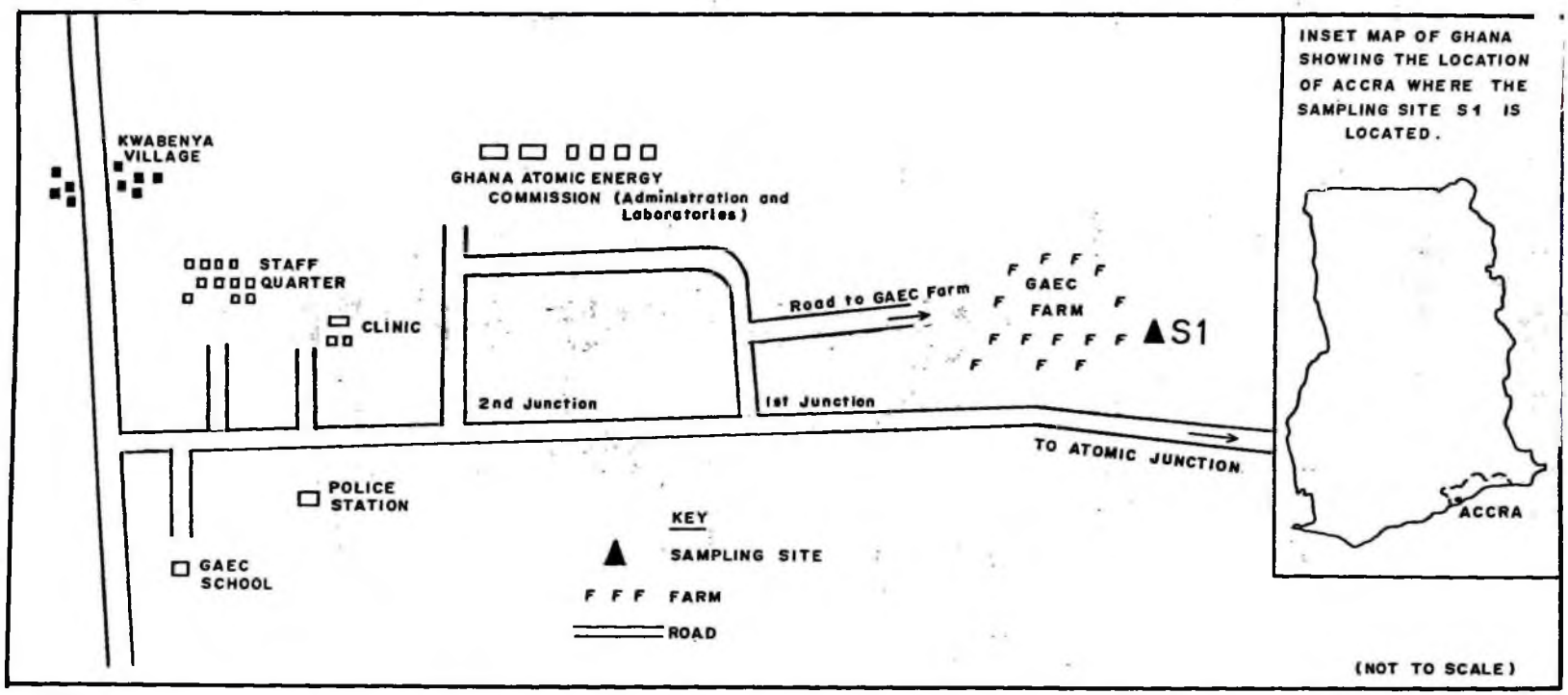

Fig. 1 ; A sketch map of a section of GAEC showing the Sampling Site S1

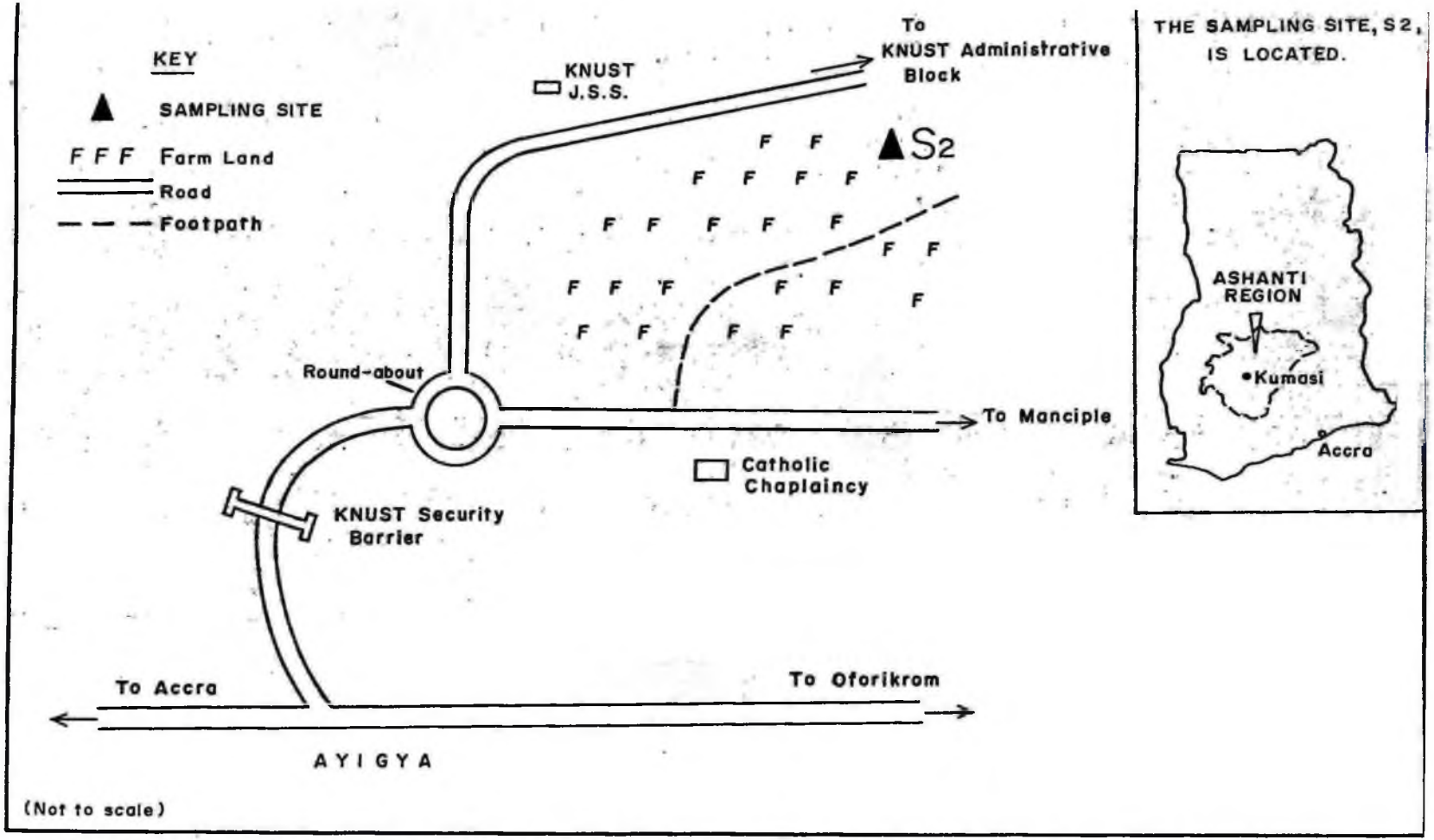

Fig. 2: A sketch map of a section of KNUST showing the Sampling Site S2

at the time of sampling but there were few cassava plants scattered on the plot. No pesticide was used during cultivation and there was no history of pesticide application on the plot.

3.3.1. SAMPLING AND SAMPLE TREATMENT

About 50 m × 50 m-plot size on each field was demarcated for sampling. Soil samples were taken randomly on the demarcated plots with an auger to a depth of 10 cm. Samples were mixed thoroughly and wrapped in an aluminium foil, and then placed in black polythene bags and the polythene taped. In the laboratory, part of the sampled soil was taken a day after sampling for the determination of the soil moisture content. The rest of the soil was wet sieved with 2mm mesh-size sieve aperture to remove stones and other debris, and this was used for the herbicide depletion studies.

