THE INFLUENCE OF GYPSIFEROUS WATER ON THE BEHAVIOUR OF SELECTED HERBICIDES

by

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August 2003

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LIST OF ABBREVIATIONS

ai - Active ingredient
α - Alpha
ARC - Agricultural Research Council
CV - Coefficient of variance
EC - Emmulsifiable concentrate
Df - Degree of freedom
ha - Hectares
LD_{50} - Median lethal dose: amount that kills 50% of the test organism
LSD - Least significant difference
MS - Mean square
ns - Not significant
% - Percentage
ppm - Parts per million
SC - Suspension concentrate formulation
* - Significant
** - Highly significant
SAS - Statistical Analysis System
g - gram
mg - milligram
l - litre

ABSTRACT

The influence of gypsiferous water on the behaviour of selected herbicides

by

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Gypsiferous water, i.e water containing high levels of calcium sulphate, is used to irrigate crops such as maize and wheat on a commercial scale, in some parts of South Africa due to shortage of irrigation water. Gypsiferous water is saline due to the presence of Ca^{2+}, $SO_4{}^{2-}$ and sometimes Mg^{2+} in solution. These salts may have a detrimental effect on herbicide performance. Possible herbicide injury to crops has been reported in areas irrigated with gypsiferous water, which suggested interaction between herbicides and gypsiferous water. Bioassay experiments were conducted in the greenhouse to assess whether the bioactivity and persistence of the selected herbicides were affected by the presence of gypsum in the soil. The biological activity of atrazine was significantly increased in the presence of gypsum, and in the case of 2,4-D the same effect was even more pronounced. In contrast, the activity of metolachlor was reduced in the presence of gypsum. These differential effects on herbicide activity would have important practical consequences for herbicide performance, i.e weed control efficacy, selectivity, and behaviour in soil. In the persistence experiment, the activity of all herbicides decreased with time. However, the presence of gypsum had a significant effect on the rate of dissipation of only atrazine. In the presence of gypsum the rate of loss of atrazine was significantly less compared to its rate of loss in soil that did not contain gypsum. The rate of loss of metolachlor was not significantly affected by the presence of gypsum, and in the case of 2,4-D the trends were not clear at all. A further experiment was conducted to determine the leaching potential of metolachlor and atrazine in soil irrigated with either gypsiferous water or uncontaminated water. In the leaching experiment metolachlor showed a significant growth reduction of 39% in the first 10 cm column zone in soil irrigated with gypsiferous water and no further losses were observed in deeper column segments, and this indicated that there would be no threat of

groundwater contamination. However, the results showed that weeds with shallow roots would be effectively controlled in this column. Similar results were observed in soil without gypsiferous water irrigated. The findings of this experiment appear to contradict the findings of Chapter 4 for metolachlor, however it is to be considered that in Chapter 4 the "close system" was used and in the leaching experiment an "open system" was used. In addition, the calcium sulphate concentrations for the two experiments were different. In the atrazine experiment it was found that 0.1 mg kg^{-1} would pose a serious threat to groundwater contamination and sensitive follow-up crops. There is a need to reduce the atrazine dosage rate to 0.05 mg kg^{-1} because no threat to groundwater was observed at this rate. However, weed control efficacy at this low rate maybe poor.

ACKNOWLEDGEMENTS

The author would like to thank the following people and institutions that made this research possible:

- Almighty God, for the strength and protection.
- Professor C.F. Reinhardt for his leadership and guidance, and for granting me this life time opportunity.
- Research was made possible by the financial support of Water Research Commission through Professor J.G. Annandale.
- Mr R. Gilfillan, Mr J. Marneweck and Mr A. Beyers for technical assistance and data analysis.
- Dr. L. Kanyomeka for the initial work and proposal.
- Small Grain Institute (ARC) Management for allowing me the time to work on my thesis.

DEDICATION

This work is dedicated to my family: Parents Nelly and Botilo Masike, brothers Bruno, Billy and Itumeleng, and sisters Lebogang, Mmabotilo, Nomsa, Mpho and Queen, and all my in-laws and not forgetting all my nephews and nieces, for all the support and love they gave me over the years of my studies. This work is further dedicated to my son, Ofentse and his loving mom, Tsholofelo.

INTRODUCTION

The utilization of gypsiferous water, i.e. water containing high levels of calcium sulphate, for irrigation of crops is one major potential methods that promises to reduce the problem of effluent mine drainage disposal and also the shortage of irrigation water (Jovanovic *et al.,* 1998; Annandale *et al.,* 1999). Gypsiferous water is already used for irrigation of crops, such as maize (*Zea mays*) and wheat (*Triticum aestivum* L.), on a commercial scale. The area under this kind of irrigation will likely increase in future, since there are many mines in South Africa and other parts of Africa that are likely to adopt this novel technology of utilizing mine effluents.

The use of gypsiferous water for irrigation, however, could only be successful if other crop management practices are done correctly and are not affected adversely by this practice (Jovanovic *et al.*, 1998). Thusfar the interaction of gypsiferous water with crop production factors such as fertilizers and herbicides has been ignored. Gypsiferous water is saline due to the presence of high levels of Ca^{2+}, SO_4^{2-} and sometimes Mg^{2+} in solution. Jovanovic *et al.* (1998) explained the sources of these ions and their salts. These ions may have the potential to interact with other crop production factors like pesticides, including herbicides.

Glyphosate antagonism by dissolved salts with ions such as calcium and magnesium was verified with natural water as carrier and with water to which salts were added (de Villiers, 2002). Salt antagonism of glyphosate occurred from the formation of complexes that plants were less able to absorb than the formulated isopropylamine glyphosate. Ammonium sulphate increased the absorption of glyphosate from both distilled water and water containing chloride. De Villiers (2002) further found that there is large variation in the absorption of glyphosate salts, which is indicative of glyphosate antagonism due to the formation of complexes with certain ions in carrier water. These glyphosate salts are relatively insoluble and would precipitate first from spray solutions, and as a result would not be available for absorption into plant leaves. Liu *et al.* (1992) reported that in the case of imidazolinone herbicides, ammonium sulphate increased the phytotoxicity of imazaquin and imazethapyr in late postemergence treatments to pitted morning glory *(Ipomoea lacunosa* L.).

Antagonism of glyphosate toxicity to wheat by 2,4-D increased when sodium bicarbonate, calcium chloride, and ferric sulphate were in the spray carrier (Nalewaja *et al.*, 1992). They further found that spray carrier cations appeared to antagonise both 2,4-D and glyphosate phytotoxicity, and 2,4-D also antagonised glyphosate. Therefore, interactions that reduce glyphosate phytotoxicity could occur when all the three aforementioned components are present in spray mixtures.

Nalewaja *et al.* (1991) found that calcium, magnesium, sodium, potassium and iron salts, but not sulphate and phosphate salts of calcium and sodium, were antagonistic to 2,4-D amine. Salts in spray carrier water antagonised weed control with the amine formulation of 2,4-D. Water often contains many salts that antagonise the phytotoxicity of several herbicides. Various ammonium, calcium and sodium salts have also synergised phenoxy herbicides such as 2,4-D. Kochia control with 2,4-D decreased with increasing concentrations of sodium and magnesium sulphates and chlorides, as well as calcium chloride combined with sodium bicarbonate. Specific salt adjuvants have potential for increasing the efficacy of 2,4-D amine for weed control when spray water carriers contain sodium and calcium (Nalewaja et al., 1991; Nalewaja & Matysiak, 1991).

Herbicides are widely used to control weeds in crop production systems, particularly in commercial farming. Chemical weed control represents the most practical way of coping with unwanted vegetation in this type of farming, and probably at all levels of crop production (Ashton & Crafts, 1981; Klingman *et al.*, 1982; Akobundu, 1987). The behaviour of herbicides in soil and in plants is influenced by climatic and soil factors, type of herbicide, and plant species. The combined effect of these factors will determine the fate of a herbicide in the environment (Akobundu, 1987; Walker, 1994; Weaver & Reinhardt, 1998). Of particular importance is how interactions could influence the following important criteria for herbicide performance: weed control efficacy, the tolerance of crop species, and the potential for herbicides to leach to groundwater. Soil solution characteristics, in addition to pH and soil colloids, to a large extent govern herbicide behaviour (Walker, 1994). Clay *et al.* (1995) reported the effect of fertilizer application on herbicide activity or efficacy. Application of ammonia fertilizer to the soil increases soil pH and dissolved organic carbon.

Herbicide interaction with gypsiferous water is very possible due to the high concentration of electrolytes in this type of water. The quality of water used as a carrier in herbicide application is known to influence the efficacy of certain herbicides (De Villiers, 2002).

Possible consequences of interactions between herbicides and ions in carrier water are the following:

(a) Increased herbicide leaching that leads to groundwater contamination, and poor weed control due to reduced herbicide availability in the root zone;

(b) Increased herbicide concentration in the soil solution due to decreased adsorption which may result in reduced selectivity and crop injury;

(c) Stronger adsorption of herbicides on soil colloids, resulting in reduced weed control and increased persistence that could promote environmental pollution and / or cause damage to follow-up crops.

(d) Inactivation of herbicides due to complex-formation with ions contained in either carrier water or in soil.

No knowledge apparently exists about the influence of gypsiferous water on the behaviour of herbicides. Suspected herbicide injury to crops has been reported on a limited scale in South Africa where gypsiferous water was used for irrigation, suggesting a possible interaction between herbicides and gypsiferous water. Should such interactions exist, findings of the present study will be useful in making recommendations for herbicides and their use in situations where gypsiferous water is used to irrigate crops. Ultimately, results should contribute towards reducing risks involved in herbicide use, if in fact they do exist in this case, particularly in terms of the following herbicide performance criteria: weed control efficacy, crop tolerance (selectivity), and the risk for contamination of groundwater.

The objectives of this study were to determine the influence of gypsiferous water on three key performance criteria for atrazine, 2,4-D and metolachlor, namely: biological activity, persistence and leaching potential. Answers to these questions would contribute to the development of guidelines for herbicide use in situations where gypsiferous water is used to irrigate crops.

CHAPTER 1: LITERATURE REVIEW

1.1 Background on gypsiferous mine-water and its uses

South Africa has a very low percentage of arable land, of which only a third (4.5 million ha) is regarded as being of high potential (Annandale *et al.*, 2001). In addition, South Africa has a low and variable rainfall and 66% of the country is classified as semi-arid to arid. In view of the steadily increasing human population, it is vital that agricultural land be maintained and utilized effectively and responsibly.

Coal mining in Mpumalanga is beset by a major problem, namely the generation of large quantities of mine water saturated with gypsum ($CaSO_4$) and high levels of magnesium sulphate ($MgSO_4$) (Pretorius *et al.*, 1999). Discharge of gypsum-rich water from mining operations in an environmentally acceptable manner is of importance to the country as a whole, because it assists in making utilization of mineral resources more attractive.

Gypsum is the chemical amendment most often used in soil reclamation for crop production because it is nearly always effective in liming the soil and is relatively cheap. It is moderately soluble in water and supplies soluble calcium to replace sodium in soil (Branson & Fireman, 1980). Gypsum has been shown to prevent soil degradation and to improve and maintain adequate soil hydraulic and other physical properties in cultivated soils (Levy & Summer, 1998). Structural stability and pore size distribution influence infiltration rate, water retention characteristics, and unsaturated and saturated hydraulic conductivity. Aggregates of low stability fracture and disintegrate easily into smaller units, which may reduce hydraulic conductivity (Nepfumbada *et al.*, 1999).

Sodic soils are characterized by the occurrence of excess sodium to levels that can adversely affect soil structure and disrupt availability of some nutrients to plants. Such changes ultimately affect crop growth and yield (Qadir *et al.*, 2000). The presence of excessive SO_4^- and gypsum in the active root zone can also have nutritional implications mainly due to the effect on nutrient availability. Laboratory and field research by Qadir & Oster (2002), as well as farmers' experiences indicate that calcareous sodic soils can also be reclaimed without the application of amendments through the cultivation of certain salt-tolerant crops. This vegetative reclamation strategy is generally known as bioremediation, phytoremediation, or biological reclamation. The principal contributing mechanisms include: (a) enhanced CO_2 partial pressure in the root zone because of root and microbial respiration, which increases the solubility of calcite, and (b) improved soil physical properties due to root growth. Vegetative bioremediation can provide financial benefits from the crops grown which in turn help to support farming operations.

Rennenberg (1984) stated that it is improbable that excess SO_4^- *per se* could influence plant growth through ion toxicity. Although plants generally absorb excess sulphur, injury is prevented by a series of metabolic processes in the plant. Significant amounts of saline water are to be found in the workings of closed and existing mines. The generation of this salinity is usually associated with the exposure to oxidation of sulphide minerals, the end result of which is the generation of sulphuric acid where water is present. The potential for use of this water for crop irrigation was first brought to the attention of the South African coal industry some years ago by a visiting delegation from Israel – literature reveals that this application had initially been explored in South Africa by Du Plessis (1983).

