# FATE STUDIES OF CHLORSULFURON IN SELECTED SOILS WITHIN THE MARA RIVER BASIN OF KENYA

BY

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# DECLARATION

## BY THE CANDIDATE

I certify that this thesis has not been previously presented for a degree of Maseno University or in any other university. The work proposed herein is my original work and all sources of information have been supported by relevant references.

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# ABSTRACT

Chlorsulfuron is a sulfonylurea herbicide used in the control of broadleaf and grass weeds in wheat farms as is practiced within the Mara River Basin. Though it is assumed to be environmentally safe due to its low application rates, relatively shorter half-life and low mammalian toxicity, it can persist in soil and has been detected in ground and surface waters. Its low $pK_a$ makes it anionic in soil thus highly mobile which increases the risk of its residues to be carried into the subsoil and underground aquifers. Physicochemical parameters like soil organic matter, clay content, pH and environmental factors like soil moisture, application rates and temperature affect the dissipation of chlorsulfuron in soil. Moreover, Mara River Basin soils have been classified as copper deficient, so copper foliar fertilizers are normally required. The effect of soil parameters and copper ions on the adsorption, degradation and leaching of chlorsulfuron in the wheat growing soils within the Mara River Basin is unknown. The objective of this study was to investigate the adsorption-desorption, leaching, degradation patterns and field dissipation of chlorsulfuron in wheat growing soils within the Mara River Basin. Field dissipation studies were laid as completely randomized design on 4m x4m plots. Soil was sampled from five wheat growing areas within the Basin and their physicochemical parameters established. Chlorsulfuron adsorption-desorption pattern was determined using batch equilibrium model and column leaching studies in a laboratory, after which solid phase extraction was done and herbicide and its metabolites quantified using HPLC-MS. The soil physicochemical parameters varied significantly ($p \leq 0.05$) in all the five locations. The soils were mainly sandy loam and clay soils with pH below 6.0. Chlorsulfuron sorption onto the five soils was low as indicated by the low $K_d$ values (0.59 – 2.52). Adsorption was positively correlated to soil organic matter and negatively correlated to the soil pH. Increasing $Cu^{2+}$ concentration increased the sorption of chlorsulfuron in all the five soils possibly due to decreased equilibrium solution pH and formation of Cu-chlorsulfuron complexes which were preferentially sorbed via the carboxylic and hydroxylic functional groups of the soil. Chlorsulfuron leached to a depth of 30 cm in all the soil columns. The leaching pattern varied significantly ($p\leq 0.05$) with location, depth of the soils and rate of application. However more residues were found in Mau East B soils as compared to other soils. This was due to its higher pH and lower organic carbon content. Influence of biotic degradation was faster in all the soils as compared to the sterile soils as shown by shorter degradation half-lives. Similarly, chlorsulfuron degradation was faster in soils with repeated application of chlorsulfuron than in single application. Field dissipation of chlorsulfuron was faster with half-lives of 13.5 and 13.6 days in Olokurto and Mau East regions. The low sorption and high mobility raises the leaching potential of chlorsulfuron. On the contrary, coexistence of chlorsulfuron and copper ions reduced the chances of underground water contamination while reducing the bioavailability of the herbicide for weed protection.

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| a.i | active ingredient |
| ALS | Acetolactate synthase |
| CEC | Cation Exchange Capacity |
| Cu | Copper |
| C.V | Coefficient of Variation |
| DDT | dichlorodiphenyltrichloroethane |
| $DT_{50}$ | Degradation time for 50% of a compound |
| ESARPO | Eastern and Southern Africa Regional Program Office |
| GLOWS | Global Water for Sustainability |
| HFS | Herbicide Fact Sheet |
| HSDB | Hazardous Substances Data Bank |
| LVBC | Lake Victoria Basin Commission |
| Meq | milli equivalent |
| MoA | Ministry of Agriculture |
| SOM | Soil Organic Matter |
| WSSA | Weed Science Society of America |
| WWF | World Wide Fund for Nature |
| WWF-TPO | World Wide Fund for Nature – Tanzania Program Office |

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background to the study

The Mara River Basin is one of the river basins within the Lake Victoria catchment area and is shared between Kenya (65%) and Tanzania (35%) (LVBC and WWF-ESARPO, 2010). It is located between longitudes 33°47'E and 35°47'E and latitudes 0°38'S and 1°52' S and spans approximately 13750 km$^2$ across the two East African countries (Mutie *et al.*, 2006). The 395 km long Mara River has for a long time been considered one of the pristine rivers draining into Lake Victoria, which forms part of the upper catchment of the Nile Basin. Its main perennial tributaries, rivers Amala and Nyangores, drain from the upper Mau Escarpment. Other prominent tributaries are Talek River, Engare Engito and the Sand River (Abulude *et al.*, 2007). Agriculture is the mainstay of people within the Basin, (Aboud *et al.*, 2002). Although majority of the population rely on wheat production as the main economic farming activity, other crops grown include maize, tea, banana, tomatoes, cabbages, onions, eggplants, watermelon and varieties of vegetables. These crops are supported using agrochemicals, particularly pesticides (WWF-TPO, 2004). Indeed, some pesticides and their metabolites have been detected in the surface waters within the basin (GLOWS, 2007). This poses a threat to over half of the households in the Mara River Basin who rely on its water for domestic and livestock needs (Aboud *et al.*, 2002).

The use of pesticides is crucial and has been used in farming systems adopting minimum tillage to improve production through elimination of pests and weeds in many parts of the world (Tang and Robson, 2000). Within the Mara River Basin, the herbicides used in wheat production include; 2, 4-Dichlorophenoxyacetic acid, flufenacet, glyphosate, flurosalam, bromoxynil, fenoxaprop-p-ethyl, chlorsulfuron, clodinafop-propargyl and glycine among others (MoA, 2012). From a reconnaissance survey, chlorsulfuron, a sulfonylurea was one of the most widely used herbicide within the basin. Chlorsulfuron is selective and controls both broadleaf and grass weeds. The herbicide has been widely used both as a pre- and post-emergent herbicide to control broadleaf weeds in cereal crops because of its low application rates and low mammalian toxicity (Beyer *et al.*, 1988; Battaglin *et al.*, 2000; Cessna *et al.*, 2006).

Sulfonylureas have replaced other herbicides which are more toxic to animals (Beyer *et al.*, 1988). However, the wide-spread use of sulfonylureas could have a devastating impact on the productivity of non-target crops and the makeup of the natural plant communities and wildlife food chains (Fletcher *et al.*, 1993). Off-site transport of sulfonylureas by drift or leaching may have severe ecological impacts due to their phytotoxicity. Sulfonylureas can persist in the soil and may affect plant reproduction and rotational crop yields beyond the application season (Lee *et al.*, 2002; Bedmar *et al.*, 2006; Hollaway *et al.*, 2006; Kjær *et al.*, 2006). Also, sulfonylureas are considered relatively mobile compounds in soils because of their anionic character, and may leach to the undergroundwater. Sulfonylureas, in general, have been reported in both surface water and underground water (Battaglin *et al.*, 2000; Cessna *et al.*, 2006). These chemicals pose potential risks to soil microorganisms, non-target organisms, and mammalian laboratory animals (Arnold *et al.*, 2001; Sabater *et al.*, 2002; Healy *et al.*, 2004). Chlorsulfuron is a relatively new sulfonylurea herbicide which causes a reduction in the growth of *Pseudomonas* soil bacteria (Boldt and Jacobsen, 1998) resulting in major changes in species composition and diversity thus affecting soil fertility. The potential hazards due to widespread use of chlorsulfuron in the wheat farms within Mara River Basin have not been evaluated.

Chlorsulfuron dissipation in soil is affected by several physicochemical parameters including soil organic matter (SOM), clay content, pH, and environmental factors including soil moisture and temperature (Walker and Brown, 1983;Thirunarayan *et al.*, 1985; Barriuso *et al.*, 1992). The herbicide dissipates faster in soils with high sand content, high temperature and moisture and low organic matter. However, these studies above were for temperate countries where temperatures can be low, and, chlorsulfuron dissipation in Mara River Basin, which falls within the tropics has not been established.

Within the Mara River Basin, wheat is majorly grown in two regions; upper highlands and the lower highlands. These two regions vary in their altitudes, annual precipitations, types of soils and other environmental conditions and these affect the wheat planting seasons and other agronomic activities within the Basin. Although, the wheat growing soils in the Mara River Basin have been classified as cambisols, andisols and phaezeoms (Jaetzold *et al.*, 2010), the soil types have not been characterized for their physico-chemical parameters. These parameters may be changing from one location to another. The influence of variations in soil characteristics on chlorsulfuron dissipation in the wheat growing areas within the Mara River Basin has not been quantified.

The adsorption-desorption behavior of a herbicide applied to soil governs its bioefficacy and persistence (Gajbhiye and Gupta, 2001). When a herbicide enters soil environment, it is first partly adsorbed resulting in its distribution into soil adsorbed herbicide and soil solution herbicide. The herbicide present in solution is bioavailable and may be subjected to various dissipation processes like degradation, volatilization and leaching. If the adsorbed herbicide is in equilibrium with the soil solution herbicide, it will be partially desorbed when the concentration in the soil solution decreases (Gajbhiye and Gupta, 2001). Thus adsorption capacity of the soil determines the effective dose as well as persistence of a herbicide. However, adsorption is affected by the soil properties besides the herbicide characteristics.

Chlorsulfuron has been found to adsorb differently on different types of soils (Joshi *et al.*, 1985; Thirunarayan *et al.*, 1985; Mersie and Foy, 1986; Shea, 1986) though in all these soils, the adsorption patterns fit in a Freundlich adsorption isotherm. However, the adsorption pattern of chlorsulfuron in wheat growing soils of the Mara River Basin is not known.

Chlorsulfuron majorly undergoes first-order degradation kinetics in the temperate regions (Strek, 1998; Marucchini *et al.*, 1991; Vischetti and Businelli, 1992; Vicari *et al.*, 1994). The half-life ranges from 10 days to more than 365 days (Chrzanowski and Priestler, 1991; Strek, 1998) depending on the pH of the soil. Soil temperatures also influence the half-life with shorter persistence at higher temperatures (WSSA, 1983). It is unknown how soil physicochemical properties would affect the degradation kinetics of chlorsulfuron in the Mara River Basin.

The dissociation constant ($pK_a$) of chlorsulfuron is low, therefore it mainly exists in anionic form in the soil making it highly mobile (HFS, 2000). This increases the risk of its residues being carried into the subsoil and underground aquifers. One of the main breakdown pathways is through hydrolysis which decreases exponentially with increasing soil pH, while solubility in water increases with increasing pH (Beyer *et al.*, 1988). This greater solubility and decreased rate of breakdown could increase the rate of leaching and persistence of chlorsulfuron in alkaline soils. Chlorsulfuron also dissipates quickly in warm, moist soils with low pH. The number of days the soil surface remains moist determines the carryover. In a year with low rainfall, significant carryover of chlorsulfuron can occur (McMullen, 2006). Wheat farms in Mara River Basin use heavy doses of nitrogenous fertilizers which reduces the soil pH. In the recent past the rainfall within the region has been unpredictable (MoA, 2012). Chlorsulfuron degradation kinetics under these conditions has not been determined.

Field leaching experiments in other countries reported that in an alkaline sandy loam, 46% of chlorsulfuron leached beyond 50 cm nine weeks after application with 103 mm of rain, and residues in the range of 0.1- 0.4 µg $g^{-1}$ soils were detected in the top 50 cm of soil 77 weeks after application (Stork, 1995). Chlorsulfuron was also detected in 40-50 cm alkaline soil at 6 weeks after 56 mm cumulative rainfall and in the top 0-50 cm soil at 32 weeks after 134 mm rain (Noy, 1996). Moreover, increasing application rates of herbicides above recommended levels increases the risk of carryover and the leaching potential (Barrett, 2009). The downward mobility of chlorsulfuron in the soils of Mara River Basin, which may be different from the above reports due to differences in soil properties and environmental factors, has not been established.

Copper, is an essential micronutrient for normal growth and metabolism of plants (Kumar *et al.*, 2009). This element plays a role in protein and carbohydrate metabolism as well as enzymatic systems (Boorboori *et al.*, 2012). The soils within Narok County (part of Mara River Basin) have been classified as copper deficient (Mailu, 1997; Ogutu, 1998; Muya *et al.*, 2014), and therefore the use of copper based fertilizers is recommended to optimize the wheat yields (MoA, 2012). Different types of foliar copper fertilizers such as copper sulphate, copper oxychloride and copper chelate are sprayed in Narok County to correct copper deficiency in wheat (Mailu, 1997; Hassan *et al.*, 1993). The timing of foliar fertilizer is crucial for maximum grain yield and as such it is recommended that it be applied early, not later than the 6th leaf stage (Brennan, 2005). This timing could coincide with the pre-emergent application of chlorsulfuron herbicide therefore causing an interaction in Cu-soil-chlorsulfuron matrices leading to a change in the adsorption pattern of chlorsulfuron. The level of interaction between copper ions and chlorsulfuron in the soils within Narok County of the Mara River Basin is not known.

Chlorsulfuron leaching in soils is affected by the soil type, soil pH, organic matter, application rate and rainfall amounts (Flury, 1996). Chlorsulfuron leaches more in acidic soils where it is less adsorbed, in high rainfall and high application rates (Kotoula-syka *et al.*, 1993). Within the Mara River Basin chlorsulfuron is applied at varied doses from 20 to 40 g a.i ha$^{-1}$ (MoA, 2012). The effect of application rates with location of application on the downward mobility of chlorsulfuron within the Mara River Basin has not been established. Herbicide degradation in soil is influenced by both biotic and abiotic factors, which act in tandem and complement one another in the microenvironment (Singh *et al.*, 2003). Microorganisms play important roles in the degradation of sulfonylureas in soil (Andersen *et al.*, 2001). Yet, when herbicides are applied repeatedly, they may be degraded more rapidly by the microorganisms, making weed control less effective after repeated applications (Arbeli and Fuentes, 2007). Chlorsulfuron has been used in wheat farms within the Mara River Basin for more than five years (MoA, 2012) and this may have resulted in adaptation of the microorganisms to it as energy source. The extent of microbial degradation and effect of repeated applications of chlorsulfuron within the Mara River Basin is yet to be established.

## 1.2 Statement of the problem

Chlorsulfuron, a sulfonylurea is one of the most popular herbicides used in wheat farming within the Mara River Basin. Although, it is assumed to be environmentally safe due to its low application rates, relatively shorter half-life in the environment and low mammalian toxicity, several studies have raised concerns over its possible environmental contamination. The soil dissipation of chlorsulfuron is highly influenced by the soil physico chemical parameters which may vary from one location of wheat production to another. Adsorption and desorption affect the bioavailability of the herbicide for weed control and its leaching potential. High adsorption lowers the amount available for weed control and increases the chances of the herbicide damaging successive crops whereas low sorption increases the risks of the herbicide leaching. The adsorption - desorption pattern of chlorsulfuron in soils within the Mara River Basin has not been established. Application of copper foliar fertilizers coincides with the application of the pre-emergent chlorsulfuron. This may lead to interaction between the copper cations and the herbicide, changing its adsorption pattern and reduce its bioavailability for weed action. It is still unknown if there is any interaction between chlorsulfuron and the copper ions in the wheat growing soils of Mara River Basin. Chlorsulfuron has a low pKa, therefore it exists mainly in anionic form in the soil making it mobile. This increases the risk of its residues being carried into the subsoil and underground aquifers. The variation in leaching chlorsulfuron in the soils within the Mara River Basin with location and rate of application is not known. Chlorsulfuron degradation in soil is influenced by biotic, abiotic and environmental factors. Microorganisms play important roles in its degradation, yet when chlorsulfuron is applied repeatedly; it may be degraded more rapidly by the microorganisms, making weed control less effective. The influence of biotic degradation and repeated application on chlorsulfuron degradation in soils within Mara River Basin is still unknown. Moreover, its field dissipation pattern and half-life within the Basin is not known.

## 1.3 General objective

To investigate the fate of chlorsulfuron in selected soils within the Mara River Basin of Kenya.

## 1.4 Specific objectives

1. To establish the equilibrium adsorption and desorption of chlorsulfuron in selected soils within the Mara River Basin.

2. To establish the effect of copper ions on equilibrium adsorption and desorption of chlorsulfuron in selected soils within the Mara River Basin.
3. To assess the effect of location and rate of application on leaching patterns of chlorsulfuron herbicide in selected soils within the Mara River Basin.
4. To determine the influence of microbial activity and repeated application on the degradation of chlorsulfuron in selected soils within the Mara River Basin.
5. To determine the field dissipation pattern and soil half-life of chlorsulfuron in selected soils within the Mara River Basin.

### 1.5 Null Hypothesis, Ho

1. Chlorsulfuron will not adsorb on the selected soils found within the Mara River Basin.
2. Copper ions do not have any effect on the adsorption-desorption pattern of chlorsulfuron
3. Chlorsulfuron leaching in the selected soils found within the Mara River Basin is not affected by location and rate of application.
4. Microbial activity and history of application do not influence the degradation of chlorsulfuron in the selected soils within the Mara River Basin.
5. Chlorsulfuron does not dissipate in the selected soils within the Mara River Basin.

   If the null hypothesis is rejected the alternative $H_1$ shall be accepted.

### 1.6 Significance of the study

Assessment of the effect of physicochemical parameters on the dissipation pattern of chlorsulfuron in the wheat growing soils has increased understanding on its' fate within the Mara River Basin by availing baseline data. This will help the farmers and extension staffs make informed decisions on the safe use of this herbicide in their regions. Investigation of the degradation kinetics, adsorption - desorption patterns and leaching potential of chlorsulfuron will provide data on the persistence and efficacy of chlorsulfuron in the soils within the Mara River Basin. This will lead to development of policies on the safe use of the herbicide in the region with the aim of protecting the surface and ground waters therefore providing safe environment for the aquatic animals besides provision of safe water for domestic use. This study would provide a recommendation on the herbicide use by the farmers to minimize crop losses, protect the environment and safeguard Mara River water quality.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Agricultural activities within the Mara River Basin

The Mara River Basin may be facing serious environmental and water resources problems. This is primarily due to the intensive settlement and cultivation that has been witnessed in the last decade (WWF-TPO, 2004) leading to loss of vegetation cover, widespread soil erosion, decreased water infiltration capacity, decreased soil fertility, and increased sedimentation and water pollution in the rivers (GLOWS, 2007). Intensive settlement has been accompanied by extensive crop cultivation. The major crops grown in the basin include maize, wheat, tea, beans and potatoes (WWF-TPO, 2004), for which agrochemicals are applied to increase the production and reduce weeds. Wheat is the second most important cereal crop after maize in Kenya and the highest economic earner. Within the Mara River Basin, wheat is mainly grown in Narok North and South sub counties, mostly on large scale basis (MoA, 2012).

Lots of agrochemicals are used in wheat farming. For large scale farming, herbicides are used on the farms for clearing the fields before land preparation. Pre and post emergent herbicides are sprayed to the farms to control weeds . The herbicides used in wheat production include; 2,4-Dichlorophenoxyacetic acid, flufenacet, glyphosate, flurosalam, bromoxynil, fenoxaprop-p-ethyl, chlorsulfuron, clodinafop-propargyl and gylcine among others (MoA, 2012). Some of these agrochemicals and their metabolites have been detected in the surface waters within the Basin (GLOWS, 2007), therefore posing a threat to the habitat. From a reconnaissance survey carried out, chlorsulfuron was one of the most widely used herbicides within the basin. It is a selective herbicide controlling both broadleaf and grass weeds (Beyer *et al.*, 1988). Despite widespread use in the wheat farms, the presence of chlorsulfuron and its metabolites in the ground and surface waters within the Mara River Basin has not been evaluated.

## 2.2 Chlorsulfuron and its application

Chlorsulfururon, 2-chloro-N-[(4-methoxy-6-methyl-l,3,5-triazin-2-yl) aminocarbonyl] benzenesulfonamide (Figure 1) is a sulfonylurea herbicide widely used in wheat crops for its good agronomic characteristics and low toxicity to mammals (HFS, 2000). It is selective and effective in controlling both pre and post emergence at field doses as low as 10-40 g active ingredient (a.i.) ha$^{-1}$ against a wide spectrum of broad-leaved weeds (Beyer *et al.*, 1988). The herbicide is a member of the triazinylsulfonyl urea group of herbicides. It is absorbed by the foliage and roots where it is rapidly translocated. Its activity is due to a very specific mechanism of inhibition of protein synthesis (Blair and Martin, 1988). The herbicide inhibits acetolactate synthase (ALS), an enzyme in the biosynthetic pathway of the branched amino acids (valine, isoleucine and leucine) present in plant and micro-organisms, but not in animals (Blair and Martin, 1988; Brown, 1990). The physical properties of chlorsulfuron are as shown in Table 1.

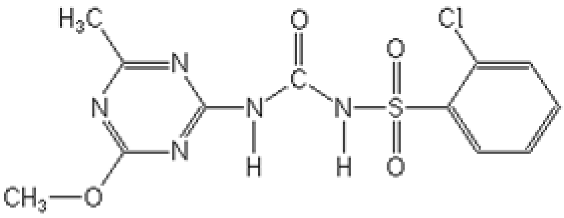

**Figure 1**: Chemical structure of chlorsulfuron (HFS, 2000)

**Table 1**: Physicochemical properties of chlorsulfuron herbicide

| | |
|---|---|
| Molecular formula | $C_{12} H_{12} Cl N_5 O_4 S$ |
| Molecular weight: | 357.8 |
| Physical state: | White crystalline solid (pure compound); the technical material has a minimum purity of 94%. |
| Odor | Odorless |
| Melting point | 174-178$^o$ C |
| Decomposition temperature | 192$^o$ C |
| Solubility in water | At 25$^o$C: 100-125 mg/l water (solution has pH 4.1), 300 mg/l (at pH 5), 27.9 g/l (at pH 7). Pure compound |
| $K_{oc}$ | 33 (13–54) |
| p$K_a$ | 3.6 |
| Log $K_{ow}$ | 2.13 (pH 5), 0.10(pH 7), 0.04 at pH9 (25 $^o$C) |
| Vapour pressure | 3x10$^{-9}$ Pa (25 $^o$C) |

Source: (HFS, 2000)

Within the Mara River Basin, chlorsulfuron whose trade name is Glean is used as both pre and post emergence herbicide (MoA, 2012). The fate of chlorsulfuron from the wheat growing soils within the Mara River Basin is unknown.

### 2.3 Soil physico chemical parameters affecting degradation of chlorsulfuron

Chlorsulfuron degradation in the environment is affected by several physico-chemical parameters (Thirunarayan *et al.,* 1985; Spark and Swift, 2002). These parameters include, soil type, soil moisture content, medium pH, soil organic matter, microbial content and temperature (Thirunarayan *et al.,* 1985; Spark and Swift, 2002; Walker and Brown, 1983). The parameters vary from one region to another. Wheat in the Mara River Basin is grown in two major different regions; the upper highlands and the lower highlands. The regions vary in their altitudes (with the upper highlands having altitudes rising above 2000 m while the lowlands ranging from 1000-1500m), the annual precipitations(averages of 500mm in the lowlands to 1800mm in the highlands), types of soils (classified as andisols, cambisols or phaezeoms) and other environmental conditions also differ within the Basin (Jaetzold *et al.,* 2010). It has not been quantified if these two regions vary in physico-chemical parameters.

### 2.3.1 Soil types

Soil type is defined by its texture, organic matter content, organic carbon levels, clay, sand and silt and its cation exchange capacity. The sorption interactions of pesticides in soil may involve either the mineral or organic components or both (Delle, 2001). In soils that have higher organic matter levels (> 5%), pesticide adsorption depends on organic matter content, rather than the nature of organic matter (Delle, 2001; Spark and Swift, 2002; Arienzo and Buondonno, 1993). In soils with low organic matter contents, the adsorption of pesticides often depends on active components of the inorganic fraction (Welhouse and Bleam, 1992), which is predominantly the clay fraction. An increase in clay content increases adsorption of a pesticide (Barriuso *et al.,* 1992; Welhouse and Bleam, 1992; Baskaran *et al.,* 1996). Chlorsulfuron degraded faster in soils with high sand content and low organic carbon content and slowly in soils with higher organic content and clay content (Sarmah *et al.,* 1999). The slow degradation of chlorsulfuron in the clay soil with high organic content was due to adsorption of the herbicide onto the clay and organic matter (Wu *et al.,* 2011). Upon increasing the organic matter in the soils with peat and manure, the quantity of adsorbed herbicide increased resulting in a decrease in the availability of the herbicide (Kolupaeva and Gorbatov, 2001). The sorption of tribenuron-methyl, chlorsulfuron and imezamethabenz-methyl positively correlated with clay and organic matter (OM) content (Alvarez-Benedi *et al.,* 1998). The wheat growing soils within the Mara River Basin have been classified as clay loam to clay ando-luvic phaeozems and sandy loam to clay loam mollic andosols. However, the organic matter content, cation exchange capacity and organic carbon content have not been established. The rate at which chlorsulfuron would adsorb and desorb on the Mara River

Basin wheat plantation soils are not known since these soil physicochemical parameters have not been studied.