3.4 PHYSICAL AND CHEMICAL SOIL PROPERTIES ANALYSIS

3.4.1 Determination of Soil Moisture

A known mass of the soil was placed in a previously weighed crucible. The container and its contents were placed in an oven set at 105°C for two hours. It was then cooled in a desiccator. This procedure was repeated until the mass of the crucible and its content became constant and the percentage soil moisture was calculated as

$$\frac{\text{mass of soil moisture}}{\text{mass of soil used}} \times 100$$

3.4.2. Determination of Water Holding Capacity (WHC)

About 100 g of air –dried soil sample was placed in a funnel clogged with cotton wool, with a rubber tube fixed at the stem and a clip attached at the middle of the rubber tube. About 100 mL of distilled water was measured and poured unto the soil in the funnel. It was allowed to soak the soil for 15 minutes. The clip holding the water was opened to allow the water to drain freely into a dried 100 mL measuring cylinder, which had been placed underneath. The amount retained was taken as the difference between the amount drained through and that added and this was used to calculate the percentage water holding capacity. Percentage water holding capacity was calculated as

$$\frac{\text{Amount of water retained by soil}}{\text{Air-dried mass of soil used}} \times 100$$

3.4.3 Determination of Particle size

The particle size analysis was done using the hydrometer method [87]. About 40 g of the soil sieved through to the 2 mm sieve was transferred into a one-litre beaker and 200 mL distilled water and 10 mL of 5 % calgon added. The suspension was shaken over-night with a shaker to obtain a uniform dispersion, which was filtered through 45 μm pore size sieve into a graduated litre

measuring cylinder. The residue was washed with distilled water until the filtrate leaving the sieve became clear. By doing this, the sieve allowed the clay and silt to pass through leaving the sand behind. The sand portion was then transferred into a previously weighed container, oven dried at 105°C and cooled in desiccator. It was weighed to obtain the amount of the sand in the soil. The filtrate in the graduated cylinder was then stirred with stirring paddle for about one minute. The paddle was removed and the swirling motion was allowed to settle. The hydrometer was carefully immersed and after two minutes, the reading was taken. The reading of the hydrometer estimated the amount of clay. The composition of silt was determined by difference between the amount of soil used for analysis and the sum of composition of sand and clay.

3.4.4 Determination of Soil pH

The soil pH was determined using the method of Campbell and Baver[88] in both water and 0.01M Potassium Chloride solution. About 20 g of the soil was weighed into 100 mL beaker and 20 mL deionised water was added. The suspension was stirred for 30 minutes with a magnetic stirrer and allowed to stand for 15 minutes. The pH of the partly settled suspension was then measured with the pH meter. Another 10 g of the soil sample was weighed into a 100 mL beaker, to which was added 20mL of 0.01M potassium chloride solution. The suspension was stirred for 30 minutes and allowed to stand for 15 minutes before the pH was measured.

3.4.5 Determination of Organic Carbon and Organic Matter

Analysis of organic carbon was based on the method of Walkley and Black[89]. About 1 g of the soil sample was weighed into 250 mL Erlenmeyer flask. 10 mL of 1M K_2CrO_7 solution was added to the soil in the flask and shaken to disperse the samle. 20 mL of concentrated sulphuric acid was then added, and the content of the flask shaken vigorously for about one minute. The mixture was then allowed to stand for thirty minutes after which, 10 mL distilled water and 10 mL orthophosphoric acid was added. Finally, 2 mL Barium diphenyl amine was added as indicator. The content was titrated against 0.2 M ammonium ferrous sulphate($Fe(NH_4)_2(SO_4)_2.6H_2O$) solution until the colour changed to green. Blank determination was carried out similarly, but without the sample. Triplicate analysis was performed. Percentage organic matter was obtained by multiplying the percentage carbon by 1.724. This is the Van Bemmelen factor. It is used because organic matter contains 58 % carbon.

This method is basically the reduction of $Cr_2O_7^{2-}$ by organic compounds and subsequent reduction of the unreacted $Cr_2O_7^{2-}$ by redox titration with Fe^{2+}

$$2Cr_2O_7^{2-} + 3C + 16H^+ + Fe^{2+} \rightarrow 3CO_2 + Fe^{3+} + 8H_2O + 4Cr^{3+}$$

3.4.6 Determination of Available Phosphorus

Available phosphorus was determined using modified Murphy and Riley method[90]. 2.5g of soil sample was weighed into 100 mL conical flask and 50 mL of 0.5M sodium hydrogen carbonate ($NaHCO_3$) of pH 8.5 was added. The

suspension was shaken for 30 minutes on a mechanical shaker. After filtration, the phosphorus was determined in the filtrate using spectrophotometer (Unicam Sp 1800 Ultraviolet spectrophotometer) at 712nm. Calibration was done with phosphorus standard of concentration 5, 10, 15 and 20 ppm.

3.4.7 Determination of total Nitrogen

This was based on the method of Page, modified by Kalkra and Maynard[91].

Two gramme of air-dried soil sample were weighed and placed in a kjedahl flask, 2 mL of distilled water was added to moisten the soil. 2 g-selenium catalyst (digestion accelerator) and 20 mL of concentrated sulphuric acid was also added. The mixture was digested for about 2 hours, transferred to 100 mL volumetric flasks and made up to the mark. 5 mL of the sample solution were pipetted into kjeldahl distillation flask, and 5 mL of 40% sodium hydroxide added. This was distilled, and the distllate collected into 20 mL of 2 % boric acid to which has been added 2 drops of mixed indicator solution. The distillate was then titrated with 0.01M HCl from light green to a pink colour end point. A duplicate analysis was performed and blank determination was carried out.

The nitrogen, which is in the soil sample as sulphate of ammonia, is converted to ammonia by the alkaline solution, sodium hydroxide. This then form a complex with boric acid H_3BO_3. i.e $H_3BO_3 + NH_3 \rightarrow NH_3H_3BO_3$

The ammonium ion is then released upon addition of HCl

i.e $NH_3H_3BO_3 + HCl \rightarrow H_3BO_3 + NH_4^+ + Cl^-$

3.5 HERBICIDE DEPLETION STUDIES

3.5.1 Preparation of the Herbicides Standards

To prepare 1 mg/mL (1000ppm) stock solution of the herbicides about 0.1 g of the reference herbicides standard was accurately weighed into 100 mL volumetric flask. except atrazine where 0.05 g was weighed, This was then dissolved with acetone and made up to the mark. These served as the stock solutions of the various herbicides. The stock solutions were diluted ten fold and used as working solutions.

3.5.2 Selection of Elution System for Analysis of the Herbicides

Two elution systems were used for the determination of the R_f of the herbicides. i.e. Silica gel-ethyl acetate and Silica gel-dichloromethane systems.

Ready made silica gel 60 plates were activated in an oven at 105°C for thirty minutes. Development tank was saturated by the vapour from the developing solvents by using 50 mL of one of the solvents for each determination. Saturation was achieved by lining the walls of the tank with filter paper cut to the size of the tank, and allowing the vapour to soak the tank for about 3 hours with the tank closed. 5 µl of each of the working herbicide solutions was then applied to the sorbent layer of the activated ***tlc*** plates with 10 µL calibrated microsyringe. The plates were then developed by dipping them into the saturated tank, where the eluent rise by capillary action. The eluent was allowed

to move more than two-third of the length of the ***tlc*** plate. It was then taken out of the tank and the eluting layer was allowed to dry and the spots detected as described below. Triplicate determination was carried. The concentrations at which the herbicides were spotted for the determination of R_f is as shown in Table 4.