In 1992 it was estimated that some 130 000 tons of salt were discharged from coal mines, and 200 000 tons from gold mines. At an average total dissolved solids concentration of 2000 mg l^{-1}, this would indicate discharge of some 65 million cubic meters of water from the coal mines, and 100 million cubic meters from the gold mines. These figures will increase with time. This amount of water is sufficient to sustain an irrigation system of some 500 to 700 hectares, depending on the cropping system used. Excessive groundwater that has been contaminated through open-cast mining operations, is used to irrigate rehabilitated land at Kleinkopje mine near Witbank in Mpumalanga, South Africa. Due to the high calcium and magnesium sulphate concentrations in the water, it can be expected that hundreds of tons of $CaSO_4$ will precipitate in the soil in a few years, if irrigated with this water (Annandale *et al.*, 1997). Concerns over the deteriorating water quality of streams due to waste drainage water that contains high levels of SO_4^-, Ca^{+2} and in some cases Mg^{+2}, originating from coal mines in Mpumalanga Province, have necessitated the need for alternative disposal options. Filtering this water through the soil and precipitating gypsum in the profile could limit environmental hazards while profits could be achieved through "on-site" use of this water for agricultural production (Strohmenger *et al.*, 1999). Disposal of mine wastewater is a worldwide problem occurring wherever coal and gold mines, as well as old underground workings are found. The use of gypsiferous mine-water for irrigation of agricultural crops is a promising technology which could solve problems related to both the shortage of irrigation water and the disposal of effluent mine drainage (Jovanovic *et al.,* 1998).

Large amounts of wastewater could possibly be utilized for irrigation of high potential soils in the Highveld region of South Africa. Contamination of downstream water supplies for other users could also be reduced. Gypsiferous mine-water has proved to be an additional resource in mining areas, particularly where prolonged drought periods are likely to occur. In an experiment conducted by Jovanovic *et al.* (1999), considerable increases in yield of irrigated sugar beans and wheat where observed, compared with rainfall cropping. No symptoms of foliar injury due to overhead irrigation with gypsiferous water were observed. According to the long-term simulation for a sugar beans / wheat rotation, irrigation with gypsiferous mine-water is not expected to cause irreparable damage to the soil. One of the advantages of irrigation with gypsiferous mine-water, according to Tanner *et* al. (1999), is its ability to neutralize subsoil acidity. Gypsiferous mine-water can be regarded either as one of the mining's greatest problems, or as a potential asset.

Initial research by Annandale *et al.* (1999) has indicated that there is potential for long-term sustainable irrigation for a range of crops with this water, and current research is directed at field verification of this premise. The utilization of mine wastewater must be balanced with the conservation of soil and water resources in such a way that the resources and associated ecosystems are not degraded to such an extent as to render them useless and unproductive (Annandale *et al.,* 1999). According to Du Plessis (1983), the possible utilization of this water for irrigation of agricultural crops shows some promise. Large amounts of wastewater could possibly be utilized for irrigation. Disposal of mine wastewater has become a problem of increasing importance from an ecological point of view. If freely discharged into the natural environment, lime-treated acid mine drainage could cause salinization of soils, rivers, dams and catchment areas. Jovanovic *et al.* (1998) concluded that if the subsoil problems experienced in the field could be solved, maize harvested for silage would likely be a more economical crop choice. They furthermore found that soil pH could be considerably increased when gypsiferous water is used for irrigation. Nepfumbada *et al.* (1999) found that treatment of soil with gypsum improves aggregation at least in the short-term. Therefore, regular tilling of the soil may in fact improve the soil structure.

1.2 Concise review of selected herbicides: metolachlor, atrazine and 2,4-D

Herbicides are used for weed control both on agricultural and non-agricultural lands. They are applied onto the soil or on aerial plant parts but irrespective of the method of application a significant portion of the applied herbicide ultimately reaches the soil. In the soil, herbicides are subject to transfer and transformation and their availability for these processes influence their rate of dissipation. Following release onto the land, herbicides may be in any of the compartments of the environment. They may be washed away with run-off water to surface waters or may vaporize into the atmosphere or leach down the soil profile into subsurface water, or may be degraded whether they are transported or not.

Due to the nature of this study, this review considered that portion of applied herbicides that reaches the soil and the subsequent behaviour thereof. This review is focused on three important herbicides that are frequently used in South Africa to control a wide spectrum of weeds, namely: atrazine, 2,4-D and metolachlor.

1.2.1 Metolachlor

Metolachlor (2 – chloro - 6’- ethyl – N – (2 – methoxy – 1 – methylethyl) acet – o – toluicide) along with other chloroacetanalides has become an important herbicide for the control of grass weeds in maize and other crops (Vencill e*t al.*, 2002). It is a pre-emergence herbicide that is applied onto the soil surface, but can also be incorporated shallowly. Its activity and therefore its phytotoxicity in maize can be influenced by many factors. Climatic and soil conditions as well as soil properties are important in this regard. Metolachlor is a member of the α-chloroacetamide group of herbicides. The Monsanto Company at St. Louis, U.S.A., discovered the highly effective herbicidal activity of the α-chloroacetamides in 1952. These herbicides represented a unique advance in weed control. In contrast to 2,4-D, which controls broadleaf weeds with little effect on annual grasses, the α-chloroacetamides control grasses with limited effect on broadleaf weeds. In 1972 Ciba–Geigy Ltd. discovered metolachlor (Dual®) and it became commercially available in 1975. Two emulsifiable concentrate formulations are available. Metolachlor is a white to tan liquid, odourless with a water solubility of 530 ppm at 20^0C (Klingman, 1982, Vencill *et al.,* 2002). The pure ingredient of metolachlor is a colourless liquid with a boiling point of 100^0C at 0,001 mg Hg and vapour pressure of $1{,}3 \times 10^{-5}$ mm Hg at 20^0C. Propachlor, alachlor, metolachlor, metazachlor and other related herbicides are also chloroacetanilides.

Uses

Metolachlor is applied as a pre-emergence or pre-plant soil-incorporated treatment for control of annual grasses, yellow nutsedge and certain broad-leaved weeds in maize, soybeans, peanuts, grain sorghum and woody ornamentals. It is also combined with several other herbicides, as well as applied in liquid dry bulk fertilizer or as a granule added to certain granular fertilizers (Vencill *et al.,* 2002). Additional crop labels are possible pending submission and/or registration for use in tomatoes, sunflowers and sugarbeets.

Site of uptake

Selectivity of soil-applied herbicides depends on several mechanisms. One such mechanism is the position of roots or other organs, such as the shoot, which absorb the herbicide, in relation to the locality and availability of the herbicide in the soil. This type of selectivity, based on the fact that the herbicide is not present at the site of uptake, is often referred to as depth protection or placement selectivity. This basis of selectivity is probably not adequate in all crop-herbicide situations (Holly, 1976). Several studies have been carried out to determine the site of uptake of chloroacetanilide herbicides. Placement studies by Gerber *et al.* (1974) indicated that the shoot is the predominant site of uptake of metolachlor in grasses. Grass weeds are highly sensitive to low concentrations of herbicides in the shoot zone, while much higher concentrations in the root zone cause only limited stunting effect. Le Court de Billot & Nel (1977) found that the emergent coleoptile of the maize seedling was the principal site of metolachlor uptake. The coleoptile absorbed more than the mesocotyl. Dixon & Stoller (1982) demonstrated that metolachlor applied in soil above the seed significantly reduced maize shoot growth, but the same concentration around or below the seed had no effect. Most of the evidence concerning the uptake of metolachlor by maize indicates shoot uptake. Since the shoot does not take up appreciable amounts of water below the soil surface, herbicides must enter mainly by diffusion (Schmidt & Pestemer, 1980). This appears to be the case for most herbicides acting on the shoot (Hartley, 1976). Root uptake of soil-applied herbicides dissolved in water is by passive mass-flow. The amount of herbicide absorbed is usually proportional to the herbicidal concentration and to the amount of water transpired by the plant.

Mode of action

These compounds inhibit early seedling growth; this effect is most evident on root growth. These responses appear to be associated with an interference with cell division and/or cell enlargement. They also control weeds by inhibiting the biosynthesis of several plant components such as fatty acids, lipids, proteins, isoprenoids and flavonoids. Their herbicidal action seem to involve conjugation with acetyl coenzyme A (Vencill *et al.*, 2002).

Soil influences

A thorough understanding of the behaviour of herbicides in soil is essential in order to predict their biological activity and environmental fate (Le Baron *et al.*, 1980). Results from numerous glasshouse experiments have confirmed the inverse correlation between herbicide activity and soil organic matter or clay content, and have usually shown organic matter to be the most important predictor of herbicide activity (Walker, 1980). Weber & Peter (1982) and Reinhardt & Nel (1984) reported that alachlor, acetochlor and metolachlor were adsorbed on montmorillonite and organic matter in similar amounts and that adsorption and bioactivity correlated with organic matter and clay content. This was further substantiated by Peter & Weber (1985), who found that the adsorption of alachlor and metolachlor correlated positively with soil organic matter content, clay content and surface area but correlated negatively with herbicidal activity. The relative ease with which a herbicide moves vertically in soil is important in determining its efficacy, suitability for use where placement selectivity is required, and in predictions of the potential for contaminating groundwater or drainage effluent (Weber & Peeper, 1977). The selectivity of metolachlor in maize depends amongst other things, on placement selectivity (Gerber *et al.* 1974).

The primary factors affecting soil degradation of chloroacetanilide herbicides are adsorption and microbial decomposition (Zimdahl & Clark, 1982). The bioactivity of these herbicides is reportedly related to the organic matter content (% C), clay content (% clay), cation exchange capacity (CEC) and particle surface area of soils (Peter & Weber, 1985). Soil pH apparently has little effect on the activity of chloroacetanilides. The rate of herbicide required to achieve a certain level of weed control on a particular soil has often been related to the capacity of the soil to adsorb the herbicide. Movement of metolachlor through soil is controlled by degradation, sorption, and desorption factors (Burgard *et al.,* 1993). Zimdahl *et al.* (1982) found that the rate of metolachlor degradation in clay loam soil was at least double than in sandy loam soil. Degradation was more rapid with increasing soil moisture content. They also pointed that it is reasonable to suggest that with analytical techniques that are precise and accurate, the ultimate detector (i.e. plant or chemical analysis) is not important. The rate of degradation and half-life can be predicted adequately for normal field conditions from laboratory experiments using known temperature and soil moisture conditions, which suggests that extensive field studies may be unnecessary. Gaynor *et al.* (2000), concluded that environmental factors such as rain affect herbicide residues more than cultural practices. Persistence of metolachlor in the field varies widely depending on temperature, soil type, soil water and depth below the soil surface. Metolachlor is also considered to have low to moderate persistence, but it is the most persistent of the acetanilide herbicides (Burgard *et al.*, 1993).

Leaching

Metolachlor, once it is applied to the soil, is subjected to many dissipation processes. Leaching, a process where a herbicide is dissolved in soil solution and carried downward through soil by gravitational forces, merits attention since it is a transport mechanism for potential non-point contamination of groundwater (Ahrens, 1994). Organic matter is the main constituent determining the leaching behaviour of metolachlor. Obrigawitch *et al.* (1981) demonstrated that metolachlor is sufficiently mobile in soils low in organic matter to cause possible crop injury. Jordan (1978) and Peter & Weber (1985) reported that the mobility of the chloroacetanilides was inversely related to their adsorptivity and, furthermore, that mobility was greater in coarse-textured soils with low organic matter content.

Leaching is restricted by adsorption onto clay and organic colloidal particles, with organic matter being the more important restraint. Dissipation of metolachlor in soils is relatively rapid. Based on the amount of desorption, metolachlor should be highly available for plant uptake. On coarse-textured soils and soils low in organic matter, metolachlor may be leached into or beyond the crop root zone resulting in crop injury or loss of herbicidal efficacy (Obrigawitch, 1981).

1.2.2 Atrazine

The s-triazines dominate broadleaf weed control in maize in South Africa (Reinhardt, 1993). Atrazine (2 - chloro - 4 – ethylamino – 6 – isopropylamino – 1,3,5 – triazine) alone is the most important herbicide in the maize-producing regions. It is a selective soil–applied herbicide (Vencill *et al.*, 2002). It is a white crystalline solid with a water solubility of 33 g l^{-1} at 22°C, and vapour pressure of 3,9 x 10^{-5} Pa at 25°C. It is formulated as a wettable powder, flowable liquid suspension, or in granular form (Klingman & Ashton, 1982; Vencill *et al.*, 2002). Atrazine has frequently been detected in groundwater. Its presence in groundwater is probably due to widespread use in the past three decades, limited sorption properties, and high relative persistence. Sandy soils and soils low in organic matter are particularly susceptible to leaching of atrazine and other herbicides (Mervosh *et al.*, 1995). The discovery that trace amounts of certain agrochemicals such as atrazine can occur in ground and surface waters has heightened public awareness and interest in the environmental behaviour of pesticides (Riley, 1991). Knowledge of factors that govern herbicide uptake by plants, crop sensitivity and the active lifetime of compounds in soil are required to properly manage the use of potentially persistent herbicides.

Uses

Atrazine is widely used to control annual grass and broadleaf weeds in maize, sorghum and sugarcane. It is effective when applied pre-emergence to annual weeds. It is also widely used as a non-selective herbicide on non-cropland. Atrazine is moderately persistent but can still injure sensitive plants the next season (Klingman & Ashton, 1982; Reinhardt, 1993; Vencill *et al.*, 2002).