### 2.3.2 Soil moisture content

Degradation rate of chlorsulfuron increased with increase in moisture content (Thirunarayan *et al.*, 1985; Walker and Brown, 1983). Since major route of chlorsulfuron degradation in soil is non-enzymatic hydrolysis, increasing soil moisture content can increase degradation. Increasing the moisture content of the soil from 25% to 50% of its field capacity increased degradation rate of chlorsulfuron by 46% due to favored chemical hydrolysis and microbial degradation (Thirunarayan *et al.*, 1985). Chlorsulfuron degrades quickly in warm, moist soils with low pH. The number of days the soil surface is wet determines the degradation rate of chlorsulfuron. This can mean that in a year with low rainfall, the degradation rate of chlorsulfuron will be slow therefore significant carryover of chlorsulfuron can occur (McMullen, 2006). Unpredictable rainfall is one of the reasons for decreased wheat production in the basin (MoA, 2012). The effect of moisture content on the degradation of chlorsulfuron in the wheat growing soils is unknown.

### 2.3.3 Temperature

An increase in soil temperature from 25$^{o}$C to 40$^{o}$C increased chlorsulfuron degradation therefore reducing the half-life by seven fold depending on the water content (Ravelli *et al.*, 1997). A two to threefold decrease in half-life was brought about by an increase in temperature from 20 to 30$^{o}$ C at moisture content of 12% (Walker and Brown, 1983). Soil temperature influences length of half-life with shorter persistence at higher temperatures (WSSA, 1983). In the wheat growing regions within the Mara River Basin, temperatures vary from 10.1$^{o}$ C to 15.0$^{o}$C in the high altitude region and from 15.1$^{o}$C to 17.6$^{o}$C in the lower altitude areas (Jaetzold *et al.*, 2010). The differences in the degradation rates of chlorsulfuron in the two regions have not been established.

## 2.4 Sorption and desorption of chlorsulfuron in soil

The sorption on the solid matrix of the soil is one of the most important processes which control transport, persistence, bioavailability and degradation of organic pesticides on soil. Many theories and models have been presented to describe the different types of sorption isotherms (Czimkota *et al.,* 2002). The most commonly used adsorption isotherm equations for organic contaminants on soil are the Langmuir (Zanini *et al.,* 2006) and the Freundlich isotherms (Stumm, 1992).

A sorption or desorption isotherm shows the relationship between the herbicides concentration in the soil solution (C) and the amount adsorbed (q) at constant temperature at equilibrium (Stumm, 1992). Isotherms are only descriptions of macroscopic data and do not definitively prove a reaction mechanism (Sparks, 2003). Mechanisms must be gleaned from molecular investigations, for instance, the use of spectroscopic techniques. However, the fit of experimental data with theoretical and/or empirical equations for adsorption isotherms is useful in determining some parameters that provide information on the strength of soil-herbicide interaction, which explains the bioavailability of the herbicide in a particular soil. There are several adsorption-desorption isotherm equations applied to soils and sediments (Haws *et al.,* 2006; Hinz, 2001).

The desorption processes of herbicides in soil is important since it determines the release rate and the potential mobility of the herbicides in the soils (Liu *et al.*, 2010). Herbicides with slower desorption rates may possess higher risk to successive crops. Like adsorption, desorption of chlorsulfuron in soils is affected by the soil physicochemical parameters. In acidic soils desorption of chlorsulfuron is low as compared to neutral soils. In sandy loam soil of pH 4.2 desorption of chlorsulfuron was found to be low with an average of 14.2% while in a neutral sandy loam of pH 7.0 31.2% was desorbed (Mersie and Foy, 1985). Similarly soils with high organic carbon content have slow desorption processes due to the binding of chlorsulfuron unto the organic carbon (Alvarez-Benedi *et al.*, 1998).

Desorption hysteresis is the evident asymmetry of sorption-desorption processes (Pignatello and Xing, 1995). There are several reasons resulting in the observed desorption hysteresis phenomena. These reasons were probably attributed to steric hindering of adsorbents and chemosorption. First, for chemosorption with specific interaction, ligand interaction or covalent attraction is prevailing. Second, with respect to the adsorption-desorption of organic compounds on solid matrixes such as soils, sediments and charcoal, desorption hysteresis is attributed to irreversible pore deformation of the adsorbent by the sorbate and the formation of meta-stable states of sorbate in the fixed mesopores (Kan *et al.*, 1994; Huang and Weber, 1997; Braida *et al.*, 2002).Besides, the desorption hysteresis can be as a result of the artifacts, such as non-equilibrium adsorption, or loss of sorbate due to solubility enhancement and solution replacement (Huang *et al.*, 1998; Pan *et al.*, 2008).

Isotherms for adsorption and desorption of organic solutes are divided into four main classes, according to the nature of slope of the initial portion of the curve, and thereafter into sub-groups (Aranovich and Donohue, 1998). The main classes are: (i) *S*curves, indicative of vertical orientation of adsorbed molecules at the surface. (ii) *L* Curves, the normal or “Langmuir” isotherms, usually indicative of molecules adsorbed flat on the surface, or, sometimes, of vertically oriented adsorbed ions with particularly strong intermolecular attraction. (iii) *H* Curves (high affinity) (commencing at a positive value on the “concentration in solid” axis), are often given by solutes adsorbed as ionic micelles, and by high-affinity ions exchanging with low affinity ions. (iv) C Curves (“constant partition”), linear curves, are given by solutes which penetrate into the solid more readily than does the solvent (Aranovich and Donohue, 1998). The most common models that describe non-linear sorption-desorption isotherms are the Freundlich, Langmuir , Temkin and Brunauer, Emmett and Teller (BET) equations. The equilibrium adsorption-desorption isotherms of chlorsulfuron in the selected soils has not been determined in the Mara River Basin.

### 2.4.1 Freundlich model

The Freundlich equation is perhaps the most widely applied model in environmental soil chemistry to describe nonlinear sorption-desorption behaviour (Valverde-García *et al.*, 1998; Kibe *et al.*, 2000). It is an empirical sorption model (Stumm, 1992; Sparks, 1986) and it can be written as:

$$q = K_f C^{1/n} \qquad (1)$$

Where, q is the amount adsorbed, $K_f$ is the distribution coefficient, n is a correction factor and C is the concentration of the solute. When C is equal to unity the distribution coefficient gives the amount adsorbed at that concentration.

The Freundlich isotherm equation (1) can be linearized to give

$$\log q = \log K_f + \frac{1}{n} \log C \qquad (2)$$

Various studies have demonstrated that sulfonylurea herbicides adsorption and desorption on soil best fit in a Freundlich model (Delgado and Pena, 1997; Norhayati and Jee-Singh, 2007; Ismail and Ooi, 2012; Wu *et al.*, 2011). However, all these studies have been carried out in the temperate regions and on soils that have different physicochemical properties from those found in Kenya, especially Mara River Basin.

### 2.4.2 Langmuir model

This model has been employed in many fields to describe sorption-desorption on colloidal surfaces (Zanini *et al.*, 2006; Xi *et al.*, 2010). The Langmuir sorption equation can be written as:

$$Q_e = \frac{Q_m K_L C_e}{1+K_L C_e} \qquad (3)$$

Where $Q_e$ is the equilibrium adsorption capacity (mg/g), $C_e$ is the adsorbate concentration at equilibrium (mg/L), $K_L$is a constant related to the binding strength (L/mg) and $Q_m$ is the maximum sorption capacity (mg/g).

This equation (3) can be rearranged to be

$$\frac{C_e}{Q_e} = \frac{1}{Q_m} C_e + \frac{1}{K_L Q_m} \qquad (4)$$

A plot of $\frac{C_e}{Q_e}$versus $C_e$ gives a straight line with a slope of $1/Q_m$ and an intercept of $1/K_LQ_m$. This equation had several assumptions made in its development (Liu, 2006). Most of these assumptions were not valid for the heterogeneous surface found in soils. However, many researchers used this model to describe adsorption on soils (Gimsing *et al.*, 2007; Ketelsen and Meyer-Windel, 1999). As with $K_f$above, $K_L$is useful for comparative purposes but they do not provide information on reaction mechanisms. Nevertheless, adsorption-desorption of sulfonylurea herbicides on soil has also been modeled using the Langmuir isotherm (Blacklow and Pheloung, 1991; Metzger *et al.*, 1996).

### 2.4.3 Temkin Isotherm

Temkin isotherm model is given by the following equation

$$X = a + b \ln C \qquad (5)$$

Where, X is the amount of adsorbate adsorbed per gram weight of adsorbent (mg/g), a and b are constants related tosorption capacity and intensity of sorption respectively, C is the equilibrium concentration of solution (mg/L) (Abdel-Ghani *et al.*, 2008).

### 2.4.4 BET Isotherm

The BET isotherm was developed by Brunauer, Emmett and Teller as an extension of Langmuir isotherm which assumes that first layer of molecules adheres to the surface with energy comparable to heat of adsorption for monolayer sorption and subsequent layers have equal energies (Sharma *et al.*, 2009). In linearized form the BET model is expressed as

$$\frac{C_f}{(C_f - C_s)q} = \frac{1}{Bq_{max}} - \left(\frac{B-1}{Bq_{max}}\right)\left(\frac{C_f}{C_s}\right) \qquad (6)$$

Where $C_s$ is the saturation concentration (mg/L) of the solute, $C_f$ is the solute equilibrium concentration. B and $q_{max}$ are constants

Most researchers fit the experimental data with both Langmuir and Freundlich equations to compare methodological approaches (Campbell and Davies, 1995; Martínez-Villegas *et al.*, 2004). The exact adsorption isotherm of chlorsulfuron in the wheat growing soils of Mara River Basin has not been established.

## 2.5 Interaction of copper with chlorsulfuron

Copper is a common ingredient present in a range of fungicides and is often present in some animal manures and biosolids that are applied to agricultural soils as fertilizers. Elevated and increasing levels of copper in agricultural soils are of concern both in Kenya (Dharani *et al.*, 2007) and internationally (Bunemann *et al.*, 2006; Gaw *et al.*, 2006). Copper based fungicides are among the most toxic pesticides to soil microorganisms (Bunemann *et al.*, 2006) and have been widely used in agriculture over a number of decades. Although pesticides are commonly applied concurrently or used on land that has previously received applications of copper fungicides , the majority of reports on pesticide persistence in soil have not considered potential effects of copper and other metal ions on formulated pesticides. Only a limited number of studies have investigated the impacts of trace elements co-

contamination on the degradation of pesticides in agricultural soils. Copper has been reported to inhibit microbial degradation of several pesticides including coumaphos (Jindal *et al.*, 2000), thiobencarb (Gunasekara *et al.*, 2005), DDT (Gaw *et al.*, 2006), cypermethrin and cyhalothrin (Liu *et al.*, 2007) and glyphosate (Kim *et al.*, 2011). Conversely, the presence of heavy metals in the soil enhances degradation of glyphosate (Kools *et al.*, 2005). Soil microorganisms can be adversely decreased by trace element contamination. These effects include inhibition of enzyme activities, reduced biomass, and alteration of the microbial community.

Studies have shown that copper interacts with organic herbicides in soils (Ren *et al.*, 2011). These interactions of Cu with organic pesticides can also influence their toxicity. Cu (II) increased the sorption of metsulfuron-methyl (Me) to peat and soil by either decreasing the negative charge on the surface of peat and soil or through the formation of Cu-Me complexes (Pei *et al.*, 2008). Application of chlorsulfuron depressed the concentration of Cu in wheat plants receiving either inadequate or adequate Cu. In plants with inadequate Cu supply, chlorsulfuron increased the severity of Cu deficiency (Tang and Robson, 2000). Similarly, a study on the joint effects of chlorimuron-ethyl and Cu on wheat at biochemical levels found that the addition of Cu alleviated the inhibitory effect of chlorimuron-ethyl on the biosynthesis of chlorophyll. It also alleviated the inhibitory effect of the activity of superoxide dismutase but aggravated that on the content of soluble protein (Wang *et al.*, 2009). The presence of Cu (II) also increased the sorption of chlorimuron on two soils, which can be attributed to decreased pH in the equilibrium solution and Cu-bridging (Ren *et al.*, 2011). In addition, chlorimuron-ethyl suppressed Cu (II) desorption from the two soils, decreasing the mobility and bioavailability of Cu (II). Increased Cu concentrations reduced the degradation rate and transport of diuron in soil profile (Jacobson *et al.*, 2005). The changes were probably caused by different composition of microbiological populations, which could lead to subsequent changes in activities involved in their degradation. However, no studies have explained the influence of Cu (II) on the adsorption and desorption of chlorsulfuron in wheat growing soils within Mara River Basin soils.

## 2.6 Leaching of chlorsulfuron in soils

Sulfonylurea herbicides exist in anionic form in agricultural soils with pH greater than 6.0 and are weakly adsorbed (Walker *et al.,* 1989; Sarmah *et al.,* 1998). Herbicides have high potential to leach under conditions of high rainfall and consequently may reach the zones of low organic matter and little biological activity (McMullen, 2006). Due to the slower rate of degradation underneath, leaching can result in their longer persistence in the subsoils and may increase the risk of phytotoxicity to succeeding crops. For instance, from a laboratory incubation study, the rate of chlorsulfuron degradation was 4 times slower at 30-40 cm depth than in the topsoil (0-10 cm) ( Sarmah *et al.*, 1998). This was attributed to the low organic matter content and low biological activity of microorganisms. The pH of the soils used in the study ranged from 8.7 in the 0-10 cm layer to 9.4 at 30-40 cm depth. Chlorsulfuron had a potential for leaching in coarse-textured soils (neutral to slightly acidic) with low organic matter content in a laboratory study, using intact soil cores (Beckie and McKercher, 1990). The soil type used had a pH range of 5.5-7.0 and organic matter content of 1.8-5.1 and 1.0-2.1% in the 0-10 and 10-30 cm depths, respectively. About 46% of the applied chlorsulfuron leached below 50 cm at nine weeks after application with 103 mm of rain in a gradationally textured alkaline sandy loam soil (pH 8.5-9.5) (Stork, 1995). Although there were differences in the analytical techniques in these studies, all showed that sulfonylureas moved to significant depths, and their greater mobility in alkaline soils than in acidic or neutral soils was apparent. Moreover, the application rate of chlorsulfuron influences the amounts that leach. Chlorsulfuron leaching was found to increase with increase in application rates (Kotoula-syka *et al.*, 1993). The extent of chlorsulfuron leaching in the wheat growing soils of Mara River Basin is still unknown.

## 2.7 Biodegradation of chlorsulfuron in soil

The rate of microbial degradation of pesticides in soil is a function of the following: availability of chemicals to microorganisms or enzyme systems, which can degrade them; types and quantity of these microorganisms or enzyme systems; and, their activity level (Frehse and Anderson, 1983). Soil moisture, temperature, aeration, pH and nutrient status can influence both the availability of a pesticide to soil microbes and the ability of soil microbes to degrade a pesticide (Monteiro and Boxall, 2009). If a pesticide is readily degraded by soil microbes (that is it is a good substrate for enzymatic activity) and if it is available to microorganisms and if the organisms are metabolically active, then in general, a high correlation between microbial biomass and degradation rates can be expected (Sagan, 1999). If only a small proportion of the biomass can attack the compound (that is it is a poor substrate for enzymatic activity) its degradation and dissipation will be more dependent on external physical forces such as light, and the chemical and physical properties of both the chemical and the soil (Sagan, 1999).

Sulfonylurea herbicides are degraded both chemically and biologically, with both types of degradation being promoted by high soil temperatures and moisture (Thirunarayan *et al.*, 1985, Beyer *et al.*, 1988, Smith and Aubin, 1992). Availability of a pesticide for degradation is determined by the extent of its adsorption and its ease of desorption on soil solids (Briggs, 1976; Anderson, 1981; Burkhard and Guth, 1981; Soulas, 1982).

The quantity of herbicide available for degradation is approximately equivalent to the amount dissolved in the soil solution. Increasing the ratio of water to soil solids increases the total amount of herbicide in solution resulting in an increase in both the metabolic rate and quantity of microflora in the soi1 (Anderson, 1981).

Hamaker (1972) suggested that an increase in organic matter content may increase the rate of microbial degradation in mineral soils up to a limiting value above which the rate of loss would be retarded. Chlorsulfuron dissipated more rapidly in non-sterile soils than in sterilized soils (Joshi *et al.*, 1985, Oppong and Sagar, 1992) with differences between degradation in sterile and non-sterile soil being more pronounced in higher soil pH where chemical hydrolysis is often limited. In alkaline soils of pH 7.1 - 9.4 and moisture content representing 70% field capacity (-33kPa), disappearance of chlorsulfuron was faster in non-sterile soils than in sterile soils demonstrating the importance of microbes in the breakdown process (Sarmah *et al.*,1999). In two non-sterile soils, chlorsulfuron had half-lives of 5 and 15 days at 30ºC compared to 21 and 28 days in sterile controls (HSDB, 2003); Biodegradation accounted for 79% of the total initial degradation in a silt loam soil (pH 5.9, organic matter 5%), 50% in a sandy loam soil (pH 6.5, organic matter 1%), and 91% in a silt loam soil (pH 8.0, organic matter 5%). Biodegradation accounted for only 25% of the total loss in a silty clay loam soil (pH 5.6, organic matter of 2.4%) (HSDB, 2003). The extent of biodegradation of chlorsulfuron on the wheat growing soils in the Mara River Basin could depend on several environmental factors that have not been investigated.

## 2.8 Enhanced degradation of chlorsulfuron

Biodegradation of a herbicide is said to be enhanced when it is more rapid in soils previously treated than in soils not previously treated with the herbicide (Rouchaud *et al.*, 2000).Microbial degradation of pesticides applied to soil is the principle mechanism which prevents the accumulation of these chemicals in the environment. Yet, when pesticides are degraded too rapidly, pest control may be less effective (Rouchaud *et al.*, 2000). One or more previous applications of the same pesticide or another pesticide with a similar chemical structure increase the rate of microbial degradation of pesticides in soil (Xu *et al.*, 2014; Xie *et al.*, 2004).In general, microorganisms are considered as the principal agents of xenobiotic degradation. Since soil harbours high numbers of diverse microbes species, soil applied pesticides are more likely to both induce and be adversely affected by accelerated degradation (Arbeli and Fuentes, 2007), resulting to loss of pesticide efficacy in some cases.

Accelerated biodegradation has been observed for several herbicides. Persistence of ureas, linuron (Rasmussen *et al.*, 2005), isopruturon and the acetanilide metalachlor (Liu *et al.*, 1988) and alachlor was decreased by prior applications. Diuron, chlorotoluron and simazine showed significant enhanced biodegradation when applied repeatedly on pear tree orchards (Rouchaud *et al.*, 2000). Carbetamide degradation was greatly enhanced by repeated applications (Hole *et al.*, 2001).

For sulfonylureas, chlorimuron- ethyl had its field half-life reduced from 54.6 days to 37.1 days after 3 years of repeated applications (Xu *et al.*, 2014). Similarly, repeated application of bensulfuron-methyl reduced the $DT_{50}$ from 16 days to 9 days due to adaptation of soil bacteria to the herbicides as a source of energy (Xie *et al.*, 2004). Chlorsulfuron had enhanced biodegradation when applied to three Brazilian oxisols (Ravelli *et al.*, 1997). Repeated applications of chlorsulfuron resulted in a reduction in the growth of microbiological structure, leading to a change in the quality of the soil (Boldt and Jacobsen, 1998). This change could lead to persistence of chlorsulfuron. On the contrary, repeated application of chlorsulfuron did not cause accelerated breakdown of chlorsulfuron (Weise *et* *al.*, 1998). The effect of repeated application of chlorsulfuron on its degradation in the Mara River Basin soils has not been evaluated.

### 2.9 Dissipation of Chlorsulfuron in soil

The degradation pathways of sulfonylurea herbicides include chemical hydrolysis and microbial degradation. In alkaline soils, hydrolysis of sulfonylureas is very slow (Sabadie, 1996; Sarmah *et al.*, 1998), leaving biodegradation as the dominant pathway that determines their persistence in such soils (Ravelli *et al.*, 1997; Sarmah *et al.*, 1998). Degradation of chlorsulfuron in soil is mainly through biotic and abiotic mechanisms. These mechanisms depend on the pedoclimatic conditions and differ from one soil to another (Joshi *et al.*, 1985; Walker and Brown, 1983; Busenelli *et al.*, 1985). Chemical hydrolysis of chlorsulfuron depends on time, temperature, humidity, pH, texture and type of the soil (Mersie and Foy, 1986).

Chlorsulfuron dissipated rapidly in a silt loam soil (pH 6.4) at 25 °C under aerobic laboratory conditions, with estimated time required for 50% and 90% dissipation ($DT_{50}$ and $DT_{90}$ values) of 20 and 50 days, respectively (Rapisarda *et al.*, 1982). Soil pH had a significant impact on the rate of chlorsulfuron degradation. The $DT_{50}$ of chlorsulfuron in a silty clay loam was 20 days at pH 5.6 and 70 days at pH 7.5 (Fredrickson and Shea, 1986). The degradation rate of chlorsulfuron in a flooded pond sediment/water system (pH 6.7–7.4) under anaerobic conditions was significantly reduced ($DT_{50}$>365 days) when compared to anaerobic metabolism study using a test soil of similar characteristics ($DT_{50}$ 20 days) (Chrzanowski and Priester, 1991).

Thirunarayan *et al.*, (1985) reported two sets of first order half-lives by excluding and including time-zero data. Half-lives of 38, 60, 82 and 99 days were observed in soils of pH 6.2, 7.1, 7.7 and 8.1 respectively when time zero data were included. Half-lives of 88, 105,135 and 143 days were observed beginning on day 15. A half-life of 19 days in a sandy soil (pH 7.1), 34 days in a silt clay soil (pH 7.8), 47 days in sandy loam (pH 7.9), 62 days in clay soil (pH 8.5) and 42 days in sandy soil (pH 8.7) has also been reported (Sarmah *et al.*, (1998). A field study in Italy, Vicari *et al.*, (1994) reported the half-lives of chlorsulfuron to vary from 51 days to 149 days in 4 locations (pH 7.8-8.2). In contrast, the half-life of chlorsulfuron was only 18 days in an irrigated Californian soil (pH 6.3-6.9 in the 0-90 cm depth); presumably because of an acidic pH range and a high soil temperature of 33°C at the site (Strek, 1998). Mara River Basin has varied agro-ecological zones which may have different soil types and physicochemical parameters. The degradation half-lives of chlorsulfuron in these wheat plantation soils have not been established. In addition, the dissipation of chlorsulfuron from various soils under field conditions may depend on environmental factors (rainfall, wind, solar radiation), its adsorption in soil and its degradation mechanisms.

Aqueous hydrolysis of chlorsulfuron was pH-dependent and followed pseudo-first-order degradation kinetics at 25°C, with faster hydrolysis occurring at pH 5 (half-life 24 days) than at either pH 7 or 9 (half-lives >365 days) (Strek, 1998).

Degradation occurred primarily by cleavage of the sulfonylurea bridge to form the major metabolites; chlorobenzenesulfonamide (2-chlorobenzenesulfonamide) **(2)** and triazine amine (4-methoxy-6-methyl-1,3,5-triazin-2-amine) **(3)** as shown in Figure 2.

