

**The influence of different management practices on soil faunal  
activity in vineyard soils.**

By

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# Declaration

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I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously, in its entire or in part, submitted it at any other university for a degree.

**Signature:**

**Date:**

# Abstract

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Food demands for the ever-increasing human population is increasing the pressure on the agricultural sector to produce more food. In order to satisfy these demands, farmers are turning to chemical biocides for the control of pest species to produce greater crop yields. All pesticides must be toxic or poisonous to the target species they intend to control. Unfortunately, most pesticides are toxic or poisonous to non-target organisms as well, with detrimental effects on their health. Organic farming was developed to enhance the overall health of the farm's natural soil-microbe-plant-animal biodiversity. No synthetic fertilisers and/or pesticides are used when farming organically. Life in the soil consists of intricate food webs and interactions between the soil dwelling invertebrates. The soil-organisms are divided into three main groups, viz., Micro-organisms (e.g. protozoa, bacteria and fungi) mesofauna (nematodes, Collembola and Acari) and macro-fauna (e.g. millipedes, isopods, insects, molluscs and earthworms). The invertebrates are very susceptible to chemical contamination by chemical biocides in natural and agro-ecosystems. The soil invertebrate communities are responsible for the decomposition of organic material in soil, thereby remineralising the soil. The decomposition processes start with comminution of the large pieces of organic material by meso- and macro-fauna and ends with the micro-fauna and microbial organisms that complete these processes by returning the nutrients in an inorganic form to the soil. The aim of this study was to investigate whether, and to what extent the soil organisms are influenced by different management practices viz., organic management practices versus conventional management practices. A vineyard on the farm Plaisir de Merle, in Simondium, Western Cape was used for the present study. One half of a one hectare vineyard was managed organically and the other half conventionally. Within each vineyard block six different treatments were performed. Three of the treatments were strictly organic and the other three were strictly conventional. Four replicates of each management treatment were performed. The bait-lamina technique was used to assess the feeding activity of the soil organisms exposed to the different management treatments. In addition to the bait-lamina trials in the vineyard itself, bait-lamina tests were performed in microcosm studies with soil from the organically and conventionally managed vineyard blocks under controlled conditions. In order to

assess the impact of the various pesticides that are used in the vineyards in the conventional way, on the soil fauna, standard acute toxicity tests and behavioural tests were performed on *Eisenia fetida*, the compost worm. The bait-lamina tests in the vineyard revealed that the moisture content of the soil plays an important role in the biological activity of soil fauna. The different management treatments did affect the biological activity of the soil fauna, but seasonal changes also proved to be one of the important factors governing biological processes in the soil. The acute toxicity tests showed that the active ingredients (mancozeb, penconazole and trifloxystrobin) of three of the pesticides that were tested in this study, had negatively affected *E. fetida* at their recommended application concentrations. The remaining two pesticides' active ingredients (glyphosate and N-acetyl salicylic acid) did not affect the earthworms negatively at the recommended application concentrations. The preference behavioural trials showed that *E. fetida* could detect and avoid contaminated substrates at the LC<sub>50</sub>-concentrations of the different pesticides. All the earthworms were influenced positively in the preference behaviour experiments. Because of certain limitations of the bait-lamina technique, it was difficult to formulate conclusions on what happens in the soil. A possible explanation for the differences in feeding activity of soil fauna could be attributed to the migration of the soil fauna to more habitable soil horizons during the dry summer conditions, when most of the pesticides are applied. The ecological relevance of the acute toxicity tests conducted need to be investigated further. It is clear that the acute toxicity tests provided important information that should be considered, but care should be taken and the necessary safety factors be determined and considered when doing risk assessment studies. The results of the preference behaviour studies showed that for certain pesticides *E. fetida* can be a sensitive bioindicator of acute and/or sub-acute lethal toxicity testing but this might not necessarily be the case for other pesticides. The goal of doing laboratory studies is to gain as much information to make reliable extrapolations to field situations from laboratory data. Laboratory-to-field extrapolations are very complicated because of the physico-chemical composition of soil, the unpredictable way pesticides behave within soil and the reaction of soil organisms to the soil and to the chemical biocides that are used. Further studies need to be done in order to fully understand to what extent the soil fauna were affected by the different management practices applied to the vineyard at Plaisir de Merle.

# Samevatting

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Voedsel aanvraag vir die toenemende menslike bevolking plaas groot druk op die landbou sektor om meer kos te produseer. Om aan die voedsel eise te voldoen, gebruik boere al meer chemiese biosiede om pessesies te bestry. Alle pestisiede moet toksies of giftig wees vir die teiken organisme waarvoor dit bestem is. Ongelukkig is die meeste pestisiede ook toksies of giftig vir nie-teiken organismes, wat tot nadelige effekte op hul gesondheid kan lei. Organiese boerdery is ontwikkel om die algehele gesondheid van die plaas se natuurlike grond-mikrobe-plant-dier biodiversiteit te bevorder. Geen sintetiese bemestingstowwe en/of pestisiede mag gebruik word wanneer daar organies geboer word nie. Die lewe in die grond bestaan uit ingewikkelde voedselwebbe en interaksies tussen die grondlewende invertebrate. Die grond invertebrate word verdeel in drie hoof groepe, nl. mikro-organismes (bv. Protozoa, bakterieë en fungi) mesofauna (Nematoda, Collembola en Acari) en makrofauna (bv. Millipoda, Isopoda, Insecta, Mollusca en erdwurms). Die Invertebrata is die mees vatbaarste vir chemiese kontaminasie deur chemiese biosiedes in natuurlike en landbou ekosisteme. Die grond invertebraat gemeenskap is verantwoordelik vir die afbreek van alle organiese materiaal in die grond en dus vir remineralisering van die grond. Die afbreekproses begin by die komminusie van groter stukke organiese materiaal deur die meso- en makrofauna en eindig met die mikrofauna en mikrobies wat die prosesse voltooi deur die nutriente terug te plaas in die vorm van anorganiese produkte in die grond. Die doel van hierdie studie was om te ondersoek of, en tot watter mate, grond organismes geraak word deur verskillende grondbestuurpraktyke, nl. Organiese grondbestuurpraktyke teenoor die konvensionele grondbestuurpraktyke. 'n Wingerd op die plaas Plaisir de Merle, in Simondium, Wes-Kaap, was gebruik vir die huidige studie. Een helfte van 'n een hektaar wingerd is organies bestuur en die ander helfte is op die konvensionele manier bestuur. Op elk van die twee wingerd blokke is ses verskillende behandelings toegepas. Drie van die behandelings was streng organies en die ander drie was streng konvensioneel van aard. Vier replikate van elke behandeling is toegepas op elk van die twee wingerdblokke. Die bait-lamina metode is gebruik om die voedingsaktiwiteit van die grond organismes te assesser. As toevoeging tot die bait-

lamina proewe in die wingerd self, is bait-lamina toetse ook in mikro-kosmosse in die laboratorium gedoen met grond afkomstig vanaf die twee wingerdblokke. Om die impak van die verskillende pestisiede op die grondorganismes te ondersoek, is standaard akute toksisiteitstoetse en gedragstoetse uitgevoer met die komposerdwurm, *Eisenia fetida*. Die bait-lamina resultate in die wingerd het getoon dat die voginhoud van die grond die belangrikste rol speel wat die biologiese aktiwiteit van die grondorganismes beïnvloed. Die verskillende behandelings het die biologiese aktiwiteit van die grond fauna geïmmuniseer, maar seisoenale veranderinge is ook uitgesonder as een van die bepalende faktore wat die biologiese prosesse in die grond stuur. Die akute toksisiteitstoetse het getoon dat die aktiewe bestandele van drie van die pestisiede (mancozeb, penconazole en trifloxystrobin), *E. fetida* negatief beïnvloed het teen die aanbeveelde konsentrasies wat toegedien is. Die aktiewe bestandele van die ander twee pestisiede (glyphosate en N-asetiel sallisiel suur) het nie die erdwurms nadelig beïnvloed teen die aanbeveelde konsentrasies wat toegedien is nie. Die gedragstoetse het getoon dat *E. fetida* die LC<sub>50</sub>-konsentrasies van al die verskillende pestisiede kan waarneem en vermy. Al die erdwurms is positief beïnvloed in die gedragseksperimente met die verskillende pestisiede. Omdat die bait-laminametode sekere beperkings het, was dit moeilik om tot gevolgtrekkings te kom oor wat presies in die grond gebeur. 'n Moontlike verklaring vir die verskillende voedingsaktiwiteite van die grond fauna kan toegereken word aan die migrasie van die grondorganismes na meer leefbare grondhorisone gedurende die droë somer toestande, wat toevallig met die spuit van die meeste pestisiede ooreenstem. Die ekologiese relevansie van die akute toksisiteitstoetse wat uitgevoer is, moet meer deeglik ondersoek word. Die belangrikheid van die akute toksisiteitstoetse is duidelik en het waardevolle informasie gelewer, maar sorg moet geneem word, en die nodige veiligheids faktore moet bepaal word en in ag geneem word, wanneer riskobepalingstudies gedoen word. Die gedragstoetse het getoon dat vir sekere pestisiede *E. fetida* 'n sensitiewe bioïndikator van akute en/of sub-akute letale toksisiteits toetse kan wees, maar nie noodwendig vir ander pestisiede nie. Die doel van laboratoriumstudies is om so veel as moontlik inligting te versamel om vertroubare ekstrapolasie te kan maak na situasies in die veld vanaf laboratorium data. Laboratorium-na-veld ekstrapolasies is dikwels baie gekompliseerd as gevolg van die fisies-chemiese samestelling van die grond, die onvoorspelbare manier waarop

chemiese pestisiede met die grond reageer en die reaksie van die grondorganismes op chemiese biosiede in die grond. Verdere studies moet gedoen word om so deeglik moontlik die mate van die impak wat die verskillende bestuurspraktyke op die grond fauna het, te verstaan op Plaisir de Merle.

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# 1. Introduction

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Over the last decades food demands on the agricultural sector have increased dramatically due to the world-wide population increase. Farmers turned to chemical biocides to prevent and eliminate pests such as weeds and disease to produce greater crop yields. The variety of pesticides available to farmers today does benefit the control of pests, but may also have negative effects on non-target organisms (NTO's). The Food and Agriculture Organisation (FAO) (1998) use the word "pest" as an umbrella term referring to all forms of life that affect the farm's productivity negatively and include nematodes, fungi, insects, etc. The word "pesticide" refers to all agricultural biocides such as nematicides, fungicides, insecticides, herbicides, etc. According to Hock & Brown (1999) all pesticides must be toxic, or poisonous, to be effective against the pests they are intended to control. The main use of pesticides is for conventional agricultural purposes and vector control (Dikshith 1990). Pesticides have acted in an advantageous manner by saving millions of people from hunger and disease, but have also been disadvantageous by causing poisoning and death to a large number of people around the world (Krishna Murti 1990).