Table 4: Concentrations and Amount of Herbicides applied for the determination of R_f

Herbicides	Conc.(μg/mL)	Amount applied.(μg)
Atrazine	55.59	0.278
Simazine	100.70	0.504
Ametryn	103.66	0.518
Diuron	99.78	0.499
Metobromuron	101.58	0.508

3.5.3 Detection and measurement of Herbicides

The O-tolidine + potassium Iodide method(OTKI) and Photosynthesis inhibition method were used.

a) For the O-tolidine + potassium Iodide method, developed ***tlc*** plates were air dried and placed in a tank saturated with chlorine for 30 seconds. The chlorine solution was made by placing a 25 mL beaker containing about 1 – 2 g potassium permanganate at the bottom of a developing tank and adding concentrated HCl. Excess chlorine was removed in a fume hood after which the

plates were sprayed with the OTKI reagent. Spots were seen as darkish blue in colour on grey-white background. Spots could stay over-night before disappearing.

b) For the Photosynthesis inhibition method, spots were visualized by spraying with the spray reagent, and the plate placed about 20 cm below 60 W ordinary electric bulb for about 2 minutes. Spots were seen as blue in colour in greenish background. Spots disappeared within 1hour. Distances moved by the solvent and chemicals were measured and these were used to calculate the R_f

R_f were calculated as

$$\frac{\text{Distance of centre of spot from starting point}}{\text{Distance of solvent front from starting point}}$$

3.5.4 Selection of Extraction Solvents for the Herbicides

Acetone, acetonitrile, hexane, methanol and acetone/hexane(4:1) were investigated for their efficiency in extracting the herbicides from the soil samples spiked with known amount of the herbicide. Extracts were analyzed by ***tlc*** and the recovery of the various herbicides by each solvent as well as the purity of the soil extracts determined. Each herbicide was analyzed three times.

3.5.4.1 Procedure for spiking

About 5 g of the soil sample was accurately weighed into individual extraction flasks. 2 mL of 100 μg/mL of herbicide standard was added and mixed with the

soil to generate a concentration of 40 μg/g. In the case of atrazine. 2 mL of 50 μg/mL was used to yield a concentration of 20 μg/g. The spiked soil in the extraction flask was allowed to stand for 30 minutes.

3.5.4.2 Extraction

Extraction was performed by adding 20 mL of each solvent unto the spiked soil and mechanically shaking the mixture on a flask shaker for 2 hours. Filtration was carried out by use of whatman No. 42. filter paper. The residue on the filter paper was washed three times with 3 mL of the solvent and washings were added to the filtrate. The extract was then dried over anhydrous sodium sulphate and filtered. Streams of air were gently blown from a hand-dryer to remove the solvent. The unclean extract was redissolved in 10 mL of acetone and analyzed for recovery and purity.

3.5.4.3 Clean-up of extracts

The extraction procedure was repeated, but this time the uncleaned filtrate was clean up as follows. SPE cartridge equipped with C-18 as adsorbent was earlier preconditioned with 2 mL acetone/water(1:9). Filtrate was passed through the preconditioned cartridge. The cartridge and its content were dried for 15 minutes by vacuum pump. The herbicide was then eluted with 10 mL of acetone into 10 mL flask. Eluate was adjusted to the mark with acetone. This is the clean up

extract. The clean up extract was also subjected to ***tlc*** method of analysis. The procedure was repeated for all the solvents.

3.5.4.4 TLC Analysis of Extracts for Purity and Recovery

A constant 5 µl of each extract was applied to the sorbent layer of the ***tlc*** plates with a calibrated mircosyringe. The same volume of the standard solution was analyzed concurrently. To spot the standard, 2 mL of the herbicide standard as used to spike the soil samples was pipetted into 10 mL volumetric flask and made up to the mark and 5 µl of the solution used for the analysis.
After detection of spots, diameters of the spots of the standards and extracts were measured to estimate concentration. For both the unclean and clean up extracts only one spot was detected, which in each case corresponded to the herbicide analyzed. Triplicate analysis was carried out.

Percentage recovery was calculated as

$$\frac{\text{Amount recovered}}{\text{Amount added}} \times 100$$

Amount recovered was calculated as shown in appendix 1(sample calculation 6)

3.5.5 Soil treatment and incubation

The method adopted was similar to the one used by G. A. El Zorgani[92].

To determine the rate of the herbicide depletion, 200 g of the soil sample was weighed into 500 mL of individual incubation flasks, and treated with 20 mL of 100 μg/mL of the herbicide stock solution to generate herbicide-soil concentration of 10 μg/g(10ppm). The soils were thoroughly mixed with the herbicide standard solution by the use of a stirring rod. The soils were then moistened to 50% field capacity by treating the KNUST and GAEC soils in the flasks with 40.1and 34.5 mL of distilled water respectively. The soils were then incubated at room temperature (28-30.5°C). Control experiment, without herbicide was also set up. Incubation was done for 12 weeks.

3.5.5.1 Sub-sampling and Extraction procedure

Sub-sampling was done at a regular interval of one week after soil treatment. 5 g was of the soil was withdrawn from the incubation flasks into 200 mL extraction flask to determine the rates of the herbicides depletion.

To the 5 g of the sub-sampled soil in the extraction flask, 20 mL of acetone/hexane (4:1) was added. This was shaken mechanically on a flask shaker for 2 hours, followed by filtration with whatman No 42 filter paper. After filtration, the residue was washed 3 times with 3 mL of the solvent. The filtrate was then dried over anhydrous sodium sulphate (Na_2SO_4). The filtrate was concentrated to dryness by gently blowing in streams of air from hand dryer. The residue was then redissolved in 5 mL of acetone and then analyzed by ***tlc.***

3.5.5.2 Thin Layer Chromatographic (TLC) Analysis

The qualitative and quantitative determination of the extracts was done by thin layer chromatography as described in section **3.5.2** above. 5 µL of the sample extract, extract from control and standard herbicide solution were analyzed concurrenty. Each extract was analyzed two times.

Photosynthesis inhibition method as described per section **3.5.3(b)** was used for the detection of the spots. The diameters of the spots of the sample extracts were measured, for quantification and the distances moved by the spots of the extracts and the standards herbicide solution was also measured for identification.

3.5.5.2.1 Quantification of residues

Calibration curves of average diameters of two replicates measurement of the spots was plotted against the herbicides standard concentration in the range of 1- 16 ng as indicated in appendix 2 (graphs 1, 2, 3, 4 and 5) and this was used for quantification of the amount of herbicide in the extracts.

The amount extracted (residue level), C was calculated as

$$\frac{\text{Concentration in final extract x dilution factor}}{\text{Weight of the sample analyzed.}}$$

3.5.6 Determination of Limit of Detection (LOD)

Two milli litre of each of atrazine, ametyne, simazine, diuron and metobromuron standard solutions of concentrations 55.59, 103.66, 100.70, 99.78 and 101.59 µg/mL respectively was each spiked with the 5 g of the soil in the extraction flasks, to generate spiking level of 22.23, 41.46, 40.2839.90 and 40.64 µg/g for atrazine, ametryne, simazine, diuron and metobromuron respectively. Extraction was done with acetone/hexane (4:1) as described per the extraction procedure in section 3.5.5.1 above. Extracts were then subjected to ***tlc*** method of analysis. To determine the limit of detection, the extracts volume in the range of 10-0.1µL was spotted on the ***tlc*** plate for analysis, and the least detectable volume noted. Both unclean and clean up extracts were analyzed. Photosynthesis inhibition and O-tolidine+Potassium Iodide methods used for detection. However, for the clean-up extracts only photosynthesis inhibition method was used for detection. Duplicate determination was done. Only the GAEC soil was used for the investigation.

Limit of detection was calculated as:

$$\frac{V_1(\mu l) \times \text{Level of spiking } (\mu g/g)}{V_2 \text{ (ml)} \times 1000}$$

V_1= least detectable volume, V_2= final volume of extract in each case.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 PHYSICO-CHEMICAL SOIL PROPERTIES

The physical and chemical properties of the soils are presented in Table 5 below.

Table 5: Physical and Chemical properties of the soils

Properties	GAEC soil	KNUST soil
Soil moisture(%)	1.82	10.24
Water holding capacity(%)	40.1	34.5
Texture	Sandy Clay Loam	Sandy Loam
Soil pH(water)	5.5	7.5
Soil pH(0.01M KCl)	5.2	7.3
Organic carbon(%)	0.633	1.023
Organic matter(%)	1.091	1.764
Available phosphorus(µg/g)	13.06	14.67
Total nitrogen(%)	0.093	0.106

The coastal savannah soil from GAEC had lower % soil moisture but higher % water holding capacity (WHC). The moisture content of 1.82% compared to water holding capacity of 40.1% is an indication that at the time of sampling the soil was quite dry. This was expected since sampling was done in early February. In Ghana the dry season in February is at its peak. However, for the forest zone soil sampling from KNUST, the moisture content of 10.24% compared to water holding capacity of 34.5% indicates that the soil was not too dry at the time of

sampling. This observation may be due to the fact that in the forest zone, the land in the dry season is not as bare as it is in the coastal savannah zone.