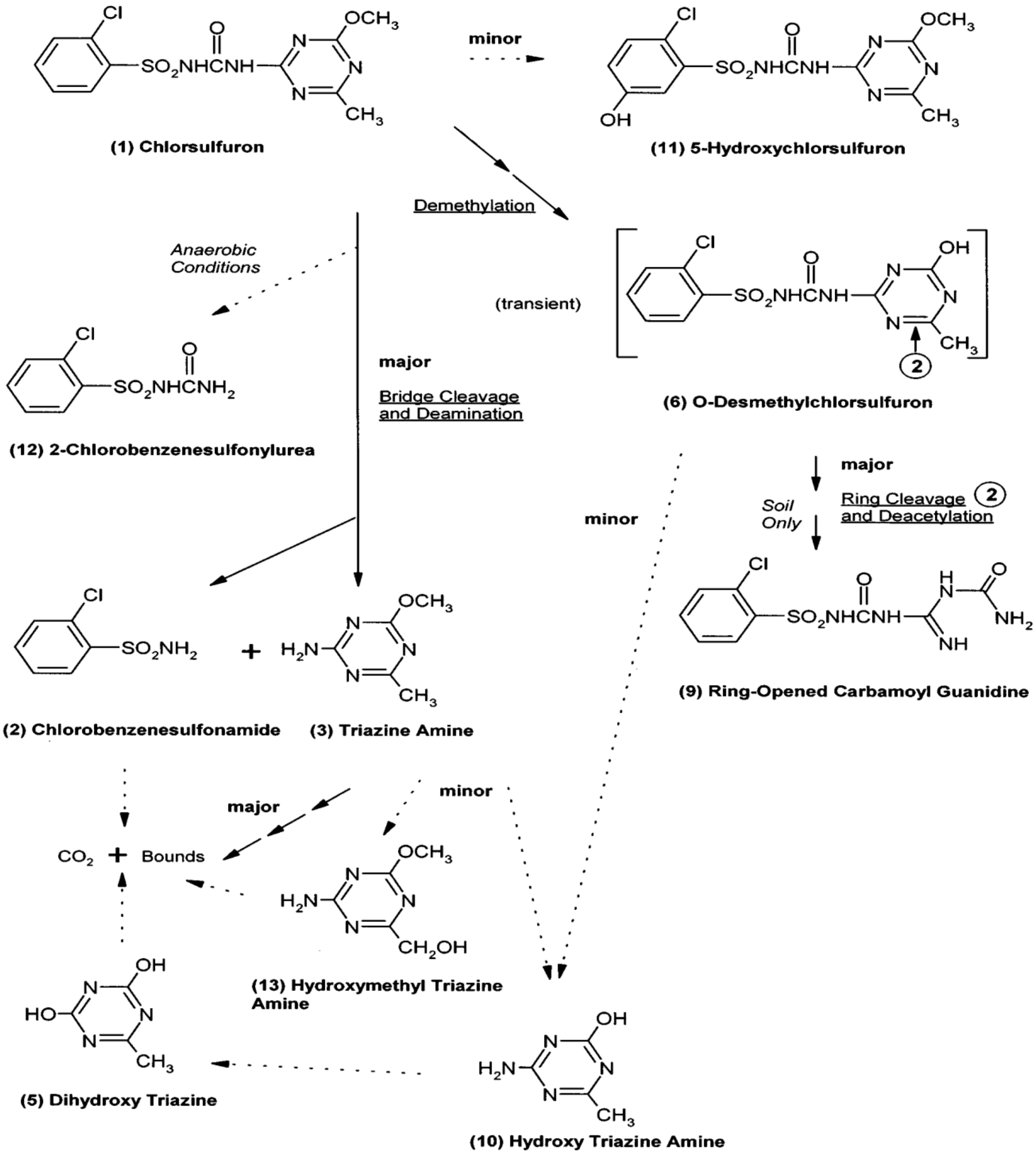

**Figure 2**: Degradation pathway of chlorsulfuron in soil (Strek, 1998)

First order kinetics for chlorsulfuron has also been reported by various researchers (Ravelli *et al.,* 1997; Hollaway *et al.,* 2006; Delle, 2001; Marucchini *et al.,* 1991; Vischetti and

Businelli, 1992; Vicari *et al.*, 1994).The degradation pathways of chlorsulfuron within the Mara River Basin has not been established.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Chemicals

Chlorsulfuron (99% purity, analytical grade) was supplied by Sigma- Aldrich (Darmstadt, Germany). The solvents methanol, HPLC water, acetonitrile and formic acid were of ultra-high performance liquid chromatography (UHPLC) grade from Biosolve, (Valkenswaard, Netherlands). $NaOH$, $NaH_2PO_4$, $Na_2HPO_4$, $CaCl_2$, $HCl$ and $NaN_3$ were of 99% purity analytical grade supplied by Sigma- Aldrich (Darmstadt, Germany. Copper (II) ions were used as analytic grade copper nitrate salt.

Chlorsulfuron (10 mg) was taken in a 10 mL volumetric flask and dissolved in methanol, and the volume was made up to the mark to obtain a stock solution containing 1,000 $\mu$g $mL^{-1}$. From this stock solution, working standards of 20, 10, 5, 2.5, 1.0, 0.5, and 0.1 $\mu gmL^{-1}$ concentration of chlorsulfuron were prepared by serial dilution.

### 3.1.2 Instruments

The instruments used were a Bandelin Sonorex RK 510H sonicator (Berlin, Germany), Edmund Bühler SM-30 orbital shaker (Hechingen, Germany), Biofuge Primo Heraeus centrifuge (Waltham, MA, USA), Mettler Toledo HB43-S moisture analyser ( Leicester, UK) LECO RC612 multiphase carbon analyser (Mönchengladbach, Germany), Biolab BAVT-101 autoclave (Ontario, Canada), Agilent 1100 Module HPLC (Palo Alto, CA, USA) equipped with a Kinetex™ 2.6 µm Biphenyl 100 Å column, Thermo Scientific iCAP6500 Inductively Coupled Plasma with Optical Emission Spectrophotometer (ICP-OES) and a RX FTIR spectrometer (Perkin-Elmer, Germany).

### 3.2 Sampling site

The wheat growing regions of the Mara River Basin are found in Narok North and South sub counties, which lie between latitudes 0°50' and 2°05' South and Longitudes 35°58' and 36°05' East (Figure 3). The districts occupy an area of over 17,128 km$^2$ and are divided into eight administrative divisions; Mau, Olokurto, Narok Central, Ololunga, Mulot, Osupuko, Mara and Loita.

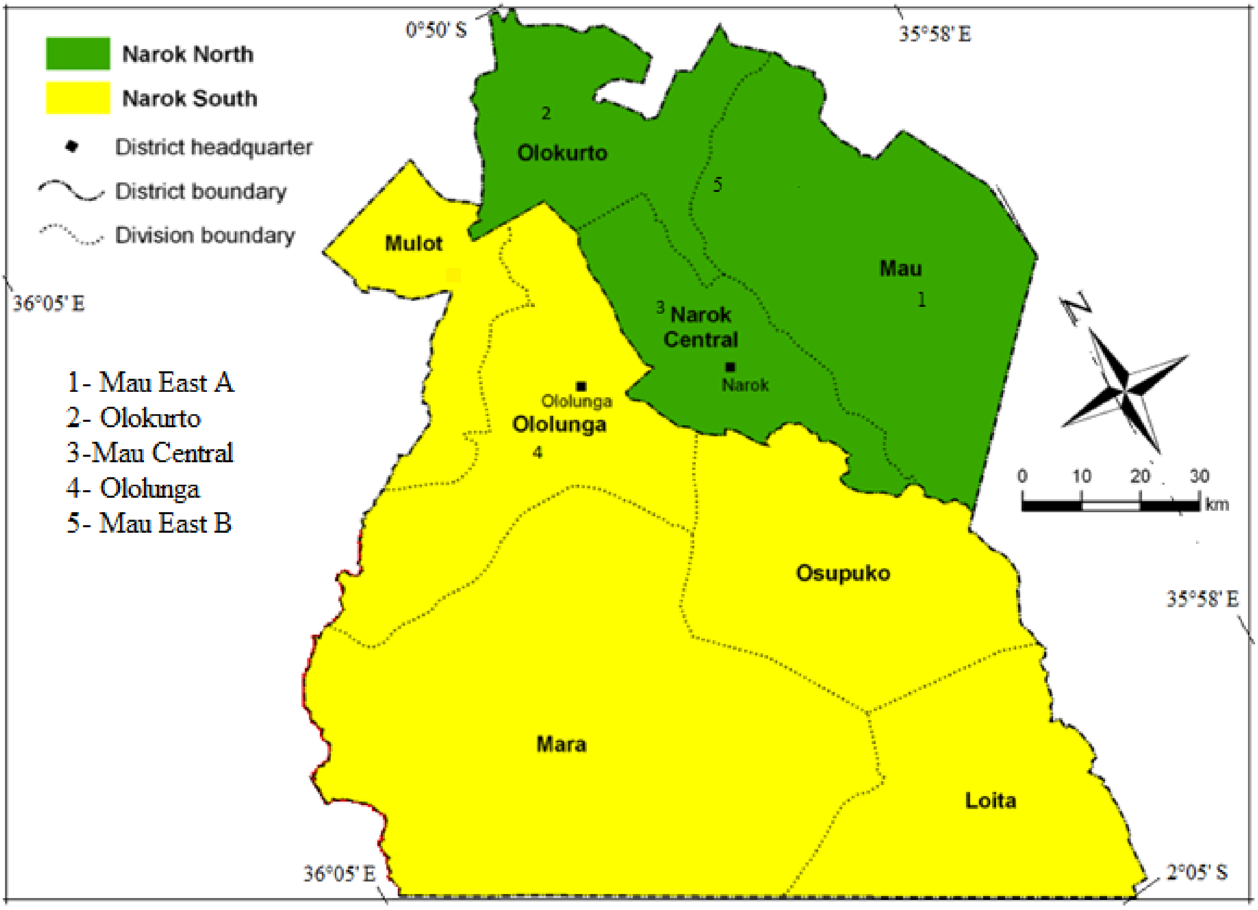

**Figure 3**: Map showing the sampling sites within Narok sub counties (Jaetzold et al., 2010)

The topography of the districts consists of highlands rising over 2000 m and lowlands of 1000 m-1,500 m above mean sea level. This varied topography determines the districts' climatic conditions. The highlands have rich volcanic soils suitable for intensive agricultural production. The lowlands on the other hand cover parts of Osupuko, Olololunga and Mara areas and the main economic activities here include cattle, goats, and sheep rearing and tourism. The highlands cover the upper Mau, Olokurto, Narok central and Mulot Divisions while the lowlands include Olololunga, Loita, Osupuko and Mara (WWW-TPO, 2004)

Soil samples were obtained from five wheat growing locations; Mau Central (latitude 1$^o$06'S, longitude 35°92'E, altitude, 1902 m amsl), Olokurto (latitude 0$^o$80'S, longitude 35°89'E, altitude 2788 m amsl), Mau East A (latitude 1$^o$04S', longitude 35°16'E, altitude 2296 m amsl), Olololunga (latitude 1$^o$01'S, longitude 35°64'E, altitude 2132 m amsl) and Mau East B (latitude 1$^o$10'S, longitude 35°15'E, altitude 1869 m amsl), within the Mara River Basin, Kenya. The soils were sampled from randomly selected points within the wheat farms by taking 0- 20 cm depth using a soil auger and kept in polyethene bags and transported to the laboratory. The soil samples were air dried and homogenized using a mortar and pestle and sieved through 2-mm sieve and thereafter stored in sealed polyethene bags in the laboratory ensuring no possibility of exposure. The soil pH was measured with a glass electrode in 1:2.5 1M $CaCl_2$ (Kalra and Maynard, 1991). The soil particle size analysis was done by bouyocous hydrometer method (Gee and Or, 2002). The organic carbon was determined by the LECO RC612 multiphase carbon analyser (Mönchengladbach, Germany), and total copper contents of the soils in a Thermo Scienitific iCAP6500 Inductively Coupled Plasma with Optical Emission Spectrophotometer (ICP-OES) after HF-$HClO_4$-HCl digestion (USEPA, 1996).

## 3.2 Soil physico-chemical parameters determination

### 3.2.1 Moisture content determination

Soil samples (10 g) in three replicates were placed in a Mettler Toledo HB43-S moisture analyser at 105$^o$C until constant weight was achieved. The percent moisture content recorded as the difference between the weight of the sample and the oven dry weight.

### 3.2.2 Determination of soil organic carbon

The soil organic carbon was determined by weighing 0.5 gram of each soil sample separately into ceramic crucibles and 2 ml of concentrated HCl was added. The crucibles with weighed soil samples were then dried in an oven at 105°C for two hours and thereafter placed in a LECO RC612 multiphase carbon analyser and the percent organic carbon recorded.

### 3.2.3 Determination of cation exchange capacity (CEC)

The cation-exchange capacities were obtained by the Gillman and Sumpter, (1986) method. Three (2.00 g) aliquots of each soil were measured into a funnel with Whatman No 1 filter paper. Slowly the soils were leached with 20 mL of 0.1 M $BaCl_2.2H_2O$ allowing each addition to soak into the soil before adding more and the pH of the slurry measured. Further 60 mL of 2 mM $BaCl_2 .2H_2O$ was used to leach each soil sample in six 10 mL portions, again allowing each addition to soak into the soil. After completing the leaching process, the filter plus soils were carefully transferred to a pre-weighed 125 mL flask and 10.0 mL of 5 mM $MgSO_4$ added. After 1 hour of occasional swirling, the conductivity of the slurry was determined and compared to conductivity of 1.5 mM $MgSO_4$ solution (it should be ~300 S or mhos). When conductivity of the sample solution was not 1.5 times that of 1.5 mM $MgSO_4$ solution, 0.100 mL increments of 0.1 M $MgSO_4$ were added until it was. The pH of the solution was also determined. If it was not within 0.1 units of the previous measure, addition of 0.05 M $H_2SO_4$ was done dropwise until pH was in appropriate range. The flask was then weighed for a final solution weight. The cation exchange capacity was then calculated as;

*a. Total solution (mLs) [assumes 1 mL weighs 1 g]*

= final tube weight (g) - tube tare weight (g) - 2 g [weight of soil used]

*b. Mg in solution, not on CEC in milliequivalents(meq)*

= total solution (mLs) x 0.003 (meq/mL) [1.5 m$M$ $MgSO_4$ has 0.003 meq/mL]

*c. Total Mg added (meq)*

= 0.1 meq [meq in 10 mLs of 5 m$M$ $MgSO_4$] + meq added in 0.1 $M$ $MgSO_4$

[mLs of 0.1 $M$ $MgSO_4$ x 0.2 meq/mL (0.1 $M$ $MgSO_4$ has 0.2 meq/mL)]

*d. CEC (meq/100g)*

= (c - b) x 50

[Total Mg added - Mg in final solution; 50 is to convert from 2 g of soil to 100 g]

(Gillman and Sumpter, 1986)

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### 3.2.4 Classification of soil texture

Soil samples texture was characterized by the Bouyocous hydrometer method (Gee and Or, 2002). Fifty grams of oven dried (110$^{o}$C) was weighed into a 600 ml beaker and 250ml of distilled water added to it. To the soil mixture, 100 mL of 50g/L sodium hexametaphosphate (HMP) was added and the mixture soaked for 12 hours. Thereafter the soil/HMP mixture was transferred to a dispersing cup and mixed for 5 min. The mixture was poured into a sedimentation cylinder and topped with distilled water up to the 1L mark. The temperature of the samples was recorded and thereafter the mixture was stroked thoroughly using a plunger .Gently a hydrometer was placed into the suspension and a reading (R) taken at 30s and 60 s. The hydrometer was rinsed and dried and further readings were taken at 90 min and 1440 min. A calibration blank was also prepared by adding 100mL HMP to 900 mL of distilled water in a sedimentation cylinder to make 1L by volume solution. The solution was mixed using a plunger and the temperature recorded. Gently a hydrometer was lowered into the cylinder and a reading ($R_L$) was recorded where the upper edge of the meniscus surrounds the stem of the hydrometer.

The concentration of soil in suspension (C) was calculated as

$$C = R - R_L$$

Where C is the concentration of the soil in suspension, g/L; R is the uncorrected hydrometer reading, g/L and RL is the hydrometer reading of blank solution in g/L.

The summation percentage (P) for each time interval was calculated as

$$P = C/C_o \text{ x } 100$$

Where C is the concentration of the soil in suspension, g/L and Co is the oven dried weight of the soil sample.

The particle diameter (X) in solution at each time interval was determined as

$$X = \theta\ (t)^{-1/2}$$

Where X is the mean particle diameter in suspension µm at time t, θ is the sedimentation parameter in µm min $^{½}$ and t is time in minutes.

For clay fraction, the percent clay ($P_{2\,\mu m}$) was determined as

$$P_{2\mu m} = m \ln (2/ X_{24}) + P_{24}$$

Where $X_{24}$ is the mean particle diameter in suspension at 24h, $P_{24}$ is the summation of percentage at 24 h, $m$ is the slope of the summation percentage curve between $X$ at 1.5h and $X$ at 24 h calculated as

$$m = (P_{1.5} - P_{24})/ \ln (X_{1.5}/ X_{24}).$$

For sand fraction, the percent sand ($P_{50\mu m}$) was calculated as

$$P_{50\mu m} = m \ln (50/ X_{60}) + P_{60}$$

Where $X_{60}$ is the mean particle diameter in suspension at 60 s, $P_{60}$ is the summation of percentage at 60 s, $m$ is the slope of the summation percentage curve between $X$ at 30 s and $X$ at 60 s calculated as

$$m = (P_{30}- P_{60})/ \ln (X_{30} / X_{60}).$$

For the silt fraction, the percent silt was calculated as

% silt = 100 - (% sand + % clay)

From the calculated percent particle sizes of clay, sand and silt the soils were classified according to "Texture AutoLookup (TAL) for Windows 4.2 (International)", a computer program for the automatic classification of soil texture (Zhang *et al.*, 2006).

### 3.2.5. Soil bulk density determination

Soil bulk densities were determined using McKenzie *et al.*, (2002) method. A metal ring (7cm i.d; 10 cm height) was gently hammered into ground till the whole 10 cm was at level with the soil surface. The ring carefully excavated and the soil transferred into a plastic bag and sealed for weight measurements.

A ceramic container was weighed and its weight ($w_1$) recorded. Carefully the soils was transferred into the ceramic container and the soils dried at 105°C in an oven for 2 h. thereafter the container and its contents were weighed and recorded as $w_2$.

The dry weight of the soils was calculated as

Dry soil weight = $w_2$-$w_1$

The volume of the soil was calculated as the volume of the ring

Ring volume ($cm^3$) = 3.14 x $r^2$ x ring height

The soils bulk densities were calculated as

$$\text{Bulk density (g cm}^{-3}\text{)} = \frac{Dry\ soil\ weig\,ht\ (g)}{Soil\ volume\ \ (cm3)}$$

The soil physicochemical parameters from the selected soils within the Mara River Basin determined are as shown in Table 2.

**Table 2**: Physicochemical parameters of wheat growing soils within Mara River Basin

| Location | pH | % MC | CEC (meq/100g) | % OC | % Sand | % Clay | % Silt | Cu (ppm) | BD g/cm$^3$ |
|---|---|---|---|---|---|---|---|---|---|
| **Mau Central** | 5.53 | 10.48 | 22.95 | 2.89 | 27.00 | 38.00 | 35.00 | 5.67 | 1.58 |
| **Mau East A** | 5.47 | 13.24 | 17.54 | 3.88 | 45.67 | 33.00 | 21.33 | 7.33 | 1.52 |
| **Olokurto** | 6.13 | 11.17 | 22.68 | 2.83 | 33.33 | 32.00 | 34.67 | 6.33 | 1.57 |
| **Olololunga** | 5.62 | 11.78 | 17.64 | 3.18 | 31.33 | 36.67 | 32.00 | 4.00 | 1.58 |
| **Mau East B** | 6.57 | 9.116 | 28.24 | 2.71 | 22.33 | 44.00 | 33.67 | 9.33 | 1.57 |
| **Mean** | 5.87 | 11.16 | 21.81 | 3.10 | 31.93 | 36.73 | 31.33 | 6.53 | 1.56 |
| **C.V %** | 3.24 | 5.88 | 2.68 | 4.37 | 10.16 | 2.53 | 9.65 | 14.25 | 5.03 |
| **LSD ($p \leq 0.05$)** | 0.12 | 1.14 | 0.99 | 0.02 | 0.56 | 1.63 | 0.53 | 0.18 | NS |

**Key:** MC-Moisture content; CEC- cation exchange capacity; OC-organic carbon ; BD-bulk density; NS- not significant

### 3.3 Laboratory adsorption-desorption studies

### 3.3.1 Selection of optimal soil/solution ratio

Two soil types and three soil solution ratios were used in this study. For ratio 1:1, 50 g of each soil type was added to 50 mL of 10 mg/L in 0.01M $CaCl_2$ chlorsulfuron solution. For ratio 1:5, 10 g of each soil type was added to 50 mL of 10 mg/ L in 0.01 M $CaCl_2$ chlorsulfuron solution. Finally for ratio 1:25, 2 g of each soil type was added to 50 mL of 10 mg / L in 0.01M $CaCl_2$ chlorsulfuron solution. A blank sample, containing the same amount of soil as the test samples, in 50mL of 0.01M $CaCl_2$ solution and a control of only 50mLof 10mg /L chlorsulfuron in 0.01M $CaCl_2$ were also prepared. These were to account for prior presence of chlorsulfuron in soils and adsorption to the walls of the container respectively. A millimeter of 0.01M $NaN_3$ was added to each of the set-ups to restrain microbial degradation. The mixtures were agitated for 4 h in an orbital shaker at 150 rpm. The soil suspensions were then separated by centrifugation at 4000 rpm for 10 min and aqueous phase filtered and analyzed using a HPLC/MS. HPLC-MS analyses were performed using an Agilent (Palo Alto, CA, USA) 1100 Module equipped with a quarternary pump, an autosampler and a column oven. Separation was achieved on a Kinetex™ 2.6 µm Biphenyl 100 Å column (Phenomenex, Germany, 100 mm × 4.6 mm) at 27°C. The mobile phases were 100% water (A) and acetonitrile-0.1% formic acid (v/v) (B).

The HPLC was calibrated by running 10µM chlorsulfuron external standard serially diluted. Recalibration was done before any batch of samples was run. Chlorsulfuron was eluted with the following gradient program: from 95% A at $t$ = 0 to 5% A at $t$ = 15 min, maintaining at 5% for 5 min and returning to 95% A up to $t$ = 26 min at a constant flow rate of 700 µL $min^{-1}$. The injection volume was 10 µL.

The HPLC was interfaced with a 4000 Q-TRAP (ABSciex, Darmstadt, Germany) fitted with aESI-Turbo Ion spray Source. Quantification of chlorsulfuron was done using the multiple reaction monitoring (MRM) mode. The transitions were m/z 358 →167, 358→ 141, 360→167 and 360 → 141. All analyses were performed in the positive mode using the following settings: de-clustering potential 50 arbitrary units; ion spray 5500 eV, curtain gas (nitrogen) 20 arbitrary units, temperature of the source 500°C, nebulizer gas (nitrogen) 50 psi and heater gas (nitrogen) 50 arbitrary units at a flow rate of 0.8 L $min^{-1}$; collision energy 20 eV and cell exit potential of 10 V. MultiQuant 2.0 was used for MS data processing.

The amount of test substance adsorbed on the soil sample was calculated as the difference between the amount of test substance initially present in solution and the amount remaining at the end of the experiment (OECD, 2000).

### 3.3.2 Sorption kinetic study

Using the best soil/solution ratio obtained above for the two soil types, three concentrations 5mg/L, 10mg/L and 20mg/L of chlorsulfuron were added to sets of samples for each soil type into 50mL polyallomer centrifuge tubes and centrifuged. The solution was then filtered through a 0.45-µm filter; the aqueous phase of each tube was recovered completely and analyzed using HPLC-MS as described above. Samples were recovered at 0, 1, 2, 4, 6, 12 and 24 hours. The percentage adsorption was calculated at each time point on the basis of the nominal initial concentration and the measured concentration at the sampling time, corrected for the value of the blank. Plots of the adsorption percentage versus time were generated in order to estimate the achievement of equilibrium time.

### 3.3.3 Adsorption-desorption equilibrium study

The batch equilibrium technique (OECD, 2000) was used to determine the soil adsorption constants of chlorsulfuron for all the soils samples. Triplicate samples of 10 g of air-dried soil and 10 mL of aqueous chlorsulfuron solutions at concentrations of 0.625, 1.25, 2.5, 5.0 and 10.0 mg/L were added to 50 mL polyallomer centrifuge tubes. $CaCl_2$ (0.01 M) was added as the background electrolyte, to keep the ionic strength of the soil solution constant and to facilitate flocculation. One milliliter $NaN_3$ (0.01 M) was used to restrain microbial degradation. Blanks of the solutions without soil were also prepared. The tubes were shaken at 150 rpm in the dark for 24 hr at (25± 2) °C using an overhead mechanical shaker. After equilibration, the suspension was centrifuged at 4000 r/min for 10 min, and then the supernatant solutions decanted and filtered through a 0.45-µm filter. Chlorsulfuron quantitation was done using HPLC-MS. The amount of herbicide adsorbed by each soil was calculated from the difference between the initial and final concentrations of chlorsulfuron in solution.

The distribution coefficient ($K_d$) was calculated as

$$K_d = \frac{C_s}{C_e}$$

Where $C_s$ is the amount sorbed in g $kg^{-1}$ and Ce is the equilibrium concentration of the solution in g $L^{-1}$

The normalized sorption coefficient ($K_{oc}$) was calculated as

$$K_{OC} = \frac{K_f}{OC} * 100$$

Where $K_f$ is the Freundlich adsorption coefficient and $OC$ is the organic carbon content of the soil.

Desorption experiments were performed immediately after decanting the solution and adding same volume of 0.01 M $CaCl_2$ solution into the tubes. The soils were re-suspended by shaking the tubes at 150 rpm for a further 24 h. After equilibration, the suspension was centrifuged at 4000 r/min for 10 min, and then the supernatant solutions decanted, filtered through a 0.45-µm filter and chlorsulfuron present in the samples quantified by HPLC – MS/MS as described above. The process was repeated three times. The amount of chlorsulfuron desorbed was calculated as amount of pesticide released into the solution during the process and expressed as a percentage of the amount adsorbed. Desorption isotherms were obtained by plotting the amount of chemical desorbed per unit weight of soil at equilibrium versus chlorsulfuron concentration at equilibrium.

Hysteresis coefficient, H, was calculated for the adsorption–desorption isotherms according to the following equation

$$H = \frac{n_{des}}{n_{ads}}$$

where $n_{des}$ and $n_{ads}$ are the Freundlich constants obtained for desorption and adsorption isotherms, respectively.