Farming in South Africa is divided into two major systems, i.e. the large-scale, export driven industrial agriculture that concentrate mainly on maximising produce and the small-scale subsistence farming mainly concentrated in the rural areas (Raath 2000). Conventional farming refers to the high-technology industrial agricultural practices that make use of synthetic fertilisers and pesticides. Conventional farming management often does not benefit the rural-based small-scale farmer because of the lack of money and the

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necessary knowledge to implement such management systems. This is especially the case in the local viticulture of South Africa (Raath 2000).

In recent years the concept of organic farming practices were developed to decrease or even eliminate the use of pesticides and to revitalise our soils with organic matter. It was also developed to create sustainable production levels without sacrificing economic concerns (Njoronge 1997). Organic agriculture focuses on maintaining and improving the overall health of the farm's natural soil-microbe-plant-animal biodiversity (FAO 1998). One of the fundamental aspects of organic farming is not to make use of any synthetic fertilisers and/or pesticides (FAO 1998). Instead of using synthetic fertilisers and pesticides, farmers rely on local biological resources and environmental friendly techniques to combat pests and keep the soil fertile (Planck 1998), thereby eliminating the risk of poisoning NTO's and thus promoting the natural soil processes for efficient nutrient turnover. The loss of nutrients through leaching is potentially harmful to the environment (Danish Directorate for Development 1999). The imbalance created by this loss of nutrients causes some functional groups of soil fauna and flora to increase or decrease, because each functional group fills a specific trophic level (Swift *et al.* 1979). Fungi and bacteria act as important sinks and sources of minerals and nutrients (Jenkinson & Ladd 1981). A reduction in the numbers of such fundamental decomposers makes nutrients and minerals more likely to be leached out of the soil. Certain pesticides used in conventional agricultural methods create an imbalance in the population numbers of certain soil organisms (Beare *et al.* 1992). The decomposition rate of organic matter was reduced when fungi and bacteria decreased (Beare *et al.* 1992), showing that the conventional chemical pest control has detrimental effects on the nutrient turnover in

soils. The time and amount of nutrients released from organic matter are determined by the microbial and soil faunal activities (Swift *et al.* 1979).

Although organic farming does manage to improve the health of the soil in agro-ecosystems, the damage had often already been done by decades of pesticide use before. The conversion from conventional to organic farming can take up to five years or more (Planck 1998). Farmers cannot afford to have lower or no crop yields for five or more years and many are therefore turning to Integrated Pest Management (IPM) practices.

Integrated Pest Management is a pest management system where all suitable techniques are applied in as compatible a manner as possible to maintain the pest population levels below those causing economic injury (Dent 1995). To develop these techniques, each aspect of a pest's taxonomy, biology and ecology is considered in depth by specialists from different disciplines, such as entomologists, pathologists, nematologists or weed scientists (Dent 1995). The goal of IPM is to provide the farmer with an economically viable and suitable means of controlling crop pests (Dent 1995). The IPM research and development therefore tends to be pest or control centred.

Management of the soil organisms in agro- or natural ecosystems can be one of the pathways by which productivity can be optimised and the loss of nutrients prevented (Pokarzhevskij *et al.* 1989). Therefore decomposition - the chemical breakdown of organic compounds by the microbial biomass (Wagner & Wolf 1998), and mineralisation - the conversion of an organic form of an element to an inorganic form as a result of microbial decomposition (Wagner & Wolf 1998) became the main focus areas of terrestrial research. These two processes became the subjects of research for many soil scientists, bacteriologists, mycologists, invertebrate zoologists, ecologists (Seastedt 1984)



and more recently, also ecotoxicologists. According to Seastedt (1984), the great amount of energy that is obtained by plants and animals eventually becomes incorporated into dead organic matter, or detritus. Decomposition and mineralisation of the detritus and organic matter are important for the continued productivity of terrestrial ecosystems (Seastedt 1984).

Soil organisms such as bacteria and fungi play a very important role in ensuring the fertility of soils. They biochemically transform organic matter into nutrients, making it available to plants (Jenkinson & Ladd 1981). In many soils earthworms incorporate organic matter into the soil while bacteria and fungi do the final breakdown of the material (Swift *et al.* 1979). The relationship between soil fauna and micro-organisms is a critical factor in the processes responsible for the release of organically bound nitrogen (N), phosphorus (P) and potassium (K) to the soil (Pokarzhevskij *et al.* 1989). The soil micro-arthropods also contribute to N availability by direct N mineralisation or indirectly by grazing on the N-fixing bacteria and in that way keeping them in a more active state (Hassink *et al.* 1993). An increase in micro-arthropod densities has been correlated with increased foliage, root and microbial productivity (Lussenhop 1981). Over a short term, comminution reduces nutrient availability by the conversion of nutrient ions to organic compounds as a result of assimilation of the ions by the microbial biomass (i.e. immobilising) (Myrold 1998). Over the long term comminution increases the net loss of organic material and indirectly the availability of nutrients by stimulating the decomposing activity of the micro-organisms (Anderson 1988a, b). This means that nutrients are released slowly over a longer period of time. In natural ecosystems the organic input and output is significantly lower than the matter circulating within the

ecosystem and the nutrient turnover is relatively closed. In agro-ecosystems the nutrient circulation is largely open because most of the nutrients are taken away with the harvested yield and fertilisers are added to compensate for nutrient loss (Pokarzhevskij *et al.* 1989). A number of other factors such as leaching and the complex-forming of nutrients, which make nutrients non-available to plants, contributes to the degradation of the soil in agro-ecosystems.

Acari and Collembola are by far the two most important groups of micro-arthropods in the soil (Swift *et al.* 1979) and account for up to 95% of the soil arthropod fauna (Seastedt 1984). Seastedt (1984) also found that 69% of the total decomposition of organic matter in the soil was a result of micro-arthropod activity. Swift *et al.* (1979) state that an important contribution of soil arthropods is the breakdown of organic material into smaller pieces, comminution, enabling the fungi and bacteria to accelerate decomposition processes. According to Anderson (1988a, b), the direct role of the soil arthropods in the soil is to change the non-available nutrients into available nutrients for plants and also indirectly by affecting the functioning of the fundamental decomposers – the bacteria and fungi. Bacterial mass in soils, studied by Hassink *et al.* (1993), was nine times as much as the combined mass of fungi, protozoa and nematodes. These authors also found that the total number of bacteria correlates positively to the total amount of pore spaces between 0.2  $\mu\text{m}$  and 1.2  $\mu\text{m}$ . Other soil organisms such as Collembola were strongly affected by the amount of pore space (Christiansen 1964). This means that the soil pore size and number of pores play an important role in defining the population structure of the soil organisms, forming an intricate soil food web, created by the abiotic and biotic components of the terrestrial environment. The fungi and bacteria serve as

food sources for protozoa, nematodes and other soil arthropods, like many mites (Acari), woodlice (Isopoda), millipedes (Diplopoda) and various insects (Insecta) e.g. ants (Swift *et al.* 1979). Setälä *et al.* (1990) found that the soil fauna significantly increased the levels of N and P. The micro-arthropods are also important for their contribution of dispersing fungal spores and bacteria (Visser 1985).

To assess the activity of the decomposer organisms, various techniques have been developed over the years (Helling *et al.* 1998). One of the easiest methods is the use of bait substances that attract the organisms. The bait-lamina technique, introduced by Von Törne (1990), is a quick and simple screening method for studying the biological activity in soils. This technique is based on visual feeding lesions on small portions of thin laminated bait substrate after exposure to decomposition processes in the soil (Von Törne 1990). Larink (1994a) suggested that the bait-lamina test could be used to evaluate newly developed pesticides by using it as a first screening method in field experiments. Since the initial stages of the development of the bait-lamina test in 1989 (Von Törne 1990), many studies have been carried out using this method (Helling *et al.* 1998; Irmeler 1998; Kratz 1998; Potthoff & Loftfield 1998; Paulus *et al.* 1999). The tests require the insertion of plastic strips with bait filled holes into the soil and taking them out approximately after 10 – 14 days. The feeding activity of the soil fauna reflects the soil biological activity and can also be seen as a measure of the rate of decomposition. The “feeding activity” quantitatively reflects a number of decomposition processes including the decomposition of the bait by micro-organisms; a process not normally regarded as ‘feeding’. It is not clear from the bait-lamina test which taxa are responsible for consuming the bait substrate or in what proportions (Helling *et al.* 1998).

## **1. The Pesticides**

In this study a range of different pesticides, mostly fungicides, were applied to a vineyard in the Western Cape of South Africa. A scheduled spraying programme on a conventionally and organically managed vineyard block was followed and studied, using the bait-lamina technique.

### ***1.1. The Conventionally Managed Vineyard Block***

On the conventionally managed vineyard block the fungicides used included Tridex® (mancozeb), Demildex® (copper oxychloride), Topaz® (penconazole) and Flint® (trifloxystrobin).

#### ***1.1.1. Demildex® (Copper oxychloride)***

Copper containing pesticides are the second highest used chemicals after sulphur used for agricultural purposes in South Africa (London & Meyers 1995). Copper oxychloride is such a fungicide and it is used against brown rot, anthracnose, black spot and downy mildew. It is considered one of the cheapest fungicides in its price-class (Van der Merwe 1991; Walker *et al.* 1997). This fungicide is diluted in water and sprayed directly on the leaves of vines and can therefore contaminate the soil both directly and indirectly (Malkomes 1997). In South Africa copper oxychloride is applied several times during the growing season of grapes (De Klerk 1988), at intervals of two to three weeks after the winter rain at a rate of between 1.25 and 7.5 kg per hectare (Krause *et al.* 1996). The general toxicity of copper oxychloride is considered to be low (Thomson 1978; Roark & Dale 1979; Högger & Ammon 1994), but Helling *et al.* (2000)

found that copper oxychloride does affect the growth and reproduction of the earthworm species *Eisenia fetida* at relatively low concentrations

### **1.1.2. Tridex® (Mancozeb)**

Mancozeb is an ethylene bisdithiocarbamate that is placed in toxicity class IV (Appendix A) by the EPA and is registered as a General Use Pesticide (GUP) (Edwards *et al.* 1991). Mancozeb contains two physiologically essential heavy metals viz., manganese (20%) and zinc (2.5%). A reported acute oral LD<sub>50</sub> of greater than 1 000 mg.kg<sup>-1</sup> in rats, indicates that mancozeb is practically nontoxic via the oral route (Edwards *et al.* 1991, Kidd & James 1991). Mancozeb is rapidly absorbed into the body of animals through the digestive tract and distributed to a number of target organs, including the thyroid gland (US EPA 1988). Carcinogenic- (US EPA 1988), teratogenic (Edwards *et al.* 1991) and mutagenic (US EPA 1988) effects have been observed in rats at high concentrations. Reinecke *et al.* (2002) found that despite the low toxicity of mancozeb, earthworms (*E. fetida*) demonstrated avoidance response at relatively low concentrations (8 mg.kg<sup>-1</sup>). Vermeulen *et al.* (2001) found that mancozeb had no significant negative effect on growth and reproduction of *E. fetida* at 8 mg.kg<sup>-1</sup> (recommended single dose) or at 44 mg.kg<sup>-1</sup> (estimated environmental concentration). Mancozeb is highly toxic to fish and aquatic invertebrates (DuPont de Nemours 1983), but because mancozeb is practically insoluble in water, it is unlikely to infiltrate groundwater (Wauchope *et al.* 1992). Mancozeb has a half-life of one to seven days and is of low soil persistence (Wauchope *et al.* 1992). In water, mancozeb has a half-life of

one to two days under slightly acidic to slightly alkaline conditions (Dupont de Nemours 1983).