Adequate water is not essential for microbiological activity, but in addition, water acts as a solvent and transporting agent, a reaction medium for both biological and non-biological processes and is a reagent in hydrolytic reactions[107].

The soil pH of the coastal savannah soil was 5.5 in water and 5.2 in 0.01M potassium chloride solution indicating acidic character, whilst for the forest soil zone the values were 7.5 and 7.3, indicating slightly basic character. Soil pH is affected by the presence of such inorganic species such as hydroxides, bicarbonates which tend to increase pH. The presence of carbon dioxide, oxides of sulphur, nitrogen, the presence of unionised portion of the weakly ionising acid such as carbonic acid lower pH

The major nutrients status of the two soils was not too different. Available phosphorus and total nitrogen were only slightly higher in the KNUST soil. However, the organic matter content of the KNUST soil was about 40% higher. This variation was expected since in the forest zone the relative high rainfall pattern promotes decomposition of more organic materials. Soil organic matter might be expected to have some effect on depletion of herbicides since microbial activity is often higher in more organic soils. However, it must be noted that adsorption of most herbicides also increases with increase in soil organic matter and since adsorption reduces the amount of herbicides available in the soil solution, it might provide protection for degradation.

Results obtained from the particle size analysis (Figures 3 and 4) gave an indication that the soil particle size is dorminated by sand particularly, the GAEC soil with 61.92 % sand content. The proportions of clay and silt are approximately equal for the GAEC soil, but are quite different for the KNUST soil.

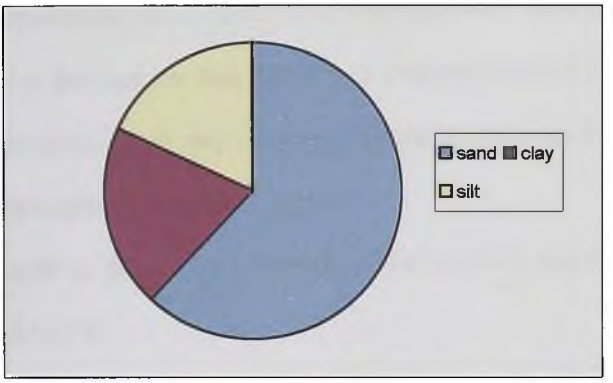

Figure 3: % composition of sand, clay and silt in the GAEC soil.

Sand=61.92 %, Clay=20.00 % and Silt=18.08 %.

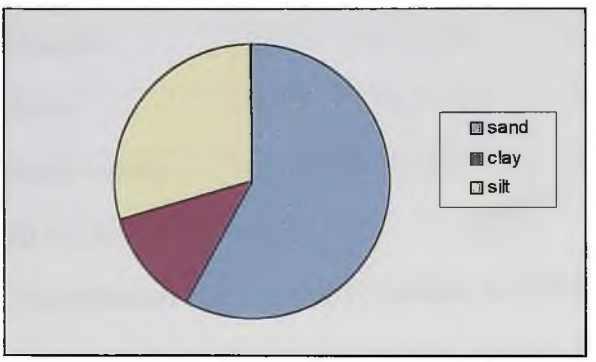

Figure 4: % composition of sand, clay and silt in the KNUST soil.

Sand=57.78 %, Clay=12.20 % and Silt=29.63 %.

4.2 SELECTION OF ELUTION SYSTEM FOR THE ANALYSIS OF THE HERBICIDES

The R_f values of the pesticides tested with the two elution systems are in agreement with the findings of Lowor *et. al*[94]. They reported of R_f values of 0.61, 0.61, 0.41 and 0.59 for atrazine, ametryne, diuron and metobromuron respectively at 32±3°C, using silica gel-ethyl acetate elution system. It is obvious from the results that the three triazine herbicides i.e. atrazine, ametryne and simazine, have very close R_f, (Table 6) particularly, atrazine and ametryne under silica gel-etyyl acetate system.

Table 6: **R_f** of the herbicides, using silica gel-ethyl acetate elution system at 30.5±2°C

Herbicides	R_f values	**R_f(mean)**	SD
Atrazine	0.622, 0.620, 0.625	**0.622**	0.004
Ametryne	0.610, 0.610, 0.617	**0.612**	0.006
Simazine	0.578, 0.580, 0.588	**0.581**	0.006
Diuron	0.425, 0.430, 0.432	**0.429**	0.005
Metobromuron	0.574, 0.580, 0.577	**0.577**	0.004

SD = Standard deviation

This indicates that this elution system could not be very useful for the analysis of these chemicals in a multi-residue procedure involving a mixture of these pesticides. This is because their spots would overlap and resolution would be very difficult. However, for a sample known to contain only one of these

chemicals, the system could conveniently be used. In the case of the substituted urea herbicides, i.e. diuron and metobromuron, their Rf values were quite distinct from each other and therefore this elution system is recommended even if these herbicides are administered in a mixture.

In the case of silica gel-dichloromethane system, ametryne and diuron, have the same Rf (Table 7).

Table 7: R_f of the herbicides, using silica gel-dichloromethane elution system at 30.5± 2°C

Herbicides	R_f values	**R_f(mean)**	SD
Atrazine	0.064, 0.064, 0.058	**0.062**	0.006
Ametryne	0.088, 0.085, 0.078	**0.083**	0.009
Simazine	0.056, 0.048, 0.056	**0.053**	0.008
Diuron	0.088, 0.088, 0.075	**0.083**	0.011
Metobromuron	0.258, 0.258, 0.251	**0.256**	0.006

It is therefore obvious that this elution system could not be very useful to separate these two chemicals if they are in a mixture, as their spots would overlap. It is of interest to note that all the herbicides except metobromuron moved very little from the origin with the silica gel-dichloromethane system. Thus, the Rf values of atrazine, ametrye, simazine and diuron were almost zero. This indicates the high affinity between these chemicals and the stationary phase on one hand and the low affinity between the chemicals and the mobile phase. However, with the silica gel-ethyl acetate system, all the chemicals moved to an

appreciable height from the origin. This also means that the stationary phase has less affinity for the chemicals compared to the mobile phase. It therefore appears that perhaps, the silical gel-dichloromethane system might not be suitable for the analysis of these chemicals. For the two systems studied, silica gel-ethyl acetate system appears to be more suitable and was selected.

Detection Methods

The O-tolidine + potassium iodide was not sensitive to metobromuron as spots appeared very faint. Spots were seen as darkish brown in grey- whitish background. At the concentrations at which the standards were injected, spots could stay overnight before disappearing.

With regard to the photosynthesis inhibition method, spots were visualized as blue black in greenish background. Spots stayed for about 45 minutes after detection before disappearing. Comparing the size and intensities of the spots obtained, the photosynthesis inhibition method appeared more sensitive than the O-tolidine + potassium iodide method.

4.3 SELECTION OF EXTRACTION SOLVENTS FOR THE HERBICIDES

Recovery of the herbicides investigated showed almost the same trend of recovery in the two soil ecosystems(compares Tables 8 and 9 below), i.e. highest efficiency of extraction for all the herbicides was achieved with acetone and acetonitrile when used as single solvents and results showed that they both have approximately equal efficiency as extraction solvents for the herbicides.

Table 8: Percentage recovery (mean of 3 replicates) of the herbicides in the GAEC soil. (Unclean extracts)

Herbicides	Acetone	Acetonitrile	Hexane	Methanol	Acetone/hexane (4:1)
Atrazine	96.2±3.2	92.6±3.5	84.8±3.5	86.0±3.9	93.6±2.3
Ametryne	95.1±4.2	93.4±3.0	82.9±3.7	84.6±3.7	92.7±2.4
Simazine	94.3±3.9	92.8±3.3	82.1±4.3	85.6±5.1	92.8±3.3
Diuron	92.6±4.2	95.0±4.2	81.6±3.1	83.3±5.3	90.9±3.8
Metobromuron	94.6±3.7	90.6±4.2	79.7±4.1	78.8±3.8	89.7±2.9

Table 9: Percentage recovery (mean of 3 replicates) of the herbicides in the KNUST soil. (Unclean extracts).