### 3.4. Effect of copper ions on the adsorption and desorption of chlorsulfuron

The batch equilibrium sorption experiments (OECD, 2000) were carried out in triplicate by mixing 2g of soil samples with 10 mL background solution containing various concentrations of chlorsulfuron (0.625–15mg $L^{-1}$) and $Cu^{2+}$ (0, 50 and 100 mg $L^{-1}$) in 50-mL glass centrifuge tubes sealed with Teflon-lined screw caps. The background solution was 0.01 M $CaCl_2$ solution containing100 mg $L^{-1}$ $NaN_3$ as a biocide. The initial pH of the solutions were recorded and the tubes shaken in an overhead shaker at 25°C for 24 hours in the dark. After equilibration, the tubes were centrifuged at 4000 r/min for 10 minutes. The pH of the equilibrium solution was recorded and a 5-mL aliquot of solution was removed and filtered through 0.45-µm pore-size cellulosic membrane filters, transferred into vials and stored at 4°C before analysis. The concentration of chlorsulfuron in the supernatant was determined by HPLC-MS as described earlier. Control samples constituting 50 and 100 mg/ L solutions of copper ions and chlorsulfuron (0.625-15 mg/L) were also treated in the same way to account for sorption of chlorsulfuron unto the walls of the container. The amount of chlorsulfuron sorbed to the soils was calculated from the difference between the initial and equilibrium concentrations in solutions per unit weight of oven dry soils.

The desorption experiments were conducted by adding an extra amount (5 mL) of freshly prepared background solutions to the soils remaining in the centrifuge tubes and maintaining the total amount of the solution exactly at 10 mL. The soil samples in the centrifuge tubes were re-suspended using a vortex mixer and then the suspensions were agitated in an overhead mechanical shaker at 150 rpm for 24 hours to attain a desorption equilibrium. Subsequently, the desorbed solutions were centrifuged, decanted and analyzed as described for the sorption experiment.

### 3.4.1 Fourier Transform Infrared (FTIR) Analysis

In order to identify the adsorption sites of chlorsulfuron and $Cu^{2+}$ on the soil matrix, an FTIR spectral study was performed (Ren *et al.*, 2011). To 100 mg of the soils, 1 ml of 100 mg $l^{-1}$ initial concentration of both chlorsulfuron and $Cu^{2+}$ was added. Controls of 100mg $l^{-1}$ chlorsulfuron mixed with the soils and 100 mg $L^{-1}$ $Cu^{2+}$ solutions mixed with soils respectively, and a blank of the soils with 0.01 M $CaCl_2$ solutions were also prepared. The suspensions were equilibrated for 16 hours, and the aqueous solutions decanted. The sorption process was repeated three times and after the third process the aqueous suspensions were filtered through a 0.45µm cellulosic membrane. The soil samples on the filter were freeze-dried and the soil removed from the filter by running a knife edge over it. FTIR spectra were recorded on an RX FTIR spectrometer (Perkin-Elmer, Germany) equipped with a deuterated L-alanine doped triglycine sulfate detector and a KBr beam splitter in the range of 700- 4000 $cm^{-1}$ with a resolution of 2.0 $cm^{-1}$ from a collection of 500 scans per sample.

## 3.5 Effect of location and application rate on leaching of chlorsulfuron in soils

### 3.5.1 Experimental design

Column leaching studies were laid down as Randomized Complete Block Design and conducted according to the OECD, (2004) method at ambient temperature. Triplicate samples of 580g each soil types were each packed in a 45 cm glass column with an internal diameter of 4 cm, up to a height of approximately 30 cm. The soils were filled in the columns manually in three batches and after each batch the soils were tamped down using a plunger at 1 bar up to 30cm to ensure uniform density. The columns were shielded from light by wrapping with aluminium foils. The columns were saturated with artificial rain (drops of 0.01 M $CaCl_2$) overnight in order to compact and stabilize the soils. Amounts representing rates of

30 g $ha^{-1}$ and 60 g $ha^{-1}$ of chlorsulfuron respectively were applied evenly over the surface of the soil columns, and the soil surface was covered with round filter papers (Whatman No. 1) to distribute the artificial rain evenly over the entire surface. Rain event of 160 mm (equivalent to 200 mL of 0.01 M $CaCl_2$) was simulated at the rate of 0.05mL $min^{-1}$. The intensity of the simulated rainfall was adjusted to fall over a period of 96 h with 50mL applied daily and the leachate collected. At the end of the experiment the columns were left to drain and soil samples collected from 0-10, 10-20 and 20-30 cm segments.

### 3.5.2 Extraction of the samples

Extraction of the soil samples was done according to Bossi *et al.*, (1999). The soil sections were weighed and 50 mL of extractive solution (pH 7.8, 0.2 M phosphate buffer: methanol (8:2, V/V)) was added to 10g of soil in a centrifuge tube with cap. The mixture was vortexed for 3 min, ultra-sonicated for 5 min, and centrifuged at 4000 r/min for 10 min. The extraction procedure was repeated twice and the supernatants were combined and the pH adjusted with 85% phosphoric acid to 2.5. The sample solution was purified with Chromabond C18 cartridge (100 mg/3 mL). The SPE cartridge was activated by soaking the cartridge for 30 min in 5 mL of methanol and then rinsed using 5 mL of extractive solution (pH adjusted to 2.5 with 85% phosphoric acid). The sample extract was loaded onto the cartridge at the flow rate of 1 mL /min, after which the cartridge was dried under vacuum for 10 min during which time the entire sample had flowed out. Elution of the analyte was carried out using 3 mL of acetonitrile/phosphate buffer (pH 7.8) (9/1, v/v). The eluate was collected and evaporated under nitrogen stream to 1 mL, for HPLC-MS analysis. The amount of chlorsulfuron in the segments was given as percentage of applied initial dose for each soil segment and leachate fraction. A graphical presentation was given for each soil plotting the percentages found as a function of the soil depths.

## 3.6 Laboratory soil biodegradation studies

### 3. 6.1 Effect of microbes on degradation of chlorsulfuron

To establish the role of micro-organisms on the degradation of chlorsulfuron, the experiments were conducted on sterile and non-sterile soils according to Li *et al.*, (1999) and laid down as Randomized Complete Block Design (RCBD). A total of 30 glass bottles each containing 50 g of soil were taken. Fifteen bottles were plugged with cotton and autoclaved for 1 h for three consecutive days at a temperature of 121◦C and pressure of 15 atmospheres to obtain the sterilized soil. An aliquot of 2 mL of 1 µg $mL^{-1}$ chlorsulfuron was added to 50 g of in each bottle to obtain 4µg $g^{-}$ and mixed thoroughly. The field water capacities of the soils were determined by placing dry soils into ceramic plates and then saturated with water and left to equilibrate for 12 h. Thereafter the plates were placed in an Eijkelkamp pF 0684 (Giesbeek, Netherlands) pressurized container at -33kPa for 24 hours. The plate were weighed and then placed in an oven at 105°C for 12 hours. The field capacity was calculated as a percentage of the difference in moisture contents before and after drying.

The field capacities for the soils were 22.2 %, 24.5%, 17.8%, 28.5% and 23.1% for Mau East A, Mau Central , Olokurto, Mau East B and Olololunga respectively. Sterile water was added to bring the water content of the soil to 50% of the field capacity, and the bottles were shaken for thorough mixing of the herbicide and the soil. The samples were handled in a laminar flow to maintain sterility. Immediately after fortification the soil with the herbicide, 5g triplicate samples (day 0) were extracted as explained in section 3.5.2 and stored at -14° C for later analysis. The remaining bottles were incubated in the dark at 20 ± 2° C. The water content of the soils was maintained by occasional weighing of the flasks and any loss in weight corrected by adding an accurate amount of sterilized and shaking the bottles. Sampling was done at 3, 7, 14, 21, 30, 40 and 60 days after application. One replicate from each soil type was dried overnight at 110°C for final residue calculations on an oven-dry-weight basis. The amount of chlorsulfuron in the samples was quantified using HPLC-MS as described earlier.

### 3.6.2 Laboratory studies of the effect of repeated application on degradation of chlorsulfuron

The experiments were laid as Randomized Complete Block Design (RCBD) and soil incubation done according to Cox *et al.*, (1996). Five hundred grams of each soil sample was treated with 10 ml of 10µg $ml^{-1}$ chlorsulfuron in quartz sand to attain 4µg $g^{-1}$ and the soils were mixed thoroughly. Two sets (each with three replications) of 50 g fortified soils were packed in Sigma Aldrichs' Duran Z3051 (Darmstadt, Germany) glass bottles (5.6 cm i.d and 10.5 cm height)to a height of 1.5 cm and incubated at 20°C. Moisture contents were maintained at a constant level throughout the experiment by adding distilled water as necessary. For the first set, 5g was sampled after 0, 3, 7, 14, 21, 30, 40 and 60 days, extracted and analyzed for chlorsulfuron by HPLC (see Section 3.5.2). After 60 days, 50 g of each soil from the second set were retreated with 2ml of 1µg $ml^{-1}$ chlorsulfuron and the replicates sampled periodically during the next 60 days.

### 3.6.3 Identification of degradation products

A portion (5 g) of the soil samples from section 3.6.1 and 3.6.2 were extracted with 10 mL of PBS- acetonitrile (80:20, v/v) on an Edmund Bühler SM-30 orbital shaker for 30 min and further ultrasonicated for 15 min (Bossi *et al.*, 1999). The slurry was centrifuged at 4000 rpm on a Biofuge Primo Heraus centrifuge for 10 min and the supernatant was decanted into glasstubes. The supernatant was concentrated on a Savant DDA speed vac at 35°C and 40 mbar pressure to remove organic acetonitrile.

The aqueous extract was concentrated with SPE. A HR-X cartridge was conditioned with 10 mL dichloromethane followed by 10 mL methanol and finally the cartridge was washed with

20 mL of water. The extract was then passed through the cartridge and the residual water was removed by drying the cartridge under vacuum for 30 min the analytes were eluted with 10 mL dichloromethane. The solvent was evaporated to dryness under a stream of nitrogen and the sample reconstituted in 1mL methanol-water (10:90, v/v). The degradation metabolites identification was performed by positive mode LC-ESI-MS. The operating conditions for ESI were: sheat gas (nitrogen) (10:90, v/v) pressure 65 p.s.i.; auxiliary gas (nitrogen) flow 2.1 L 21 min; spray voltage 5 kV and capillary temperature 250$^{o}$C at 1 p.s.i. and confirmed by comparison with 10µ M authentic standards based on spectral data.

## 3.7 Chlorsulfuron field dissipation experiment

### 3.7.1 Field experimental design

Field experiments on persistence of chlorsulfuron in wheat farm soils were conducted at Enengepire National Cereal and Produce Board farms in Olokurto and at Nairegia Ngare in Mau East from1st April 2015 to 31st July 2015.The experiments were laid as completely randomized block design (CRB) with three replications and a control on a plot size of 4 m × 4 m. The plots were prepared by digging to remove stones and weeds then 48 PVC pipes of 10 cm diameter and 45 cm length were driven into the soils with 3 cm left protruding above the soils surface in each plot. The pipes were then left undisturbed for one week before the application of chlorsulfuron. Thereafter, 0.05g of chlorsulfuron was mixed in 5l of water (amount equivalent to 30 g a.i. ha$^{-1}$) and sprayed with the help of knapsack sprayer on each plot. The amount of rainfall, ambient temperature and percent relative air humidity were also recorded before taking the samples for the entire study period.

### 3.7.2 Soil core sampling and extraction

The samples were collected at 0 (3 h), 1, 3, 7, 14, 21, 30 and 60 day time intervals after treatment (DAT) and at approximated crop harvest (115 DAT) time from all the treated and control plots. Samples were mixed air dried and thoroughly ground using a pestle and mortar, and passed through a 2 mm sieve. Each soil sample (4 kg) was spread on a glass plate (30 cm i.d and 5 cm height) and divided into four quarters. Soil of two opposite quarters was retained, rejecting the remaining two. The process was further repeated to obtain 100 g of representative sample for the final analysis. One replicate from each sample was dried overnight at 110°C, and all residues data are reported on an oven-dry-weight basis.

To the 100 g soil 100 mL of extractive solution (pH 7.8, 0.2 M phosphate buffer: methanol (8:2, V/V)) was added to it in a centrifuge tube with cap (Bossi *et al.*, 1999). The mixture was vortexed for 3 min, ultrasonicated for 5 min, and centrifuged at 4000 r/min for 10min. The extraction procedure was repeated twice, the supernatants combined and the pH adjusted with 85% phosphoric acid to 2.5. The sample solution was then purified with Cleanert C18 cartridge (100 mg /3 mL): The SPE cartridge was activated by soaking the cartridge for 30 min in 5 mL of methanol and then rinsed using 5 mL of extractive solution (pH adjusted to 2.5 with 85% phosphoric acid). Sample was loaded the onto the cartridge at the flow rate of 1 mL/min, after which the cartridge will be dried under vacuum for 10 min after all the sample flows out. Elution of the analyte was carried out using 3 mL of acetonitrile/phosphate buffer (pH 7.8) (9/1, V/V). The eluate was collected and evaporated under nitrogen stream to 1 mL, for HPLC analysis. HPLC analyses were conducted with a Hewlett-Packard HP-1050 quaternary pump and equipped with a HP-1050 Variable Wavelength Detector (HPLC-VWD). Data were processed with a HP Chemstation (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separation was achieved on a Lichrocart C-18 column (250 mm x 4 mm i.d, Merck, Darmstadt, Germany). The analyses were carried out using a linear gradient: from 20:80 methanol 1% acetic acid in water to 100: 0 methanol/1% acetic acid in water, over 50 min, then 5 min isocratic; the flow rate was 1 mL/min. Chromatograms were recorded at 254 nm.

### 3.8 Analytical performance, recovery studies and statistical analysis

#### 3.8 1.Analytical performance and Recovery studies

Validation of the method on HPLC-MS was performed with different known concentrations of chlorsulfuron in methanol by diluting the stock solution of the analytical standard in the range of 0.01-5 mg/L. The linearity of the calibration curves was generated by plotting peak area (y) versus the concentration (x), mg $L^{-1}$). The response function was found to be linear with correlation coefficients (r) higher than 0.996. Recovery study was conducted by spiking 0.4µg $g^{-1}$ and 4 µg $g^{-1}$ chlorsulfuron in 50 g of each soil retaining a control. The soils were extracted as explained in section 3.5.2 and recoveries of 72.1%, and 88.3%, respectively were achieved. The limit of detection was determined as the lowest concentration based on the signal to noise ratio of 3:1 defined from the analysis of the control and it was established as 0.003µg $g^{-1}$ in soil. For the degradation products, the three replicates of 0.05µg $g^{-1}$ and 0.5 µg $g^{-1}$ of both 2- amino-4-methoxy-6-methyltriazine (product 1) and 2-chlorobenzenesulfonamide (product 2) each were spiked to 50 g of soil and extracted.as explained in section 3.6.3 The aqueous extract was analyzed and the average recoveries for product 1 were 72% and 86% for 0.05µg $g^{-1}$ and 0.5 µg $g^{-1}$ respectively. For product 2 the recoveries were 84% and 73% for 0.05µg $g^{-1}$ and 0.5 µg $g^{-1}$ respectively. The limits of detection were 0.01 µg $g^{-1}$ and 0.025 µg $g^{-1}$ for product 1 and 2 respectively.

#### 3.8.2 Statistical Analyses

The influence of soil properties on chlorsulfuron dissipation rates under field conditions was evaluated using the Pearson correlation and regression procedures in GraphPad Prism 6.

Analyses of variance were conducted as factorial one in Randomized Complete Block Design using MSTAT C statistical package (Michigan State University, MI) for the experiments on the effect of location and rate of application on leaching of chlorsulfuron and the effects of sterility and repeated applications of chlorsulfuron on its degradation. The field study analysis of variance was done as completely randomized block design. Comparisons of mean values in this study were accomplished using least significant difference (LSD) methods at a confidence level (P) of 0.05 for the interaction effects. The calculation and determination of the half-lives ($t_{1/2}$) of the chlorsulfuron were obtained by first order kinetics, through regression analysis. Significant differences between regression coefficients were examined.

### 3.9 Limitations of the methods

1. In adsorption-desorption studies, centrifugation changes the particle size of the soils therefore creating a totally different soil.
2. In the influence of biotic degradation studies, autoclaving of the soils at $121^{o}$ C may not eliminate some soil bacteria which withstand very high temperatures

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Sorption and desorption of chlorsulfuron

### 4.1.1 Sorption kinetics

Sorption of chlorsulfuron with time was plotted as shown in Figure 4. The sorption of chlorsulfuron in all the soils was biphasic. The first phase for each soil was characterized by quick increase in sorbed chlorsulfuron during the first 3 hours of soil-chlorsulfuron solution contact followed by slower progress towards equilibrium at 12 hours.

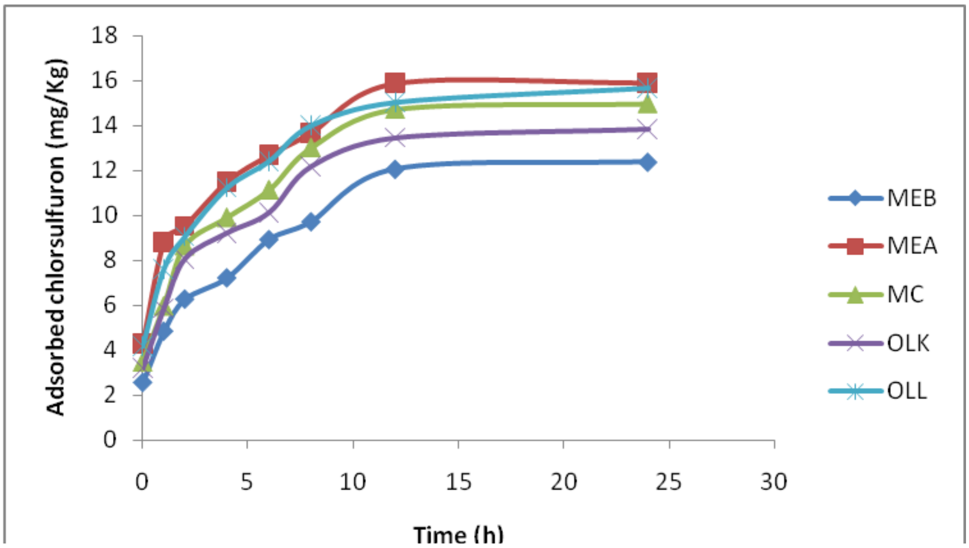

**Figure 4:** Kinetics of chlorsulfuron (10 mg/L initial concentration) sorption in the soils at 25 ± $2^{o}$ C. MEA- Mau East Central; MEB-Mau East B; MC-Mau Central; OLK- Olokurto; OLL-Olololunga

After 12 h, chlorsulfuron sorption slowed and chlorsulfuron concentration stayed constant even after 24 hours. The adsorption equilibrium was reached after 12 h for all soils, although the amounts adsorbed per unit mass of dry soil varied and increased from Mau East B < Olokurto < Mau Central < Olololunga < Mau East A, indicating that the sorption kinetics of chlorsulfuron in the wheat growing soils of Mara River basin was dependent on soil physicochemical parameters. Indeed Mau East A with high % OC had a higher adsorption capacity to chlorsulfuron than Mau East B with higher clay content. This finding is inconsistent with earlier reports where soils with higher clay contents exhibited higher adsorption capacities for pesticides than those with lower clay content (Barriuso *et al.*, 1992; Welhouse and Bleam, 1992; Baskaran *et al.*,1996; Villaverde *et al.*, 2008; Azcarate *et al.*, 2015).

Cation exchange capacity (CEC) determines the ability of soils to adsorb positively charged compounds (Kah and Brown, 2006). As the CEC increases, more herbicide is expected to be bound to the soil but if the herbicide is anionic it would be less bound due to repulsion (Kodešová *et al.*, 2011). Mau East B had the highest CEC (Table 2) yet it had the least adsorbed amounts after 12 hours. This is due to the fact that chlorsulfuron is mainly anionic in acidic solutions with pH greater than its $pK_a$ and would be repelled from the soil surface. This observation has been reported for chlorimuron-ethyl where it was less adsorbed in Bentonite clay which had a high CEC (70 cmol $kg^{-1}$) and adsorbed more in kaolinite clay with CEC of 8 cmol $kg^{-1}$ (Suyal *et al.*, 2013). This was attributed to retention of the anionic species of the herbicide on to the positively charged kaolinite surface rather than repulsion.

Chlorsulfuron was rapidly adsorbed during the first stage since the soils had readily available vacant adsorption sites. When a pesticide is mixed with the soil it reacts with the active sites that are available on the surfaces of the soil surfaces (Gilchrist *et al.*, 1993). Some of the soils were very active, and held the pesticide strongly, while others had lower reactivity which could be attributed to low organic carbon content and cation exchange capacity. This resulted in different adsorption kinetics (Fig 4). With continued adsorption of chlorsulfuron, the loosely bound pesticide molecules would desorb, so that the adsorption and desorption processes occur concurrently, but the adsorption efficiency was greater than that of desorption as had also been observed elsewhere (DiVincenzo and Sparks, 1997) .The soil adsorption-desorption behavior of pesticides reported by DiVincenzo and Sparks, (1997) were similar to the soil adsorption kinetics of chlorsulfuron observed here. The results imply that if low levels of chlorsulfuron are applied in the Mara River Basin soils, most of it will be adsorbed onto the soil within 12 hours decreasing the amount of herbicide available for the control of weeds in wheat.

### 4.1.2 Sorption isotherm

The sorption of chlorsulfuron in the soils fitted best the Freundlich model as compared to the Langmuir model (Appendix 1). The adsorption coefficients $K_{f\text{-}ads}$ were between 0.46 (Mau East B), 0.57 (Olokurto), 0.61 (Mau Central), 0.73 (Ololulunga) and 0.75 (Mau East A), respectively, for chlorsulfuron (Table 3).

**Table 3**: Chlorsulfuron: adsorption isotherms, coefficients of determination ($r^2$), adsorption coefficient ($K_{f\text{-}ads}$), distribution coefficient ($K_d$), organic carbon-normalized distribution coefficients ($K_{oc}$) and $n_{f\text{-}ads}$ is a describer of adsorption isotherm curvature

| **Soil** | **$K_d$** | **$K_{OC}$** | **$K_{f\text{-}ads}$** | **$1/n_{f\text{-}ads}$** | **$r^2$** | **P** |
|---|---|---|---|---|---|---|
| Mau Central | 0.88±0.03 | 21.03 | 0.61 ± 0.07 | 0.87± 0.11 | 0.978 | 0.00 |
| Mau East A | 2.52±0.07 | 19.54 | 0.75 ±0.13 | 0.91 ±0.08 | 0.890 | 0.04 |
| Olokurto | 1.87±0.11 | 21.58 | 0.57 ± 0.11 | 0.99±0.21 | 0.983 | 0.00 |
| Ololulunga | 2.35±0.03 | 22.93 | 0.73 ± 0.03 | 0.86±0.10 | 0.939 | 0.01 |
| Mau East B | 0.59±0.09 | 15.91 | 0.46 ±0.08 | 0.99 ±0.05 | 0.986 | 0.00 |

Mau East A soils showed the maximum sorption possibly due its low pH, high organic matter content and high CEC (Grey and McCullough, 2012; Földėnyi *et al.*, 2013). Chlorsulfuron is a weakly acidic herbicide and exists in the anionic form in mildly acidic agricultural soils. Since the pH values of the soils were greater than their $pK_a$ (3.3), especially for soils at Olokurto and Mau East B, there were increased amounts of anionic molecules in the solutions leading to lack of charged molecules and higher lipophilicity of the neutral molecular species thus reducing its sorption. High organic carbon content in Mau East A soil compared to the rest could be responsible for the high adsorption coefficient (Grey and McCullough, 2012).

Increase in soil pH increased solubility of molecules due to either particle dispersion increase or the repulsion of the increasing negative charges on both organic matter and soil inorganic solids (You *et al.,* 1999). Sorption of sulfonylureas has been shown to be favoured in low pH soils and soils with high organic matter (Wu *et al.*, 2011). The extent of monosulfuron adsorption on soils was at rather high levels under low pH value conditions, and it decreased with increasing pH value. The content and chemical characteristic of the soil organic matter was one of the important factors leading to differences in sorption capacity of monosulfuron ester (Tang *et al.*, 2009).

Generally, chlorsulfuron sorption onto the five soils from the Mara River Basin was low as estimated by the low $K_d$ values (0.59 – 2.52). Alvarez-Benedi *et al.*, (1998) reported low sorption for chlorsulfuron in three Spanish soils with increase in ionic strength. Similarly, low sorption of chlorsulfuron was reported in fresh and amended soils with composted olive cake (Delgado and Pena, 2008). $^{14}$C- chlorsulfuron sorption in fourteen Chinese soils was also found to be low (Zuyi *et al.*, 1995). The low sorption of chlorsulfuron in the wheat growing soils raises concerns about its leaching potential during high rainfall seasons.

Sorption of chlorsulfuron in all the five soils were nonlinear, since all the 1/ n values were less than unity (Table 3). This predicted the L-type adsorption isotherm usually characterized by strong interaction between the adsorbent and adsorbate (Aranovich and Donohue, 1998), implying that chlorsulfuron could adsorb to the clay fraction of the soils as well as the organic matter in the soils. Nemeth- Konda *et al.*, (2002) described this type of curve in the case of cultivated soils. For the Mara River Basin soils, the L-type curve could have been caused by decrease of accessibility of the free adsorption sites in the soils with time and subsequently decreased adsorbed amounts even when chlorsulfuron concentration was high. This means that the leaching potential of chlorsulfuron increases with increase in its concentration within the Mara River Basin.