Persistence

Because most research with herbicides has focused on the parent compounds, little information is available on the behaviour of herbicide degradation products in soil. To assess the impact of pesticides on groundwater, it is important to understand the formation and persistence of degradation products and their mobility in soil. The potential for a herbicide or its degradation products to move through soil is affected by a number of factors. The most important of the factors appears to be persistence of the compound and its soil adsorption-desorption characteristics (Sorenson *et al.*, 1993). Atrazine is moderate to highly persistent in soil, with an average half-life of 60 days (Meisner *et al.*, 1993). While longevity in soil increases the effectiveness of atrazine as a pre-emergence herbicide, its persistence can limit farmers' options in crop rotations and may result in groundwater and surface water contamination. Because atrazine inhibits photosynthesis, the vulnerability of algae to atrazine may affect the food chain at some contaminated sites. Although specific risks of atrazine to human health are not well-understood, potential toxic effects from atrazine metabolites, particularly from adducts of nitroso derivatives that may be produced in mammalian systems (Meisner *et al.*, 1993). Persistent herbicides should be administered in ways that acceptably limits residual biological activity, thereby lowering the risk of damage to susceptible follow-up crops. In addition, herbicide residue should be prevented from reaching surface water and groundwater below the rooting zone. Atrazine has been detected in groundwater more frequently than any other herbicide. Numerous studies have shown that atrazine can move through the soil profile and contaminate groundwater supplies (Sorenson *et al.*, 1993). Most rotational crops can be planted one year after application of atrazine at selected rates, except under an arid or semi-arid climate. Persistence is increased by higher soil pH as well as by cool, dry soil conditions (Vencill *et al.*, 2002). Herbicidal bioactivity and persistence are both determined by the following four factors: characteristics of the compound, application rate, plant sensitivity, and rate of dissipation in the environment (Leistra & Green, 1990). Forecasts of the presence and bioactivity of atrazine residues are often perplexing. Behavioural inconsistencies locally and abroad have occasionally manifested in either poor weed control by the herbicide, loss of selectivity that leads to crop injury, or excessive persistence that causes damage to sensitive following crop.

Residual soil-applied herbicides such as atrazine are an essential component of many crop production systems. The time that herbicides persist in the soil is of particular importance as this has already been shown to have serious implications for the safety of sensitive crops (Wischnewsky & Zhao, 1991; Reinhardt, 1993). From an agronomic viewpoint, the ideal herbicide should persist long enough to provide season-long weed control but not so long that its residue harm sensitive follow-up crops. Carry-over problems are likely to occur when soil and weather factors which reduced herbicide degradation prevail periodically or for a significant length of time subsequent to herbicide application, and when the particular follow-up crop is markedly sensitive to the specific herbicide residue. Excessive persistence tends to occur more frequently in some seasons than in others, reflecting the influence of climatic factors on rates of loss (Eagle, 1983b; Reinhardt, 1993). Some soils are also more prone to carry-over problems than others. These problems are exacerbated by errors in initial application that leads to overdosing. High enough residual amounts to damage sensitive plants may be present in soil, but unavailability of the total amount due to adsorption may prevent plants being affected. However, should a particular set of environmental factors render the residue available for plant uptake in high enough amounts, a phytotoxic effect is likely to occur.

Soil characteristics

Soil is a dynamic and complex biological and chemical medium in which numerous variables interact to determine the degradation rate of a compound, and hence, its persistence. In this context, factors such as temperature, soil texture, organic and pH are regarded as important variables. Degradation processes usually involve direct chemical transformation of the herbicide and the activities of soil microorganisms. Atrazine half-life was 335 days in water at 12-45°C and pH 7 with natural light (Vencill *et al.*, 2002). Organic matter content of soil could be of particular importance in determining the degradation rate and thus the persistence of atrazine. The clay content of soil may also be important in determining its persistence, because it is an important predictor of atrazine availability in the soil solution. When working with natural soils, it can be difficult to separate the effect of clay and organic matter since they are often correlated (Reinhardt & Nel, 1989). Clay content is an important component of soil structure, and soil texture can have a marked effect on other soil properties. Coarse-textured soils with low clay content tend to have less organic matter and lower microbial biomass than fine-textured soils (Walker, 1989). Soil texture will also influence pore size distribution in soil and consequently water relations and aeration characteristics.

Soil pH

Soil pH can have a pronounced effect on atrazine degradation rates. pH also exerts a direct effect when the stability of the chemical is pH dependent, or it has an indirect effect through changes in microbial populations, or through colloidal adsorption of the compound. Atrazine is moderately adsorbed to soil and this increases at lower pH (Vencill *et al.,* 2002). Changes in soil pH influences the behaviour of herbicides. The atrazine molecule is stable under neutral pH conditions, but rapid chemical hydrolysis occurs under highly acid or alkaline conditions (Reinhardt, 1993). Chemical hydrolysis of atrazine to hydroxyatrazine is regarded as the main avenue of inactivation of the herbicide in the soil (Gamble & Khan, 1985). According to Weber (1970a), the Cl^- ion of the atrazine molecule is replaced with an OH^- ion through direct nucleophilic substitution under alkaline conditions. Under acid conditions, protonation of a ring or a side chain N atom is followed by cleavage of the C–Cl bond by water. Protonation of N would increase the electron deficiency of the C bonded to Cl, thus increasing the tendency for nucleophilic displacement of Cl by H_2O. Ammonia-based fertilizers increase pH, and at high pH, atrazine sorption is decreased. This results in high atrazine in the soil solution, thus increasing the possibility of atrazine leaching, as well as the risk to sensitive follow up crops. The principle behind this is that some herbicides compete with charged electrolytes for adsorption sites on the soil colloids. The replacing power of cations on clay and humus generally follows the order $C^+ < C^{2+} < C^{3+}$ (Brady, 1974). The divalent ions are more competitive and more strongly adsorbed than monovalent ions.

Leaching

Detection of atrazine in ground and surface water supplies has raised concern about atrazine degradation products and their potential to contaminate water supplies (Fleming *et al.*, 1992; Sorenson *et al.*, 1994; Jenks *et al.*, 1998). Leaching studies with atrazine and its degradation products indicate the compounds most likely will leach in soil and contaminate water supplies, but they do not describe the formation of the compounds and their distribution in the soil profile (Sorenson *et al.*, 1993). In field studies, unless ^{14}C-labelled atrazine is included, the amount of unextracted residues left in the soil and the total atrazine load in soil cannot be determined. Research with ^{14}C-atrazine by Sorenson *et al.* (1993) using packed columns under controlled conditions in the laboratory does not accurately assess atrazine movement or duplicate temperature and moisture regimes observed in the field. To accurately model the fate of atrazine in soil and assess the potential for atrazine and its degradation products to contaminate groundwater, these data are needed. Long-lived residues have the potential to leach to groundwater. However, during a 16-month column leaching study, ^{14}C-atrazine residue were not detected deeper than 40 cm in a sandy loam, and no radioactivity was detected in leachate collected from a 1-m monitoring column (Sorenson *et al.* 1994). They further found that atrazine moved deeper in silt loam than sand following application to packed field lysimeters, even though the silt loam had nearly double the organic content and much greater atrazine adsorption than the sand. Generalizations regarding pesticide leaching with respect to soil type have suggested that coarse-textured soils have greater potential for pesticides to leach than fine-textured soils. However, rapid water infiltration and short water residence time in coarse-textured soil may result in less pesticide being leached than in finer textured soils (Sorenson *et al.*, 1994). Rates of atrazine leaching depend on soil parameters such as the retardation factor, which is a measure of how rapidly atrazine moves through the soil profile. The extent of leaching also depends on alternative fates such as runoff, volatilization and biodegradation, which control the available pool of atrazine in the surface soil horizon. Rate of biodegradation is a function of the type and population density of atrazine-degrading microbes and bioavailability. Bioavailability depends on the affinity of specific atrazine-degrading strains for atrazine and soil solution concentration (Shelton *et al.*, 1995). Consequently, the crop residue/organic matter complex plays a role in controlling transport of atrazine to groundwater by affecting rates of biodegradation through bioavailability and by providing potential substrates for atrazine-degrading microorganisms. Fleming *et al.* (1992) found that soil-applied herbicides are subject to many types of losses including degradation, sorption, volatilization and leaching. To overcome these losses, herbicide application rates provide an initial soil concentration in excess of that needed for effective weed control. Because atrazine degrades more slowly in the lower soil profile where organic matter, microbial populations and temperature are lower than at the soil surface, the controlled-release formulations that keep most of the herbicide near the soil surface may favour herbicide dissipation via degradation rather than leaching (Fleming *et al.*, 1992).

1.2.3 2,4-D

2,4-D is the common name for (2,4-dichlorophenoxy) acetic acid (Vencill *et al.*, 2002). It is a white crystalline solid with a water solubility of about 600 ppm and odourless. Its salts, however, are quite soluble in water, and the acute oral LD_{50} of its various formulations range from 300 to 1000 mg kg^{-1}. The first reference to 2,4-D in the literature is an article by Pokorny in 1941. Zimmerman & Hitchcock (1942) first described it as a plant-growth regulator. 2,4-D is formulated as an emulsifiable acid, amine salts, mineral salts and esters (Klingman & Ashton, 1982). The phenoxyacetic acid 2,4-D and its growth regulatory properties were discovered in the USA in 1941. Research on 2,4-D compounds started during World War II, under wartime secrecy (Vencill *et al.,* 2002). The American Chemical Paint Company "weedone" soon realized its agricultural potential with the marketing of 2,4-D in 1945. Since this molecule could kill many broadleaf weeds in narrow-leaf crops, it is the first truly selective, non-toxic organic herbicide that was effective at low doses. This herbicide is best regarded as synthetic auxins, in that the morphological symptoms produced are indicative of an exaggerated auxin response, leading to disorganised growth and death in susceptible species. The discovery of 2,4-D as a herbicide precipitated the greatest single advance in the science of weed control and one of the most significant in agriculture. It has been reported that 2,4-D and its derivatives were the most active compounds in inducing cell elongation (growth) (Bovey & Young, 1980). 2,4-D is rapidly inactivated in moist soil and it persists for longer periods in dry than moist soils. Other factors found to affect 2,4-D disappearance included soil temperature, leaching and soil organic matter.

Uses

2,4-D is a systemic, foliar-applied non-selective herbicide and is widely used for control of broadleaf weeds in cereal crops, sugarcane, turf, pastures, and non-croplands (Vencill *et al.,* 2002). Most dicot crops are susceptible at normal herbicide rates. In low concentrations it is used as a growth regulator to reduce fruit drop, increase fruit size and increase storage life in certain citrus fruits (Klingman & Ashton, 1982; Humburg *et al.*, 1989; Morillo *et al.,* 2001). The determination of phenoxy acids in soils is of concern because they have harmful effects on the microflora of the soil when they are not degraded quickly enough. Moreover, because of their high solubility in water, they can be present in underground water (Jesus Santos-Delgado *et al.*, 2000).

Mode of action

The primary action of these compounds appears to involve cell wall plasticity and nucleic acid metabolism (Vencill *et al.,* 2002). The phenoxy herbicides induce several abnormalities in growth and plant structure. Epinastic bending is rapid following foliar applications, subsequently, tumors, secondary roots and fasciated structures often develop in susceptible species. They cause differentiation and initiation of cell division in certain mature cells and inhibit cell division in primary meristems (Ashton & Craft, 1981).

Soil influences

Soil type and formulation of 2,4-D influences its leaching in soil (Vencill *et al.,* 2002). Water-soluble forms leach more readily than those that are slightly soluble. It is adsorbed by soil colloids, and less leaching occurs in clay and organic soils than in sandy soils. Low rates of 2,4-D will normally be decomposed in one to four weeks in a warm, moist loam soil. 2,4-D undergoes microbial breakdown in warm, moist soil. Rate and breakdown increases with increased temperature, moisture, pH and organic matter content. There is no risk of the chemical accumulating in the soil from one year to the next under such conditions. In very dry soil or in frozen soils, the rate of decomposition may be inhibited considerably (Klingman & Ashton, 1982, Vencill *et al.,* 2002). 2,4-D is an acid with pKa 2.80 (Vencill *et al.,* 2002). As a result, this compound may occur either as an anion or as an undissociated charged molecule in the pH range of concern for practical applications of herbicides.

Negatively charged anionic herbicides are not readily adsorbed to soil colloids since they have the same negative charges as the soil particle. Weber *et al.* (1965) indicated that when adsorption of 2,4-D exists in soils it must be attributed to organic matter, iron and aluminium hydrous oxide or possibly diffusion into fine pores of inert material. However, in most cases a small amount of herbicide is bound to positively charged soil colloids. Average persistence of phytotoxicity is generally 1-4 weeks in warm, moist soil, and the average field half-life is 10 days (Vencill *et al.,* 2002). Wilson *et al.* (1997) found that 2,4-D has a relatively short half-life and is rather immobile in the soil. The acid form of 2,4-D, as well as the amine and ester chemical groups, metabolized to compounds of non-toxicological significance and ultimately to forms of carbon. Thus, 2,4-D is considered a biodegradable compound. Under normal conditions, 2,4-D residues are not persistent in soil, water and vegetation.