Herbicides	Acetone	Acetonitrile	Hexane	Methanol	Acetone/hexane (4:1)
Atrazine	96.7±3.4	92.7±3.7	83.6±1.9	86.8±2.2	93.4±1.1
Ametryne	95.9±2.1	93.3±2.9	81.1±2.3	85.7±2.4	92.8±1.7
Simazine	95.1±2.9	93.9±1.8	82.4±1.4	85.9±3.8	93.7±5.3
Diuron	94.8±4.8	95.3±3.6	80.8±4.5	83.5±4.6	90.5±4.0
Metobromuron	95.9±3.7	89.1±4.4	79.7±1.6	79.3±1.8	90.3±3.1

For instance, the recovery efficiencies of atrazine in the GAEC soil were 96.2 % and 92.6 % (Table 8) using acetone and acetonitrile respectively. In the case of the KNUST soil the respective values for the two were 96.7 and 92.7 %(Table 9). Extraction with methanol and hexane as single solvent gave lower recovery efficiencies, particularly, with the clean up extracts (Table 10). However, no clear reason could be assigned to this trend.

Table 10: Percentage recovery (mean of 3 replicates) of the herbicides in the GAEC soil (Clean up extracts)

Herbicides	Acetone	Acetonitrile	Hexane	Methanol	Acetone/hexane (4:1)
Atrazine	84.8±2.7	82.1±5.4	71.4±3.3	74.4±3.3	81.7±5.5
Ametryne	83.6±4.5	83.9±4.2	72.3±3.0	73.3±4.7	80.8±3.7
Simazine	82.3±4.4	82.5±2.9	73.5±2.3	74.0±4.9	82.8±5.5
Diuron	81.3±5.1	85.2±4.3	68.4±2.5	72.5±4.9	81.7±5.3
Metobromuron	83.7±4.4	80.0±3.9	69.3±3.5	69.1±5.6	80.9±3.3

Performance of acetone/hexane (4:1) was not surprising as it was between those of acetone and hexane used as single solvents, but closer to that of acetone. Recovery for the herbicides in the soil ecosystems studied with acetone, acetonitrile and acetone/hexane (4:1) in almost all cases yielded efficiencies of 90 % and above with the unclean extracts (Table 8 and 9). The highest efficiency of 96.7 % was achieved for atrazine in the coastal savannah soil. The two solvents, methanol and hexane used as single solvents could also be used for the extraction of these herbicides since their recovery efficiencies for the unclean extracts were above 80 % (Table 8 and 9). It is accepted that an extraction solvent for a given pesticide should give a minimum of 80 % recovery efficiency[95]. For the two solvents the highest efficiencies of 86.8 was obtained for atrazine using methanol in the KNUST soil while the lowest efficiency of 78.8 % was obtained for metobromuron in the GAEC soil. However, on cleaning up

the GAEC soil with solid phase extraction (SPE) cartridge equipped with C-18 as adsorbent, recovery efficiency in the range of 80.0 – 85.2 % was obtained with acetone, acetonitrile and acetone/hexane (4:1) as shown in Table 10. The highest recovery of 85.2 % was achieved for diuron with acetonitrile. In the case of methanol and hexane, low recovery of 68.4 % was attained for diuron with hexane. Thus, with the methodology employed in this study clean up might not be necessary to achieve the desired results.

With regard to the purity of the extracts, only one spot, the spot of the analyte was detected for all the extracts both the unclean and the clean up, hence it could be deduced that all the solvent extracts have the same level of purity based on the detection method.

4.4 THIN LAYER CHROMATOGRAPHY ANALYSIS OF EXTRACTS

Results of the ***tlc*** analysis indicated one spot detected for each of the extracts of the samples, and in each case, the spot corresponded to the herbicide being investigated with the same R_f as the standard herbicides solution analysed concurrently. The R_f values were 0.62, 0.61, 0.58, 0.57 and 0.42 for atrazine, ametryne, simazine, metobromuron and diuron respectively. In the case of the extracts from the control experiments no spots were detected. This confirmed that the spots detected were that of the herbicides being investigated. Although the R_f values were very close, the elution system was conveniently used since each sample contained only one of the herbicides in question. However, for a multi residue analysis the elution system would have to be changed.

4. 5 DEPLETION OF THE HERBICIDES

Decline of all the herbicides shows slightly faster disappearance in the forest zone soil sampled from KNUST than the coastal savannah soil sampled from GAEC. This can be attributed to the difference in the soil moisture, organic matter content and the difference in the clay compositions of the two soils, although other factors such as soil pH might be involved. Both the organic matter and moisture contents were higher in the KNUST soil than the GAEC soil (Table 5). Higher organic matter facilitates microbial activity and with increased microbial activity, decomposition of the chemicals would be enhanced, ultimately leading to an increase in the rate of herbicide depletion, but up to a limiting value[

96]. Soil moisture is not only essential for microbial activity, but in addition water acts as solvent and transport agent, a medium for both biological and non-biological processes[97]. Hence adequate water contributed to the relative faster depletion of the chemicals in the KNUST soil.

Clay content was higher in the GAEC soil compared to the KNUST soil (Figures 3 and 4). Soils with low amount of clay are known to greatly facilitate dissipation of pesticides[98] and could be one of the factors that accounted for the relatively slow depletion of the herbicides in the soil sampled from GAEC.

The rate of disappearance of all the chemicals was observed to be very rapid in the first two weeks of incubation as indicated in the herbicide depletion curves (Figures 5, 6, 7, 8 and 9) below. This behaviour is inevitable and can be attributed to the high herbicide concentration at the beginning of the experiment.

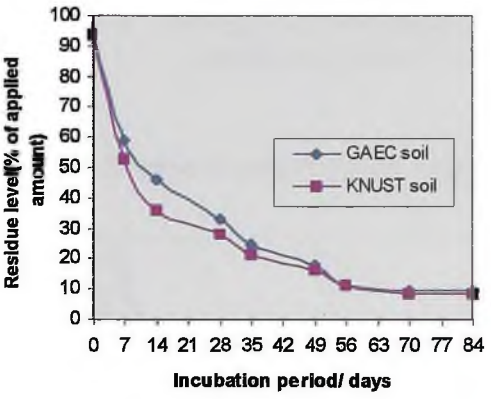

Figure 5: Decline of atrazine from treated soils.

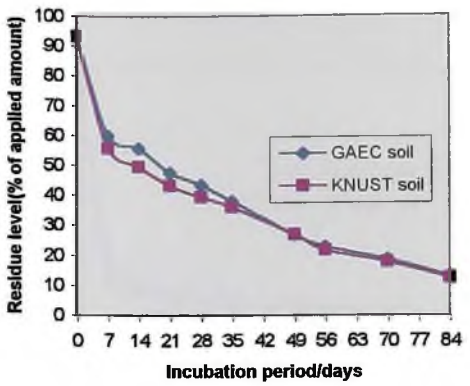

Figure 6: Decline of ametryne from treated soils

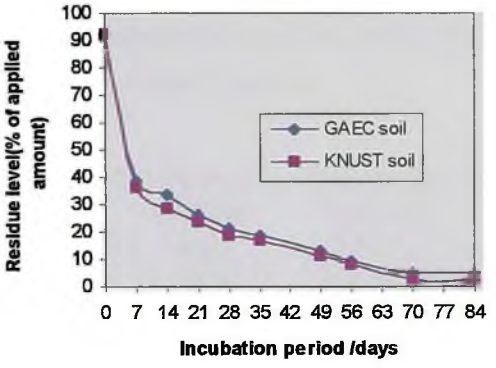

Figure 7: Decline of simazine from treated soils.