The sorption coefficient $K_{oc}$ is commonly a measure of pesticide sorption to soil organic carbon and is useful in predicting the mobility of soil contaminants. Higher $K_{oc}$ values (50-150) correlate with less mobile organic chemicals while lower $K_{oc}$ values (< 50) correlate to more mobile organic chemicals (Grathwohl, 1990). The $K_{oc}$ values calculated by normalizing chlorsulfuron sorption to soil organic carbon in all the five soil types were below 50, suggesting that chlorsulfuron could be relatively mobile in these soils which could lead to its leaching into the sub soils.

The correlation of the sorption coefficient $K_{fads}$ and the soil properties was done to determine the factors responsible for chlorsulfuron adsorption in the five soils. Sorption of chlorsulfuron showed a significant ($p \leq 0.05$) inverse correlation with the soil pH and a positive correlation ($p \leq 0.05$) with the organic carbon content (Table 4).

**Table 4**: Coefficient of correlation (r) values for chlorsulfuron sorption

| | *pH* | *CEC* | *OC* | *Sand* | *Clay* | *Silt* |
|---|---|---|---|---|---|---|
| $K_f$ | -0.8111* | -0.3163 | 0.7882* | -0.7719 | -0.6286 | -0.6607 |
| P | 0.004 | 0.3732 | 0.0068 | 0.1262 | 0.2560 | 0.2248 |

*Significant at P = 0.05; these values were obtained by determining the correlations between Freundlich coefficient ($K_f$) and the soil properties.

This further confirms that soil organic carbon and pH affected the sorption of chlorsulfuron in the soils. Similar observations have been reported for other sulfonylureas. A negative correlation (-0.993, $p \leq 0.05$) was reported between soil pH and metsulfonyl-methyl sorption but not with organic matter (Wu *et al.*, 2011). Monosulfuron sorption was inversely correlated to pH ($r = -0.783$, $p \leq 0.01$) and positively correlated to organic matter ($r = 0.848$, $p \leq 0.05$) (Tang *et al.*, 2009). Similarly, a correlation coefficient of -0.87 ($p \leq 0.05$) between pH and sorption of metsulfuron methyl has been reported (Tahir and Sing, 2012). The sulfonylureas adsorptions have been found to be higher in low pH soils with high organic matter (Said-Pullicino *et al.,* 2004; Walker *et al.,* 1989). Although Alvarez-Benedi *et al.* (1998) observed that the sorptions of chlorsulfuron, tribenuron-methyl and imazamethabenz-methyl were positively correlated with organic matter and clay content, no positive sorption correlation with clays was observed in this study.

Mau East B soils with the highest clay content had a lower adsorption coefficient (Table 3). Due to anionic nature, sulfonylureas generally exhibit very low adsorption on clay minerals especially at a pH values above their $pK_a$, since more of the herbicide is in anionic form and repelled by the negatively charged soil colloids. However, there have been instances of binding of sulfonylurea herbicides to clay. Soils containing amorphous iron oxides have a high capacity for adsorption of sulfonylureas although the forces are relatively weak (Borggard and Streibig, 1989). These results imply that chlorsulfuron applied on the wheat growing soils within the Mara River Basin is lowly sorbed on the soils therefore the herbicide is available for herbicidal activity. On the contrary, this low sorption could make the herbicide easily available to the soil solution thus increasing the leaching potential to the surface and underground waters.

### 4.1.3 Desorption isotherms

Desorption isotherms were fitted in the Freundlich isotherm and the constants are as shown in Table 5.

**Table 5**: Chlorsulfuron desorption isotherms, coefficients of determination ($r^2$), desorption coefficient ($K_{fdes}$) and hysteresis coefficient (H) obtained for the top soils of Mara River Basin.

| **Soil** | **$K_{f\text{-}des}$** | **$1/n_{\text{-}f\ des}$** | **$r^2$** | **p- values** | **Hysteresis (H)** |
|---|---|---|---|---|---|
| Mau Central | 1.16 | 0.89 | 0.98 | 0.02 | 1.02 |
| Mau East A | 2.49 | 0.73 | 0.96 | 0.00 | 0.80 |
| Olokurto | 1.44 | 1.10 | 0.98 | 0.01 | 1.11 |
| Ololulunga | 1.90 | 0.55 | 0.97 | 0.01 | 0.63 |
| Mau East B | 1.03 | 0.72 | 0.95 | 0.05 | 0.72 |

The $K_{f\text{-}des}$ (Table 5) values of chlorsulfuron in all the five soils were higher than the $K_{f\text{-}ads}$ (Table 3) implying that the sorption process was not fully reversed and some sorbed chlorsulfuron was retained in the soils (Singh and Singh, 2012). Soils from Mau East A had the highest $K_{f\text{-}des}$ suggesting it retained more chlorsulfuron compared to the other soils, while the Mau East B soil had the lowest. Desorption isotherms were nonlinear ($1/n_{des}<1$) (Table 5) for all the soils except Olokurto. The two soils from Mau Central and Olokurto had higher values of $1/n_{des}$ than their respective $1/n_{ads}$ suggesting the rates of desorption for these soils were faster than adsorption (Mamy and Barriuso, 2007). Studies by Mersie and Foy, (1986) also reported nonlinearity of chlorsulfuron desorption in soils. This observation has also been reported for other sulfonylureas such as bensulfuron (Delgado and Pena, 2008). Soils from Mau East A, Olololunga and Mau East B had lower $1/n_{des}$ than their respective $1/n_{ads}$, implying that chlorsulfuron adsorbed on these soils were not easily desorbed. Therefore the herbicide applied in these soils will have decreased herbicidal activity and a greater potential for carryover, hence injuring susceptible crops in the future.

The hysteresis coefficient, H, is a measure of the extent of hysteresis in desorption. A value of H >1 means that desorption proceeds as fast as adsorption and no hysteresis occurs. However, a value of H < 1 indicates that the rate of desorption is slower than the rate of adsorption, and therefore hysteresis occurs (Pusino *et al.*, 2004). The H values for the soils ranged from 0.63-1.11 (Table 5), with Mau East A, Olololunga and Mau East B showing hysteresis effect.

Desorption hysteresis has been reported for several sulfonylureas with varying H values including monosulfuron which had hysteresis effects in eight Chinese soils with H values ranging from 0.14 to 0.63 (Tang *et al.*, 2009), metsulfuron-methyl (0.79 - 0.98) and sulfosulfuron (H= 0.87) (Singh and Singh, 2012) and H of 0.35-0.64 for monosulfuron-methyl (Wu *et al.*, 2011), triasulfuron (Pusino *et al.*, 2003) and chlorimuron-ethyl (Zhang *et al.*, 2007). Hysteresis effect can be as a result of irreversible binding and sequestration of solute to the OC and/ or clay mineral of soil aggregates (Bhandari *et al.*, 1996; Neville *et al.*, 2000) and entrapment of sorbed molecules in meso and microporous structures within the mineral structures and OC matrix of soil aggregates. Chemical or biological transformation, non-equilibrium conditions and high–energy bonding have also been proposed as explanation for hysteresis for a number of soil organic compound systems (Tang *et al.*, 2009; Zhang *et al.*, 2007). These results imply that Mau Central and Olokurto, had a higher leaching potential for chlorsulfuron in to the surface and underground waters as a result of faster desorption than adsorption suggesting that the chlorsulfuron that was initially sorbed is readily released into the solution.

## 4.2 Interaction of copper ions with chlorsulfuron

### 4.2.1 Effect of copper on sorption and desorption

Sorption isotherms of chlorsulfuron in the five soils in the absence and presence of Cu (II) were fitted to the Freundlich equation. The presence of Cu (II) ions in the solution increased the adsorption of chlorsulfuron in all the soil types and the effect was more pronounced with increase in the concentration of $Cu^{2+}$ (Figure 5). However, these increases caused by $Cu^{2+}$ ions were higher at equilibrium concentrations > 5 mg/L.

The adsorption constants $K_f$ which give an estimate of the extent of adsorption at a concentration and $n$ which represents the variation in adsorption with concentration of the herbicide are presented in Table 6.

**Table 6**: Freundlich constants ($K_f$ and n), hysteresis (H), coefficient of determination ($r^2$) for chlorsulfuron sorption on Mau Central (MC), Mau East A (MEA), Olokurto (OLK), Ololunga (OLL) and Mau East B (MEB) soils treated with $Cu^{2+}$.

| | $Cu^{2+}$ mg/l | Sorption | | | | Desorption | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $K_f$ | n | $r^2$ | P | $K_f$ | n | $r^2$ | P | H |
| MC | 0 | 0.55±0.04 | 0.76±0.07 | 0.98 | 0.02 | 0.77±0.04 | 0.71±0.03 | 0.98 | 0.21 | 0.93 |
| | 50 | 0.64±0.03 | 0.73±0.04 | 0.96 | 0.05 | 0.91±0.02 | 0.69±0.05 | 0.94 | 0.02 | 0.95 |
| | 100 | 0.81±0.09 | 0.64±0.08 | 0.97 | 0.06 | 1.04±0.05 | 0.66±0.03 | 0.98 | 0.01 | 1.03 |
| MEA | 0 | 0.79±0.07 | 0.77±0.04 | 0.99 | 0.04 | 1.01±0.05 | 0.71±0.05 | 0.96 | 0.05 | 0.92 |
| | 50 | 1.12±0.13 | 0.81±0.10 | 0.99 | 0.04 | 1.36±0.09 | 0.78±0.09 | 0.98 | 0.07 | 0.96 |
| | 100 | 1.49±0.24 | 0.75±0.05 | 0.99 | 0.32 | 1.65±0.05 | 0.79±0.04 | 0.99 | 0.04 | 1.05 |
| OLK | 0 | 0.64±0.05 | 0.72±0.05 | 0.93 | 0.04 | 0.89±0.04 | 0.67±0.04 | 0.98 | 0.03 | 0.93 |
| | 50 | 0.82±0.03 | 0.75±0.07 | 0.98 | 0.03 | 1.02±0.07 | 0.76±0.05 | 0.99 | 0.04 | 1.01 |
| | 100 | 1.14±0.05 | 0.76±0.02 | 0.96 | 0.02 | 1.29±0.09 | 0.77±0.11 | 0.98 | 0.05 | 1.01 |
| OLL | 0 | 0.71±0.06 | 0.84±0.09 | 0.99 | 0.04 | 0.99±0.07 | 0.77±0.07 | 0.96 | 0.03 | 0.91 |
| | 50 | 0.93±0.05 | 0.91±0.11 | 0.98 | 0.03 | 1.13±0.12 | 0.87±0.05 | 0.97<br>0.99 | 0.04 | 0.95 |
| | 100 | 1.37±0.16 | 0.77±0.03 | 0.95 | 0.03 | 1.48±0.06 | 0.78±0.05 | 0.98 | 0.11 | 1.01 |
| MEB | 0 | 0.51±0.23 | 1.01±0.08 | 0.91 | 0.05 | 0.69±0.08 | 0.98±0.02 | | 0.02 | 0.94 |
| | 50 | 0.59±0.09 | 1.14±0.13 | 0.93 | 0.14 | 0.75±0.03 | 1.14±0.04 | 0.99 | 0.05 | 1.00 |
| | 100 | 0.72±0.06 | 0.99±0.05 | 0.99 | 0.03 | 0.97±0.05 | 1.04±0.06 | 0.98 | 0.04 | 1.05 |

Mau East A soil showed the highest sorption for chlorsulfuron while soil Mau East B had the lowest sorption. The sorption of chlorsulfuron both in the absence and presence of Cu (II) ions for the five soils was in the order Mau East A > Ololunga > Olokurto > Mau Central > Mau East B. For all the soils, the $K_f$ values in the presence of copper ions were greater than those obtained in their absence (Table 6), indicating increase in sorption. Increase in sorption of herbicide in the presence of cations has also been observed by Ren *et al.* (2011) for Cu ions which significantly increased the sorption of chlorimuron-ethyl in two Chinese soils. Similarly, the presence of $Cu^{2+}$ in soil solution increased the sorption of anionic metsulfuron-methyl in soil and peat by decreasing the repulsion between soil surface and metsulfuron-methyl herbicide (Pei *et al.*, 2008). Also the sorption of glyphosate increased when Cu ions were present in solution treatments (Morillo *et al.*, 2000). On the contrary, the presence of Cu ions in soil solutions suppressed the sorption of cationic difenzoquat on peat and soils due to competitive sorption of the two for the same sorption sites (Pei *et al.*, 2008). The desorption coefficient $K_f$ values were greater than those for sorption indicating that some amount of chlorsulfuron was held in the soils after desorption cycles at all the concentrations. The results imply that the existence of both copper ions and chlorsulfuron in the Mara Basin soils lowers the effectiveness of the herbicide in eradicating the broadleaf and grass weeds in the wheat farms since the increase in sorbed amounts means more of the herbicide is not available for herbicidal activity. Besides the increased sorption may affect non-target rotational crops due to carry over effects.

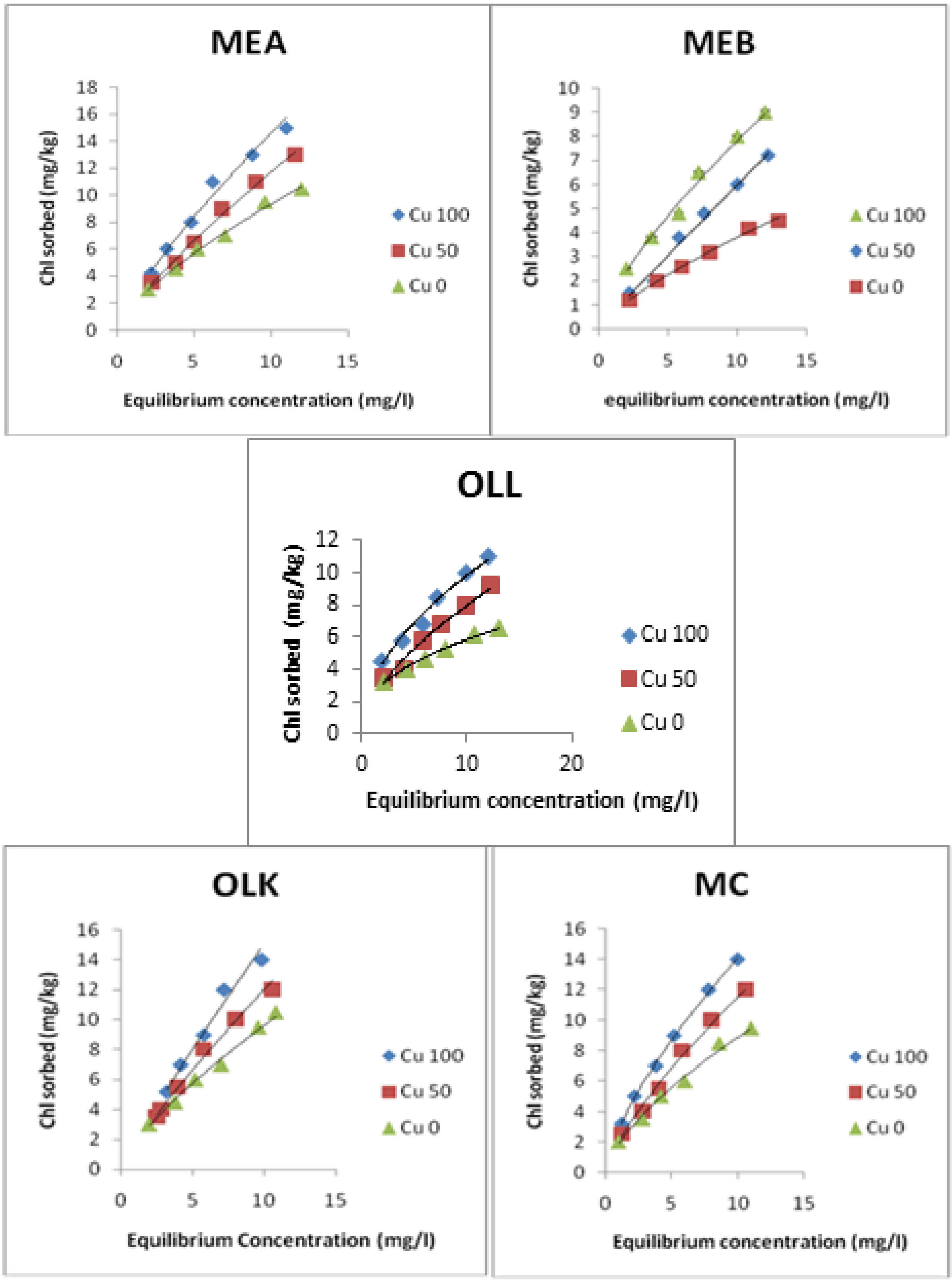

**Figure 5**: Sorption isotherms of chlorsulfuron in Mau Central (MC), Mau East A (MEA), Olokurto (OLK) and Mau East B (MEB) soils in the presence and absence of Cu (II).

There was a slight decrease in pH of the equilibrium solution with the introduction of Cu$^{2+}$ (Table 7). This observation could explain the increase in sorption since the decreased pH caused the variable charge surface of the soil oxides to be more protonated thus reducing the soil surface negative charges (Morillo *et al.*, 2000). Since chlorsulfuron is anionic in nature, the repulsion between the herbicide and the soil surface decreased thus increasing the adsorption. This behavior has also been observed by Ren *et al.* (2011) for Cu ions which decreased the soil solution pH therefore increasing the sorption of chlorimuron-ethyl. In fields with large concentrations of copper ions the pH of the soil will be lowered, increasing the sorption of chlorsulfuron herbicide in the soils thus compromising its efficiency in eliminating weeds. Farmers will therefore use higher application rates, increasing the amounts of chlorsulfuron into the soils and potential to leach in soils.

**Table 7**: Soil solution pH for the five soils after equilibration with and without Cu$^{2+}$

| Soil | Initial Cu (II) (mg L$^{-1}$) | Initial chlorsulfuron concentration (mg L$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | 0.625 | 1.25 | 2.5 | 5.0 | 10 |
| MC | 0 | 5.55 | 5.57 | 5.53 | 5.56 | 5.53 |
| | 50 | 5.53 | 5.55 | 5.49 | 5.52 | 5.49 |
| | 100 | 5.53 | 5.52 | 5.49 | 5.49 | 5.47 |
| MEA | 0 | 6.01 | 6.03 | 5.99 | 6.00 | 5.88 |
| | 50 | 5.88 | 5.85 | 5.88 | 5.85 | 5.76 |
| | 100 | 5.71 | 5.68 | 5.65 | 5.67 | 5.61 |
| OLK | 0 | 6.44 | 6.41 | 6.38 | 6.40 | 6.37 |
| | 50 | 6.39 | 6.41 | 6.33 | 6.37 | 6.33 |
| | 100 | 6.33 | 6.36 | 6.29 | 6.37 | 6.31 |
| OLL | 0 | 5.83 | 5.81 | 5.80 | 5.81 | 5.83 |
| | 50 | 5.79 | 5.77 | 5.79 | 5.75 | 5.76 |
| | 100 | 5.71 | 5.75 | 5.79 | 5.71 | 5.69 |
| MEB | 0 | 6.64 | 6.60 | 6.49 | 6.57 | 6.44 |
| | 50 | 6.41 | 6.43 | 6.30 | 6.29 | 6.33 |
| | 100 | 6.29 | 6.28 | 6.20 | 6.17 | 6.15 |

MC-Mau central, MEA-Mau East A, OLK-Olokurto, OLL-Olololunga, MEB- Mau East B

Formation of complexes between chlorsulfuron and cationic Cu$^{2+}$ could also increase adsorption since the complexes could be sorbed more easily than chlorsulfuron itself. The organic amine and compounds containing the NH-group like chlorsulfuron are efficient ligands for divalent metal ions (Cáceres *et al.*, 2010). $Cu^{2+}$ can coordinate with the N-H in the ureic bridge and the pyrimidine ring of chlorsulfuron to form a six membered ring chelate, with reduced negative charges compared to chlorsulfuron. This would favour the adsorption of the chelate to the negatively charged soil surface. Previous study ( Pei *et al.*, 2014) on the effect of pH on the adsorption of anionic sulfathiozole on peat and soil and demonstrated that sulfathiozole adsorption was increased on soil at a pH greater than 5.0 since sulfathiozole could form a complex with Cu or through the formation of Cu bridge which enhanced its adsorption.

To verify if Cu (II) acts as a bridge between chlorsulfuron and soil and to demonstrate the sorption sites of chlorsulfuron and Cu (II), FTIR spectroscopic analysis (Ren *et al.*, 2011) was carried out using soil from Mau East A. The strong peak at 1003 $cm^{-1}$ corresponded to C-O stretch, the peak at 1633 $cm^{-1}$ was assigned to the C═O while the broad peak at 3406 corresponded to -COOH or –OH stretch vibrations of the soil (Figure 6). With the introduction of both copper (II) and chlorsulfuron, the shift at C-O stretch was not significant. However, for the sorption of chlorsulfuron, Cu (II) or a mixture of chlorsulfuron and Cu (II), the peaks at 1633 $cm^{-1}$ and 3406 $cm^{-1}$ shifted to 1634 and 3372 $cm^{-1}$, 1635 and 3369 $cm^{-1}$ and 1637 and 3357 $cm^{-1}$, respectively, suggesting interaction between chlorsulfuron, copper and the soil. Increase in the intensity of O-H band (3600-3300 $cm^{-1}$) could be attributed to the adsorption of the copper herbicide ($Cu^{2+}$-chlorsulfuron) complex onto the soil. The displacement of C ═ O band (1633 $cm^{-1}$ ) to higher wavenumbers(1635 and 1637 $cm^{-1}$ ) suggests an increase in the double-bond character of the C = O group, more likely owing to the electron-withdrawing effect from a protonated neighboring group in this case Cu(II), or to the loss of intermolecular hydrogen bonding. Similar red shifts on the C = O group on the addition of cations was reported for the sorption of $Zn^{2+}$ and *p*-nitrophenol on wheat ash when C = O band (1592 $cm^{-1}$) on ash was shifted to 1622 $cm^{-1}$ and 1632 $cm^{-1}$ on addition of $Zn^{2+}$ and *p*-nitrophenol respectively (Wang *et al.*, 2011). Similarly, the addition of $Cu^{2+}$ and chlorimuron-ethyl on a Chinese soil caused a blue shift on the –OH vibration from 3418 $cm^{-1}$ to 3428 $cm^{-1}$ and 3432 $cm^{-1}$ respectively (Ren *et al.*, 2011). Complexation of soil surface with exchangeable multivalent cations has been suggested as a sorption mechanism for sulfonylurea and many ionic herbicides (Pei *et al.*, 2006). In another study, Cu-bridging was reported to be the main sorption mechanism for chlorimuron-ethyl in soil (Ren *et al.*, 2011). Similarly, rimsulfuron was adsorbed mainly on to Cu (II)-clays due to formation of a complex with the saturating Cu (II) ion (Calamai *et al.*, 1997). Interaction of copper ions with chlorsulfuron in the Mara River basin soils will lower the activity of chlorsulfuron on the targeted weeds due to the formation of complexes. Besides non availability of the copper ions to the wheat plant uptake will affect the yields.

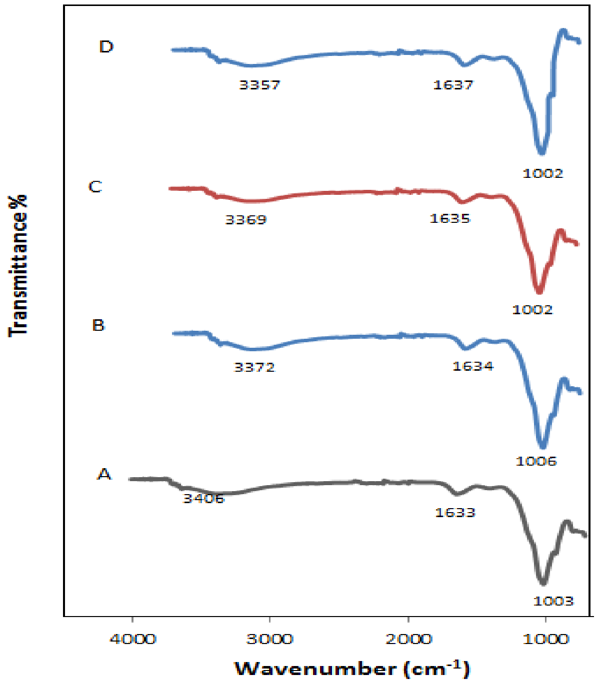

**Figure 6**: FTIR spectra of (A) soil, (B) soil with chlorsulfuron, (C) Soil with Cu (II), and (D) soil with chlorsulfuron and Cu (II).

### 4.3. Effect of site and application rate on leaching of chlorsulfuron in soils

A number of factors influence the leaching of a pesticide and its degradation products in soil under field conditions including pesticide chemical properties (for instance, sorption coefficient and degradation half-life), application rate, soil characteristics (for example, organic carbon, water-holding capacity, structure and surface management) and climatic influences (such as amount and timing of precipitation/irrigation, evapotranspiration and temperature) (Flury *et al.*, 1996). Within the Mara River Basin, the wheat growing regions differ in their soil characteristics, climatic patterns and agronomic practices such as application rate which may affect the leaching pattern of chlorsulfuron within the basin.