#### **1.1.3. Topaz® (Penconazole)**

Penconazole is one of the so-called sterol biosynthesis inhibitor (SBI) fungicides (Halleen & Holtz 2001). It is used especially for powdery mildew in several viticulture areas in South Africa. Penconazole is a systemic fungicide that acts on specific sites within the fungal cells (Schnabel & Jones 2001). Penconazole is a very effective fungicide, but intensive use can lead to the development of resistance in certain medicinal (Hitchcock 1993) and agriculturally important (Walmsey-Woodward *et al.* 1979) fungi. Where multiple applications are performed regularly, the fungicide becomes useless at the recommended application rate (Halleen *et al.* 2000). The recommended application rate is usually what is considered a “safe” concentration when beneficial organisms are taken into account. Another related effect of the SBIs to target pests is the possibility of cross-resistance (Halleen & Holtz 2001). When a species is cross-resistant to a range of pesticides, it means that the organism can be more tolerant to a pesticide without ever having been exposed to it.

#### **1.1.4. Flint® (Trifloxystrobin)**

Trifloxystrobin works as a fungicide by interfering with the respiration of the plant pathogenic fungi (EPA 1999). When used at recommended application concentrations, trifloxystrobin will not cause an adverse impact on groundwater through leaching (New York State Department of Environmental conservation 2000). The New York State Department of Environmental Conservation (2000) also states that trifloxystrobin should only be used in ground based applications, because this chemical

poses a high level of risk to aquatic organisms when applied aerially. Trifloxystrobin is presumed to be highly toxic to fish and aquatic invertebrates (EPA 1999; New York State Department of Environmental Conservation 2000), but adverse effects are limited because of the low exposure concentrations when used at recommended application concentrations. Trifloxystrobin degrades rapidly in water and soils (half-life of hours – days) by mechanisms of metabolism, photolysis and hydrolysis (EPA 1999). The aggregate risk of trifloxystrobin does not exceed the EPAs level of concern (EPA 1999).

## **1.2. *The Organically Managed Vineyard Block***

The use of pesticides in the organically managed vineyard block was limited to Sting® (glyphosate), Bio-build® (N-acetyl salicylic acid) and Demildex® (copper oxychloride). The herbicide, Sting was only applied on one treatment plot where a full chemical weed control was performed (Appendix B).

### **1.2.1. *Sting® (Glyphosate)***

Glyphosate [N-(phosphonomethyl) glycine] is a colourless crystal at room temperature (Kidd & James 1991). It is used as a broad-spectrum, non-selective systemic herbicide for the control of grasses, sedges, broad-leaf weeds and woody plants. This chemical is commonly used in salt form and is generally distributed as water-soluble concentrates and powders (Kidd & James 1991). Glyphosate has an oral LD<sub>50</sub> of 5600 mg.kg<sup>-1</sup> in rats (Weed Science Society of America 1994). In chronic toxicity tests conducted on rats, no adverse effects were observed in rats given doses of 400 mg.kg<sup>-1</sup> per day (Weed Science Society of America 1994). Glyphosate does not appear to be teratogenic (US EPA 1992), mutagenic (Weed Science Society of America 1994), carcinogenic (US EPA 1992). Micro-organisms exhibit a wide range of tolerance and

microflora are generally not affected by it (Sassman *et al.* 1984). Because glyphosate is non-lipophilic, it does not easily bioaccumulate in animal tissue (Sassman 1984) and is poorly absorbed in the digestive tract of mammals (Malik *et al.* 1989). With an estimated half-life of 47 days (Weed Science Society of America 1994), glyphosate is moderately persistent in soil. Although it is highly soluble in water, glyphosate does not leach appreciably and it is estimated that less than 2% of the applied chemical is lost through runoff (Malik *et al.* 1989).

### **1.2.2. *Bio-build*® (*N-acetyl salicylic acid*)**

Salicylic acid is a phenolic acid found naturally in insects and plants (EPA 1998). When used as a fungicide, e.g. in *Bio-build*®, it is used in conjunction with other natural compounds and a light oil which activates the plant's natural resistance mechanisms and is an approved product that may be used by producers in the Société Générale de Surveillance (SGS) organic certification programme (SGS South Africa Pty LTD). Salicylic acid has been tested on numerous species in long term dietary studies and at a range of concentrations with no observed adverse effects (EPA 1998). With an acute oral toxicity of greater than 3 000 mg.kg<sup>-1</sup> in rats, salicylic acid falls under Toxicity Category III of the EPA toxicity classification (EPA 1998) (Appendix A). Salicylic acid is highly regulated in plants and animals and because it is applied at concentrations less than or equal to those concentrations found naturally in plants, it will not accumulate easily in animal tissue (EPA 1998).



## 2. Ecotoxicological Tests

To evaluate the effects of pesticides on soil organisms in conventional viticulture, a good understanding of the toxicity of the pesticides is necessary. Most biological assays involving the evaluation of chemicals concentrate on LC<sub>50</sub> or LD<sub>50</sub> data of the chemical for a particular organism (Vermeulen *et al.* 2001). According to Ma (1984), Reinecke (1992) and Gibbs *et al.* (1996), the one objection to using only the above tests is that mortality as a measure of a population's sensitivity to a chemical is not a sensitive or relevant ecotoxicological parameter. The organisms in the population can suffer from negative growth and/or reproductive effects before mortality occurs (Gibbs *et al.* 1996). Another objection is that organisms are normally exposed to low concentrations of a toxic substance over long periods of time (Ecobichon 1992). In acute toxicological tests, the test organisms are exposed to abnormally high concentrations of the test substance for only a short period of time (Vermeulen *et al.* 2001). The results obtained in this manner are not accurate predictions of what might happen under natural conditions. Yeardley *et al.* (1996) proposed that avoidance behaviour could be a more sensitive method to assess toxic stress in animals and could be used as a range finder for acute and chronic toxicity tests. Chemoreceptors in the prostomium of earthworms and sensory tubercles on their bodies are highly sensitive to certain chemicals in the environment (Reinecke *et al.* 2000). If earthworms can detect and avoid certain chemicals, they can minimise their actual exposure to such chemicals.

The aim of this study was to determine whether the activity of soil organisms are influenced differently by the different management practices viz., organic management

versus conventional management. The bait-lamina test was used to assess the feeding activity of the soil organisms in the field under prevailing environmental conditions. Indirectly this could be related to the amount of decomposition in the soil (Von Törne 1990). The influences of different organic and conventional management treatments on the soil fauna were also investigated to determine which favours the soil organisms more.

To eliminate the variable influence of temperature and moisture on the soil organism feeding activity, soil microcosms with bait-laminae were also studied under laboratory conditions. The results were compared to those of the field trials.

Acute toxicity tests ( $LC_{50}$ ) were performed to determine the acute effects, if any, of penconazole, trifloxystrobin, glyphosate and N-acetyl salicylic acid on the mortality and biomass of *E. fetida*. Acute toxicity tests are designed to determine the concentrations of a chemical that produces lethal responses on a test animal during continuous short-term exposure. Acute toxicity tests with *E. fetida* in an artificial substrate (artificial soil) with mortality as endpoint, is an internationally recognised test of the Organisation for Economic Co-operation and Development (OECD 1984).

The preference behaviour of *E. fetida* was also investigated under different concentrations of penconazole, trifloxystrobin, mancozeb, copper oxychloride, glyphosate and N-acetyl salicylic acid. Acute toxicity tests often do not reflect the situation that occurs in the field. The sublethal effects of low concentrations of a chemical on the behaviour of animals are not revealed in  $LC_{50}$  tests. The avoidance or non-avoidance behaviour of earthworms to a pesticide is an important sublethal endpoint in ecological testing because pesticides with a repelling effect could have an effect on the population densities of the earthworm species.

## 2. Materials & Methods

### 2.1. Field Trials

#### 2.1.1. The Vineyard

The field trials were conducted on a one and a half hectare vineyard block on a 974 ha farm in the Paarl valley, Western Cape, South Africa. The farm, Plaisir de Merle, of which 375 ha are used for viticulture out of a possible 402 ha, is situated in Simondium, near Paarl.

				T1R1	T4R2	T2R3	<b>ORGANIC BLOCK</b>
T3R4	T3R1	T1R2	T5R3	T6R3	T5R4	T6R4	
T2R1	T2R2	T6R1	T6R2	T1R3	T4R4	T5R1	
T3R2	T3R3	T2R4	T4R1	T5R2	T4R3	T1R4	
<b>Vines with no treatments between the Organic Block and the Conventional Block</b>							
				T2R1	T4R2	T5R3	<b>CONVENTIONAL BLOCK</b>
T5R4	T3R1	T5R2	T6R3	T1R4	T6R1	T1R2	
T2R3	T4R4	T4R1	T3R2	T1R3	T2R4	T1R1	
T6R2	T3R3	T3R4	T5R1	T2R2	T4R3	T6R4	

(a)

X	X	X	X	X	X	X	X	X	X	X	X	X
4	3	3	3	3	3	3	3	3	3	3	3	1
X	X	X	X	X	X	X	X	X	X	X	X	X
4	3	3	3	3	3	3	3	3	3	3	3	1
X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X
3	5	5	5	5	5	5	5	5	5	5	5	2
X	X	X	X	X	X	X	X	X	X	X	X	X
3	5	5	5	5	5	5	5	5	5	5	5	2
X	X	X	X	X	X	X	X	X	X	X	X	X

(b)

**Figure 2.1:** (a) A diagrammatic layout of the two vineyard blocks. T = Treatment, R = Replication. (b) An example of two adjoining trial plots extracted from the outlay. X = Side rows or barrier vines. **X** = Trial vines. The numbers represent the management treatments.