CHAPTER 2: EFFECT OF GYPSIFEROUS MINE-WATER ON HERBICIDE STABILITY IN A TANK MIXTURE

2.1 Introduction

There have been reports of suspected herbicide injury to crops in South Africa in areas irrigated with gypsiferous mine-water, which suggested interaction between gypsiferous water and herbicides. The quality of water used as a carrier in herbicide application is known to influence the efficacy of certain herbicides (De Villiers, 2002). Consequently, herbicide interaction with gypsiferous water is very possible due to the high concentration of electrolytes in these types of water. De Villiers (2002) observed that 2,4-D antagonises glyphosate, so these interactions could increase glyphosate phytotoxicity when sodium bicarbonate, calcium chloride, and ferric sulphate were in the spray carrier water.

Nalewaja *et al.* (1991 & 1992) found that calcium, magnesium, sodium, potassium, and iron salts, except sulphate and phosphate salts of calcium and sodium, antagonised the action of 2,4-D amine. Weed control was antagonised by these salts in spray carrier water containing the amine formulation of 2,4-D. Specific adjuvants have potential to increase the efficacy of 2,4-D amine when spray water carriers contain sodium and calcium. Water often contains many salts that antagonises the phytotoxicity of several herbicides. Nalewaja *et al.* (1991) and Nalewaja & Matysiak (1991) found that an increase in concentration of sodium and magnesium sulphates and chlorides, and calcium chloride combined with sodium bicarbonate, decreased kochia control with 2,4-D. A test was done to determine the stability of atrazine and 2,4-D in a tank mixture where mine-water was used as a carrier.

2.2 Materials and Methods

Two sources of water were used to prepare herbicide tank mixes. Table 2.1 shows the chemical composition of mine-water used in the herbicide tank mixture. Two herbicides, atrazine and 2,4-D were made up in each of the water types and also with distilled water that was used as a control. Jacuzzi and Tweefontein (Witbank, South Africa) water samples were collected at the respective sources of gypsiferous irrigation water. Treatments made up were: atrazine + Jacuzzi water, atrazine + Tweefontein water, atrazine + distilled water, 2,4-D + Jacuzzi water, 2,4-D + Tweefontein water, and 2,4-D + distilled water, respectively.

Table 2.1: Chemical composition of mine-water used in the herbicide tank mixture

Water source	$CaSO_4.2H_2O$ (g l^{-1})	$MgSO_4$ (g l^{-1})	NaCL (g l^{-1})
Jacuzzi	2.38	0.84	0.10
Tweefontein	1.88	0.96	0.11

Atrazine and 2,4-D were made up in the water in quantities that were equivalent to field-use rates of 1625 g ha^{-1} and 960 g ha^{-1} (active ingredient), respectively. The formulation was not analysed. The herbicide tank-mix was left to stand for three hours and then placed in a freezer with the temperature cold enough not to let the mixer to freeze. After seven days, the samples were analysed for active ingredients content by the Pesticide Analytical Laboratory (PAL), Plant Protection Research Institute, ARC. All samples were extracted using liquid extraction methods. The 2,4-D extraction included a methylation step. Atrazine analysis was done using a Gas Chromatograph fitted with a nitrogen phosphorous detector. A packed column with OV 17 as stationary phase was used (Ahmed *et al.* 1989).

2,4-D was analysed using a Gas Chromatograph fitted with a SE 30 packed column and electron capture detector. A packed column with SE 30 as stationary phase was used. Metolachlor was also analysed using a Gas Chromatograph fitted with an electron capture detector. A packed column with SE 30 as stationary phase was also used for this herbicide. Sample analyses were done following OECD GLP guidelines. This study was however not conducted as a GLP study (Ahmed *et al.* 1989).

2.3 Results and Discussion

The results given below have been corrected for recoveries. Quantitative results are presented in Table 2.2. Generally the residue levels measured were lower than expected if compared with concentrations made up of both compounds. Amongst the three mixtures of each herbicide, the highest residue levels were detected in the distilled water (control) and the lowest in the Jacuzzi water. For 2,4-D, the decrease in concentration, from the control, was 8% for Tweefontein water and 24% for Jacuzzi water. Where atrazine was added a much more severe effect was found. In the Tweefontein water, atrazine concentrations decreased by 77% from the control, while in the Jacuzzi water, atrazine residues were reduced by 90%.

The results indicate that there is rapid transformation of the herbicides in the mine-water, with atrazine having the highest transformation rate of the two herbicides. This suggests that the electrolytes in the mine-water interact with herbicide molecules to rapidly transform them. This could mean that mine-water may not be a suitable carrier for herbicide sprays.

Table 2.2: Quantitative results of the mine-water herbicide tank-mix

Water source	Atrazine (mg l^{-1})	2,4-D (mg l^{-1})
Distilled water	1602.7	528.85
Tweefontein	356.01	486.39
Jacuzzi	113.85	403.46

The retention times of the control sample (distilled water + herbicide) was the same as for the standard. However, a slight shift in retention time was found with both the Tweefontein and Jacuzzi samples. The mass spectra were the same for all samples with the exception of Jacuzzi water where many peaks were observed in the spectrum as compared to the standard and library spectrum. The spectrum fit as compared to the library spectrum for atrazine was 98% for all samples, again with the exception of Jacuzzi water where only 90% fit was rendered. Although the retention time shift found with atrazine is slight, the data indicates that the Tweefontein and Jacuzzi water may exert an effect on the adsorption characteristics of the atrazine molecule. This is not completely unexpected, as one would expect dissolved salts to have an effect on the polarity of the compound. This would mean that in the field, there is a possibility that soil retention would decrease and so leaching could increase, reducing the efficacy of the herbicides because the herbicide will not stay long enough in the top soil layer to be absorbed by plants.

2.4 Conclusions

Mine-water caused a reduction in tank-mix concentration of both atrazine and 2,4-D, particularly in the case of atrazine, and this has weed control efficacy implications. The reduction in concentration of atrazine means that the weeds would not be controlled properly, however, research has shown (Reinhardt, 1993) that small quantities of atrazine can actually lead to improved growth compared to the control. The practical relevance of this finding is not known at this stage. Although the retention time shift found with atrazine is slight, the data indicate that the Tweefontein and Jacuzzi water may exert an effect on the adsorption characteristics of the atrazine molecule. This is not completely unexpected, as one would expect dissolved salts to have an effect on the polarity of the compound. It is imperative that the biological activity of the herbicide transformation products be assessed also. The findings have such profound implications for weed control efficacy that further research is imperative. Work reported in subsequent chapters was focussed on herbicide interactions with gypsiferous ($CaSO_4$ - containing) water only.

CHAPTER 3: EFFECT OF GYPSUM ON THE BIOLOGICAL ACTIVITY OF ATRAZINE, 2,4-D AND METOLACHLOR

3.1 Introduction

The bioassay principle is that the growth response of a sensitive organism (plant species) towards a chemical (herbicide) is indicative of the biological activity of that compound. Bioassaying is a powerful tool for assessing the influence of various environmental factors on herbicide performance. Bioassays remain a major tool for qualitative and quantitative determination of herbicides. The main reasons for the wide use of bioassays in herbicide research are probably their relative simplicity and versatility (Horowitz, 1976).

The availability of soil-applied herbicides to plants generally decreases as the clay and /or organic matter content of soils increases (Best *et al.* 1975). Higher rates of soil-applied herbicides are needed on fine-textured (soil with high organic matter content) than on coarse-textured (low organic matter content) soils. They further suggested that plant uptake could contribute more to herbicide dissipation in low adsorptive soils than in highly adsorptive ones. Furthermore, different herbicides, or degradation products from a given herbicide, can display different relative availability for uptake depending upon adsorptive properties of the soil. This reflects the specific interaction between a given chemical and a certain combination of soil constituents.

The concentration of the herbicide at a vital location in the plant at any given time may determine herbicidal effectiveness. If the same amount of herbicide would be absorbed over an extended length of time it may have little or no effect. If the structure of the herbicide must be altered within the plant to an active form, or if absorption or translocation is slow, the concentration of the chemical at any one moment may be less than the lethal dose. Variation in molecular configuration of a herbicide changes its properties, which in turn modifies its effect on plants (Klingman & Ashton, 1982). The authors further found that when degradation occurs rapidly in a plant, the plant is not injured (e.g. atrazine in maize); however, if degradation occurs slowly, toxic levels of the herbicide accumulate, and the plant is injured (e.g. atrazine in *Amaranthus* spp).

Greenhouse experiments, with separate trials for each herbicide, were conducted with the aim of assessing the effects of possible interactions between herbicides and mine-water on the availability of herbicides for uptake by plants.

3.2 Materials and Methods

Herbicides registered for use in maize (*Zea mays*) and wheat (*Triticum aestivum*) were included in these experiments. These were considered priority crops since they are already commercially produced with mine-water irrigation. Test plant species were used as indicators of herbicide availability for uptake from the soil. The biological activity of atrazine, metolachlor and 2,4-D were investigated in separate pot experiments conducted in a greenhouse at the phytotron facility of the University of Pretoria. Dose response curves for the parameters dry matter yield and visual injury rating were obtained with a herbicide concentration range consisting of ten rates.

The rates for each herbicide are presented in Table 3.1. The experiments were repeated (Table 3.2) in order to obtain dose response curves that show higher definition across the concentration range, i.e. in order to avoid too low or too high herbicide activity across major portions of the concentration range. These changes were made because in the initial experiments (Table 3.2) not enough activity was found with the rates used for metolachlor, while the atrazine rates used were too high, and hence, elicited excessive bioactivity. The test species for atrazine, metolachlor and 2,4-D were wheat, sorghum and sunflower, respectively. Properties of the soil used are presented in Table 3.3. The soil factor investigated was gypsum added to the soil and no gypsum added (control). The original soil was collected from the Hatfield experimental farm (University of Pretoria) and therefore, differences in soil properties are due only to the addition of gypsum (Table 3.3).

Table 3.1: Initial herbicide rates (mg kg^{-1}) used prior to adjustment for improved definition across the range

Rate number	Metolachlor	Atrazine	2,4-D
1	0.000	0.000	0.000
2	0.050	0.025	0.100
3	0.075	0.050	0.200
4	0.100	0.075	0.300
5	0.125	0.100	0.400
6	0.150	0.125	0.500
7	0.175	0.150	0.600
8	0.200	0.175	0.700
9	0.225	0.200	0.800
10	0.250	0.225	0.900

Table 3.2: Final dosage rates (mg kg^{-1}) of herbicides used for bioassay to determine dose response relationships

Rate number	Metolachlor	Atrazine	2,4-D
1	0.000	0.000	0.000
2	0.075	0.020	0.100
3	0.100	0.030	0.200
4	0.125	0.040	0.300
5	0.150	0.050	0.400
6	0.175	0.060	0.500
7	0.200	0.070	0.600
8	0.225	0.080	0.700
9	0.250	0.090	0.800
10	0.275	0.100	0.900

Table 3.3: Properties of soil used in the experiments

Soils	Ammonium Acetate Extractable						Carbon	SO^{-2}_4	Coarse sand	Silt	Clay	Total	CEC cmol kg^{-1}
	pH water	P Bray I	Ca mg kg^{-1}	K mg kg^{-1}	Mg mg kg^{-1}	Na mg kg^{-1}	%	mg kg^{-1}					
Soil without gypsum added	6.1	25.6	531	45	158	11	0.47	154.08	68.5	8.2	22.1	98.7	3.60
Soil with gypsum added	5.7	106.2	28394	52	135	20	0.37	7095.44	68.5	8.2	22.1	98.7	3.60

Gypsum was mixed thoroughly with soil that had been air-dried and sieved through a 4-mm screen, at the rate of 10% gypsum per total volume. The control treatment consisted of unamended soil. Pots were filled with either 1 kg soil/gypsum mixture or 1 kg of unamended soil. Herbicides were applied directly to the soil and were immediately incorporated. A specific volume of herbicide stock solution was thoroughly mixed with the 1 kg soil by means of a mechanical mixer. A plastic bag in each pot prevented herbicides from leaching out. Thereafter water was added to bring the soil water content to 15%, which was the field capacity for the specific soil. Each factorial experiment consisted of the following treatments:
10 (herbicide rates) x 2 (soils = with gypsum and without gypsum) x 4 (replicates) = 80. The experimental design in the greenhouse was completely randomized.

Day/night temperatures varied between 25-30°C and 15-18°C, respectively. Watering was done to ensure that plants were not subjected to water stress. All the pots were weighed twice a week and each pot was watered with nutrient solution (Nitsch, 1972) to replace water lost. Growth responses of the test species were expressed as percentage reduction in growth from the controls (zero herbicide). The conversion to percentage reduction data was done to eliminate possible confounding influences from factors such as temperature, which varied slightly between the different experiments. According to Rayner (1969), analysis of percentages of this nature does not necessarily imply transformation, as this is merely a change of scale.

Plants were harvested four weeks after planting, and the following measurements were taken:

a. Plant count 2-3 days after emergence.
b. Visual injury rating. Rating was done relative to the untreated control. The EWRS Rating scale was used where 0 = control, 1= no effect and 10 = complete kill.
c. Shoot dry mass.