In this study, chlorsulfuron leached to a depth of 30 cm in all the sites, at 160 mm simulated rainfall condition. The mean concentration of chlorsulfuron recovered in all the sites decreased as the depth of the soil increased (Table 8). This is because chlorsulfuron is a weak acid and as such it exists as negatively charged species which react weakly with soil matrices and organic matter and thus moves freely through soils (Sondhia, 2009). The intrinsic mobility of a herbicide is inversely related to its degree of sorption to the soil surface (Gustafson, 1995). Since chlorsulfuron was weakly adsorbed to all the soil types, it was expected that it exhibits high mobility in the soils. The solubility of chlorsulfuron in water (31.8 g/ L, pH 7) could also be the other factor that contributed to its mobility. In a previous study, chlorsulfuron added to 42 cm depth soil columns was found to leach through the whole column containing sandy soil and to depth of 24 cm in an organic soil after a precipitation of 411 mm over 1.5 months (Iivanainen and Heinonen-Tanski, 1991). In another study, chlorsulfuron was detected in the top 40 cm after cumulative rainfall of 56 mm in a self-mulching clay soils (Noy , 1996). Chlorsulfuron has also been reported to have leached to a depth of 70 cm in an alkaline soil (Sarmah *et al.*, 1999). This high mobility was attributed to high soil pH which made chlorsulfuron less bound to the soil particles. These rates of leaching are comparable with rates found in this study.

**Table 8**: Effect of location and application rate on the vertical distribution of chlorsulfuron (µg $g^{-1}$ dry weight) in five wheat growing soils within Mara River Basin.

| **Site** | **Rate (g** | **Depth (cm)** | | | **Mean** | **Mean** | **C.V (%)** |
|---|---|---|---|---|---|---|---|
| **Mau East A** | | 0 -10 | 10 -20 | 20 -30 | | | |
| | 30 | 1.17 | 0.10 | 0.07 | 0.46 | 0.59 | 7.86 |
| | 60 | 1.42 | 0.58 | 0.19 | 0.73 | | |
| | Mean Depth | 1.30 | 0.34 | 0.13 | | | |
| | CV | | 7.86 | | | | |
| | L.S.D | | 0.08 | | 0.14 | | |
| **Mau Central** | | | | | | | |
| | 30 | 1.25 | 1.12 | 0.08 | 0.48 | 0.66 | 11.52 |
| | 60 | 1.38 | 0.78 | 0.32 | 0.83 | | |
| | Mean Depth | 1.32 | 0.45 | 0.20 | | | |
| | L.S.D | | 0.13 | | 0.225 | | |
| **Olokurto** | | | | | | | |
| | 30 | 1.18 | 0.90 | 0.07 | 0.72 | 0.79 | 10.04 |
| | 60 | 1.36 | 0.87 | 0.36 | 0.86 | | |
| | Mean Depth | 1.27 | 0.88 | 0.22 | | | |
| | L.S.D | | 0.13 | | 0.24 | | |
| **Olololunga** | | | | | | | |
| | 30 | 1.26 | 0.94 | 0.080 | 0.759 | 0.83 | 12.66 |
| | 60 | 1.28 | 0.88 | 0.554 | 0.905 | | |
| | Mean Depth | 1.27 | 0.91 | 0.317 | | | |
| | L.S.D | | 0.18 | | NS | | |
| **Mau East B** | | | | | | | |
| | 30 | 0.78 | 0.35 | 0.10 | 0.41 | 0.58 | 7.80 |
| | 60 | 1.23 | 0.63 | 0.40 | 0.75 | | |
| | Mean Depth | 1.00 | 0.49 | 0.25 | | | |
| | L.S.D | | 0.08 | | 0.14 | | |
| **All sites** | 30 | 1.13 | 0.48 | 0.08 | 0.56 | | 10.71 |
| | 60 | 1.34 | 0.75 | 0.37 | 0.82 | | |
| | Mean Depth | 1.23 | 0.62 | 0.22 | | | |
| | L.S.D | | 0.06 | | 0.098 | 0.05 | |
| **Interactions:** SxR=0.07, SxD=0.08, RxD= NS, SxRxD= 0.11 | | | | | | | |

Within the Mara River Basin, chlorsulfuron leached up to 30 cm depth raising concerns about risk of groundwater contamination during high rainfall seasons.

The amounts of chlorsulfuron that leached varied significantly ($p \leq 0.05$) in all the sites (Table 8). This can be attributed to the differences in the soil physicochemical parameters in all sites. The rate of application of chlorsulfuron had a significantly different ($p \leq 0.05$) effect on the leaching of chlorsulfuron in all the sites except in Olololunga. This implies that the rate of application does not affect the amount of chlorsulfuron that leaches in Olololunga. While for the other sites, applying a higher rate, more chlorsulfuron would leach as compared to application of lower rate (30 g a.i. ha$^{-1}$). This observation was also reported by Kotoula-syka *et al.* (1993) where chlorsulfuron leaching was found to increase with increase in application rates but this varied with soil type. Chlorsulfuron was retained more ($p \leq 0.05$) in the 0-10 cm depth in all the sites for the two application rates.

Significant ($p \leq 0.05$) interaction effects between rate of application and site were noted demonstrating that the pattern of leaching of chlorsulfuron for the two application rates was not the same for all the sites. Similarly, there was significant ($p \leq 0.05$) interaction between site and depth implying that the movement of chlorsulfuron down the soil profile varied from one location to another.

However, there was no significant ($p \leq 0.05$) interaction effects between the rate of application and depth implying that the pattern of movement of chlorsulfuron down the soil was the same and not affected by the application rate.

Further, the mobility of chlorsulfuron increased with increasing application rate in all the soils (Figs 7 and 8), with higher rates for 60 g a.i ha$^{-1}$. However, the more of chlorsulfuron was present within the top 1-10 cm of soil. Mau East A soils had the highest chlorsulfuron residues in the upper layer (0- 10cm) for both application rates when compared to the other soils. This may be due to the high organic matter content in the Mau East soils which favour adsorption of chlorsulfuron. Adsorption reduces the amount of chemical available to plants and slows leaching. In the 10-20 and 20-30 cm depths, Mau East B had the highest residue recovered for 30 g a.i ha$^{-1}$ implying that chlorsulfuron moved faster in that soil when compared to the others. After the study period, 43.6, 42.5, 42.4, 46.8 and 50% chlorsulfuron was recovered from Mau East A, Mau Central, Olokurto, Ololulunga and Mau East B soils respectively for 30g a.i ha$^{-1}$.

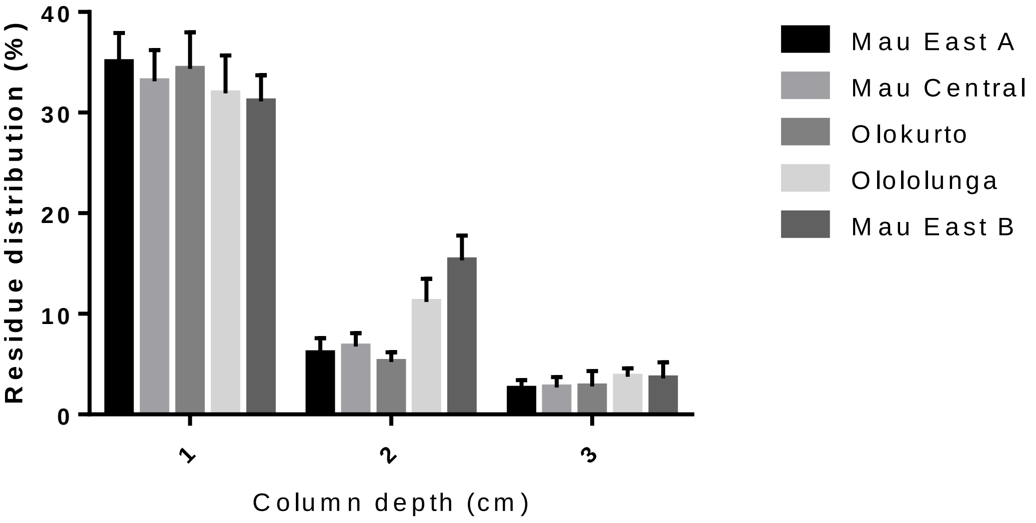

**Figure 7**: Percent distribution of 30 g a.i ha-1 chlorsulfuron in five wheat growing soils of Mara River Basin (1= 0-10 cm; 2 = 10-20 cm; 3 20-30 cm).

Generally, herbicides are more likely to leach when they are both weakly adsorbed in the soil. Since the mobility of sulfonylureas increases with increasing pH and decreasing soil organic matter (Curran, 2001), this could be the reason why higher amounts were recovered in the lower depths in Mau East B which had a higher pH and lower organic matter (Table 2). In soils with high pH, the mobility of chlorsulfuron is expected to increase due to increased solubility and ionization of the chemical as adsorption decreases. This therefore means that the possible risk of groundwater contamination could be higher in Mau East B soil as compared to the other soils which had lower pH. Moreover, this high mobility may reduce efficacy of chlorsulfuron for weed control in Mau East B when compared to other soils.

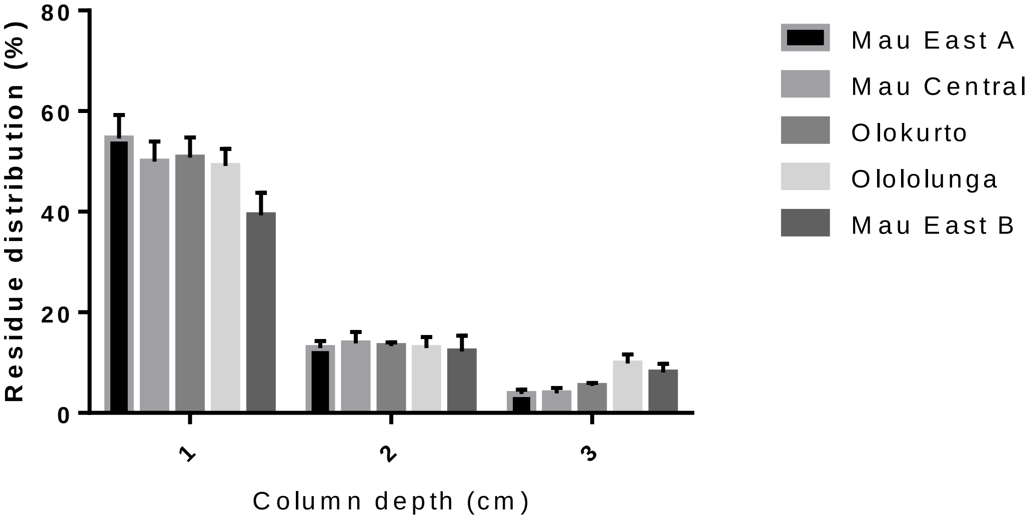

**Figure 8**: Percent distribution of 60 g a.i ha-1 chlorsulfuron in five wheat growing soils of the Mara River Basin (1= 0-10 cm; 2 = 10-20 cm; 3 = 20-30 cm)

The mobility of chlorsulfuron is enhanced at higher soil pH and/or relatively lower organic matter content, reflecting the influence of these soil properties on herbicide (Mersie and Foy, 1985; Fredrickson and Shea, 1986; Beckie and McKercher, 1990). Moreover, leaching of herbicide from the soil application zone depends on the amount of rainfall or irrigation received (Savage, 1977). Therefore for the Mara River Basin soils these results would vary depending on the amount of rainfall received in each cropping season. It is important to note that despite leaching after 4 days with concomitant precipitation most chlorsulfuron residues were still found within the top 1-10 cm layer of soil.

## 4.4 Biodegradation of chlorsulfuron in soils in bio meter flasks

### 4.4.1 Chlorsulfuron degradation in sterilized and non-sterilized soils

Chlorsulfuron degradation was determined in fresh (non autoclaved) and autoclaved soil samples. The degradation pattern for both sterile and non-sterile soils exhibited two phases: an initial rapid degradation up to the 14th day, followed by a slower degradation phase. However, the initial rapid degradation of chlorsulfuron in non-sterile soils of the Mara River Basin in the first 14 days was faster than in the sterile soils (Figure 9).

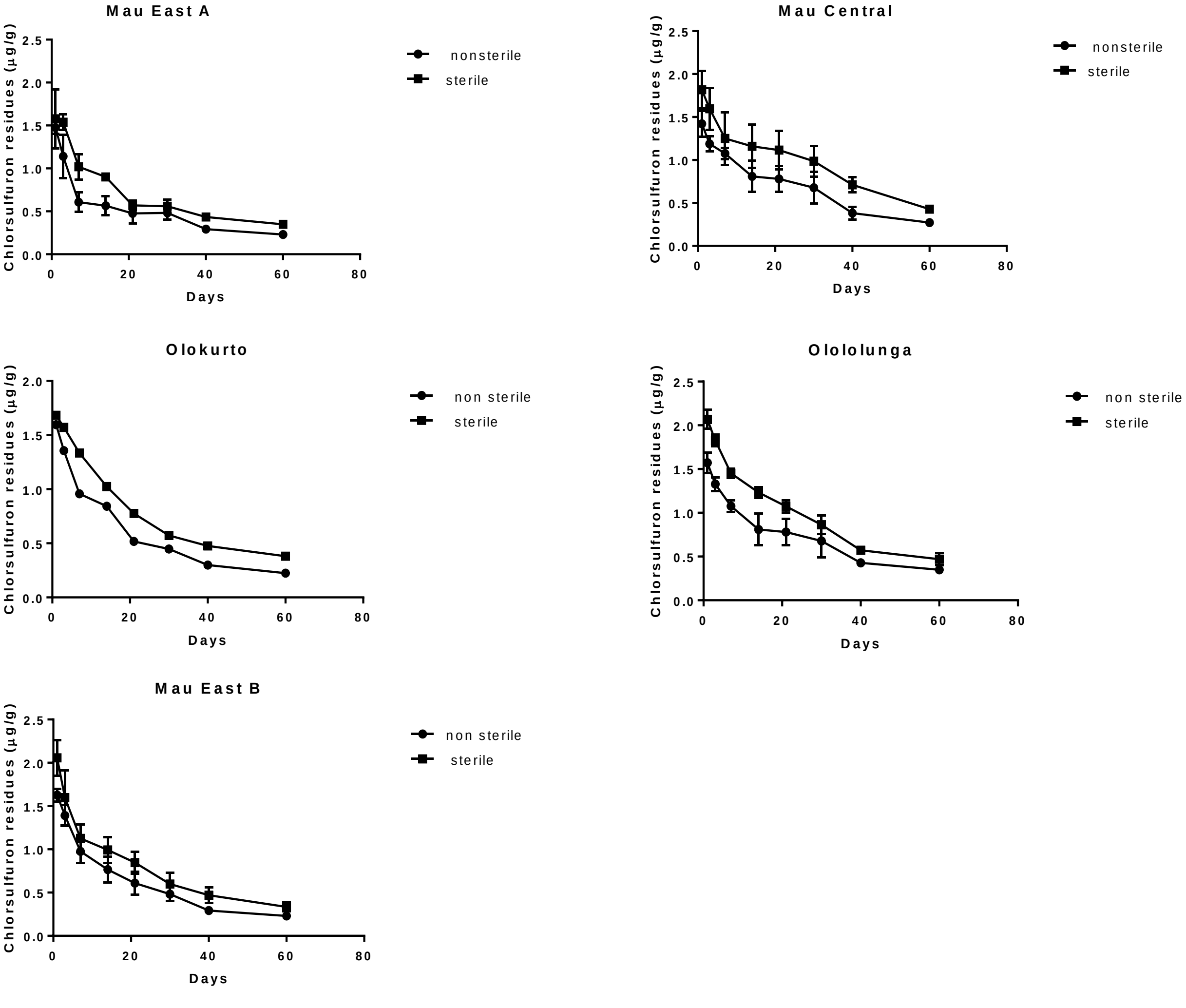

**Figure 9**: Chlorsulfuron degradation in sterile and non-sterile soils from wheat growing soils of the Mara River Basin.

Although the degradation patterns were similar for both soils, the mechanisms of degradation were different. The initial rapid degradation for the sterile soils could be attributed to chemical hydrolysis enhanced by the water content in the soils (Senseman, 2007). For the non-sterile soils the initial rapid degradation could be attributed to biotic degradation of the herbicide together with chemical hydrolysis in the soils. The second slower phase has been attributed to decrease in availability of chlorsulfuron to soil microbes due to adsorption or movement to protected compartments (Strek, 1998), where chemical hydrolysis continues at a slower rate than microbial degradation rate (Duffy *et al.*, 1993). Thus abiotic degradation mechanisms including hydrolysis contributed to the fast degradation rate in non-sterile soils.

Chlorsulfuron dissipation in sterile and non-sterile soils was significantly ($p \leq 0.05$) different in all locations (Table 9). This difference is attributed to the different soil physicochemical parameters in all the locations which affect the dissipation/ degradation of sulfonylureas in soil. The significant ($p \leq 0.05$) interaction between chlorsulfuron concentration in type of soil and days of sampling indicated that the pattern of change in chlorsulfuron concentration was not uniform in all the locations.

**Table 9**: Effect of soil sterility, location and time on the degradation of chlorsulfuron ($\mu g\ g^{-1}$) in soils within Mara River Basin

| Site | Type | Days | | | | | | | | Mean Type | Mean Site | C.V (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mau East A | | 0 | 1 | 3 | 7 | 14 | 21 | 30 | 60 | | | |
| | Non sterile | 1.97 | 1.14 | 0.61 | 0.57 | 0.47 | 0.48 | 0.29 | 0.23 | 0.66 | 0.79 | 20.46 |
| | Sterile | 1.91 | 1.54 | 1.02 | 0.90 | 0.57 | 0.56 | 0.43 | 0.35 | 0.91 | | |
| | Mean days | 1.94 | 1.34 | 0.81 | 0.73 | 0.52 | 0.52 | 0.36 | 0.29 | | | |
| | L.S.D (P≤0.05) | | | | | 0.18 | | | | 0.29 | | |
| Mau East B | Non sterile | 1.92 | 1.18 | 1.02 | 0.81 | 0.71 | 0.68 | 0.38 | 0.27 | 0.83 | 0.98 | 17.88 |
| | Sterile | 1.92 | 1.60 | 1.25 | 1.16 | 1.12 | 0.99 | 0.71 | 0.43 | 1.13 | | |
| | Mean days | 1.92 | 1.39 | 1.16 | 0.98 | 0.95 | 0.83 | 0.55 | 0.35 | | | |
| | L.S.D (P≤0.05) | | | | | 0.19 | | | | 0.32 | | |
| Olokurto | Non sterile | 1.90 | 1.36 | 0.96 | 0.84 | 0.52 | 0.45 | 0.30 | 0.22 | 0.78 | 0.88 | 5.99 |
| | Sterile | 1.98 | 1.57 | 1.33 | 1.02 | 0.78 | 0.57 | 0.48 | 0.38 | 0.98 | | |
| | Mean days | 1.94 | 1.46 | 1.15 | 0.93 | 0.65 | 0.51 | 0.39 | 0.30 | | | |
| | L.S.D (P≤0.05) | | | | | 0.06 | | | | 0.10 | | |
| Ololulunga | Non sterile | 1.97 | 1.33 | 1.08 | 0.84 | 0.78 | 0.68 | 0.43 | 0.35 | 0.88 | 1.04 | 10.34 |
| | Sterile | 1.91 | 1.83 | 1.45 | 1.23 | 1.07 | 0.86 | 0.57 | 0.47 | 1.19 | | |
| | Mean days | 1.94 | 1.58 | 1.26 | 1.03 | 0.93 | 0.77 | 0.50 | 0.41 | | | |
| | L.S.D (P≤0.05) | | | | | 0.12 | | | | 0.20 | | |
| Mau Central | Non sterile | 1.94 | 0.61 | 0.57 | 0.48 | 0.48 | 0.29 | 0.23 | 0.23 | 0.50 | 0.753 | 19.69 |
| | Sterile | 1.96 | 1.60 | 1.13 | 0.99 | 0.85 | 0.60 | 0.47 | 0.33 | 1.00 | | |
| | Mean days | 1.95 | 1.10 | 0.85 | 0.73 | 0.67 | 0.45 | 0.35 | 0.28 | | | |
| | L.S.D (P≤0.05) | | | | | 0.16 | | | | 0.27 | | |
| All sites | Non sterile | 1.94 | 1.12 | 0.86 | 0.71 | 0.61 | 0.52 | 0.33 | 0.26 | 0.73 | | |
| | Sterile | 1.91 | 1.63 | 1.24 | 1.06 | 0.88 | 0.72 | 0.53 | 0.39 | 1.04 | | |
| | Mean days | 1.93 | 1.38 | 1.06 | 0.88 | 0.74 | 0.62 | 0.43 | 0.33 | | | |
| | L.S.D (P≤0.05) | | | | | 0.07 | | | | 0.11 | 0.06 | 15.36 |
| | Interactions: SxT=0.08, SxD= 0.13, TxD= 0.09, SxTxD= NS | | | | | | | | | | | |

Chlorsulfuron degradation in all the soils was described with the pseudo first order kinetics with the rate constants *k* ranging from - $2.5 \times 10^{-2}$ to $-3.4 \times 10^{-2}$ $day^{-1}$ for the non-sterilized soils and $2.2 \times 10^{-2}$ to $2.9 \times 10^{-2}$ $day^{-1}$ for the sterilized soils (Table 10).

**Table 10**: Pseudo first order regression coefficient, rate constant and half-life of chlorsulfuron in sterile and non-sterile soils.

| **Soil** | **Type** | **$r^2$** | **K ($days^{-1}$)** | **Half-life (days)** |
|---|---|---|---|---|
| Mau East A | Non sterile | 0.7806 | -0.028 ± 0.003 | 24.75 |
| | Sterile | 0.8630 | -0.026 ± 0.002 | 26.65 |
| Mau Central | Non sterile | 0.9050 | -0.027 ± 0.002 | 25.67 |
| | Sterile | 0.8598 | -0.022 ± 0.001 | 31.50 |
| Olokurto | Non sterile | 0.9307 | -0.034 ± 0.002 | 20.38 |
| | Sterile | 0.9267 | -0.027 ± 0.001 | 25.67 |
| Ololollunga | Non sterile | 0.8828 | -0.026 ± 0.002 | 26.65 |
| | sterile | 0.9426 | -0.025 ± 0.001 | 27.72 |
| Mau East B | Non sterile | 0.9101 | -0.033 ± 0.002 | 21.00 |
| | Sterile | 0.8893 | -0.029 ± 0.002 | 23.89 |

Generally, the degradation rates were faster in the non-sterilized soils than in the sterilized for all the locations. This observation is attributed to the absence of microorganisms in the sterilized soils therefore implying the reducing chlorsulfuron concentrations was mainly through chemical degradation and volatilization while in the non-sterilized soils chlorsulfuron dissipation was a combination of microbial and chemical degradation. The estimated half-lives ranged from 20.38 to 26.65 days for the non-sterilized soils with Olokurto soils having the shortest soil half-life ($t_{1/2}$ = 20.38 days) and Ololollunga ($t_{1/2}$ = 26.65) days having the longest for the 60 day exposure data. In the sterilized soils, the soil half-life was between 23.89 and 31.50 days with soil from Mau East B having the shortest soil half-life ($t_{1/2}$ = 23.89) days while soils from Mau Central ($t_{1/2}$ = 31.50) days having the longest half-life for the 60 days exposure.

Degradation of chlorsulfuron in the sterilized soils could be mainly through hydrolysis. The estimated first order hydrolysis half-lives in this study were found to compare well those reported in other studies at almost similar experimental conditions. Chlorsulfuron had a half-life of 20 days in a silt loam soil (Strek, 1998) and a half-life of 27 days (Hemmamda *et al.*, 1994), at pH 5 and 25°C. On the contrary, hydrolysis half-life for chlorsulfuron was 1 day at pH 3 at 25 °C (Beyer *et al.*, 1987) and >500 days at pH 7.5 (Strek, 1998). These differences in the reported half-lives are due to the different pH in the studies. At low pH, chemical hydrolysis of sulfonylurea herbicides is faster and this decreases with increase in pH (Curran, 2001).

Microbial degradation of chlorsulfuron has been reported in different soils. Soil sterilization was reported to significantly reduce the breakdown of chlorsulfuron in soils (Joshi *et al.*, 1985; Grey and McCullough, 2012). Similarly, chlorsulfuron degraded faster in surface soils where there was more microbial biomass than in the sub surface soils (Anderson *et al.*, 2001). Ponia *et al.*, (2003) reported half-lives of 93 days in non -sterile soils and 120 days in sterile soils at pH 8.2 and 25°C in Indian soils, values that are much higher than those from this study and could be attributed to different soil-physicochemical characteristics and environmental factors. This large variation could be due to the alkaline soil pH in the Indian soil which does not favour the degradation of chlorsulfuron. These results mean that for wheat growing soils within the Mara basin, microorganisms play a role in the degradation of chlorsulfuron. This also implies that soils with fewer soil microbes, the degradation of chlorsulfuron would be slower therefore raising potential for carryover and leaching.

### 4.4.2 Effect of repeated application and location on the degradation of chlorsulfuron

Biodegradation of a herbicide is said to be enhanced when it is more rapid in soils previously treated than in soils not previously treated with the herbicide (Rouchaud *et al.*, 2000). High herbicide doses and repeated applications over a long period are favourable for generating accelerated biodegradation due to adaptation of the microbes on the herbicides as their source of energy. This may reduce herbicide efficiency, though on the other hand, it decreases the concern about the accumulation of herbicide in soil after repeated past applications (Hole *et al.*, 2001). The pattern of chlorsulfuron degradation in the five soil types as affected by history of application is as shown in Figure 10.