The vineyard block was divided in half, one half for organic and the other for conventional management. Both halves were subdivided into 24 plots. On each half, six different treatments were performed with four repetitions (Figure 2.1).

### 2.1.2. The Treatment Plots.

The pest and disease control practices in the organically managed vineyard block was managed to meet all criteria set out in the Organic Standards of the British Soil Association (1997). The pest and disease control practices followed in the conventionally managed vineyard block was done according to conventional management practices. The plots on both vineyard blocks received the same soil management treatments. The soil management treatments (Table 2.1) are explained in detail in Appendix B.

**Table 2.1:** The soil management practices (treatments) that were applied to the plots of both vineyard blocks.

Treatment 1	<b>T1</b>	Full surface chemical weed control.
Treatment 2	<b>T2</b>	Soil managed according to IPM (Integrated Pest Management) guidelines.
Treatment 3	<b>T3</b>	Organic management based on self-sufficiency.
Treatment 4	<b>T4</b>	Organic management based on the use of commercial organic products.
Treatment 5	<b>T5</b>	T3 with additional use of EM (Effective Micro-organism)-technology.
Treatment 6	<b>T6</b>	T2 with additional use of EM-technology.

The applications of the treatments were performed by researchers of the Nietvoorbij Centre for Vine and Wine of the Agricultural Research Council (ARC-Infruitec/Nietvoorbij). Soil analysis was performed by ARC-Infruitec/Nietvoorbij. Weather (rainfall) data was also supplied by the ARC-Infruitec/Nietvoorbij to aid this study.

### 2.1.3. Bait-lamina Tests

Bait-laminae (Von Törne 1990) were specially made with minor modifications to the specifications of Helling *et al.* (1998). The plastic strips were 140 mm long, 5 mm broad and 1.5 mm thick. The laminae were perforated in distances of 5 mm with 16 holes. The holes were filled with a bait substance to attract soil organisms.

The bait substance consisted of a mixture of cellulose powder, vine leaf powder and agar-agar in a ratio of 6.5 : 2.5 : 1. Vine leaves were collected from an organically managed vineyard in Paarl before the beginning of the field trials. The leaves were dried in an oven at 60°C for 48 hours and then ground to a powder using a liquidiser. The powder was sieved to obtain a particle size smaller than 200 µm. The bait was prepared by using the dry vine-leaf powder and mixing it thoroughly with the agar-agar and cellulose powder using the given ratios. The powdery bait substance was mixed with a little distilled water (dH<sub>2</sub>O) to form a paste that was smeared into the holes in the bait-laminae by using a knife. The filled bait-laminae were dried in an oven at 40°C for 24 hours. Any excess bait was removed from the surface by hand in order to confine the bait to the holes in the laminae. 16 bait-laminae made up a unit and each unit was wrapped in aluminium foil prior to use. Each unit was used to interpret the feeding activity of the soil organisms spatially per treatment plot.

The 16 bait-laminae were carefully inserted into the soil of each plot by using a knife to make a provisional slit in the soil to prevent the bait-laminae from breaking and/or the bait from falling out. The bait-laminae were inserted in the soil near the stem of a vine and spaced at least 5 cm from one another. Every bait-lamina was inserted to a depth of approximately 85 mm, until the uppermost hole was covered by soil. The bait-laminae were removed carefully after 13 – 17 days and stored in an aluminium foil wrapper until the evaluation of the feeding activity in the laboratory no later than the next day.

The bait-laminae from the field were cleaned individually by wiping them carefully with a damp cloth to remove soil particles and placed on the glass plate of an electronic scanner. The bait-laminae were then covered with a white paper and scanned using extra lighting from the top. This was obtained from a desk lamp. The scanned image was then electronically enhanced and inspected for holes in the bait. The number of empty holes or perforated bait plugs was counted. For better comparison, the feeding activity was expressed in a standardised period of percentage bait eaten per 10 days. To obtain this standardised period, the amount of holes made by the soil fauna was divided by the duration (in days) the bait-laminae were in the field and multiplied by 10. For each plot a control unit was prepared. This unit was placed into the soil of each plot in exactly the same way as the experimental one, but removed immediately after insertion and the holes counted. This control accounts for the holes that are made because of the handling of the bait-laminae. This was done twice, once at the beginning of the trials (Sept. 2000) and again just before the last trial (Apr. 2001).

## **2.2. Microcosm Trials**

Soil was collected from treatment plots one and three (Fig. 2.1) of both the organically and conventionally managed vineyard blocks at the end of the growing season (February 2001) and stored in five litre plastic buckets. T1 and T3 were used because T1 was full chemical control and T3 full organic management. All macro-invertebrates that were visible with the eye were hand sorted and removed from the containers and kept separately. The soil from each treatment was mixed thoroughly before dH<sub>2</sub>O was added to obtain a moisture content of between 20% and 25%. The macro-invertebrates were replaced in the containers from which they originally came from. Straw was placed on the soil of treatment three coming from both the organically and conventionally managed vineyard blocks to mimic the situation in the field. In the field, the soil directly surrounding the grapevines is covered by straw to preserve moisture. The lids of the buckets were perforated. The microcosms were

placed in a climate room at 18°C for seven days to stabilise, after-which bait-laminae (as prepared in 2.1.3) were inserted into the soil. The bait-laminae were removed after 22 days and the feeding activity evaluated in the same way as in 2.1.3. The reason for taking out the bait-laminae after 22 days was because the feeding activity of the soil fauna has to be between 40% and 60% to obtain comparable results.

### 2.3. Toxicity Tests

#### 2.3.1. Test Species

*E. fetida* was used as the test species used for the acute and preference behaviour tests. This earthworm species is prescribed by the OECD guidelines (1984) as a test species for ecotoxicological testing. The life cycle and behaviour of *E. fetida* have been well documented (Reinecke & Kriel 1981, Venter & Reinecke 1988, Edwards & Bohlen 1992). For the present study earthworms were obtained from the uncontaminated breeding cultures of the department of Zoology at the University of Stellenbosch. These cultures are kept specifically for ecotoxicological tests and are maintained in a climate room at a constant temperature of  $\pm 18^{\circ}\text{C}$ . Only clitellate earthworms were used for this study.

The classification of the species (Simms & Gerard 1985):

Phylum	: Annelida
Subphylum	: Clitellata
Class	: Oligocheata
Order	: Haplotaxida
Suborder	: Lumbricina
Superfamily	: Lumbricoidea
Family	: Lumbricidea (Ralfinesque-Schmaltz 1815)

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Subfamily : Lumbricina (Ralfinesque-Schmaltz 1815)

Genus : *Eisenia*

Species : *Eisenia fetida* (Savigny 1826)

### 2.3.2. Acute toxicity tests

The LC<sub>50</sub>-values of the different pesticides were determined for *E. fetida* using methods recommended by the OECD guideline no. 207 (OECD 1984). An artificial soil was prepared consisting of a mixture of 69% quartz sand, 20% kaolin clay, 10% peat moss and 1% calcium carbonate (CaCO<sub>3</sub>). Because no range-finding tests were performed, the concentrations of the different pesticides were selected according to their recommended agricultural application concentration. The recommended agricultural concentrations of the respective pesticides were used as the median value of a geometric series, with two concentrations lower and between two and five concentrations higher than the median value. The pesticides were diluted with dH<sub>2</sub>O to the appropriate concentrations and mixed into the substrate. The moisture content of the artificial soil was between 30% and 35%. This was measured by using a Sartorius infrared moisture detector. The pH of the substrate was monitored with a Crison micropH 2001 (KCl-electrode) pH meter using approximately 1g of substrate suspended in 40 ml of dH<sub>2</sub>O.

Ten clitellate worms were randomly selected from an uncontaminated breeding stock. The worms were individually weighed using a Sartorius balance and placed into each experimental container. Three replicates of each concentration were prepared and the containers were kept in a climate room at 18°C for 14 days. After 7 and 14 days, earthworms were handsorted out of the substrate and the biomass of the surviving earthworms and

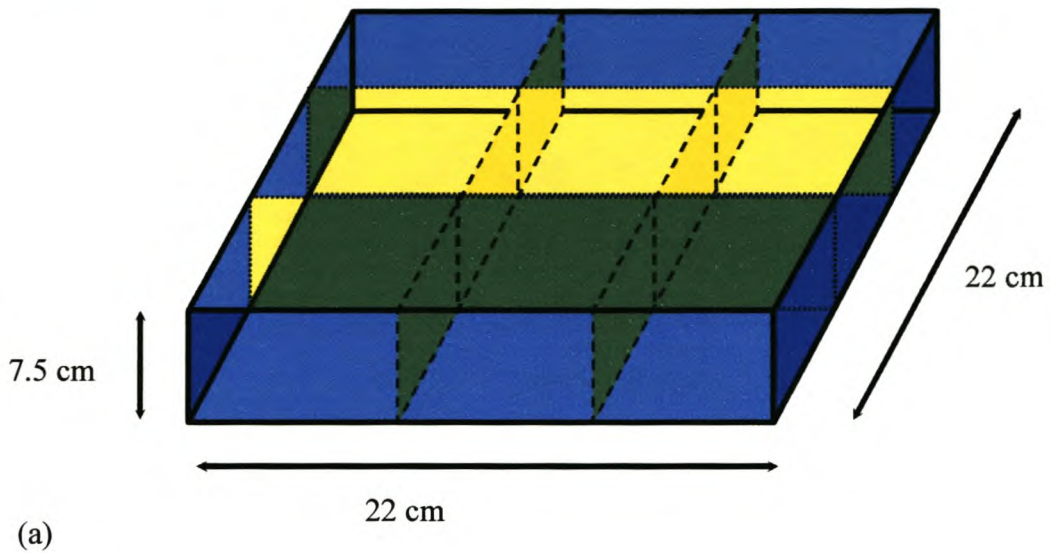


mortality recorded. Earthworms were considered dead if they did not respond to a mechanical stimulus to the anterior end of their body.

## 2.4 Preference Behaviour Tests

The effects of the different pesticides were further evaluated by carrying out avoidance behavioural tests. *E. fetida* were exposed to different concentrations of every pesticide as well as a control substrate to see if the worms had a preference for contaminated or uncontaminated substrates. Perspex boxes of 484 cm<sup>2</sup> were used with four removable Perspex partitions to divide the box into nine cubicles of 49 cm<sup>2</sup> each (Fig. 2.2a,b). Urine free cow manure substrates were used for the experiment. Fresh cow manure was collected from the field at Welgevallen Estate in Stellenbosch, air dried, milled to powder and sieved to a particle size of < 850 µm. Every cubicle was filled with 35g of the dried organic substrate mixed with 80ml dH<sub>2</sub>O. For the contaminated cubicles the substrate was mixed with the specific amount of the pesticide dissolved in the 80 ml of dH<sub>2</sub>O and was mixed thoroughly. The moisture content of both the contaminated and uncontaminated substrates was between 70% and 75%.