Analysis of variance (ANOVA) was done using the SAS® programme on the mainframe computer of the University of Pretoria. All data were subjected to analysis of variance, and means were compared at 5% level of significance. In striving for the best fit of concentration applied to effect measured, the highest R^2-value and the lowest standard error, as well as the simplest functions were chosen, keeping in mind the biological meaning of the trend.

3.3 Results and Discussion

In the case of percentage shoot dry mass reduction (SDMR) the interaction soil x herbicide rate was significant for 2,4-D only (Table 3.4). The interaction effect of soil and herbicide rate for shoot drymass (SDM) was significant in the case of all three herbicides. Table 3.5 shows percentage shoot drymass reduction for 2,4-D rates, Table 3.6 shows means for shoot drymass for atrazine and Table 3.7 shows means for metolachlor shoot drymass. Analysis of variance results for SDM and SDMR in the three experiments appear in Tables 4A to 6A and 7A to 9A, respectively, in the appendix. The 2,4-D experiment indicated significantly greater biological activity in soil with gypsum added compared to where gypsum was not added (Figure 3.1). The biological activity of atrazine was significantly increased in the presence of gypsum (Figure 3.2), and in the case of metolachlor (Figure 3.3), the effect was even more pronounced.

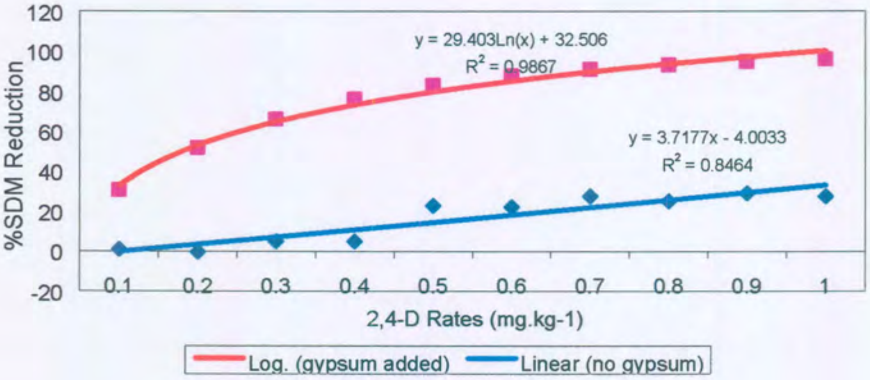

Figure 3.1: Percentage shoot drymass reduction (SDMR) in 2,4-D bioassay (test species: sunflower) on soil with or without gypsum added

Table 3.4: Means (shoot drymass) for soil x rate interaction for the 2,4-D experiment

	2,4-D rates									
Soils	1	2	3	4	5	6	7	8	9	10
Without gypsum	1.8	1.8	1.7	1.7	1.4	1.4	1.3	1.3	1.3	1.3
With gypsum	1.6	0.9	0.7	0.5	0.6	0.2	0.2	0.1	0.1	0.0

Soil x Rate LSD $_{(p.\ 0.05)}$ = 0.54

Table 3.5: Means (percentage shoot dry mass reduction) for soil x rate interaction for the 2,4-D experiment

	2,4-D rates									
Soils	1	2	3	4	5	6	7	8	9	10
Without gypsum	0.8	0.0	4.9	4.9	22.8	22.2	27.6	25.0	29.0	27.3
With gypsum	26.4	58.5	66.0	77.3	73.4	89.7	90.0	94.7	94.2	98.3

Soil x Rate LSD $_{(p.\ 0.05)}$ = 25.4

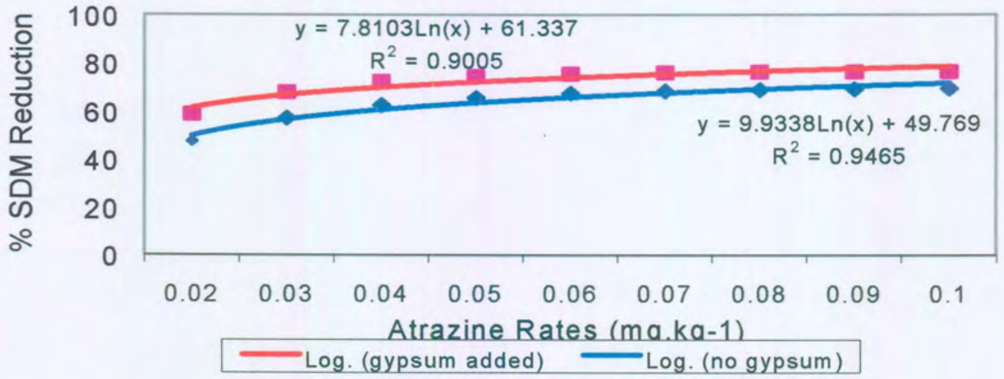

Figure 3.2: Percentage shoot drymass reduction (SDMR) in atrazine bioassay (test species: wheat) on soil with or without gypsum added

Table 3.6: Means (shoot drymass) for soil x rate interaction for the atrazine experiment

Soils	Atrazine rates									
	1	2	3	4	5	6	7	8	9	10
Without gypsum	0.18	0.09	0.07	0.08	0.05	0.05	0.05	0.06	0.06	0.05
With gypsum	0.23	0.09	0.08	0.06	0.05	0.05	0.06	0.06	0.05	0.05

Soil x Rate LSD $_{(p.\ 0.05)}$ = 0.04

The extent of reduction in growth caused by atrazine generally increased with increasing herbicide rates (Figure 3.2). Anderson *et al.* (1980), Ehlers *et al.* (1987 & 1988), and Reinhardt *et al* (1990) reported a strong negative correlation between the organic matter content and, to a lesser degree, the clay content of soils and atrazine bioactivity. Therefore it appears pointless to assign atrazine threshold concentrations to specific crop species, irrespective of the soil and climatic conditions they are grown under. Reinhardt (1993) reported that prediction of potential damage to susceptible crops should reflect the differential availability of atrazine in different soil types. Also, the amount of residues available for uptake by plants, and not merely total residue concentration in a particular soil should determine crop choice. Recropping intervals that are specified on labels of atrazine products neither reflect the variability in atrazine threshold values for sensitive crop species nor differential threshold concentrations for different soils (Reinhardt, 1993).

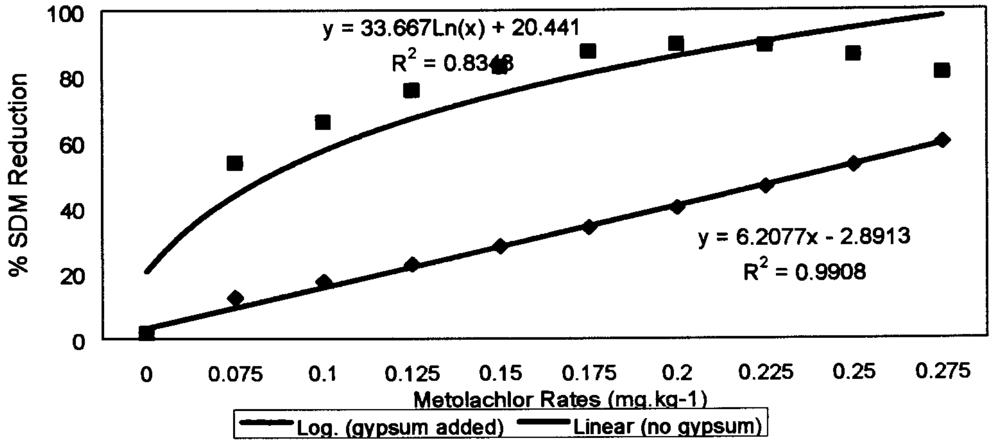

Figure 3.3: Percentage shoot drymass reduction (SDMR) in metolachlor bioassay (test species: sorghum) on soil with or without gypsum added

Table 3.7: Means (shoot drymass) for soil x rate interaction for the metolachlor Experiment

	Metolachlor rates									
Soils	1	2	3	4	5	6	7	8	9	10
Without gypsum	16.7	23.0	16.0	12.0	9.5	9.2	12.7	11.7	6.0	6.5
With gypsum	27.0	11.7	5.6	10.5	7.0	2.0	2.0	1.7	4.5	5.3

Soil x Rate LSD $_{(p.\ 0.05)}$ = 14.3

In contrast to the effect on the bioactivity of both atrazine and 2,4-D, the activity of metolachlor was reduced in the presence of gypsum (Figure 3.3). The magnitude of activity change was far greater in the case of metolachlor than for 2,4-D (Figure 3.1) and atrazine (Figure 3.2). In the case of the latter herbicide, the activity difference observed was so slight that it probably does not hold any practical consequences in terms of herbicide efficacy, selectivity or persistence. In contrast, the performance of metolachlor was dramatically affected, and has important practical consequences. Reduction of metolachlor activity in the presence of gypsum implies that weed control by this herbicide will be poor on soils irrigated with water containing high levels of gypsum (calcium sulphate). Consequently, the finding does not suggest that selectivity of metolachlor towards the crop would be a concern. The effect on herbicide selectivity is also not an issue in the case of atrazine, because of the inherent high tolerance of the main crop in which it is used, namely maize.

Using best-fit regression equations the ED_{50} values (estimated dose causing 50% growth reduction) for each of atrazine, 2,4-D and metolachlor in soil plus gypsum, or in soil minus gypsum, were calculated as:
Atrazine + gypsum: 0.017 mg kg^{-1}
Atrazine – gypsum: 0.021 mg kg^{-1}
2,4-D + gypsum: 0.22 mg kg^{-1}
2,4-D – gypsum: 3.06 mg kg^{-1}
Metolachlor + gypsum: 0.063 mg kg^{-1}
Metolachlor – gypsum: 1.12 mg kg^{-1}

3.4 Conclusions

For both atrazine and 2,4-D there was significantly greater biological activity in soil containing gypsum compared to where gypsum was not added. Increased bioactivity in the presence of gypsum suggests that the herbicide (atrazine and 2,4-D) were less strongly adsorbed to soil colloids in soil containing gypsum compared to soil without gypsum. Although this response will promote weed control efficacy, concomitant increased leaching potential poses a risk in terms of contamination of groundwater. In contrast, the activity of metolachlor was reduced in the presence of gypsum. Research reported in subsequent chapters was aimed at determining what effect, if any, the presence of gypsum has on the persistence and leaching potential of the herbicides. Should persistence and/or leaching of a herbicide be increased in the presence of salts associated with gypsiferous water, there is a risk of contamination of groundwater in particular.

CHAPTER 4: EFFECT OF GYPSUM ON ACTIVITY AND PERSISTENCE OF ATRAZINE, 2,4-D AND METOLACHLOR

4.1 Introduction

The performance of herbicides and pesticides is judged according to three criteria, *viz.,* biological activity, selectivity and persistence. Therefore, knowledge about all three attributes is crucial for predictions of the effectiveness, as well as the environmental impact of those compounds. Season-long weed control from a soil-active herbicide is often considered to be a desirable attribute. In order to manage the use of a potentially persistent herbicide in a way that acceptably limits the risk of damage to following crops, an understanding of herbicide properties and behaviour in soil is required (Reinhardt, 1993).

If the herbicide remains in the soil at concentrations above the injury threshold of a sensitive crop during the following season, injury symptoms and, in extreme situations, reduced yields, can be expected. Experience has shown that some persistent herbicides may affect a particular follow-up crop in one year but not the next, at one location and not another and/or at some application rates but not others (Barnes, *et al.*, 1989). In addition, excessive persistence of a compound in the soil would increase the risk of contamination of both surface and groundwater with residues of the chemicals.

Excessive herbicide persistence, e.g. the continuation of herbicidal activity beyond the time of planting a sensitive following crop, is determined by the interaction of three factors: application rate, crop sensitivity and rate of dissipation. The dosage, together with the sensitivity of the rotational crop, will determine how much of the applied herbicide must be removed from the root zone before recropping can be safely performed. Exact relationships between environmental factors and herbicide persistence in soil are not well quantified, because the rate at which a herbicide degrades is influenced by several soil and weather factors (Van Rensburg & Van Wyk, 1986). Soil is a complex medium in which biological, chemical and physical factors such as microorganisms, texture, pH, and organic matter are important variables (Duffy, 1991). All of these may interact to determine the degradation rate of a particular compound and hence its persistence. The rate of herbicide dissipation in soil determines how long it will take for the necessary loss to occur in order to render the compound inactive. Soil properties and climate greatly influence the process of herbicide dissipation and the bioavailability of the herbicide to the following crop. The potential for persistence can be assessed by direct measurement of the herbicide residue in a field or by means of computer simulation models.

The aim of these experiments were to investigate the possibility of increased herbicide groundwater contamination, and also the risk to sensitive follow-up crops, due to increased herbicide persistence resulting from the interaction of gypsiferous mine-water with the herbicides atrazine, metolachlor and 2,4-D.

4.2 Materials and Methods

The bioassay technique was employed to assess the presence of biologically active residues of the three herbicides at specific stages after the compounds were applied to soil. Bioassays have the advantage that they measure only the plant-available residue fraction in soil. Their disadvantages are that they are non-specific and qualitative. In contrast, the detection methods used for analytical determination of residues have greater specificity, are quantitative, and require less time to produce results (Duffy, 1991). Results from chemical analyses are however more difficult to interpret since the amount detected is not necessarily available for uptake by plants. Analytical methods are therefore ideally suited to herbicide degradation studies in which the effect of herbicide residues on crops is not the main objective.