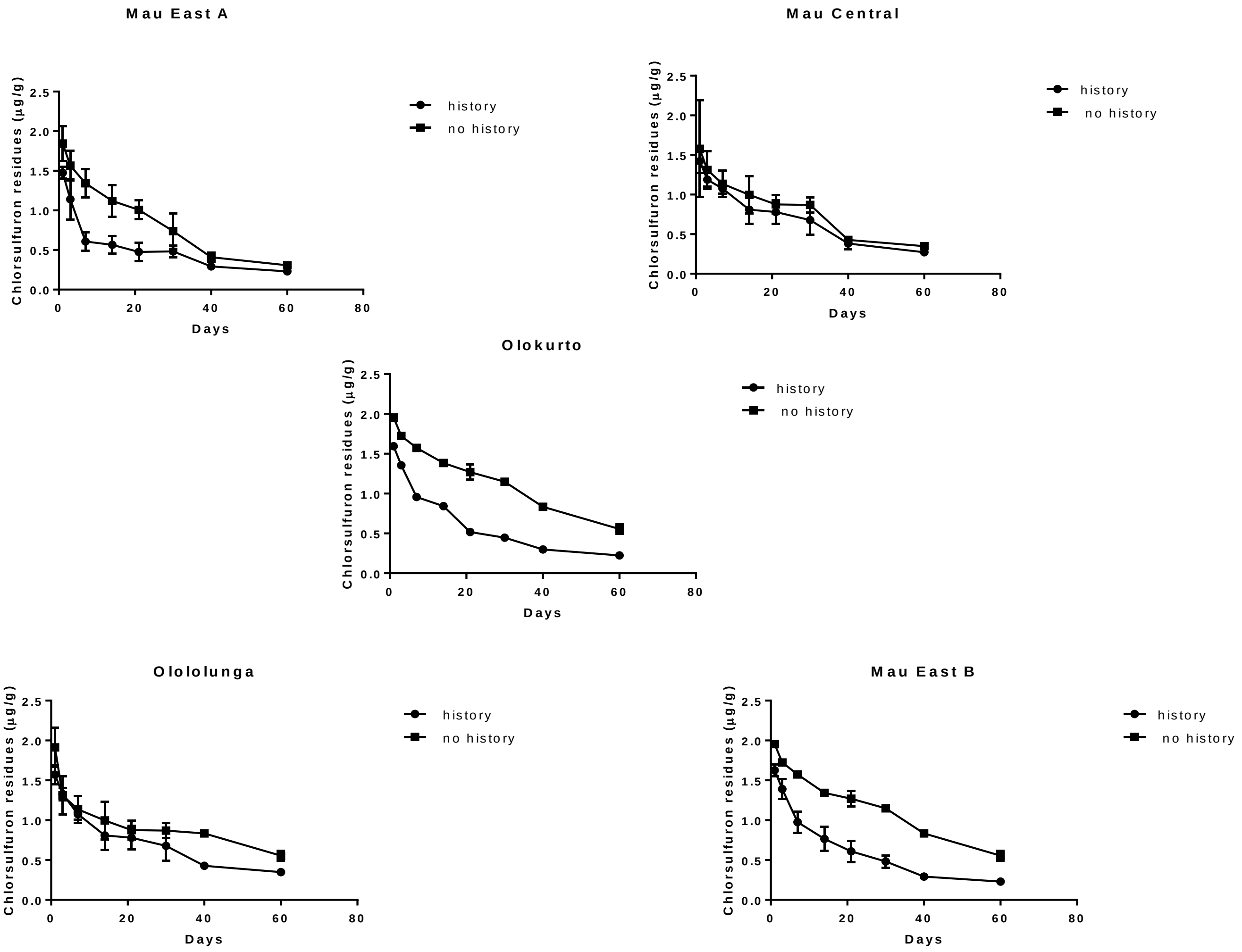

**Figure 10**: Chlorsulfuron degradation in the five soil types with and without history of application

Chlorsulfuron degraded faster in soils with history of previous application in all the soil types with up to 94% degradation for soils from Mau East A, Olokurto and Mau East B, as compared to 86% in soils without history of application from Olololunga and Mau East B after 60 days application. This increase in degradation could be caused by adaptation of the soil microbes to chlorsulfuron as a source of carbon and energy due to repeated applications. This adaptation increases the degradation activity of a limited number of microorganisms initially present due to selective gene expression or an increase in the number of degraders initially present due to microbial growth and/or lateral gene transfer and the evolution of the new enzymes necessary for use of chlorsulfuron as an energy source (Arbeli and Fuentes, 2007; Hugo *et al.*, 2014). In addition, since the degradation rate and the extent of microbial growth during degradation is influenced by the structure of the pesticide structurally simple pesticides with high water solubility and low adsorptivity can easily support microbial growth thus develop accelerated degradation more quickly, sometimes even after a single application (Arbeli and Fuentes, 2007). Chlorsulfuron has high water solubility (Table 1) and showed low sorption on the soils used in this study, therefore it is highly susceptible to accelerated degradation as it is bioavailable.

The half-life of chlorsulfuron in soils with history of application was significantly lower than for the soils without history as shown in Table 11.

**Table 11**: Linear regression coefficient, rate constant and half-life of chlorsulfuron in soils with single and repeated application.

| Soil | Application | $r^2$ | K ($days^{-1}$) | Half-life (days) |
|---|---|---|---|---|
| Mau East A | Single | 0.7806 | -0.028 ± 0.003 | 24.75 |
| | Repeated | 0.9198 | -0.031 ± 0.002 | 22.42 |
| Mau Central | Single | 0.9050 | -0.024 ± 0.002 | 28.88 |
| | Repeated | 0.8455 | -0.027 ± 0.002 | 25.67 |
| Olokurto | Single | 0.9307 | -0.020 ± 0.001 | 34.65 |
| | Repeated | 0.9714 | -0.034 ± 0.002 | 20.38 |
| Olololunga | Single | 0.8828 | -0.016 ± 0.002 | 43.31 |
| | Repeated | 0.7213 | -0.025 ± 0.002 | 27.72 |
| Mau East B | Single | 0.9101 | -0.020 ± 0.002 | 34.65 |
| | Repeated | 0.9690 | -0.033 ± 0.002 | 21.00 |

For the soils with history, the half-life was shortest in Olokurto and increased in the order Mau East B < Mau East A < Mau Central < Ololulunga. Generally soils from Ololulunga had the longest half-life in both soils with and without history of application, meaning that chlorsulfuron persistence can be more pronounced in this location as compared to the other locations regardless of the history of application.

Accelerated biodegradation has been observed for several herbicides. Persistence of ureas linuron (Rasmussen *et al.*, 2005), isopruturon and the acetanilides metalachlor (Liu *et al.*, 1988) and alachlor was slightly enhanced by prior applications. Diuron, chlorotoluron and simazine showed significant enhanced biodegradation when applied repeatedly on pear tree orchards (Rouchaud *et al.*, 2000). Similarly, carbetamide degradation was greatly enhanced by repeated applications (Hole *et al.*, 2001). For sulfonylureas there are limited reports on enhanced biodegradation. However, repeated application of chlorsulfuron accelerated its biodegradation in three Brazilian oxisols (Ravelli *et al.*, 1997) although the variation in terms of half-lives was not reported. These results suggest that repeated application of chlorsulfuron on the wheat within the Mara river basin may lead to accelerated degradation of the herbicide therefore if applied as pre-emergent then it may lead to loss in its efficacy for weed protection.

Significant ($p \leq 0.05$) variations were noted for chlorsulfuron degradation in the soils with history of application and those without for all the soil types (Table 12).

**Table 12**: Effect of history of application, location of wheat production and day of sampling on degradation of chlorsulfuron (µg $g^{-1}$)

| Site (S) | Type (T) | Days (D) | | | | | | | | Mean Type | Mean Site | C.V (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 3 | 7 | 14 | 21 | 30 | 60 | | | |
| Mau East A | Repeated | 1.841 | 1.141 | 0.608 | 0.566 | 0.476 | 0.483 | 0.294 | 0.231 | 0.659 | 0.851 | 17.32 |
| | Single | 1.874 | 1.566 | 1.343 | 1.120 | 1.008 | 0.739 | 0.409 | 0.308 | 1.042 | | |
| | Mean Days | 1.858 | 1.354 | 0.975 | 0.843 | 0.742 | 0.611 | 0.351 | 0.269 | | | |
| L.S.D ( P≤0.05) | | | | | | 0.16 | | | | 0.27 | | |
| Mau East B | Repeated | 1.822 | 1.188 | 1.077 | 0.808 | 0.780 | 0.678 | 0.383 | 0.271 | 0.826 | 0.885 | 23.21 |
| | Single | 1.879 | 1.311 | 1.136 | 0.997 | 0.877 | 0.870 | 0.428 | 0.350 | 0.940 | | |
| | Mean Days | 1.851 | 1.249 | 1.106 | 0.902 | 0.828 | 0.774 | 0.405 | 0.310 | | | |
| L.S.D ( P≤0.05) | | | | | | 0.22 | | | | NS | | |
| Ololunga | Repeated | 1.854 | 1.355 | 0.958 | 0.843 | 0.517 | 0.447 | 0.229 | 0.224 | 0.780 | 1.043 | 4.14 |
| | Single | 1.866 | 1.723 | 1.573 | 1.384 | 1.269 | 1.149 | 0.834 | 0.556 | 1.305 | | |
| | Mean Days | 1.860 | 1.539 | 1.265 | 1.113 | 0.893 | 0.798 | 0.567 | 0.390 | | | |
| L.S.D ( P≤0.05) | | | | | | 0.05 | | | | 0.08 | | |
| Olokurto | Repeated | 1.813 | 1.311 | 1.077 | 0.808 | 0.708 | 0.678 | 0.428 | 0.350 | 0.878 | 0.970 | 15.20 |
| | Single | 1.871 | 1.328 | 1.136 | 0.997 | 0.876 | 0.870 | 0.835 | 0.556 | 1.062 | | |
| | Mean Days | 1.842 | 1.320 | 1.106 | 0.902 | 0.828 | 0.774 | 0.631 | 0.453 | | | |
| L.S.D ( P≤0.05) | | | | | | 0.16 | | | | 0.27 | | |
| Mau Central | Repeated | 1.854 | 1.141 | 0.607 | 0.566 | 0.476 | 0.483 | 0.293 | 0.230 | 0.659 | | |
| | Single | 1.877 | 1.723 | 1.573 | 1.342 | 1.169 | 1.149 | 0.835 | 0.556 | 1.288 | 0.973 | 9.35 |
| | Mean Days | 1.815 | 1.432 | 1.090 | 0.954 | 0.822 | 0.816 | 0.564 | 0.393 | | | |
| L.S.D ( P≤0.05) | | | | | | 0.09 | | | | 0.17 | | |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| All 5 sites | Repeated | 1.848 | 1.124 | 0.857 | 0.706 | 0.607 | 0.516 | 0.327 | 0.261 | 0.730 |  | 14.65 |
|  | Single | 1.862 | 1.626 | 1.236 | 1.062 | 0.877 | 0.716 | 0.532 | 0.392 | 1.043 |  |  |
|  | Mean | 1.855 | 1.375 | 1.046 | 0.884 | 0.742 | 0.616 | 0.429 | 0.327 |  |  |  |
|  | Days |  |  |  |  |  |  |  |  |  |  |  |
| L.S.D ( P≤0.05) |  |  |  |  |  | 0.07 |  |  |  | 0.11 | 0.06 |  |

Interactions: SxT= 0.09, SxD= NS, SxTxD= 0.19

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Repeated application means after two successive applications simulated over 60 days

These variations were as a result of the soil physico-chemical parameters which differed from one location to the other. Since the degradation of herbicides in soils is influenced by these parameters, it is expected that the degradation pattern would vary from location to location. Similarly, the amount of chlorsulfuron decreased significantly ($p \leq 0.05$) with the days of sampling for both soils with and without history of application. There was significant ($p \leq 0.05$) interaction effect between location of production and type of soil implying that the pattern of chlorsulfuron degradation with respect to history of application was not uniform.

### 4.4.3 Chlorsulfuron degradation products in wheat growing soils of the Mara River Basin

The most common pathways of degradation of sulfonylurea herbicides are chemical hydrolysis and microbial degradation (Beyer *et al.*, 1988). In acidic soils, chemical hydrolysis is the major degradative process for the majority of sulfonylurea herbicides (Bossi *et al.*, 1999) where the sulfonylurea bridge breaks into sulfonamide and s-triazine derivatives (Fig.2). In alkaline soils, microbial degradation will play the more important role as soil pH is greater than the pKa of the compound and it is the associated form of the molecule that is mainly subject to microbial attack (Hemmamda *et al.*, 1994). In the wheat growing soils from Mara River Basin two degradation products were detected from chlorsulfuron biodegradation study. The metabolite with tR = 3.05 min showed an $[M + H]^+$ ion at m/z 141 and was identified as 2-amino-4-methoxy-6-methyltriazine **product 1** (Figure 11). The LC-MS positive ion analysis of the second metabolite with tR = 10.04 min showed a $[M + H]^+$ ion at m/z 191 and was identified as 2- chlorobenzenesulfonamide (Product 2) (Figure 12).

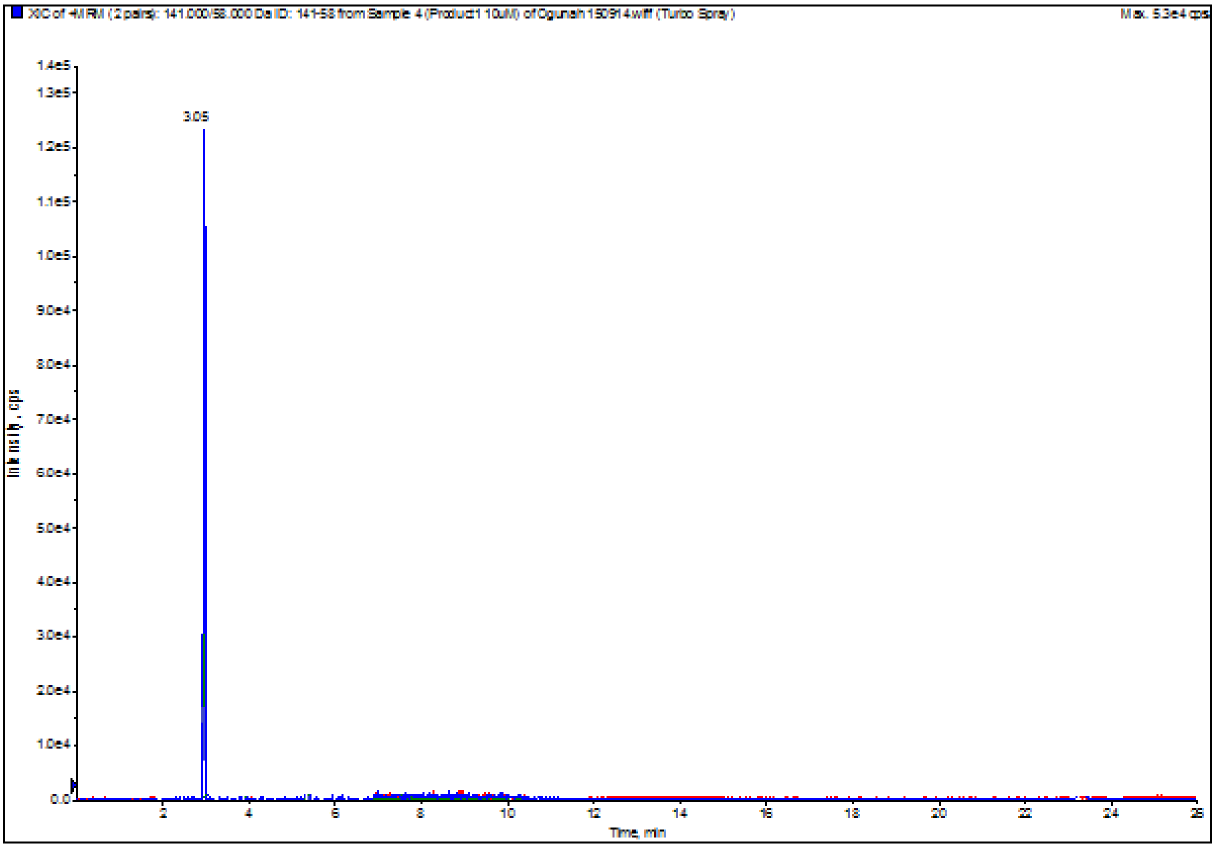

**Figure 11**: HPLC chromatogram of 2-amino-4-methoxy-6-methyltriazine standard

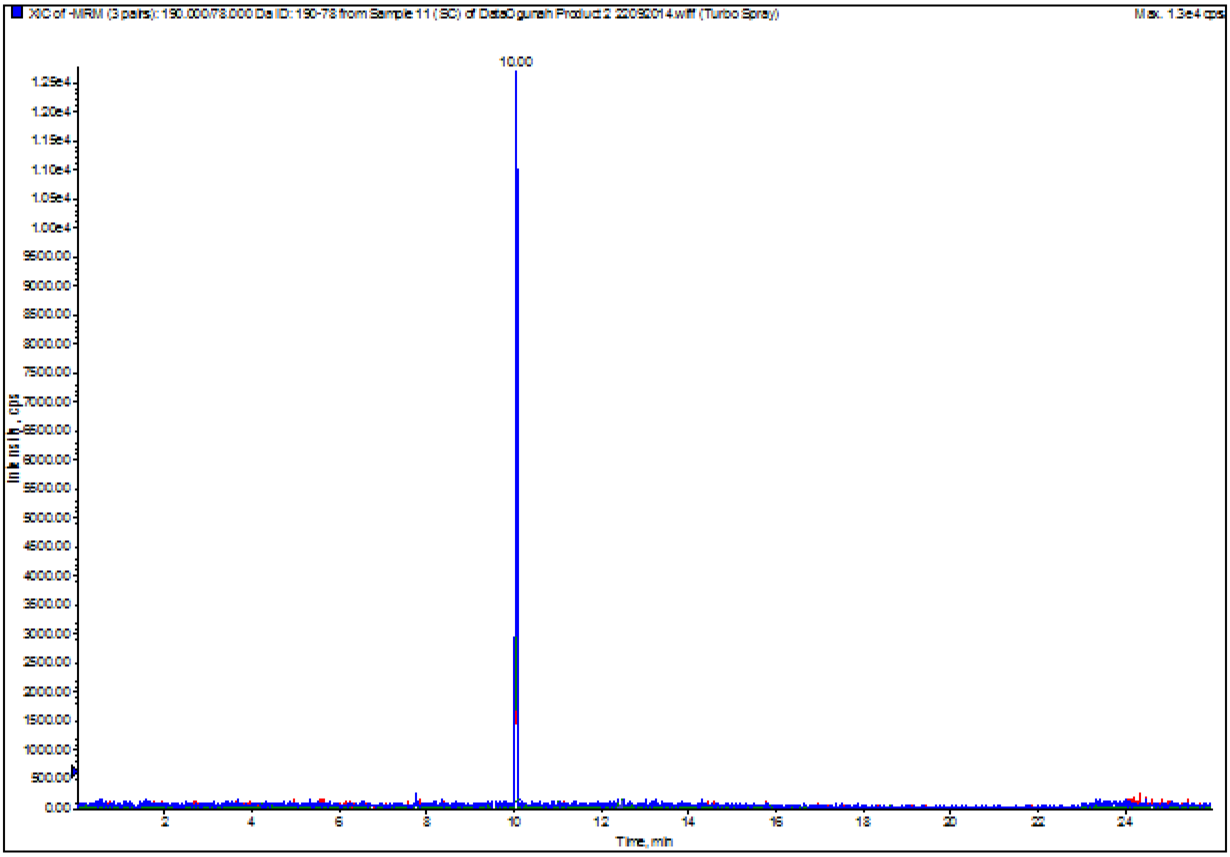

**Figure 12**: HPLC chromatogram of 2- chlorobenzenesulfonamide standard

These two degradation products were detected in both sterile and non-sterile soils indicating that both hydrolysis and biodegradation processes yielded the same products. After seven days of incubation, product 1 was detected in all the soils while product 2 was only detected in soil Mau East B (Appendix II to V). Chlorsulfuron hydrolysis in soils occurs through an acid catalysed mechanism involving protonation of the carbonyl oxygen initially (Larson and Weber, 1994), producing carbon dioxide, 2-amino-4-methoxy-6-methyltriazine, and 2-chlorobenzenesulfonamide (Figure 13).

Generally, the sulfonyl group in chlorsulfuron enhances the acidity of the proton on the adjacent nitrogen atom. Deprotonation of the sulfonylurea bridge results in a distribution of the negative charge throughout the sulfonylurea moiety (Figure 13 ), which hinders the direct nucleophilic attack of $H_2O$ or -OH to the carbonyl group by making the carbon atom less electrophilic (Zheng *et al.*, 2008).

These two metabolites have been reported as chlorsulfuron biodegradation products by *Aspergillus niger* in Spanish soils (Boschin *et al.*, 2003) and in sandy soils (Bossi *et al.*, 1999). Other studies have reported the two products from chemical hydrolysis (Ye *et al.*, 2004; Strek *et al.*, 1998).

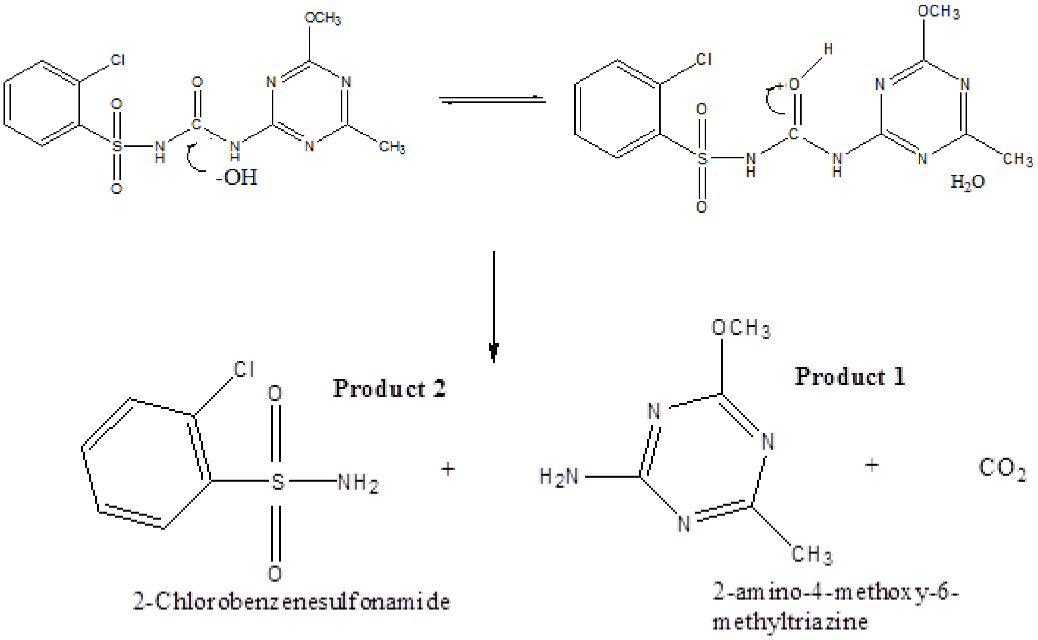

**Figure 13**: Mechanism of acid catalyzed hydrolysis of chlorsulfuron

### 4.5 Chlorsulfuron field experiments

### 4.5.1 Field dissipation of chlorsulfuron

Chlorsulfuron was applied at an application rate of 30 g a.i ha$^{-1}$ and the residues monitored up to harvest time (112 days). The dissipation of chlorsulfuron exhibited two phases in all soils with initial

rapid dissipation in seven days after treatment (DAT). Thereafter the dissipation was much slower up to 60 DAT as shown in Figure 14.

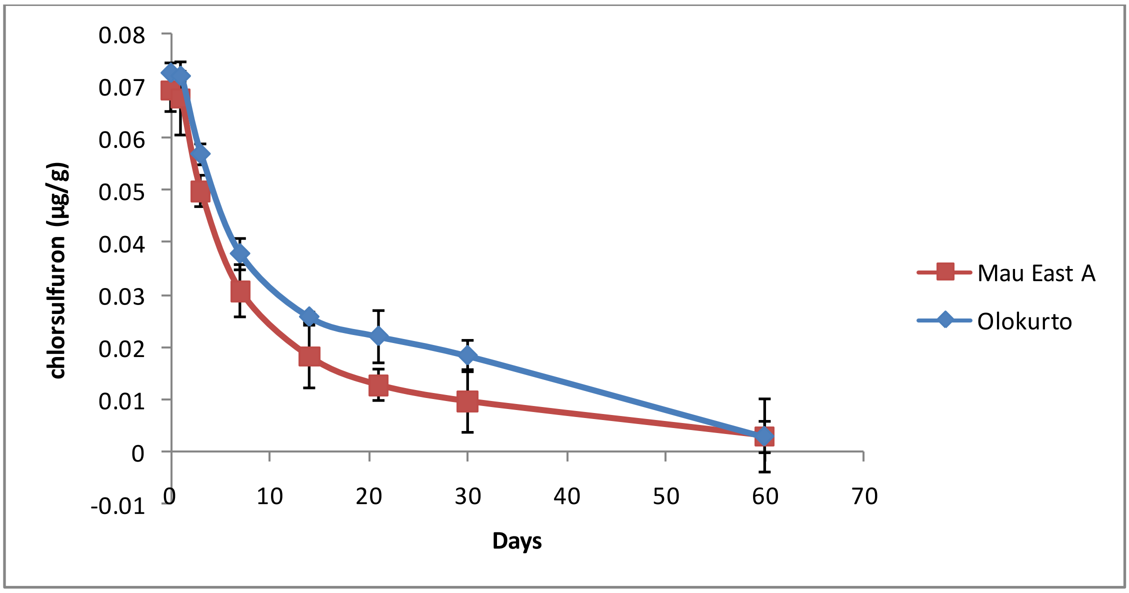

**Figure 14**: Chlorsulfuron field dissipation in Olokurto and Mau East in the Mara River Basin

In both soils, chlorsulfuron dissipated faster and was not detected after 112 days. The dissipation was faster in Mau East A with about 90% as compared to 81% in Olokurto 30 days after treatment (Table 13).