The LC<sub>50</sub> values obtained from the acute toxicity tests for each of the pesticides for *E. fetida* were used as the uppermost limits of concentrations for each test. The concentrations of the different pesticides that the earthworms were exposed to were as follow: LC<sub>50</sub>, LC<sub>50</sub> ÷ 5, LC<sub>50</sub> ÷ 10, LC<sub>50</sub> ÷ 50, LC<sub>50</sub> ÷ 100 (Table 2.2). Four control cubicles with uncontaminated substrate as shown in Figure 2.2 (a) and (b) were also used. Five clitellate worms with a biomass of between 0.3g and 0.6g were placed into each of the cubicles containing contaminated substrates. Two replicates of the experiment were done for every pesticide. The biomass of the earthworms was determined before and after the experiment.



	A	B	C
1	LC <sub>50</sub> ÷ 50 5 Worms	Uncontaminated Substrate 0 Worms	LC <sub>50</sub> ÷ 10 5 Worms
2	Uncontaminated Substrate 0 Worms	LC <sub>50</sub> 5 Worms	Uncontaminated Substrate 0 Worms
3	LC <sub>50</sub> ÷ 5 5 Worms	Uncontaminated Substrate 0 Worms	LC <sub>50</sub> ÷ 100 5 Worms

———— Sides of the experimental container.

----- The partitions that divide the container into cubicles. This is taken out after the worms were put in.

(b)

**Figure 2.2:** (a) A diagrammatic representation of the apparatus and the experimental design used in determining the preference behaviour of *E. fetida* to a range of concentrations lower or equal to the LC<sub>50</sub> for the different pesticides. (b) Earthworm numbers and substrate concentration levels in the experimental container before the partitions were removed.

The test containers were kept in a climate-controlled room at 18°C for the duration of the test.

After seven days the worms in every cubicle were counted and weighed again.

**Table 2.2:** The actual nominal concentrations ( $\text{mg.kg}^{-1}$ ) of the respective pesticides used in the preference behaviour study with *E. fetida*.

	<b>B2</b>	<b>A3</b>	<b>C1</b>	<b>A1</b>	<b>C3</b>
	<b>LC<sub>50</sub></b>	<b>LC<sub>50</sub> ÷ 5</b>	<b>LC<sub>50</sub> ÷ 10</b>	<b>LC<sub>50</sub> ÷ 50</b>	<b>LC<sub>50</sub> ÷ 100</b>
<b>Copper oxychloride</b>	882.78	176.56	88.28	17.66	8.83
<b>Mancozeb</b>	2332.50	466.50	233.25	46.65	23.33
<b>Penconazole</b>	379.00	75.80	37.90	7.58	3.79
<b>Trifloxystrobin</b>	770.00	154.00	77.00	15.40	7.70
<b>Glyphosate</b>	3737.41	747.48	373.74	74.75	37.37
<b>N-acetyl salicylic acid</b>	312.70	62.54	31.27	6.25	3.13

## 2.5. Statistical Analysis

The data obtained from the bait-lamina trials, moisture content and acute toxicity tests were analysed using Jandel Scientific SigmaStat® 2.0 software. All the raw data were analysed by the Kolmogorov-Smirnov normality test to determine whether the data were parametric or non-parametric. One-way analysis of variance (ANOVA) was performed on all parametric data. Kruskal-Wallis one-way ANOVA on ranks was performed on all non-parametric data. Student-Newman-Keuls method (all pairwise multiple comparison) was performed as a post-hoc test. A *P* value <0.05 was considered significant.

Data obtained from the preference behaviour tests were analysed by applying  $\chi^2$ -tests. This method of evaluating the data was done with the help of Prof. S.C. Maritz from the Department of Statistics at the University of Stellenbosch. The first analysis was to determine whether a statistically significant preference occurred for different cubicles within the test containers. If a statistically significant difference or preference did occur between cubicles, a second  $\chi^2$ -test was performed. In the latter  $\chi^2$ -test, the cubicles with the least or highest number of earthworms were omitted, as well as the cubicles they were in. This was done to determine whether the cubicles that did have earthworms in them showed a statistically significant difference or not. P-values were then looked up in a  $\chi^2$ -distribution table (Finney 1971).

## 3. Results

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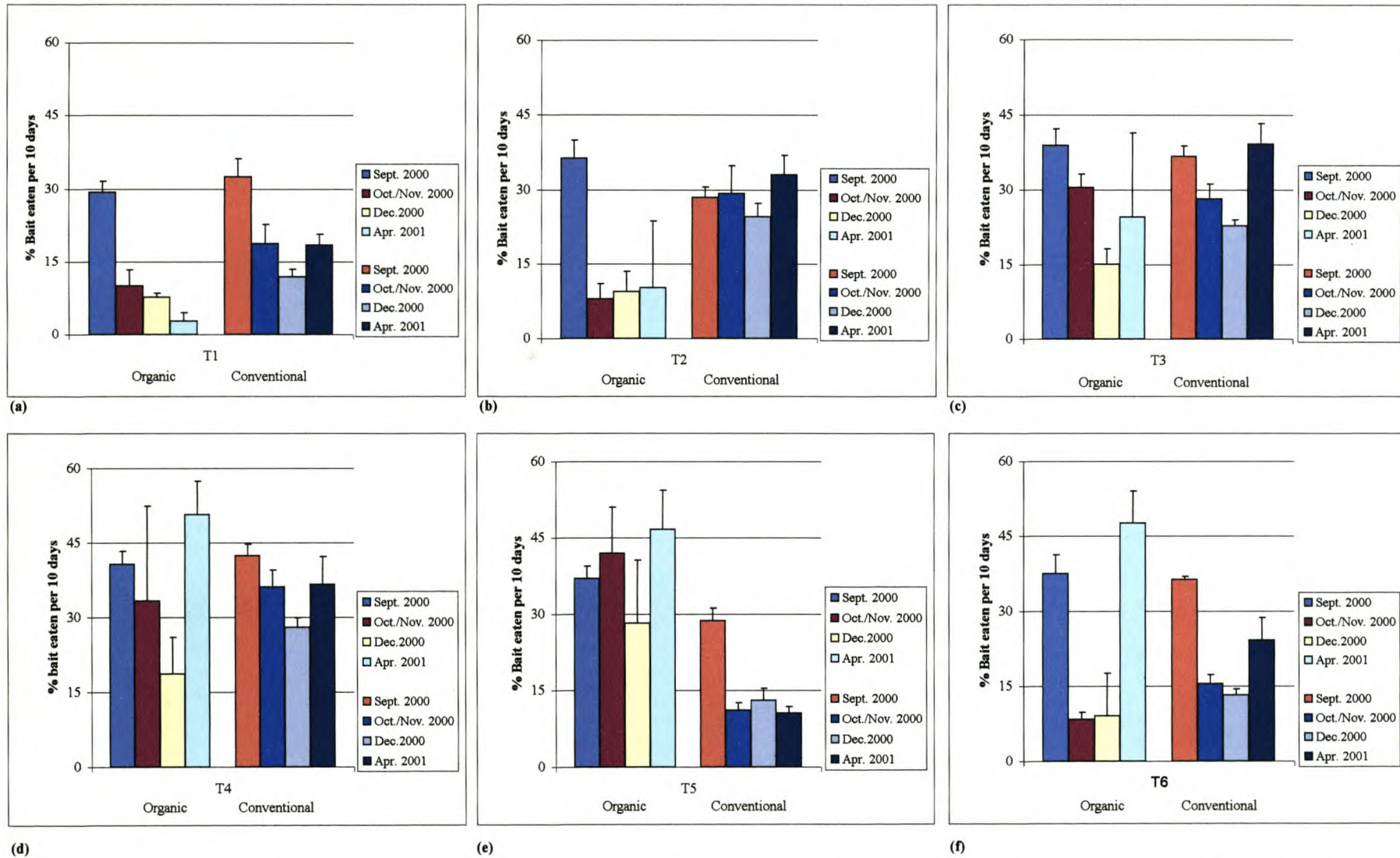
### 3.1.1. Bait-lamina Tests in the Field

#### 3.1.1.1. *The organically vs. the conventionally managed vineyard block*

The feeding activity of the soil fauna did not differ statistically significantly ( $P>0.05$ ) between all the treatment plots from both the organically and conventionally managed vineyard blocks prior to (Sept. 2000) and after (Oct./Nov. 2000, Dec. 2000 and Apr. 2000) the application of the management treatments (Fig. 3.1.1a-f, Table 3.1.1).

The bait-lamina sets (Oct./Nov. 2000, Dec. 2000 and Apr. 2001) of T1, coming from the organically managed vineyard block, had a significantly ( $P<0.05$ ) lower feeding activity (Fig. 3.1.1a) than the bait-lamina set that was exposed prior to the application (Sept. 2000) of the management treatments. Bait-lamina sets of T1, from the conventionally managed vineyard block, showed a significant ( $P<0.05$ ) decrease in feeding activity in Oct./Nov. 2000, Dec. 2000 and Apr. 2001 when compared to those of Sept. 2000 (Fig. 3.1.1a). No significant ( $P<0.05$ ) difference in feeding activity was observed between Oct./Nov. 2000, Dec. 2000 and Apr. 2001, when comparing the feeding activity within the conventional vineyard block. When comparing the feeding activity of the organically and conventionally managed vineyard blocks, with respect to T1, no significant ( $P>0.05$ ) difference in feeding activity was observed during the Oct./Nov. 2000 and Dec. 2000 sets (Table 3.1.1). A significantly ( $P<0.05$ ) higher feeding activity of the soil fauna occurred in the conventionally managed vineyard block during the Apr. 2001 set than in the organically managed vineyard block (Fig. 3.1.1a).

Treatment two (T2) of the organically managed vineyard block resulted in a significantly ( $P<0.05$ ) lower feeding activity of soil fauna in the treatment sets that followed the initial treatment set in September 2000 (Fig. 3.1.1b). No significant ( $P<0.05$ ) difference in feeding activity was observed between bait-lamina sets of T2 coming from the conventionally managed vineyard block (Fig. 3.1.1b). When comparing T2 of the organically and conventionally managed vineyards blocks, the conventionally managed vineyard block had a significantly ( $P<0.05$ ) higher feeding



**Figure 3.1.1:** The total feeding activity, measured with the bait-lamina method, in percentage ( $\pm$ SD) for the exposure period of between 13 and 17 days, normalised to % bait eaten per 10 days, for the management treatment plots of both the organically and conventionally managed vineyard blocks. The legend shows the time of year when the bait-laminae were exposed in the field. The differences in feeding activity obtained by comparing the organically and conventionally managed vineyard blocks are shown in a-f.

activity than the organically managed vineyard block in sets Oct./Nov. 2000, Dec. 2000 and Apr. 2001 (Table 3.1.1).