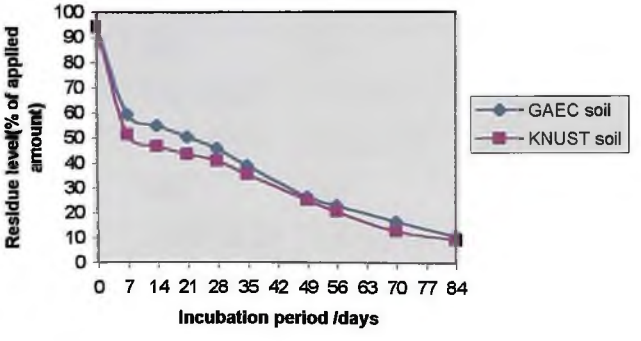

Figure 8: Decline of diuron from treated soils

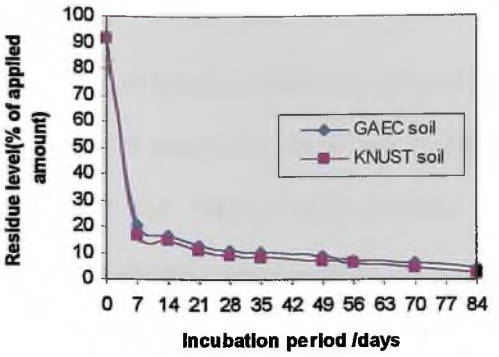

Figure 9: Decline of metobromuron from treated soils.

After 14 days of incubation, the triazine herbicides, i. e atrazine, simazine and ametryne had depleted to 4.61, and 3.38, 5.58 µg/g respectively in the GAEC soil as shown in Table 12 below.

Table 12: Concentration of the herbicides in the incubated GAEC soil.

Incubation Period/days	Atrazine .(µg/g)	Simazine . (µg/g)	Ametryne (µg/g)	Diuron (µg/g)	Metobromuron (µg/g)
0	9.36±0.75	9.28±0.69	9.27±0.31	9.09±0.33	9.07±0.44
7	5.92±0.33	3.90±0.46	5.99±0.28	5.95±0.51	2.04±0.15
14	4.61±0.31	3.38 ±0.57	5.58±0.27	5.49±0.57	1.66 ±0.13
21	3.95±0.29	2.61±0.20	4.76±0.25	5.03±0.22	1.28±0.33
28	3.29±0.26	2.09±0.32	4.35±0.24	4.75±0.33	1.09±0.19
35	2.47±0.32	1.85±0.12	3.81±0.33	3.88±0.34	1.02±0.17
42	NM	NM	NM	NM	NM
49	1.81±0.28	1.31±0.11	2.69±0.27	2.63±0.14	0.93±0.12
56	1.15±0.21	0.9±20.25	2.28±0.13	2.28±0.27	0.74±0.15
63	NM	NM	NM	NM	NM
70	0.987±0.28	0.52±0.19	1.87±0.34	1.64±0.46	0.64±0.21
77	NM	NM	NM	NM	NM
84	0.987±0.28	0.52±0.19	1.32±0.27	1.09±0.13	0.43±0.12

Similar trend was found in the KNUST soil. Thus, for the triazine herbicides, simazine depleted most while ametryne least depleted after 14 days of incubation.

In the case of the substituted urea herbicides, i.e diuron and metobromuron, after two weeks of incubation, they had respectively depleted to 5.49 and 1.66 µg/g in the GAEC soil (Table 12). However, in the case of the KNUST soil their respective values were 4.57 and 1.47 µg/g as indicated in Table 13.

Table 13: Concentration of the herbicides in the incubated KNUST soil.

Incubation Period/days	Atrazine (µg/g)	Simazine (µg/g)	Ametryne (µg/g)	Diuron (µg/g)	Metobromuron (µg/g)
0	9.34±0.85	9.37±0.34	9.28±0.32	9.09±0.67	9.01±0.81
7	5.26±0.34	3.64±0.51	5.58±0.27	5.03±0.56	1.67±0.13
14	3.62±0.55	3.38±0.57	4.97±0.40	4.57±0.50	1.47±0.25
21	3.29±0.26	2.35±0.21	4.33±0.26	4.33±0.50	1.09±0.20
28	2.83±0.45	1.85±0.16	3.95±0.23	4.06±0.25	0.91±0.26
35	2.14±0.25	1.69±0.19	3.60±0.16	3.55±0.47	0.83±0.13
42	NM	NM	NM	NM	NM
49	1.65±0.13	1.09±0.22	2.69±0.27	2.52±0.22	0.74±0.11
56	1.15±0.20	0.79±0.16	2.18±0.26	2.05±0.38	0.64±0.19
63	NM	NM	NM	NM	NM
70	0.82±0.19	0.27±0.11	1.77±0.13	1.26±0.21	0.45±0.16
77	NM	NM	NM	NM	NM
84	0.82±0.16	0.27±0.11	1.25±0.16	0.91±0.28	0.26±0.14

NM = not measured.

The rate of depletion of the chemicals after 14 days of incubation however, became slow and essentially insignificant. This was not surprising since after weeks of soil treatment, most of the applied herbicides had disappeared and many of the soil organisms might have died as a result of the influence of the chemicals.

At the end of the 12th weeks of incubation, the triazine herbicides, i.e atrazine, ametryne and simazine had respectively declined to about 9.87, 13.16 and 4.49 % of the applied amount in the GAEC soil, whiles in the KNUST soil the respective values were about 8.22, 12.48 and 2.66 % of the applied amount as indicated in Figure 10 below.

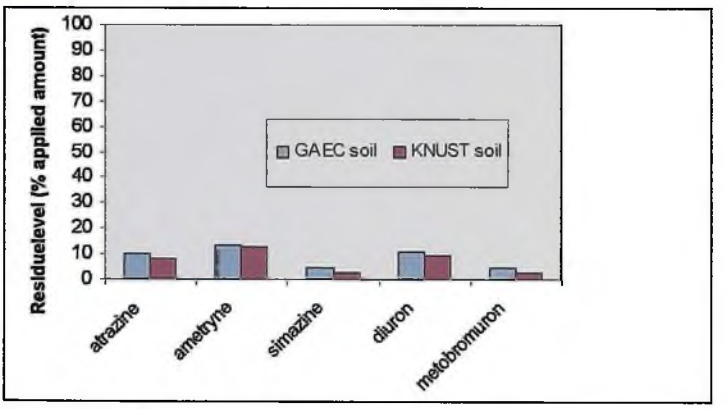

Figure 10: Concentration of the herbicides in the treated soils at the end of 12 weeks of incubation.

With regard to the substituted urea herbicides, at the end of incubation, diuron

and metobromuron have respectively declined to 10.61, 4.38 % (Table 8) of the applied amount in the GAEC soil. Similar trend of decline was also observed in the KNUST soil. Thus at the end of 12 weeks of incubation metobromuron depleted most. This is in agreement with the findings of Green *et. al.*[99] who observed that metobromuron is less persistent in the soil.

The pattern of depletion of the chemicals in the two soil ecosystems investigated showed similar trend of decline. For all the chemicals, there was a continuous decline with time, with a more rapid depletion within the first two weeks of incubation, however, the rate of loss became slow getting to the end of the incubation time.

4.6 KINETICS OF DEPLETION OF THE HERBICIDES

The data obtained indicated that the kinetics involved in the pattern of depletion of the herbicides, to a higher degree fitted well with first order reaction as indicated in the first order kinetics plots for the herbicides (Figures 11 – 15).