The persistence experiment involved three herbicides, and each was applied at different rates because the sensitivity of a particular test plant species towards a certain herbicide varied from one herbicide/plant combination to another. Wheat was used as test (indicator) species for atrazine activity, grain sorghum for metolachlor activity, and sunflower for assessing 2,4-D activity (Table 4.1). The soil type was the same as the one used in the experiments reported earlier (Chapter 3). Pots were filled with 1kg soil that either contained gypsum or where gypsum was absent (control).

Pots were lined with plastic bags to avoid herbicide leaching out of the system. As in the previous experiments, the gypsum treatment was 10% gypsum on a mass/mass basis in soil (10 g $CaSO_4$ in 1 kg soil). Herbicides and gypsum were thoroughly mixed prior to the sowing of seeds of test species. For each herbicide, there were five time intervals at which samples were removed for bioassay at a later stage. The stages at which activity was assessed were 0; 15; 30; 45 and 60 days after herbicide application.

Each of the three (atrazine/2,4-D/metolachlor) factorial experiments had the following treatments: 2 (soil with gypsum or without) x 5 (time interval) x 3 (herbicide rates) x 4 (replicates) = 120 pots.

Table 4.1: Herbicide rates (mg kg^{-1}) used in the persistence experiment

Metolachlor	Atrazine	2,4-D
0.000	0.00	0.00
0.125	0.02	0.40
0.250	0.04	0.80

At each time interval batches of 24 pots per experiment were removed from the dark room where they were incubated at 20°C and placed in a freezer and kept until the last set of samples was removed on day 60. At that stage all the samples were removed from the freezer and left until the temperature of all the samples was at room temperature before the bioassaying commenced. Test plants were grown for 21 days after seeds were sown before they were harvested and top growth dry mass was determined. The data were expressed as percentage growth reduction compared to the controls (zero herbicide treatment). Analysis of variance (ANOVA) was done by means of the SAS® programme on the mainframe computer of the University of Pretoria. All data were subjected to analysis of variance and means were compared at the 5% level of significance.

4.3 Results and Discussion

The results for shoot drymass and percentage biomass reduction are presented in Tables 4.2 to 4.7. The abbreviated analysis of variance tables for the two parameters are presented in Tables 9A-13A, respectively, in the appendix. Tables 4.2 and 4.3 show mean shoot drymass and percentage biomass reduction for atrazine, while Tables 4.4 and 4.5 contain data for 2,4-D, and Tables 4.6 and 4.7 data for metolachlor. Shoot drymass and percentage biomass reduction data for atrazine indicate that there was a significant second-order interaction between the effects: soil, rate and time. For 2,4-D and metolachlor, however, only the first-order interaction (rate x time) was significant in the case of both parameters. For the metolachlor experiment the same interaction was significant for both parameters.

Percentage biomass reduction (% SDM) results for atrazine, 2,4-D and metolachlor are presented graphically in Figures 4.1, 4.2 and 4.3, respectively. Simple linear regression was used to illustrate the rate of dissipation of herbicides in the two soils (with gypsum added and no gypsum). As was expected, the activity of all the herbicides decreased with time. This is the general trend of herbicide dissipation in soils (Walker, 1989). The presence of gypsum had a significant effect on the rate of herbicide dissipation (loss) in case of only atrazine (Figure 4.1). In the presence of gypsum the rate of loss of atrazine was significantly slower compared to its rate of loss in soil that did not contain gypsum.

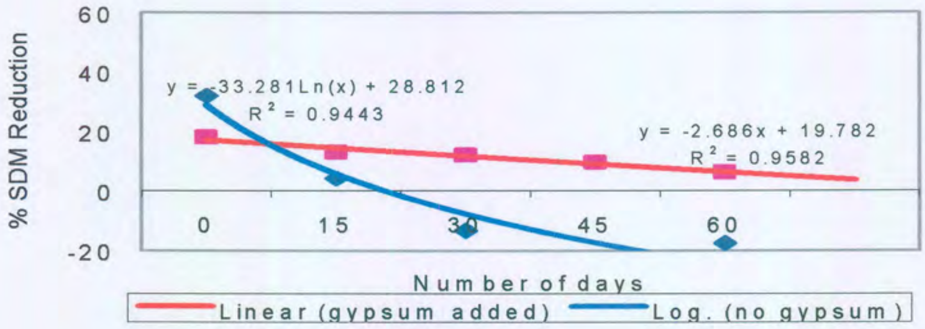

Figure 4.1 Atrazine persistence in soil with or without gypsum added over 60 day period.

Table 4.2: Means for shoot drymass (SDM) (wheat) from the atrazine experiment (g/plant)

Time in days	0			15			30			45			60		
Atrazine rates Mg kg^{-1}	0.00	0.02	0.04	0.00	0.02	0.04	0.00	0.02	0.04	0.00	0.02	0.04	0.00	0.02	0.04
Soil without gypsum	0.15	0.10	0.12	0.15	0.21	0.14	0.11	0.21	0.24	0.25	0.19	0.26	0.21	0.19	0.20
Soil with gypsum	0.21	0.20	0.24	0.27	0.19	0.24	0.30	0.19	0.21	0.22	0.26	0.24	0.31	0.25	0.19

Soil x Rate x Time LSD $_{(p. 0.05)}$ = 0.17

Table 4.3: Means for percentage biomass reduction (wheat) from the atrazine experiment (g/plant)

Time in days	0			15			30			45			60		
Atrazine rates Mg kg^{-1}	0.00	0.02	0.04	0.00	0.02	0.04	0.00	0.02	0.04	0.00	0.02	0.04	0.00	0.02	0.04
Soil without gypsum	15.3	41.7	33.3	13.9	0.00	19.4	37.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
Soil with gypsum	19.4	25.9	8.33	0.00	29.6	9.26	0.00	26.8	20.4	16.7	1.85	10.1	0.00	7.40	28.7

Soil x Rate x Time LSD $_{(p.\ 0.05)}$ = 24

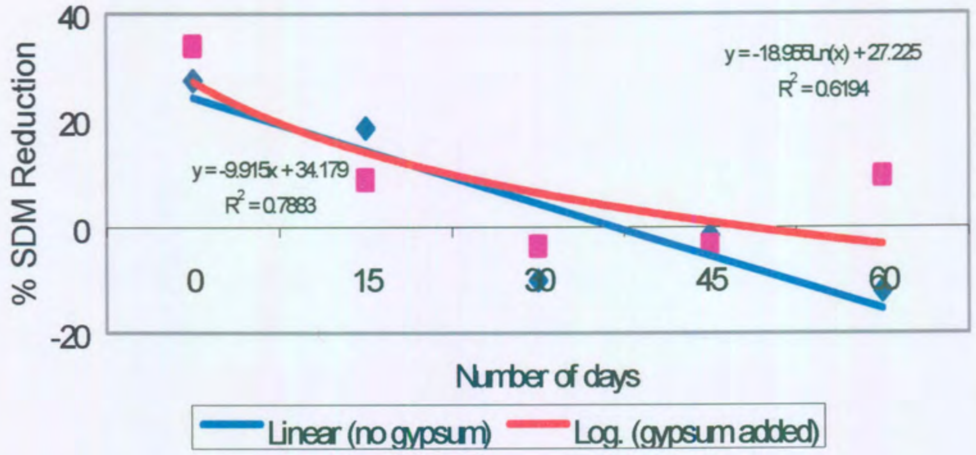

Figure 4.2: 2,4-D persistence in soil with or without gypsum added over a 60 day period

Table 4.4: Means for shoot drymass (sunflower) from the 2,4-D experiment (g/plant)

2,4-D rates g kg^{-1}	Time in days 0	15	30	45	60
0.0	0.5	0.6	0.6	0.6	0.6
0.4	0.6	0.6	0.6	0.6	0.6
0.8	0.4	0.6	0.5	0.6	0.6

Rate x Time LSD $_{(P. 0.05)}$ = 0.19

Table 4.5: Means for percentage biomass reduction (sunflower) from the 2,4-D experiment

2,4-D rates g kg^{-1}	Time in days 0	15	30	45	60
0.0	16.1	0.0	0.0	0.0	0.0
0.4	2.1	4.5	0.0	2.0	3.1
0.8	31.1	0.0	12.9	0.0	0.0

Rate x Time LSD $_{(P.\ 0.05)}$ = 30.6

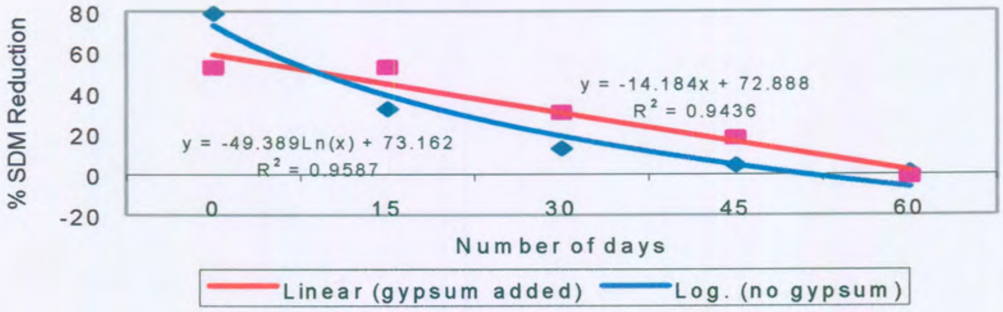

Figure 4.3 : Metolachlor persistence in soil with or without gypsum added over a 60 day period

Table 4.6: Means for shoot drymass (sorghum) from the metolachlor experiment (g/plant)

Metolachlor rates g kg^{-1}	Time in days 0	15	30	45	60
0.00	70.1	75.3	74.5	74.7	86.0
0.125	61.5	67.2	77.3	79.3	74.5
0.250	27.7	42.7	59.7	62.6	78.8

Rate x Time LSD $_{(P.\ 0.05)}$ = 23.9

Table 4.7: Means for percentage biomass reduction (sorghum) from the metolachlor experiment

Metolachlor rates g kg^{-1}	Time in days 0	15	30	45	60
0.00	9.2	1.5	2.9	1.2	0.0
0.125	17.1	11.6	0.0	0.0	2.0
0.250	65.8	41.9	19.5	17.5	0.0

Rate x Time LSD $_{(P.\ 0.05)}$ = 31

The reduced rate of loss of atrazine in the presence of gypsum implies that its persistence was increased (Figure 4.1). The positive aspect of this effect is that the herbicidal effect of atrazine will be extended in soil containing high amounts of gypsum. On the downside, this prolonged persistence would have serious ramifications as far as the potential for carry-over of phytotoxic residues to sensitive follow-up crops is concerned. Also, the longer the compound persists in soil, the greater is the risk that it may be transported to surface and groundwater. The time that herbicides persist in the soil is of particular importance as this has already been shown to have serious implications for the safety of sensitive crops (Reinhardt, 1993).

In the soil without gypsum, atrazine was apparently degraded to non-phytotoxic levels within about 30 days, and this corresponds fairly well with the half-life figures that are quoted for the herbicide. Furthermore these results also agree with the findings of Martinez *et al.,* (1996) which found that the field half-lives varied from 23 to 57 days for atrazine and from 22 to 42 days for metolachlor in the 0-10 cm soil depth. Reinhardt (1993) found that soil characteristics were clearly determining the persistence of atrazine in soil, thus explaining the longer persistence of atrazine in the montmorillonite clay soil. In the neutral state, atrazine molecule would be relatively weakly bonded to negatively charged colloids which predomonate under high pH conditions. It is also possible that atrazine molecule could be temporarily trapped inside the soil colloids.

The rate of loss of metolachlor was not affected significantly by the presence of gypsum (Figure 4.3), and in the case of 2,4-D (Figure 4.2) the trends were not clear at all. Sanyal *et al.* (1999*)* found that there was a trend for more rapid rates of degradation with increasing numbers of previous herbicide treatments, and that the 50% dissipation time (DT_{50}) of metolachlor declined from 18 days for the first spray to 2.5 days for the fourth spray. They further found that repeated application of metolachlor to soil resulted in the generation of an adapted microbial population with an enhanced ability to degrade the applied herbicide.

4.4 Conclusions

The results for metolachlor and 2,4-D showed significance only in the rate x time interaction. It seems 2,4-D did not have an effect at all and this could be attributed to the fact that it is applied in a spray form. The persistence of atrazine was significantly affected by the presence of gypsum in soil. Results suggest that the persistence of the herbicide will be extended in soil irrigated with gypsiferous mine-water, which could increase the risk of damage to sensitive follow-up crops, and also increase the potential for contamination of water resources with residues of the compound. It is crucial that the effect of gypsum on the leaching potential of all three herbicides be evaluated, in order to predict the risk of these compounds reaching groundwater. Given the effect on atrazine persistence, the leaching behaviour of this herbicide is of paramount importance because the combination of high persistence and high leaching would constitute high risk of environmental pollution.