T3 of the organically managed vineyard block showed a significant ( $P < 0.05$ ) decrease in feeding activity from the initial bait-lamina exposure set (Sept. 2000) to sets in Oct./Nov. 2000 and Dec. 2000 (Fig. 3.1.1c). The feeding activity increased again in the Apr. 2001 set, but it was not significant ( $P > 0.05$ ). No significant ( $P > 0.05$ ) difference in feeding activity of soil fauna occurred in T3 from the conventionally managed vineyard block (Fig. 3.1.1c). No significant ( $P > 0.05$ ) difference in feeding activity of T3 was observed when comparing the organically and conventionally managed vineyard blocks (Table 3.1.1).

Although a decrease in feeding activity of soil fauna was observed in T4 of the organically managed vineyard block, it was not significantly ( $P > 0.05$ ) lower than prior to the application (Sept. 2000) of the management treatments (Fig. 3.1.1d). T4 of the organically managed vineyard had a significantly higher feeding activity in the Apr. 2001 – set than the Dec. 2000 – set (Fig. 3.1.1d). T4 from the conventionally managed vineyard block showed no significant ( $P > 0.05$ ) difference in feeding activity from the beginning of the field trial in Sept. 2000 to Apr. 2001 (Table 3.1.1). No significant ( $P > 0.05$ ) difference occurred in T4 when the organically and conventionally managed vineyard blocks were compared to each other (Fig. 3.1.1d).

No significant ( $P > 0.05$ ) difference in feeding activity of T5 from the organically managed vineyard block occurred from the start to the end of the field trials (Fig. 3.1.1e). A significantly ( $P < 0.05$ ) lower feeding activity occurred after the conventional management treatment started. The low feeding activity continued until the end of the field trial in Apr. 2001. T5 of the Oct./Nov. 2000, Dec. 2000 and Apr.

2001 – sets from the organically managed vineyard block had a significantly higher feeding activity than that of the conventionally managed vineyard block (Table 3.1.1).

The feeding activity in T6 of the organically managed vineyard block was significantly lower in the Oct./Nov. 2000 and Dec. 2000 bait-lamina sets (Fig. 3.1.1f). The conventionally managed vineyard block showed a similar decrease in feeding activity for T6 in the Oct./Nov. 2000 and Dec. 2000 bait-lamina sets, but a significant increase in the Apr. 2001 set (Fig. 3.1.1f). When comparing the organically and conventionally managed vineyard blocks no significant difference was observed for the Oct./Nov. 2000 and Dec. 2000 – sets. A significantly ( $P < 0.05$ ) higher feeding activity in T6 from the organically managed vineyard block in Apr. 2001 occurred when comparing it with the conventionally managed vineyard block (Table 3.1.1).



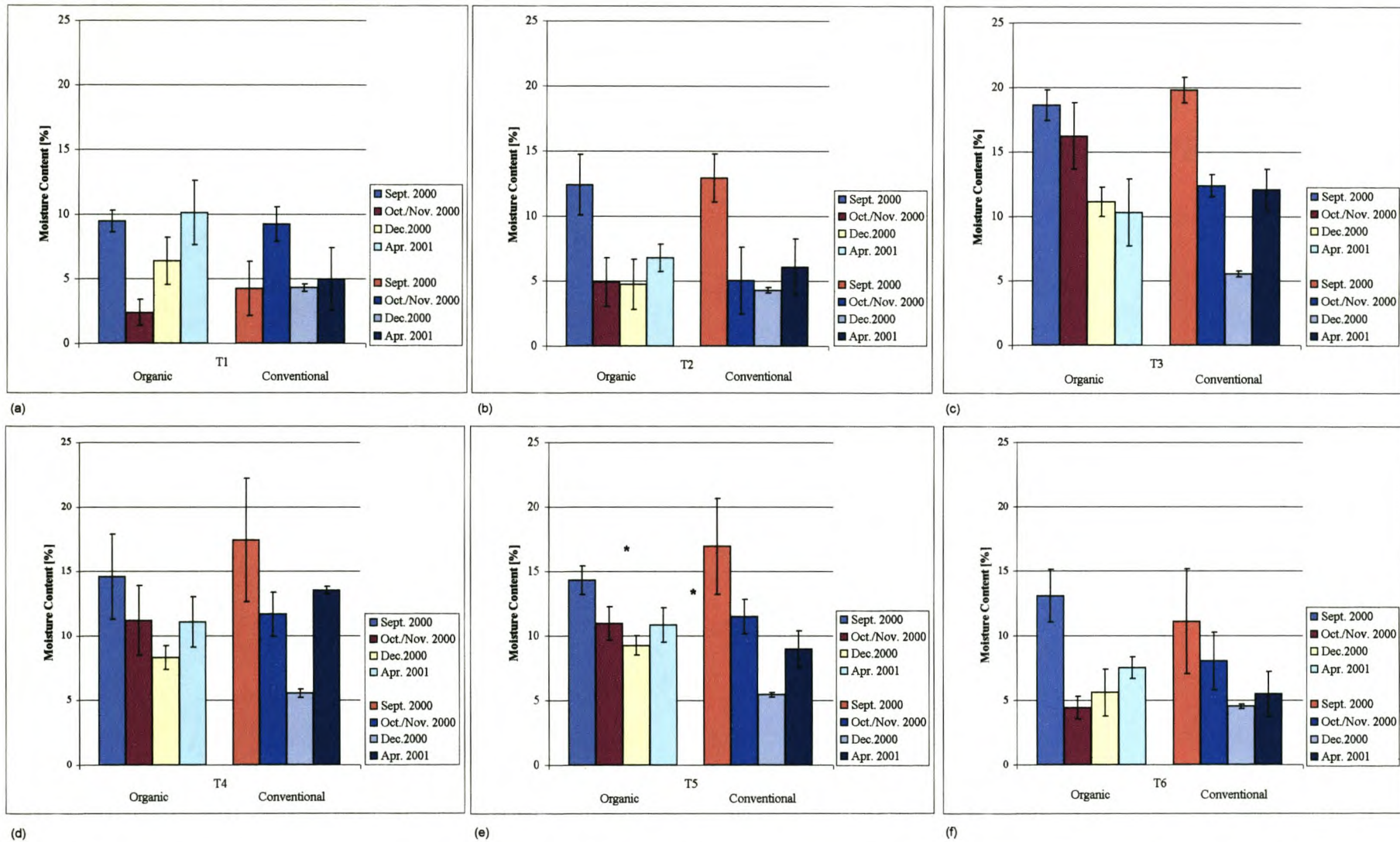
**Table 3.1.1:** The mean ( $\pm$ SD) percentage of bait eaten after field exposure of between 13 and 17 days, normalised to percentage bait eaten per 10 days, of the bait-lamina trials put out in the six treatment plots of the organically and conventionally managed vineyard blocks respectively.  $n = 4$  units of bait-laminae (one unit = 16 bait-laminae).

	Plots	Vineyard Management	Bait-lamina Exposure Trials			
			Sept. 2000	Oct./Nov. 2000	Dec. 2000	Apr. 2001
% Bait eaten per 10 day Period	T1	Organic	29.35 $\pm$ 2.3	10.11 $\pm$ 3.2	7.76 $\pm$ 0.8	2.76 $\pm$ 1.7
		Conventional	32.73 $\pm$ 3.7	18.73 $\pm$ 4.0	11.95 $\pm$ 1.5	18.44 $\pm$ 2.2
	T2	Organic	36.31 $\pm$ 3.6	7.98 $\pm$ 3.1	9.47 $\pm$ 4.1	10.26 $\pm$ 13.4
		Conventional	28.44 $\pm$ 2.1	29.30 $\pm$ 5.5	24.53 $\pm$ 2.8	33.03 $\pm$ 3.9
	T3	Organic	38.87 $\pm$ 3.3	30.45 $\pm$ 2.7	15.08 $\pm$ 3.0	24.59 $\pm$ 16.8
		Conventional	36.71 $\pm$ 2.1	28.21 $\pm$ 3.0	22.75 $\pm$ 1.2	39.18 $\pm$ 4.1
	T4	Organic	40.67 $\pm$ 2.7	33.32 $\pm$ 19.1	18.67 $\pm$ 7.3	50.67 $\pm$ 6.8
		Conventional	42.39 $\pm$ 2.3	36.13 $\pm$ 3.3	27.98 $\pm$ 1.9	36.65 $\pm$ 5.5
	T5	Organic	36.99 $\pm$ 2.4	41.99 $\pm$ 9.0	28.21 $\pm$ 12.3	46.65 $\pm$ 7.7
		Conventional	28.66 $\pm$ 2.5	11.14 $\pm$ 1.4	13.04 $\pm$ 2.3	10.53 $\pm$ 1.3
	T6	Organic	37.51 $\pm$ 3.8	8.33 $\pm$ 1.3	9.02 $\pm$ 8.5	47.65 $\pm$ 6.4
		Conventional	36.31 $\pm$ 0.6	15.51 $\pm$ 1.8	13.15 $\pm$ 1.2	24.18 $\pm$ 4.5

### 3.1.2. Soil Moisture in the Field

#### 3.1.2.1. *Moisture content of the conventionally managed vineyard block regarding sampling periods*

In Sept. 2000, the moisture content of T1 was significantly ( $P < 0.05$ ) lower than that of T2, T3 and T4 (Fig. 3.1.2). In Sept. 2000 the moisture content of T2 was significantly ( $P < 0.05$ ) lower than that of T3 (Fig. 3.1.2). During the same period, T3, T4, T5 and T6 did not have a significantly ( $P > 0.05$ ) higher soil moisture content than T2 (Table 3.1.2). T1 did not have a significantly ( $P > 0.05$ ) lower soil moisture content than T5 or T6 in Sept. 2000. Soil samples taken in Oct./Nov. 2000 showed that the soil moisture content of T2 was significantly ( $P < 0.05$ ) lower than that of T1, T3, T4, T5 and T6 (Fig. 3.1.2). The soil moisture content of T6 was significantly ( $P < 0.05$ ) lower than that of T3 and T5 in Oct./Nov. 2000, but no significant ( $P > 0.05$ ) difference in soil moisture occurred between T3, T4 and T5 (Table 3.1.2). Soil moisture content of T4 was significantly ( $P < 0.05$ ) higher than that of T1, T2 and T6 during Dec. 2000 (Fig. 3.1.2). T3 had a significantly ( $P < 0.05$ ) higher soil moisture content than T1, T2 and T6 during Dec. 2000 (Fig. 3.1.2). The soil moisture content of T5 was significantly higher than that of T1, T2 and T6 during Dec. 2000 (Fig. 3.1.2) too. No significant ( $P > 0.05$ ) differences in soil moisture content occurred between T1, T2 and T6, as well as between T3, T4 and T6 in Dec. 2000 (Table 3.1.2). A significantly ( $P < 0.05$ ) lower soil moisture content occurred in T1 and T2 compared to T3, T4 and T5 in Apr. 2001 (Fig. 3.1.2). Although T3 and T4 did not differ significantly ( $P > 0.05$ ) in soil moisture content during Apr. 2001, both did have a significantly ( $P < 0.05$ ) higher soil moisture content than that of T5 and T6 (Table 3.1.2).