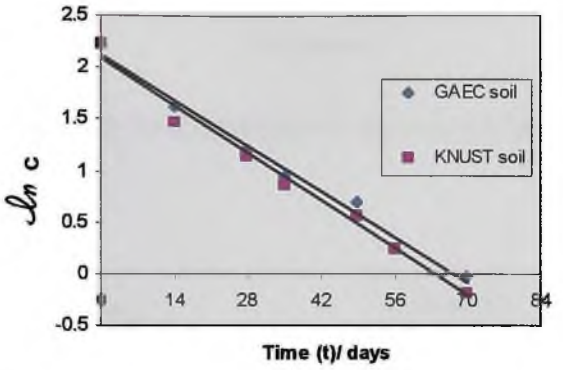

Figure 11 : First order plot for depletion of atrazine from treated soils at 28±1.5°C

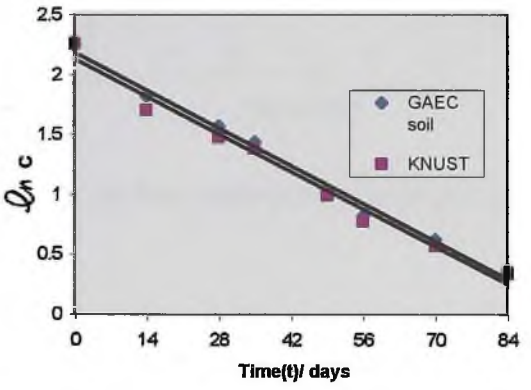

Figure 12: First order plot for depletion of ametryne from treated soil at 28±1.5°C

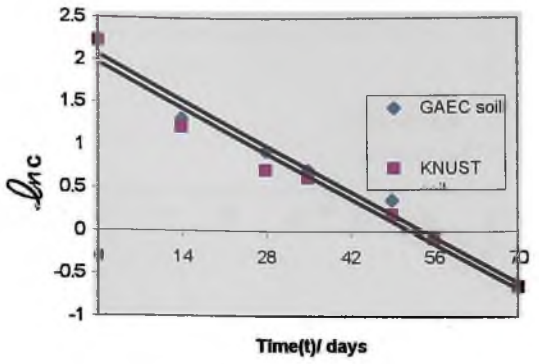

Figure 13: First order plot for depletion of simazine from treated soils at 28±°C

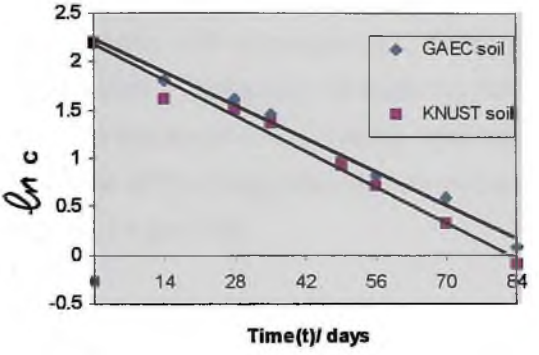

Figure 14: First order plot for the depletion of diuron at 28±1.5°C

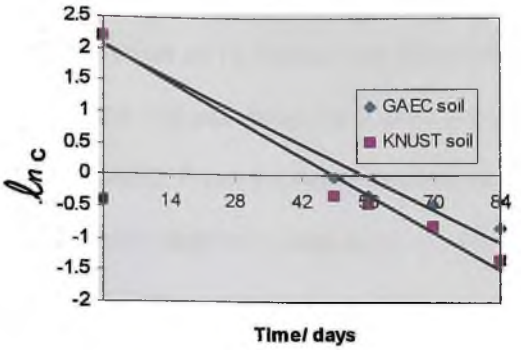

Figure 15: First order kinetics plot for the depletion of metobromuron from treated soils at 28±1.5°C

The specific rate constants (k) for the depletion of the herbicides in the soil ecosystems investigated, as deduced from the first order kinetics plots (Figures 11 – 15), is the slope of the graphs. The specific rates constants and the calculated half lives of the herbicides are as presented in herbicides depletion data below (Tables 14 and 15).

Table 14: Depletion data of the herbicides in the GAEC soil investigated.

Herbicides	Degradation equation	Rate constant (k)/ day^{-1}	Half-life/days (0.6932/k)
Atrazine	ℓn C=2.1261-0.0316t	0.0316	21.9
Ametryne	ℓn C=2.1824-0.0215t	0.0215	32.2
Simazine	ℓn C=2.0637-0.0362t	0.0362	19.1
Diuron	ℓn C=2.2325-0.0246t	0.0246	28.2
Metobromuron	ℓn C=2.0321-0.0469t	0.0469	14.8

Table 15: Depletion data of the herbicides in the KNUST soil investigated.

Herbicides	Degradation equation	Rate constant (k)/ day^{-1}	Half-life/ days (0.693/k)
Atrazine	ℓn C=2.0837-0.0328t	0.0328	21.2
Ametryne	ℓn C=2.1249-0.0223t	0.0223	31.1
Simazine	ℓn C=1.9644-0.0378t	0.0378	18.2
Diuron	ℓn C=2.1774-0.0263t	0.0263	26.0
Metobromuron	ℓn C=2.0613-0.0524t	0.0524	13.3

The half-lives of the chemicals were in the range of 14.8 – 32.2 days (Table 14) and 13.3 – 31.1days (Table 15) in the GAEC and KNUST soils respectively. The lowest value of 13.3 days was obtained for metobromuron in the KNUST soil, while the highest value, 32.2 days was obtained for ametryne in the soil sampled from GAEC. From the half-lives values obtained, it could be rationalized that the chemicals might not pose much environmental problems in terms of soil persistency.

4.7 DETECTION LIMIT

The detection limit gives an idea of the lowest practical concentration of the herbicide residue or contaminant that can be quantitatively measured and identified in a specific matrix. The results, which suggest the suitability of the method for the screening of the herbicides, are presented in Tables 16 and 17.

Table 16: Detection limits of the herbicides in the GAEC soil using the unclean extracts.

Herbicides	*Detection limit(µg/g)*[x]	*Detection limit(µg/g)*[xx]
Atrazine	0.008	0.006
Ametryne	0.008	0.004
Simazine	0.012	0.006
Diuron	0.159	0.004
Metobromuron	0.406	0.008

[x]O-tolidine + potassium iodide detection method, [xx]Photosynthesis inhibition detection method.

Table 17: Detection limit of the herbicides in the GAEC soil using the clean-up extracts and the photosynthesis inhibition detection method.

Herbicides	*Detection (µg/g)*
Atrazine	0.025
Ametryne	0.017
Simazine	0.025
Diuron	0.024
Metobromuron	0.162

Using the O-tolidine + potassium iodide method, detectability, in the range of 0.008 –0.012 µg/g (Table16), was achieved for atrazine, ametryne and simazine, (the triazine herbicides), while detectability in the range of 0.159 – 0.406 µg/g (Table 16) also was achieved for metobromuron and diuron respectively, both substituted urea herbicides. Comparing these values, it may be deduced that the O- tolidine + potassium iodide method is perhaps more suitable for the screening of triazine herbicides than the substituted urea herbicides. In the case of the photosynthesis inhibition detection method, detectability was in the range of 0.008 – 004 µg/g (Table 16). Comparing the detectability values obtained for the two detection methods, it is clear that the photosynthesis inhibition method is more suitable for the screening of the herbicides.

With regard to the clean-up extracts, detectability obtained was in the range of 0.162 – 0.017 µg/g as indicated in Table 17. The detection limits 0.025 µg/g (Table 17) obtained for atrazine and simazine compares well with the findings of

Balinova *et.al.*[100]. They reported of detection limit of 0.02 µg/g for both atrazine and simazine in the study of herbicides residue in the soil using Gas Chromatography methodolody.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

From the results just discussed, the following conclusions can be drawn.

The herbicides depleted slightly faster in the KNUST soil, (a forest zone soil) than in the coastal savannah soil sampled from GAEC. This can be attributed to the higher organic matter and moisture content, and the lower clay composition of the KNUST soil. In all metobromuron, a substituted urea herbicide depleted most by the end of the incubation experiment, while ametryne, atrazine herbicide least depleted. The pattern of decline of the chemicals in the two soil ecosystems studied was however, similar. For all the herbicides there was rapid disappearance at the early stages of the incubation experiment.

Kinetics involved in the process of depletion of the herbicides in the soils to a larger degree could be described by first order reaction. The half-lives obtained for the chemicals are an indication that the chemicals may not pose much environmental problem in terms of persistency.

Acetone, acetonitrile and acetone/hexane (4:1) are efficient solvents for the extraction of the chemicals in the soil ecosystems investigated. However, recoveries with methanol and hexane were relatively low particularly, with the clean up extract.

The ***t/c*** methodology used for the study is simple, fast and reliable and is recommended for routine analysis of herbicides in soil ecosystems.