CHAPTER 5: LEACHING POTENTIAL OF METOLACHLOR AND ATRAZINE IN SOIL IRRIGATED WITH GYPSIFEROUS MINE-WATER

5.1 Introduction

The movement of a herbicide through the soil profile has important implications with respect to its efficacy in controlling weeds, the injury risk to sensitive crops as well as its potential to reach groundwater. Factors affecting the movement of organic chemicals through the soil profile the most are the extent to which they are adsorbed to soil colloids, and their water solubility. The amount of water, its flow rate, and physical properties of the soil are also important (Beckie & McKercher, 1990).

Leaching is the downward movement of a substance with water through the soil. As indicated above, leaching may determine herbicide effectiveness, may explain selectivity or crop injury, or may account for herbicide removal from the soil. The extent to which a herbicide is leached is determined principally by: adsorptive relationships between the herbicide and the soil, solubility of the herbicide in water, and amount of water passing downward through the soil. The strength of "adsorption bonds" is considered more important than water solubility in determining the leaching of herbicides.

Organic matter content in the soil is the most important single factor determining the adsorptive capacity of the soil. The second most important is the clay fraction (Hance, 1980). Besides downward leaching with water, herbicides are known to move upward in the soil by capillary movement of the soil water. If water evaporates from the soil surface, water may move slowly upward and may carry with it soluble herbicides. Leaching of herbicides is one of the main factors governing their dissipation from soil, which not only impacts negatively on weed control efficacy but also constitutes a risk for pollution of groundwater. Atrazine is an example of a herbicide which, although benign in terms of toxicity to human and animals, has been banned in several countries due to detection of residual, non-toxic amounts in groundwater (Reinhardt, 1993). Amongst other herbicides and pesticides it has also been detected in both surface and groundwater resources in South Africa, but so far has escaped being banned. Crop producers in this country would be in even greater dire straits in the event that atrazine and related herbicides be banned, or their use restricted. Leaching of a herbicide beyond the root zone is dependent on the strength by which it is held on soil colloids, and also amount, frequency and intensity of water received. Another factor that determines the extent of leaching is a herbicide's water solubility. The driving force behind leaching is water. Greater water solubility of herbicides will ultimately lead to greater leaching potential (Van Wyk, 1997), which would be exacerbated by weak adsorption of the compounds to soil colloids.

Field studies to monitor the fate of agrochemicals are often required to meet regulatory concerns about the behaviour of a particular chemical / formulation with regard to its potential impact on the environment. Studies may also be carried out to develop a better understanding of specific processes that determine their fate. Herbicide fate in the soil environment depends upon the integrated effect of the processes such as sorption, leaching, degradation, volatilization, erosion and uptake by plants (Van Biljon, 1998). The relative ease with which a herbicide can move vertically in soil is important in determining its efficacy, suitability for use as a placement selective herbicide and potential for contaminating groundwater or drainage effluent. Factors such as the amount of water passing through the profile and the structure of the given profile also determine the extent to which leaching will take place. For a herbicide dissolved in water in the soil, movement is essentially determined by the rate of flow of the water.

The main objective of this study was to investigate whether gypsiferous water, or the presence of calcium sulphate in soil, will affect the leaching behaviour of two important soil-applied herbicides, atrazine and metolachlor. The ultimate aim was to make predictions on the potential of the herbicides to reach groundwater under those conditions.

5.2 Materials and Methods

Two separate experiments were done to investigate the leaching behaviour of metolachlor and atrazine in soil irrigated with gypsiferous mine-water. The soil used was the same one used in previous experiments. 2,4-D was not used in this experiment because it is not considered to be a soil-applied herbicide. The test species for atrazine and metolachlor were wheat and sorghum, respectively. The bioassay technique was used for assessing the extent to which the herbicides leach in soil. Bioassays are valuable tools for quantitative analysis of herbicides in soil and water and susceptible species are frequently used for this purpose (Günther *et al.* 1993). The effect of natural conditions such as intermittent rainfall, transient water flow and fluctuating water tension, all of which may have a greater or lesser influence on leaching, were not investigated.

Soil column preparation

Leaching columns, made from polyvinyl chloride (PVC) pipe (400 mm length, inside diameter 110 mm) were used. This leaching method was used since it approximates field conditions more closely than most other methods (Weber & Whitacre, 1982). Columns were hand-packed with soil collected from Hatfield experimental farm. PVC pipe sections of 100 mm length x 110 mm diameter were attached to each other with silicone rubber. Ridges of silicone rubber were placed inside the columns at the joints of the sections to prevent water moving down the sides. The columns were filled with air-dried soil by shaking the columns lightly several times while pouring in the soil. The bottom ends were closed with mutton cloth to prevent soil from falling out. The soil in the columns was brought to 12% field capacity by adding the required volume (600 ml) of water. The tops of the columns were covered with plastic bags to avoid water evaporation. The "+ gypsum" columns were leached with 600 ml of simulated gypsiferous mine-water to simulate a situation that would exist in a field irrigated with mine-water. The "- gypsum" columns were leached with 600 ml of distilled water. Leachate was collected from the bottom of columns and taken to the laboratory for EC (electrical conductivity) analysis. The EC of leachate was determined and compared to that of the simulated mine-water in order that a uniform EC be established in the columns (Table 5.1). It was determined that 600 ml of water was required before water leached out at the bottom.

Table 5.1: Means for electrical conductivity (EC) for atrazine and metolachlor (mS/m)

Water Source	Without gypsum	With gypsum
Atrazine	313.7	329.7
Metolachlor	85.9	84.6

Herbicide application

A 915 g ai L^{-1} emulsifiable concentrate of metolachlor (Dual S Gold®) and a suspension concentrate of 500 g ai L^{-1} of atrazine (Gesaprim®) were used. Three herbicide rates for each compound were established by applying a certain volume of stock solution to the surface of each soil in each column using a micro-sprayer. Rates for metolachlor and atrazine were: 0, 0.2 and 0.3 mg kg^{-1} and 0, 0.05 and 0.1 mg kg^{-1}, respectively. The herbicides were allowed to equilibrate within the soil for a period of 24 hours before the last 100 ml (500 ml had been applied earlier) was applied to each column to allow water to be collected below the columns. Columns were covered with plastic bags to prevent water evaporation. Following the equilibration period, filter paper disks were placed on top of each core to assist in uniform dispersion of the final 100 ml mine-water across the soil core surface. Application was done with the same micro-sprayer that was used to apply the herbicide. Control columns, not treated with herbicide, received 100 ml distilled water. Soil columns were allowed to drain for 36 hours after water addition was complete. Columns were kept covered with plastic bags at the top to prevent water evaporation. Leachate was collected at the bottom and tested for herbicide residues. Each treatment was replicated four times.

Bioassaying

After leaching (36 h after final 100 ml water was applied) columns of each treatment were divided into the original four 100 mm segments. Each segment was placed in a saucer to allow water to be applied from below, and seeded with both wheat and *Brassica rapa* for atrazine and sorghum for metolachlor. Each segment was covered with plastic at the top to avoid water loss. Six seeds per segment were planted to a depth of 0.5 cm, and thinned to four plants per pot (= segment) after emergence. The pots were arranged according to a completely randomised design at a constant temperature regime of 20/25°C night/day with 18 hour light. Additional water was supplied to compensate for any water loss that could have occurred. Nutrient supply of 100 ml was supplied twice a week as a complete nutrient solution (Nitsch, 1972). Harvesting took place 21 days after planting. Four plants per segment were harvested. Fresh and dry mass of shoots were recorded and expressed as percentage reduction relative to untreated controls at the corresponding soil depth.

Each factorial experiment (one for each herbicide) consisted of the following treatments: 2 (soils) x 4 (soil depths) x 3 (herbicide rates) x 4 (replicates) = 96. Analysis of variance (ANOVA) was done by means of the SAS® programme on the mainframe computer of the University of Pretoria. All data were subjected to analysis of variance and means were compared at 5% level of significance.

5.3 Results and discussions

Results that reflect the leaching potential of metolachlor are presented in Table 5.1, and for atrazine in Tables 5.2 and 5.3. The abbreviated analysis of variance tables for percentage biomass reductions are presented in Tables 14A to 16A in the appendix. The soil x depth interaction was significant for metolachlor. Table 5.1 shows the means of percentage biomass reduction in sorghum at different depths. Metolachlor leached more in soil without gypsum, indicating it was less strongly adsorbed in this soil, which is associated with higher bioactivity. In the 0-10 cm column segment of soil without gypsum the percentage loss in biomass was 10.9% and in the 10-20 cm column segment the loss was 16.3%. There were no further losses in the 20-30 and 30-40 cm columns segments implying that metolachlor was not leached beyond the 10-20 cm zone.

Table 5.1: Mean percentage biomass reduction for metolachlor (test species: sorghum)

Soils	Soil zones (cm)			
	0-10	10-20	20-30	30-40
Without Gypsum	10.9	16.3	0.0	0.0
With Gypsum	39.6	0.0	0.0	0.0

Soil x Depth LSD $_{(p.0.05)}$ = 36

Results for both soils suggest that metolachlor will not leach down to groundwater irrespective of whether irrigation water contains gypsum or not (Table 5.1). The fact that there was higher percentage biomass reduction in the 10-20 cm zone of this soil indicates that metolachlor is dissipated at a faster rate than in the soil containing gypsum. Reduction in the amount of metolachlor in the top 0-10 cm zone of soil may cause a reduction in weed control efficacy. In contrast, soil irrigated with gypsiferous water showed a significant percentage biomass reduction of 39% in the 0-10 cm zone for metolachlor. No further reductions were observed in the subsequent column segments. This implies that metolachlor essentially remained in the top 10 cm of soil where gypsiferous water was used. The reduction in percentage biomass in soil irrigated with gypsiferous water in the 0-10 cm column segment indicates that more weeds with shallow roots will be controlled in this soil. However, this signifies higher biological activity in the presence of gypsum, which contradicts findings for metolachlor that was presented in Chapter 4. The results for atrazine, as judged by the response of *Brassica rapa* (Table 5.2) showed highly significant differences in only the soil main effect, while all other main effects and interactions were not significant.

Table 2: Means for atrazine (*Brassica rapa*) for the two soils used

Soil without gypsum added	2.2
Soil with gypsum added	0

LSD$_{(p.\ 0.05)}$ Soil = 1.3

Results for atrazine on wheat showed significant differences in the depth x rate interaction (Table 5.3). Atrazine mobility in soil and not fluctuation in its concentration in soil governs herbicide availability at the root surface (Nel & Reinhardt, 1984). Most of the atrazine applied at normal rates can be leached out of the root zone, but those fractions adsorbed on the internal surface of 2:1 swelling type clays, such as montmorillonite, would be least subject to leaching. Controlled release formulations may have promise for reducing the mobility of atrazine. Fleming *et al.* (1992) showed that a starch-encapsulated atrazine formulation should result in relatively fast atrazine release and improved weed control, while still reducing the leaching potential of atrazine.

Table 5.3: Means for percentage biomass reduction for atrazine leaching
(Test species: wheat)

Depth	Rates (mg kg^{-1})		
(cm)	0	0.05	0.1
0-10	0.70	42.7	0.00
10-20	1.80	9.00	0.00
20-30	0.00	0.00	21.10
30-40	0.00	2.50	57.20

Depth x Rate LSD $_{(p.\ 0.05)}$ = 36.6

There was no reduction in the 20-30 cm zone and this could not be explained as more growth was experienced. Sigua *et al.* (1993) studied the leaching of atrazine at two different rainfall intensities, and found that after the initial higher rate of leachng at the higher rainfall regime, leaching rate slowed. The small percentage reduction in the 20-30 cm and 30-40 cm zone indicate that atrazine will probably not pose a threat to follow-up sensitive crops at the 0.05 mg kg^{-1} rate. In contrast, growth reductions at the depths 0-10 cm and 10-20 cm indicate that the weeds will be controlled at these soil levels. At the 0.1 mg kg^{-1} rate it was found that there was no growth reduction in the 0-10 cm and 10-20 cm for both soils, however the 20-30 cm and 30-40 cm zones showed a 21.1% and 57.2% growth reduction in biomass, respectively. The magnitude of these reductions suggests a greater threat for groundwater contamination when high atrazine rates are used. These findings could be as a result of the apparent competition between calcium and sulphate salts (ions) for sorption positions with the herbicide, and thus the leaching. There have been findings of atrazine groundwater contamination in other parts of the world, as well as in South Africa (Reinhardt, 1993), consequently atrazine use may be banned or restricted, which will have economical implications for farmers who are reliant on its use. Theoretically, in the column studies, leaching could be initially slow and gradually increase as water penetrated deeper into the columns.