**Figure 3.1.2:** The mean ( $\pm$ SD) moisture content in percentage of the six different management treatment plots of both the organically and the conventionally managed vineyard blocks. The legend shows the time of year when the soil samples were taken and analysed for moisture content.  $n = 4$ . \*  $n = 3$ .

### 3.1.2.2. *Moisture content of the organically managed vineyard block*

During Sept. 2000, the soil moisture content of T3 was significantly ( $P < 0.05$ ) higher than that of T1, T2, T4 and T6 (Fig. 3.1.2), but not when compared to T5 (Fig. 3.1.2). T3 had a significantly higher soil moisture content than all other treatments when sampling took place in Oct./Nov. 2000 (Fig. 3.1.2). T4 had a significantly higher soil moisture content than T1, T2, and T6 when sampled in Oct./Nov. 2000 (Fig. 3.1.2). T1, T2 and T6 did not have a significant ( $P > 0.05$ ) difference in soil moisture content in Oct./Nov. 2000, but had a significantly ( $P < 0.05$ ) lower soil moisture content than that of T5 (Table 3.1.2). T3 had a significantly higher soil moisture content than all other treatments when sampling took place in Dec. 2000 (Fig. 3.1.2). T4 had a significantly higher soil moisture content than T1, T2, and T6 when sampled in Dec. 2000 (Fig. 3.1.2). T1, T2 and T6 did not have a significant ( $P > 0.05$ ) difference in soil moisture content in Dec. 2000, but had a significantly ( $P < 0.05$ ) lower soil moisture content than that of T5 (Table 3.1.2). No significant ( $P > 0.05$ ) difference in soil moisture content occurred between all the treatments in the Apr. 2001 sampling (Table 3.1.2).

**Table 3.1.2:** The mean ( $\pm$ SD) soil moisture content of the treatment plots of the organically and conventionally managed vineyard blocks respectively. Soil samples for analysis of moisture content coincided with the time the bait-lamina trials took place.  $n = 4$ .

	Plots	Vineyard Management	Sampling Times			
			Sept. 2000	Oct./Nov. 2000	Dec. 2000	Apr. 2001
Moisture Content of the Soil [%]	T1	Organic	9.45 $\pm$ 0.8	2.38 $\pm$ 1.0	6.37 $\pm$ 1.8	10.11 $\pm$ 2.5
		Conventional	4.24 $\pm$ 2.1	9.22 $\pm$ 1.3	4.30 $\pm$ 0.3	4.96 $\pm$ 2.4
	T2	Organic	12.41 $\pm$ 2.3	4.90 $\pm$ 1.9	4.73 $\pm$ 1.9	6.77 $\pm$ 1.1
		Conventional	12.93 $\pm$ 1.9	5.03 $\pm$ 2.6	4.29 $\pm$ 0.2	6.06 $\pm$ 2.2
	T3	Organic	18.62 $\pm$ 1.2	16.24 $\pm$ 2.6	11.13 $\pm$ 1.1	10.30 $\pm$ 2.6
		Conventional	19.81 $\pm$ 1.0	12.40 $\pm$ 0.9	5.54 $\pm$ 0.2	12.08 $\pm$ 1.6
	T4	Organic	14.59 $\pm$ 3.3	11.19 $\pm$ 2.7	8.30 $\pm$ 0.9	11.06 $\pm$ 1.9
		Conventional	17.42 $\pm$ 4.8	11.67 $\pm$ 1.7	5.55 $\pm$ 0.3	13.53 $\pm$ 0.3
	T5	Organic	14.31 $\pm$ 1.1	10.97 $\pm$ 1.3	9.25 $\pm$ 0.8	10.84 $\pm$ 1.3
		Conventional	16.93 $\pm$ 3.7	11.49 $\pm$ 1.3	5.45 $\pm$ 0.2	8.97 $\pm$ 1.4
	T6	Organic	13.09 $\pm$ 2.0	4.43 $\pm$ 0.9	5.59 $\pm$ 1.8	7.50 $\pm$ 0.8
		Conventional	11.10 $\pm$ 4.1	8.03 $\pm$ 2.2	4.53 $\pm$ 1.2	5.47 $\pm$ 1.7

### 3.1.2.3. *The organically vs. the conventionally managed vineyard block.*

The soil moisture content of T1 was not significantly ( $P>0.05$ ) higher in Sept. 2000 than the samples taken in Dec. 2000 and Apr. 2000 (Fig. 3.1.2a) within the organically managed vineyard block. The soil moisture content of T1 was significantly lower in Oct./Nov. 2000 compared to all other sampling periods (Fig. 3.1.2a) within the organically managed vineyard block. No significant ( $P>0.05$ ) difference in soil moisture content of T1 was observed between sampling periods (Sept. 2000, Dec. 2000 and Apr. 2000, Oct./Nov. 2000 however did have a significantly higher feeding activity (Table 3.1.2). The organically managed vineyard block always had a significantly ( $P<0.05$ ) higher soil moisture content for every sampling period, except in Oct./Nov. 2000 when no significant ( $P>0.05$ ) difference in soil moisture content occurred between the two vineyard blocks (Fig. 3.1.2a).

T2 of the organically managed vineyard block had a significantly ( $P<0.05$ ) higher soil moisture content in Sept. 2000 than during any of the subsequent sampling periods (Fig. 3.1.2b). No significant ( $P>0.05$ ) difference in soil moisture of T2 was observed when comparing Oct./Nov. 2000, Dec. 2000 and Apr. 2001 within the organically managed vineyard block (Table 3.1.2). Within the conventionally managed vineyard block the soil moisture content of T2 was significantly higher prior (Sept. 2000) compared to samples taken in Oct./Nov. 2000, Dec. 2000 and Apr. 2001 (Fig. 3.1.2b). No significant ( $P>0.05$ ) difference in soil moisture of T2 was observed when comparing Oct./Nov. 2000, Dec. 2000 and Apr. 2001 within the conventionally managed vineyard block (Table 3.1.2). No significant ( $P>0.05$ ) difference in soil moisture content occurred between sampling periods when the organically managed vineyard block was compared to the conventionally managed vineyard block (Fig. 3.1.1b).

T3, within the organically managed vineyard block, did not have a significantly ( $P>0.05$ ) higher soil moisture content prior (Sept. 2000) to application of the management treatment compared to sampling in Oct./Nov. 2000. Both the earlier (Sept. 2000 and Oct./Nov. 2000) samples' moisture content was significantly higher than that of the two later (Dec. 2000 and Apr. 2000) samples (Fig. 3.1.2c). Within the conventionally managed vineyard block the soil moisture content of T3 was significantly ( $P<0.05$ ) higher prior (Sept. 2000) compared to samples taken in Oct./Nov. 2000, Dec. 2000 and Apr. 2001 (Fig. 3.1.2c). The soil moisture content of T3 sampled in Dec. 2000 was significantly lower than that of all the other samples taken before or after that (Fig. 3.1.2c). The organically managed vineyard had a significantly ( $P<0.05$ ) higher soil moisture content compared to the conventionally managed vineyard block during the second (Oct./Nov. 2000) and third (Dec. 2000) sampling periods (Table 3.1.2). The first (Sept. 2000) and last (Apr. 2000) sampling periods did not differ significantly ( $P>0.05$ ) in soil moisture content when comparing the organically managed vineyard block with the conventionally managed one (Table 3.1.2).

T4 of the organically managed vineyard did not show a significantly ( $P>0.05$ ) different soil moisture content for sampling periods during Sept. 2000, Oct./Nov. 2000 and Apr. 2001 (Fig. 3.1.2d). A significantly ( $P<0.05$ ) lower soil moisture content occurred between the Sept. 2000 and Dec. 2000 sampling periods (Fig. 3.1.2d). The only significantly ( $P<0.05$ ) different soil moisture content in T4 within the conventionally managed vineyard block occurred between the first (Sept. 2000) and third (Dec. 2000) sampling periods (Fig. 3.1.2d). T4 of the organically managed vineyard block had a significantly ( $P<0.05$ ) higher soil moisture content during Dec. 2000, but at no other sampling period did a significantly different soil moisture

content occur between the organically and conventionally managed vineyard blocks (Table 3.1.2).