5.2 RECOMMENDATION

It is recommended that further work should be carried out in subsequent years in other soil ecosystems, for instance in soils sampled from an interior savannah zone to show the depletion pattern of the chemicals in such soils.

Furthermore, the study should be carried out under field condition to ascertain whether the rates of depletion of the chemicals would be similar to that of laboratory incubation system. Also the half-lives of the chemicals could be determined at different herbicide-soil concentration to make the half-lives values obtained more meaningful.

In addition, the degradation products of the herbicides, which could not be identified by the photosynthesis inhibition method used, could be investigated, using other methods to ascertain their accumulation rates with time.

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APPENDIX 1

Sample calculation

1. Percentage soil moisture = $\frac{\text{mass of soil moisture}}{\text{mass of soil sample}} \times 100$

For the GAEC soil, mass of soil moisture = 0.943 g, mass of soil sample = 51.790 g.

Therefore % soil moisture = $\frac{0.943}{51.790} \times 100 = 1.821\ \%$

2. Percentage water holding capacity = $\frac{\text{amount of water retained by soil}}{\text{air-dried mass of the soil used.}} \times 100$

For the GAEC soil, amount of water retained = 40.1 g

Air-dried mass of soil used = 100 g

Therefore % WHC = $\frac{40.1}{100} \times 100 = 40.1\ \%$

3. Percentage organic carbon = $\frac{(V - v) \times M \times 0.39}{\text{Mass of soil used}}$

V = titre volume(mL) of the blank, v = titre volume(mL) of sample

M = molarity of ferrous ammonium sulphate.

For the KNUST soil , V = 48.85 mL, v = 35.75 mL

Therefore % organic carbon = $\frac{(48.85 - 35.75) \times 0.2 \times 0.39}{1} = 1.023\ \%$

4. Preparation of the herbicides standards

For example atrazine, using 100 ml volumetric flask

Mass of primary standard = 0.0565 g

% purity = 98.4

Mass of active ingredient (atrazine) = $\frac{98.4}{100} \times 0.0565$

= 0.05559 g

= 0.000555 g/mL

= 555.9 µg/mL

5. $R_f = \frac{\text{distance of centre of spot from starting point}}{\text{distance of solvent front from starting point}}$

Atrazine, using silica-gel-ethyl acetate system

First determination, Rf = $\frac{7.80}{12.45} = 0.626$

Second determination, Rf = $\frac{7.70}{12.45} = 0.620$

Third determination, Rf = $\frac{7.75}{12.45} = 0.621$

Rf (mean) = $\frac{0.626 + 0.620 + 0.621}{3}$ = 0622± 0.004

6. Percentage recovery = $\frac{\text{amount recovered}}{\text{amount added}} \times 100$

Using acetone for the recovery of atrazine,

Amount recovered = $\frac{\text{diameter of the sample extract}}{\text{diameter of standard}} \times$ conc. of standard

= $\frac{8.25 \text{ mm}}{8.75 \text{ mm}} \times 0.05 \text{ µg} = 0.0485 \text{ µg}$

Therefore % recovery = $\frac{0.0485}{0.0500} \times 100$

= 97.1 %

7. Amount of herbicides extracted (residue level) on incubation

$$= \frac{\text{concentration in final extract x dilution factor}}{\text{weight of sample analysed}}$$

Concentration in final extract determined from calibration curve.

Atrazine, 7 days of incubation

Equation of calibration curve = $Y = 0.4567x + 3.7462$

$$\text{i.e } D = 0.4567C + 3.7462$$

D = diameter of spot, C= concentration from calibration curve.

Using the GAEC soil, D = 8.25 mm

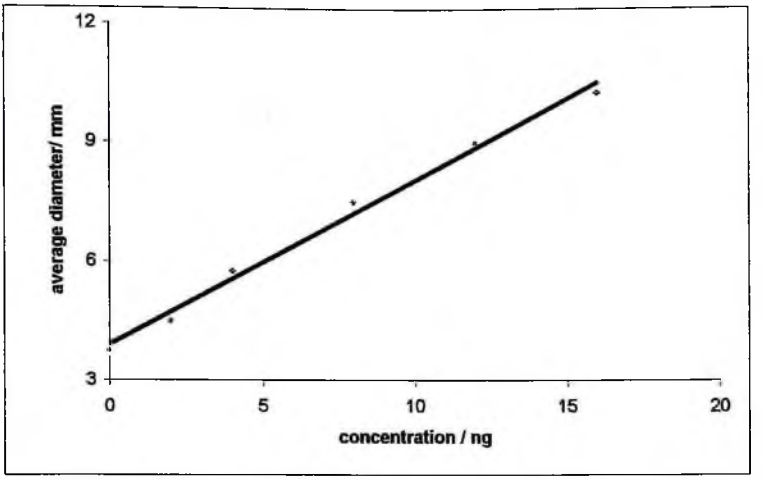
$C = 8.25 - 3.7462 / 0.4567 = 9.861$ ng/5 µL = 1.972 ng/µL

Therefore amount extracted (residue level), after 7 days of incubation is

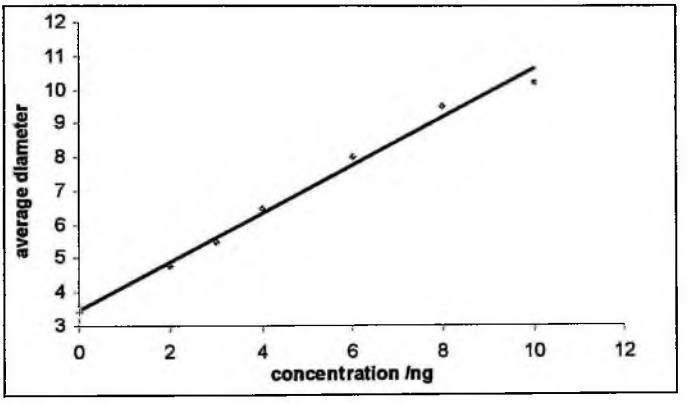
$$\frac{1.972 \text{ ng/ uL} \times 15 \times 10^3}{5 \text{ g}}$$

$$= 5.916 \times 10^3 \text{ ng/g} = 5.916 \text{ µg/g}$$

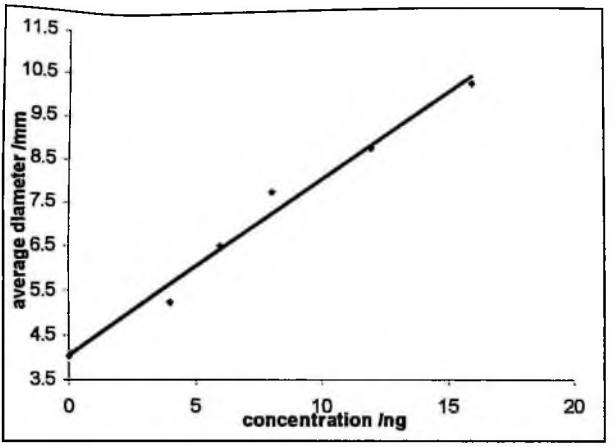
APPENDIX 2: CALIBRATION CURVES



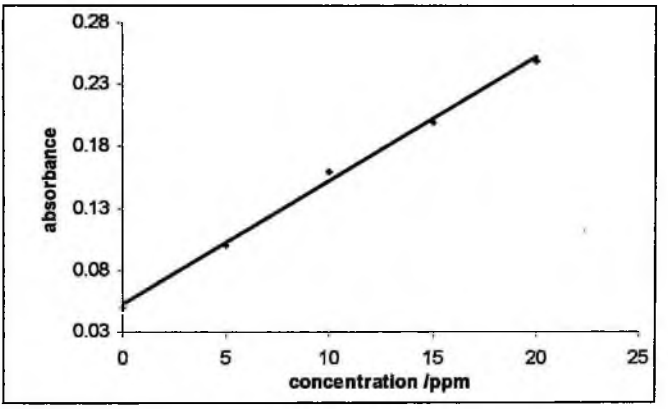
Graph 1: Calibration curve for the determination of atrazine



Graph 2: Calibration curve for the determination of ametryne.



Graph 5: Calibration curve for the determination of metobromuron.



Graph 6: Calibration curve for the determination of available phosphorus.