5.4 Conclusions

Metolachlor showed a significant growth reduction of 39% in the 0-10 cm soil segment in soil irrigated with gypsiferous water, and no further losses in subsequent zones were observed. This implies that metolachlor essentially remained in the top 10 cm of soil where gypsiferous water was used. The results also suggest that weeds with shallow roots would be effectively controlled. However, this appears to contradict the findings of reduced biological activity in the presence of gypsum, which was reported in Chapter 4. But then it should be considered that in the earlier experiments (Chapter 4) the herbicide was contained in a plastic bag and could not leach out, or away from the roots of the test species. Also, the gypsum concentration in the present experiment would have differed from that in the earlier experiment. In the atrazine experiment it was found that a rate of 0.1mg kg^{-1} could pose a threat to groundwater contamination and possibly also to sensitive follow-up crops. Atrazine dosage rates in the order of 0.05 mg kg^{-1} may pose no threat to groundwater. However, weed control efficacy at this low rate may be poor. There was no statistical difference between the two soils in terms of the leaching potential of atrazine, which indicates that gypsiferous water does not influence atrazine leaching.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

Gypsiferous mine-water affected the performance of the selected herbicides differently. The biological activity of atrazine and 2,4-D were significantly increased in the presence of gypsum, whilst that of metolachlor was reduced. The activity responses of atrazine and 2,4-D probably do not hold any practical consequences in terms of herbicide efficacy, selectivity or persistence. However, the reduction of metolachlor activity in the presence of gypsum has important practical consequences, and this implies that the weed control by this herbicide will be poor on soils irrigated with water containing high levels of calcium sulphate. These findings do not suggest that selectivity of the herbicide towards the crop would be a concern. The effect on herbicide selectivity is also not an issue in the case of atrazine, because of the inherent high tolerance of the main crop in which it is used, namely maize.

Gypsiferous mine-water significantly reduced the rate of dissipation of only atrazine, implying increased persistence. The positive aspect of this effect is that the herbicidal effect of atrazine could be expected to be high in soil containing high amounts of gypsum. In contrast, the prolonged persistence would have serious ramifications as far as the potential for carry-over of phytotoxic residues to sensitive follow-up crops is concerned. Also, the longer the herbicide persist in soil, the greater is the risk that it may be transported to surface and groundwater.

The leaching potential of both atrazine and metolachlor was not influenced significantly by gypsum. Metolachlor leaching was relatively low compared to that of atrazine. The leaching potential of atrazine was dependent upon the concentration of the herbicide, with significantly higher leaching occurring at the highest rate.

Findings with the most profound implications were that the presence of gypsum promoted the biological activity and persistence of atrazine. Both these responses could increase the risk of environmental pollution for this herbicide. Linking of the inherent moderate to high leaching potential of atrazine to increased activity and persistence in the presence of gypsum makes the high potential for groundwater contamination apparent. Atrazine has already been banned in many countries because residues of the herbicide have been detected in compartments of the environment (water and atmosphere) where its presence is regarded as unacceptable. In order that this very valuable crop protection agent remains on the market, more research is needed to understand its environmental behaviour better.

CHAPTER 7: SUMMARY

The influence of gypsiferous mine-water on key behavioural aspects of three important herbicides: atrazine, metolachlor and 2,4-D were assessed. Bioassay experiments were done to assess the bioactivity and selectivity of the three herbicides in the presence or absence of gypsum. Results showed that the activity of atrazine and 2,4-D were significantly increased in the presence of gypsum, while that of metolachlor was significantly reduced.

Subsequent research was aimed at determining what effect, if any, the presence of calcium sulphate (gypsum) has on the persistence of the same herbicides. Three dosage rates of each herbicide and five time periods were chosen. Treated soil samples were incubated in a dark room at a temperature of 20°C, and at 15- day intervals samples were taken out and frozen. At the final sampling stage (60 days after treatment) all samples were thawed to room temperature and bioassayed. As expected, the activity of all the herbicides decreased over time. The presence of gypsum had a significant effect on the rate of dissipation of only atrazine. Results showed that in the presence of gypsum the rate of loss was significantly less compared to its rate of loss in soil that did not contain gypsum. The reduced rate of loss of atrazine in the presence of gypsum implies increased persistence, which would have serious implications as far as potential for carry-over of phytotoxic residue to sensitive follow-up crops is concerned. In the soil where gypsum was not present atrazine was apparently degraded to non-phytotoxic levels within about 30 days.

With the above findings in mind, it was crucial that the effect of gypsum on the leaching of these herbicides be evaluated, in order to predict the risk of potential groundwater contamination. Only two herbicides, atrazine and metolachlor were used in this experiment because they are soil-applied herbicides. Leaching columns of 400 mm length were cut into four 100 mm sections which were attached together with silicone sealer. The columns were packed with soil, treated with respective herbicides, and then leached with gypsiferous mine-water to simulate a situation that would exist in a field irrigated with mine-water. Herbicides were applied at the top of each column and allowed to equilibrate within the soil for a period of 24 hours. Columns were covered at the top to prevent water evaporation and bioassayed when the leaching process was concluded. Metolachlor showed a significant growth reduction of the test species in the 0-10 cm column in soil irrigated with gypsiferous water and no further losses in subsequent columns were observed. This indicated that in the case of metolachlor there would be no threat of groundwater contamination. Atrazine concentrations in soil in the order of 0.05 mg kg^{-1} may pose no threat to groundwater. However weed control efficacy at this low rate may be poor. In the atrazine experiment it was found that a herbicide concentration of 0.01 mg kg^{-1} could pose a threat to groundwater contamination and possibly also to sensitive follow-up crops. There were no statistical differences between the two soils in terms of the leaching potential of atrazine, which indicates that gypsiferous water does not influence atrazine leaching. However, atrazine rate affected its leaching potential.

Further field research is needed to confirm these results, and more fundamental research should be done to explain the mechanism of these herbicides' behaviour in soil irrigated with gypsiferous water.

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APPENDIX

CONTENTS

Table 1A: Composition of the stock Nitsch (1972) nutrient solution used in the pot experiments

	Salt	Concentration (g 10 l^{-1})
A	KNO_3	610
	KH_2PO_4	310
B	$MgSO_4.7H_2O$	610
	$(NH_4)_2\ SO_4$	310
C	$Ca(NO_3)_2.\ 4H_2O$	2440
	EDTA Na_2Fe	60
D	KCL	6.1
	H_3BO_3	6.7
	$MnSO_4.H_2O$	3.8
	$ZnSO_4.7H_2O$	0.61
	$(NH_4)_6\ MO_724.4H_2O$	6.1
	$CuSO_4.5H_2O$	0.31
	H_2SO_4	0.31 ml

A, B, C and D were made separately to 10 l using distilled water. Once totally dissolved these were made up to 2219 l using distilled water in an asbestos tank.

Table 2A: Abbreviated Anova of shoot dry mass for metolachlor (sorghum) in bioassay experiment

Source	Df	MS	F
Soil	1	0.0352	11.84 *
Rate	9	0.0226	7.62 **
Soil * Rate	9	0.0086	2.90 *
Error	51	0.1519	
Total	70	0.4616	
R^2 = 67.09 %	CV = 50.72		

* significant

** highly significant

Table 3A: Abbreviated Anova of shoot dry mass for 2,4-D (sunflower) in bioassay experiment

Source	Df	MS	F
Soil	1	18.51	437.41 **
Rate	9	0.927	21.92 **
Soil * Rate	9	0.227	5.38 **
Error	55	0.042	
Total	74		
R^2 = 92.31 %	CV = 19.22		

** highly significant

Table 4A: Abbreviated Anova of shoot dry mass for atrazine (wheat) in bioassay experiment

Source	Df	MS	F
Soil	1	0.0007	ns
Rate	9	0.0179	60.50 **
Soil * Rate	9	0.0008	2.60 *
Error	59	0.0002	
Total	78		
$R^2 = 90.62$ %	CV = 21.58		

ns -not significant

*- significant

** - highly significant

Table 5A: Abbreviated Anova of % biomass reduction for metolachlor (sorghum) in bioassay experiment

Source	Df	MS	F
Soil	1	33791.47	38.84 **
Rate	9	4729.51	5.44 **
Soil * Rate	9	1619.40	ns
Error	51	869.94	
Total	70		
R^2 = 67.06 %	CV = 61.14		

ns –not significant

** highly significant

Table 6A: Abbreviated Anova of % biomass reduction for 2,4-D (sunflower) in bioassay experiment

Source	Df	MS	F
Soil	1	66462.63	707.02 **
Rate	9	2057.94	21.89 **
Soil * Rate	9	377.84	4.02 *
Error	55	94.01	
Total	74		
R^2 = 94.36 %	CV = 21.77		

* significant

** highly significant

Table 7A: Abbreviated Anova of % biomass reduction for atrazine (wheat) in bioassay experiment

Source	Df	MS	F
Soil	1	1303.05	25.10 **
Rate	9	416.13	8.02 **
Soil * Rate	9	39.03	ns
Error	53	51.91	
Total	70		
R^2 = 64.03 %	CV = 10.58		

ns – not significant

** highly significant

Table 8A: Abbreviated Anova of shoot dry mass for atrazine (wheat) in persistence experiment

Source	Df	MS	F
Soil	1	0.0924	20.46 **
Rate	2	0.0045	ns
Soil * Rate	2	0.0077	ns
Time	4	0.0174	3.91*
Soil * Time	4	0.0063	ns
Rate * Time	8	0.0019	ns
Soil * Rate * Time	8	0.0110	2.86 *
Error	90	0.0041	
Total	119	0.6888	
R^2 = 46.18 %	CV = 30.19		

ns –not significant

* significant

** highly significant

Table 9A: Abbreviated Anova of % biomass reduction for atrazine (wheat) in persistence experiment

Source	Df	MS	F
Soil	1	6556.95	8.32 **
Rate	2	562.53	ns
Soil * Rate	2	1544.01	ns
Time	4	4303.94	5.46 **
Soil * Time	4	2308.91	2.93 *
Rate * Time	8	757.75	ns
Soil * Rate * Time	8	2590.14	3.29 *
Error	90		
Total	119		
R^2 = 47.44%	CV =		

ns –not significant

* significant

** highly significant

Table 10A: Abbreviated Anova of shoot dry mass for 2,4-D (sunflower) in persistence experiment

Source	Df	MS	F
Soil	1	0.0282	ns
Rate	2	0.0201	ns
Soil * Rate	2	0.0096	ns
Time	4	0.0582	5.32 **
Soil * Time	4	0.0127	ns
Rate * Time	8	0.0237	2.17 *
Soil * Rate * Time	8	0.0160	ns
Error	90	0.0109	
Total	119		
$R^2 = 41.27\%$	CV = 16.99		

ns –not significant

* significant

** highly significant

Table 11A: Abbreviated Anova of % biomass reduction for 2,4-D (sunflower) in persistence experiment

Source	Df	MS	F
Soil	1	1.822	ns
Rate	2	488.35	ns
Soil * Rate	2	221.938	ns
Time	4	1436.74	5.28 **
Soil * Time	4	307.23	ns
Rate * Time	8	590.37	2.17 *
Soil * Rate * Time	8	408.31	ns
Error	90	272.20	
Total	119		
R^2 = 40.08 %	CV =		

ns –not significant

* significant

** highly significant

Table 12A: Abbreviated Anova of shoot dry mass for metolachlor (sorghum) in persistence experiment

Source	Df	MS	F
Soil	1	1.12	65.25 **
Rate	2	0.535	30.97 **
Soil * Rate	2	0.038	ns
Time	4	0.253	14.64 **
Soil * Time	4	0.014	ns
Rate * Time	8	0.063	3.67 *
Soil * Rate * Time	8	0.045	ns
Error	90	0.0173	
Total	119		
R^2 = 73.03 %	CV = 19.49		

ns –not significant

* significant

** highly significant

Table 13A: Abbreviated Anova of % biomass reduction for metolachlor (sorghum) in persistence experiment

Source	Df	MS	F
Soil	1	751.33	ns
Rate	2	9227.88	31.71**
Soil * Rate	2	348.27	ns
Time	4	4781.01	16.43 **
Soil * Time	4	524.21	ns
Rate * Time	8	1189.26	4.09 *
Soil * Rate * Time	8	861.87	ns
Error	90	291.05	
Total	119		
$R^2 = 68.71$ %	CV = 42.8		

Table 14A: Abbreviated Anova of % biomass reduction for metolachlor (sorghum) in leaching experiment

Source	Df	MS	F
Soil	1	681.9	ns
Depth	3	4995.1	7.42**
Rates	2	704.8	ns
Soil * Depth	3	2324.2	3.45*
Soil * Rates	2	441.8	ns
Depth * Rates	6	1439.2	ns
Soil * Depth * Rates	6	720.0	ns
Error	69	673.1	
Total	92	81043.5	
$R^2 = 56.30$ %	CV = 48.95		

Table 15A: Abbreviated Anova of % biomass reduction for atrazine (*Brassica rapa*) in leaching experiment

Source	Df	MS	F
Soil	1	7377.3	9.75**
Depth	3	499.7	ns
Rates	2	2241.4	ns
Soil * Depth	3	477.6	ns
Soil * Rates	2	2062.6	ns
Depth * Rates	6	814.9	ns
Soil * Depth * Rates	6	496.4	ns
Error	71	757.0	
Total	94	80466.7	

R^2 = 56.30 %　　CV = 48.95

Table 16A: Abbreviated Anova of % biomass reduction for atrazine (wheat) in leaching experiment

Source	Df	MS	F
Soil	1	1247	ns
Depth	3	1844	ns
Rates	2	884	ns
Soil * Depth	3	4320	ns
Soil * Rates	2	351	ns
Depth * Rates	6	7684	ns
Soil * Depth * Rates	6	2342	ns
Error	69	2801	
Total	92		

$R^2 = 49.72$ CV = 43.85