**Table 13**: Mean concentration of chlorsulfuron recovered in field soils (µgg$^{-1}$) with time.

| **Days** | **Olokurto** | **% dissipation** | **Mau East A** | **% dissipation** |
|---|---|---|---|---|
| **0** | 0.073 ± 0.021 | 27.3 | 0.069 ± 0.018 | 30.8 |
| **1** | 0.072 ± 0.011 | 28.1 | 0.068 ± 0.007 | 32.3 |
| **3** | 0.057 ± 0.032 | 43.0 | 0.050 ± 0.013 | 50.0 |
| **7** | 0.038 ± 0.009 | 62.1 | 0.031 ± 0.020 | 69.1 |
| **14** | 0.026 ± 0.010 | 74.2 | 0.018 ± 0.016 | 81.7 |
| **21** | 0.022 ± 0.015 | 77.9 | 0.013 ± 0.007 | 87.1 |
| **30** | 0.018 ± 0.013 | 81.6 | 0.010 ±0.006 | 90.2 |
| **60** | 0.003 ± 0.009 | 97.1 | 0.003 ± 0.013 | 96.8 |
| **112** | BDL | | BDL | |

This difference was due to the difference in rainfall and temperature conditions in the two regions (Appendices VI and VII). Olokurto had more rainfall amounts in the first 30 days and therefore the soils had higher moisture content which favoured faster degradation of chlorsulfuron (Sarmah *et al.*, 1999). Two stage degradation of chlorsulfuron has also been reported in Indiana soils (Strek, 1998) and was attributed to high rainfall and temperature conditions in the initial days after treatment as compared to the latter days.

The dissipation followed pseudo-first-order kinetics with half-lives of 13.5 and 13.6 days for Olokurto and Mau East A, respectively, lower than 20.38 and 24.75 days found for the degradation under laboratory conditions as shown in Table 14.

**Table 14:** Half-life, correlation coefficient and regression equation of chlorsulfuron in the wheat growing fields

| **Soil** | **$DT_{50}$ (days)** | **$r^2$** | **K ($days^{-1}$)** |
|---|---|---|---|
| **Field experiment** | | | |
| Olokurto | 13.5 | 0.98 | 0.0513 |
| Mau East | 13.6 | 0.93 | 0.0511 |
| **Laboratory** | | | |
| Olokurto | 20.38 | 0.93 | 0.028 |
| Mau East | 24.75 | 0.78 | 0.034 |

The difference in the dissipation half-lives between the field and laboratory studies may be a consequence of the relatively high temperatures at these sites, where the daily soil temperature maxima averaged 26°C for days 0 to 30 DAT and 23°C for days 30 to 60 DAT (see Appendix VII) compared to 20°C for the laboratory experiments. The high temperatures, coupled with an abundance of soil water (the sites received a total of 628 mm and 547 mm precipitation for Olokurto and Mau East respectively). This probably resulted in enhanced aqueous hydrolysis of chlorsulfuron before significant diffusion to sites of lesser reactivity or lesser availability to microbes could occur (Strek, 1998). In general, other environmental factors contributed such as volatilization, rainfall and solar radiation intensity during the day. The observed field half-lives compared well with the Kolupaeva and Gorbatov, (2001) who reported a $DT_{50}$ of 10 days in soils of pH 5.4. However the half-lives were much higher in American soils with half-lives of 39 to 192 days ( pH 6.9 to 8.0) and 18 days in pH 6.1 (Strek, 1998). This difference could be due to the higher soil pH and other environmental factors as compared to the Mara River Basin. The results imply that field dissipation of chlorsulfuron in Mara River Basin is fast and climatic conditions such as rainfall, volatilization rate and soil temperatures influence its dissipation pattern. The short half-life of chlorsulfuron means that it may not be carried over to the next planting season therefore affecting non target plants.

# CHAPTER FIVE

## 5.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This study was designed to investigate the sorption, leaching and degradation of chlorsulfuron herbicide in different wheat growing soils within the Mara River Basin, Kenya. Based on the results achieved, the following summary, conclusion and recommendations can be drawn:

### 5.1 Summary

1. The soil physicochemical parameters and nutrients varied significantly ($p \leq 0.05$) in all the five locations. The soils were mainly sandy loam and clay soils with pH below 6.0. Since chlorsulfuron dissipation depends on soil parameters these results implied that the pattern of chlorsulfuron dissipation would be different in the five locations.
2. Chlorsulfuron adsorption on the five soils was biphasic with an initial fast process followed by a much slower process. Chlorsulfuron sorption onto the five soils from the Mara river Basin was low as predicted by the low $K_d$ values ( 0.59 – 2.52). However, soils from Mau East A, Olololunga and Mau east B had lower $1/n_{des}$ than their respective $1/n_{ads}$, implying that chlorsulfuron adsorbed on these soils were not easily desorbed. This would reduce the efficacy of the herbicide for the target weed as well as the leaching potential of chlorsulfuron into the underground aquifers.
3. Copper ions increased the sorption of chlorsulfuron in all the five soils by slightly decreasing the soil solution pH which favours adsorption and by forming complexes with chlorsulfuron which were readily adsorbed. As a

consequence more chlorsulfuron would be bound to the soil and therefore reducing bioavailabilty for weed eradication.

4. Chlorsulfuron leached up to a depth of 30 cm in soil columns for all the soils. The leaching pattern varied significantly ($p \leq 0.05$) with location, depth of the soils and rate of application. However chlorsulfuron leached more in Mau East B soils as compared to other soils. This is due to its high pH and low organic matter content. This implies that groundwater aquifers in Mau East B have higher potential of being contaminated due to leaching of chlorsulfuron.
5. Chlorsulfuron degradation was faster non sterile soils as compared to the sterile soils as shown by shorter degradation half-lives. Similarly, chlorsulfuron degradation was faster in soils with repeated application of chlorsulfuron than soils that had single application of chlorsulfuron.
6. Chlorsulfuron dissipation in the field was faster with $DT_{50}$ of 13.5 and 13.6 days respectively for Olokurto and Mau East regions as compared to the laboratory incubations.

## 5.2 Conclusions

1. Chlorsulfuron dissipation within the five wheat growing regions is dependent on the soil physicochemical parameters of the soils on which it is applied. For all the soils it is positively influenced by soil organic matter while negatively correlated to the soil pH.
2. Chlorsulfuron adsorption desorption equilibrium kinetics was established after 12h. Chlorsulfuron was weakly adsorbed on to the wheat growing soils of the Mara River Basin therefore raising its leaching potential into the subsurface and ground waters.

3. Coexistence of copper ions and chlorsulfuron in soils increases the adsorption of chlorsulfuron which may reduce the bioavailability of the herbicide for weed elimination but reduce contamination of ground waters.
4. Chlorsulfuron was highly mobile in all the five soils and this raises concern about surface and underground water contamination. This high mobility also lowers the efficacy of the herbicide for the target weeds.
5. Biotic degradation has significant influence on the degradation of chlorsulfuron in soil. Similarly, repeated application of chlorsulfuron enhances its degradation in soils which implies that the herbicide may degrade too fast for effective action on weeds.
6. Chlorsulfuron dissipates faster in the field and it is influenced by rainfall and temperature conditions

## 5.3 Recommendations

1. Chlorsulfuron use should be regulated in soils with high organic carbon and low soil pH, since it is adsorbed more raising chances of persistence.
2. Use of copper foliar fertilizers on farms applied with chlorsulfuron should be discouraged since the fertilizers increase the adsorption of chlorsulfuron therefore reducing the bioavailability of the herbicide for weed action.
3. Repeated application of chlorsulfuron should be discouraged since it accelerates its degradation.

## 5.4 Suggestions for further studies

1. Joint effect of chlorsulfuron and copper ions on the plant at biochemical levels.

2. Identification of the microbes species degrading chlorsulfuron in soils within the Mara River Basin

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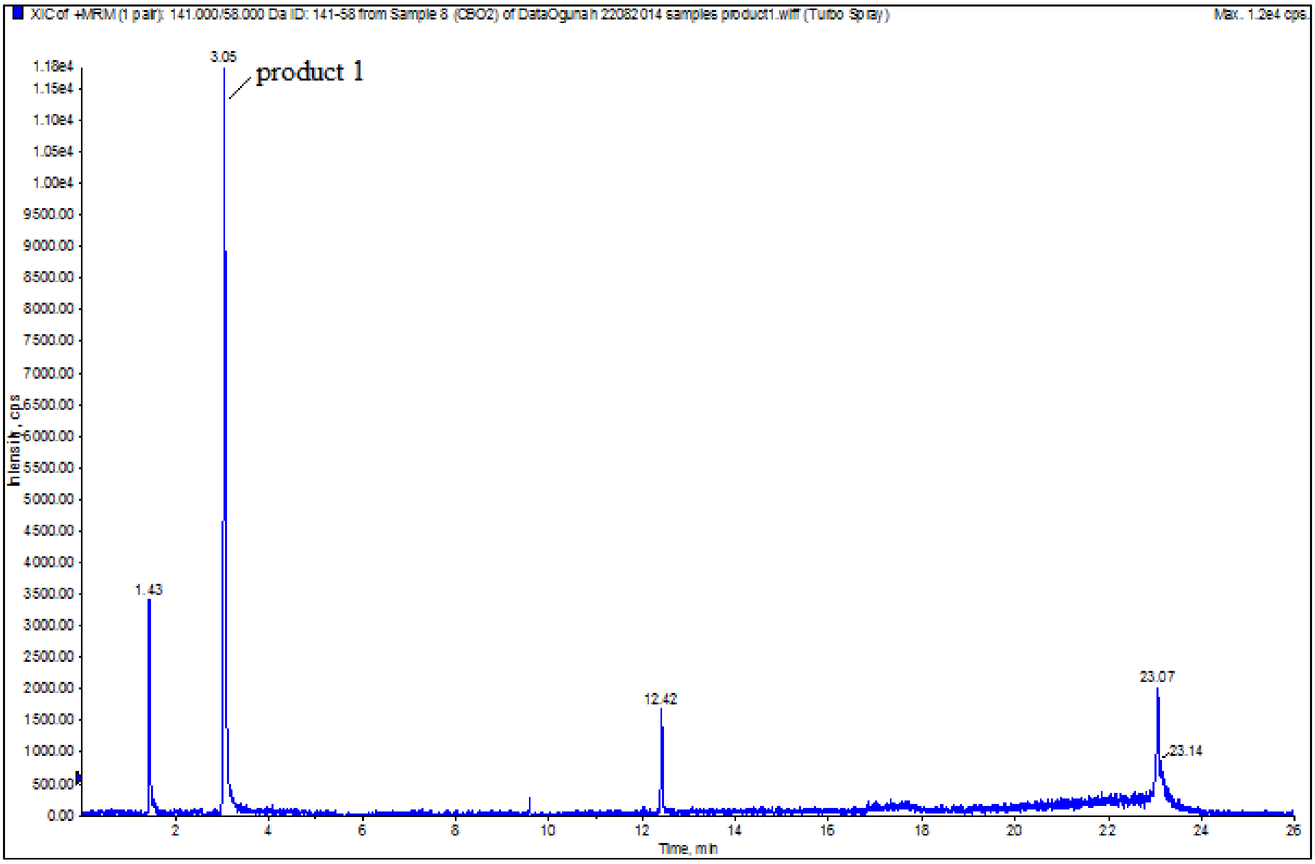
# APPENDIX

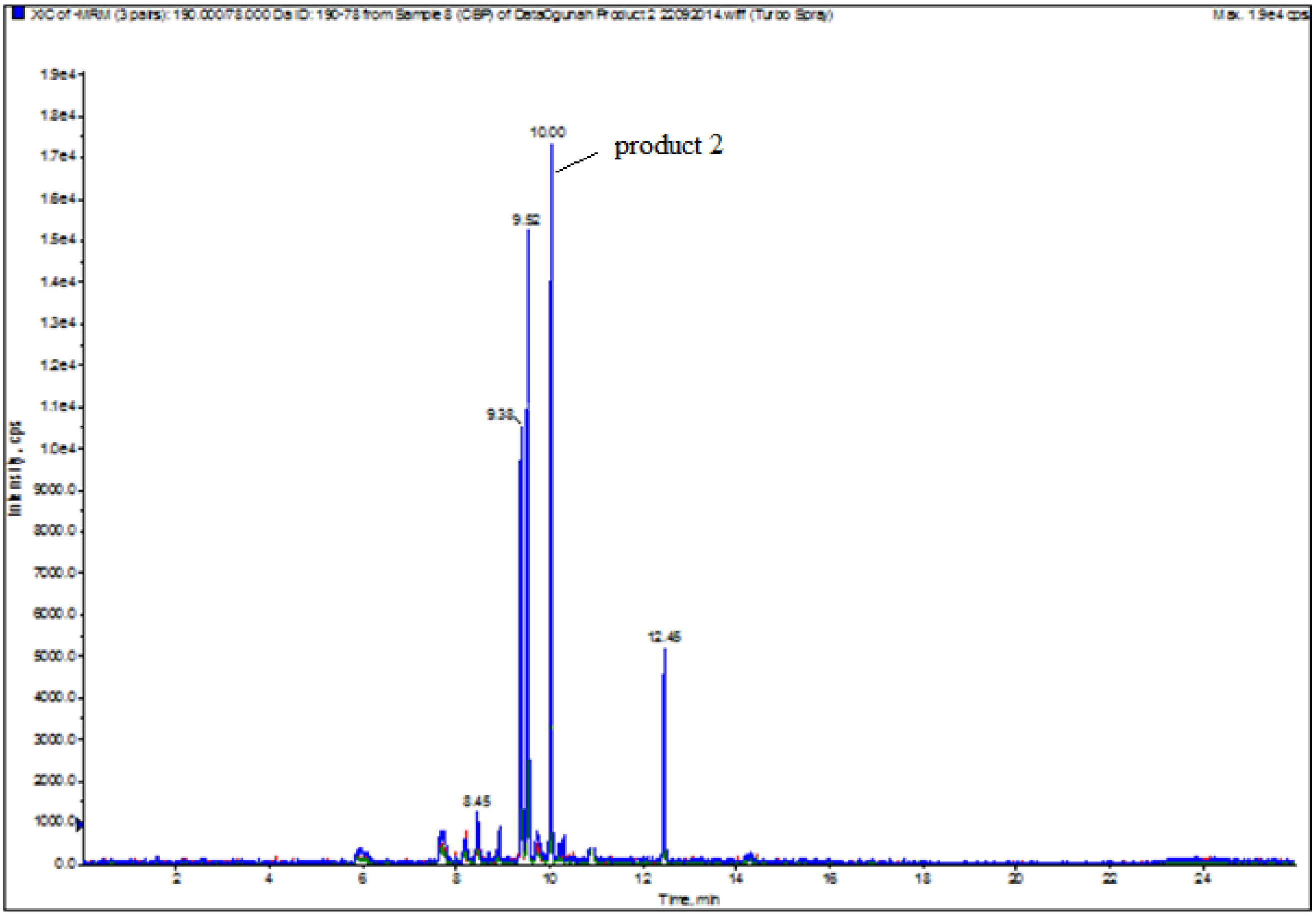
**Appendix I**: Langmuir adsorption isotherm regression coefficients

| **Site** | **$r^2$** |
|---|---|
| Mau East A | 0.5744 |
| Olokurto | 0.5895 |
| Mau East B | 0.5397 |
| Ololulunga | 0.6917 |
| Mau Central | 0.5236 |

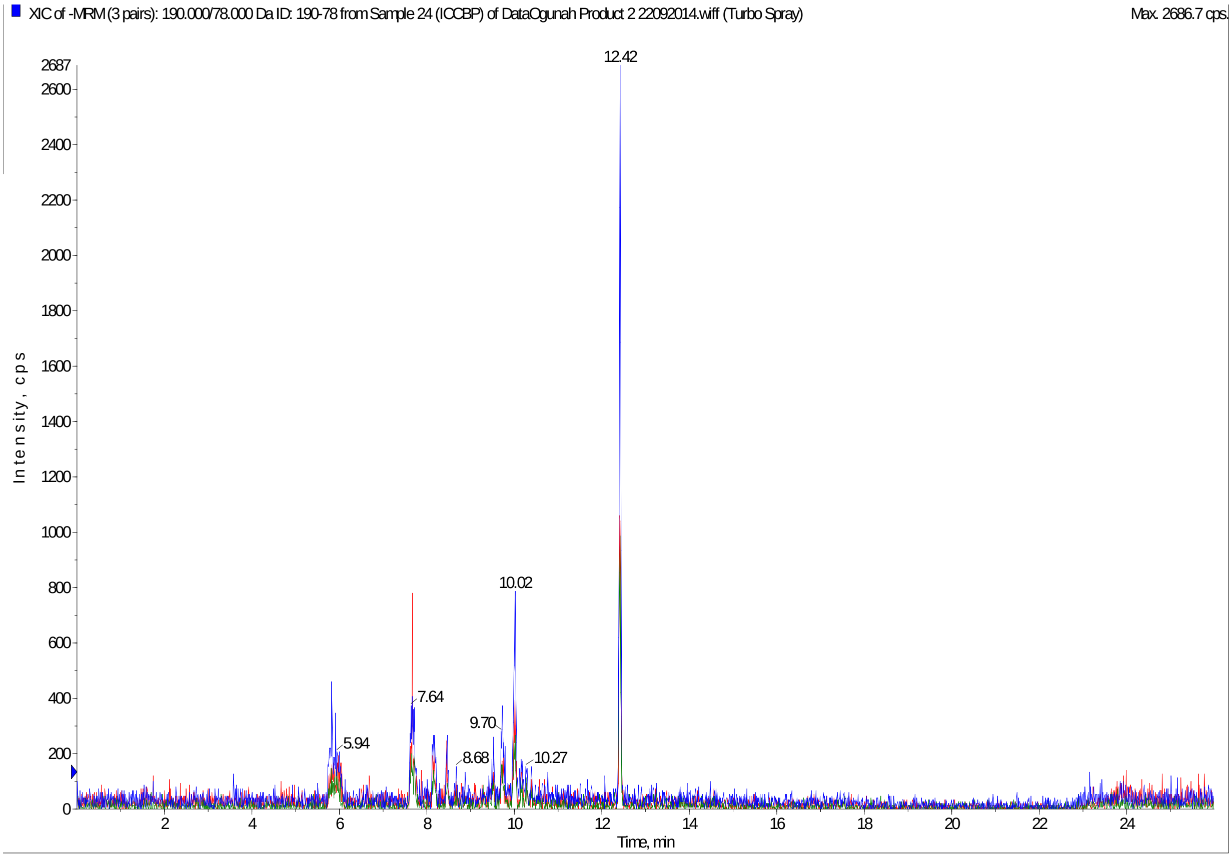
**Appendix II**: HPLC chromatogram for 2-amino-4-methoxy-6-methyltriazine



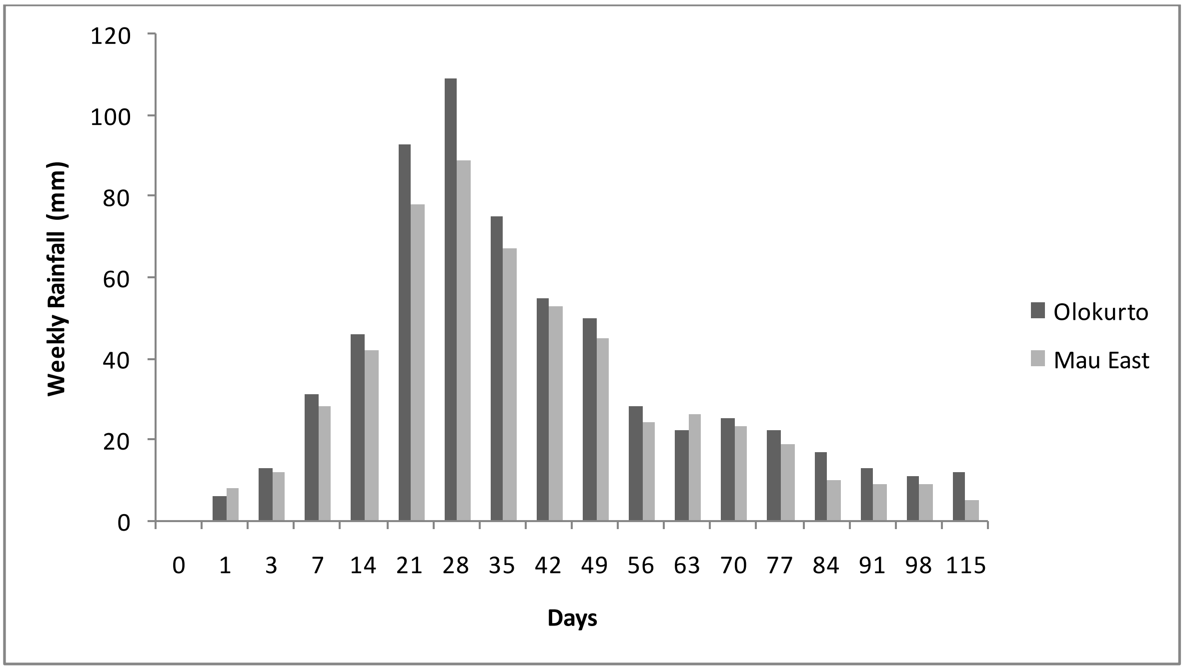
**Appendix III**: HPLC chromatogram for 2- chlorobenzenesulfonamide after 14 days



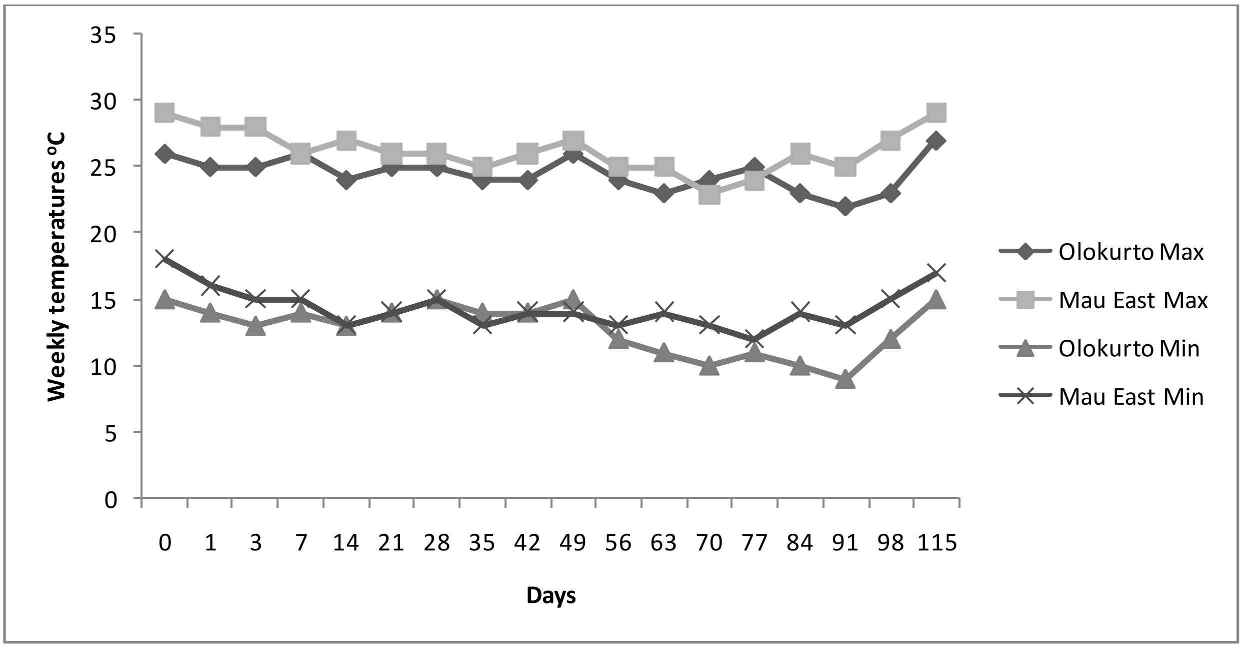
**Appendix IV**: HPLC chromatogram for 2- chlorobenzenesulfonamide after 30 days



**Appendix V**: Rainfall distribution in Olokurto and Mau East from April –July 2015



**Appendix VI**: Maxima and minima temperature in Olokurto and Mau East from April –July 2015



**Appendix VII**: Percent relative humidity in Olokurto and Mau east from April-July 2015