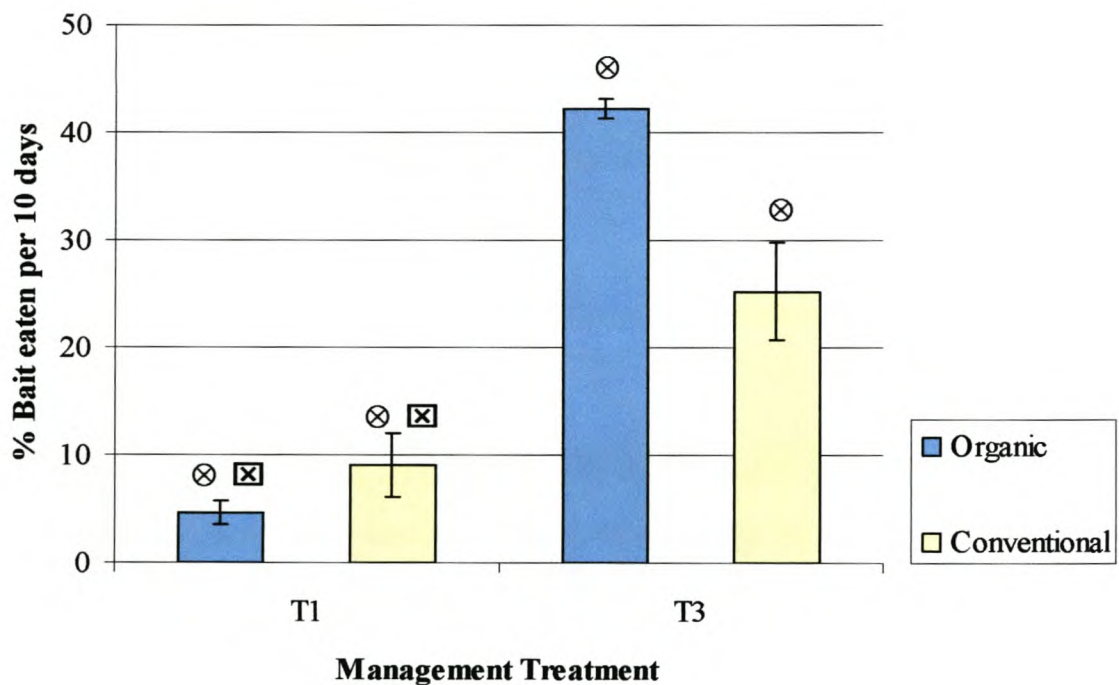
T5 of the organically managed vineyard block had a significantly ( $P < 0.05$ ) higher soil moisture content prior (Sept. 2000 to application of the treatment than in Dec. 2000 (Fig. 3.1.2e). Samples from Sept. 2000, Oct./Nov. 2000 and Apr. 2001 did not have any difference in soil moisture content within the organically managed vineyard (Table 3.1.2). T5 of the conventionally managed vineyard had a significantly ( $P < 0.05$ ) higher soil moisture content in Sept. 2000 than in the other three sampling periods (Fig. 3.1.2e). No significant ( $P > 0.05$ ) difference in soil moisture content occurred between the organically and conventionally managed vineyard blocks during Sept. 2000, Oct./Nov. 2000 and Apr. 2000. Soil moisture content was significantly lower in the conventionally managed vineyard compared to organically managed vineyard in Dec. 2000 (Table 3.1.2).

Within the organically managed vineyard block, T6 had a significantly ( $P < 0.05$ ) higher soil moisture content in Sept. 2000 than in all other sampling periods (Fig. 3.1.1.2f). Soil moisture content did not differ significantly ( $P > 0.05$ ) for T6 within the organically managed vineyard block for Oct./Nov. 2000, Dec. 2000 and Apr. 2001. Within the conventionally managed vineyard block the first two (Sept. 2000 and Oct./Nov. 2000) had a significantly higher soil moisture content than the last two sampling periods (Dec. 2000 and Apr. 2000) (Fig. 3.1.2f). At no time during the trials did the organically and conventionally managed vineyard blocks have a significant ( $P > 0.05$ ) difference in soil moisture content when comparing the two vineyard blocks (Table 3.1.2).



### 3.1.3. Microcosm Trials

A statistically significant ( $p < 0.05$ ) difference in feeding activity of soil fauna was observed between T1 and T3 in the soil of both the organically and the conventionally managed vineyard blocks (Fig. 3.1.3). After 21 days in the climate room, the highest and lowest number of holes eaten per day was measured in T3 and T1 of the organically managed soil, respectively (Table 3.1.3). No significant ( $p > 0.05$ ) difference was observed in feeding activity between the organically and conventionally managed treatment 1.



**Figure 3.1.3:** Mean ( $\pm$ SD) percentage of bait eaten per 10 days after 21 days of exposure in microcosms to soil from treatment 1 (T1) and treatment 3 (T3) of both the organically and conventionally managed plots.  $n = 3$ . ( $\otimes$  Significantly different -  $P < 0.05$ ;  $\otimes$   $\otimes$  not significantly different -  $P > 0.05$ .)

**Table 3.1.3:** Mean ( $\pm$ SD) percentage of bait eaten after 21 days, normalised to bait eaten per 10 days, of exposure in microcosms to soil from treatment 1 (T1) and treatment 3 (T3) of both the organically and conventionally management plots. n = 3.

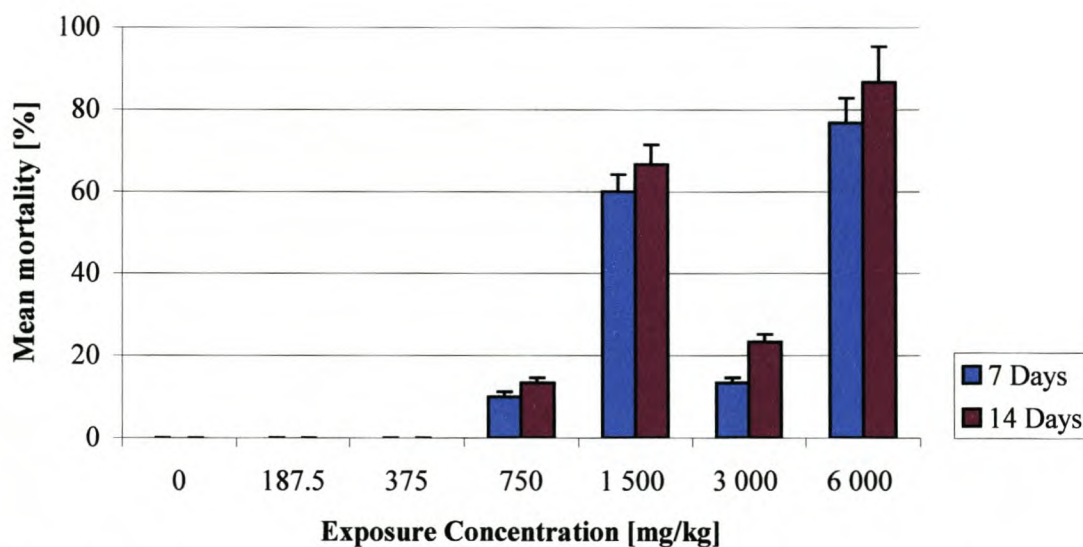
<b>Vineyard Block</b>			
<b>Organically managed</b>		<b>Conventionally managed</b>	
% Bait eaten per 10 days		% Bait eaten per 10 days	
<b>Treatment 1</b>	<b>Treatment 3</b>	<b>Treatment 1</b>	<b>Treatment 3</b>
4.71 $\pm$ 1.1	42.16 $\pm$ 0.9	9.11 $\pm$ 3.0	25.24 $\pm$ 4.6

### 3.2. Acute Toxicity Tests

#### 3.2.1. Mancozeb

##### 3.2.1.1. Mortality of *E. fetida* exposed to mancozeb

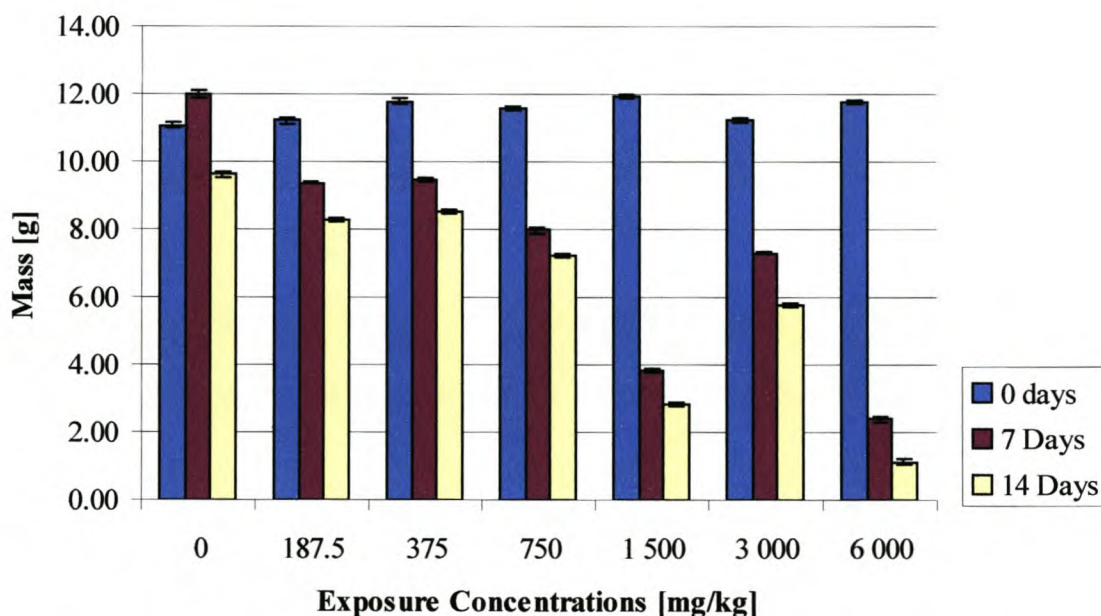
For mancozeb, earthworm mortality increased with an increase in concentration. The percentage mortality of the earthworms after seven days, was highest in the 6 000 mg.kg<sup>-1</sup> and no earthworms died at the lowest exposure concentration of 187.50 mg.kg<sup>-1</sup> (Table 3.2.1.1). No significant ( $P>0.05$ ) difference was observed in the mortality count after one week and two weeks (Table 3.2.1.1; Fig. 3.2.1.1). After 14 days, the  $LC_{50}$  of *E. fetida* for mancozeb was calculated as 2 332.50 mg.kg<sup>-1</sup>. A significantly ( $P<0.05$ ) lower mortality was observed in the worms exposed to 3 000 mg.kg<sup>-1</sup> than those exposed to 1 500 mg.kg<sup>-1</sup> after both seven and 14 days of exposure to mancozeb.



**Figure 3.2.1.1:** Percentage mortality ( $\pm$ SD) of *E. fetida* after seven and 14 days of exposure to a range of mancozeb concentrations from 0 mg.kg<sup>-1</sup> to 6 000 mg.kg<sup>-1</sup>.  $n = 30$ .

### 3.2.1.2. Changes in mass of *E. fetida* exposed to mancozeb

A significant ( $P < 0.05$ ) decrease in total mass of the earthworms was observed with increasing concentrations of mancozeb, although there was no significant ( $p > 0.05$ ) difference between the lowest ( $187.5 \text{ mg.kg}^{-1}$ ) and second lowest ( $375 \text{ mg.kg}^{-1}$ ) exposure concentrations after seven days (Fig. 3.2.1.2). A significant ( $P < 0.05$ ) increase in total earthworm mass was observed in the control after seven days, but after 14 days a significant ( $P < 0.05$ ) decrease occurred (Fig. 3.2.1.2). A significant ( $P < 0.05$ ) decrease in mean mass percentage of the earthworms was observed after 14 days of exposure in all experimental groups compared to the total mass recorded after seven days (Table 3.2.1.1).



**Figure 3.2.1.2:** Changes in total mass of surviving *E. fetida* ( $\pm$ SD) after a seven and 14 day exposure period to mancozeb concentrations ranging from  $0 \text{ mg.kg}^{-1}$  to  $6\,000 \text{ mg.kg}^{-1}$ .  $n = 30$ .

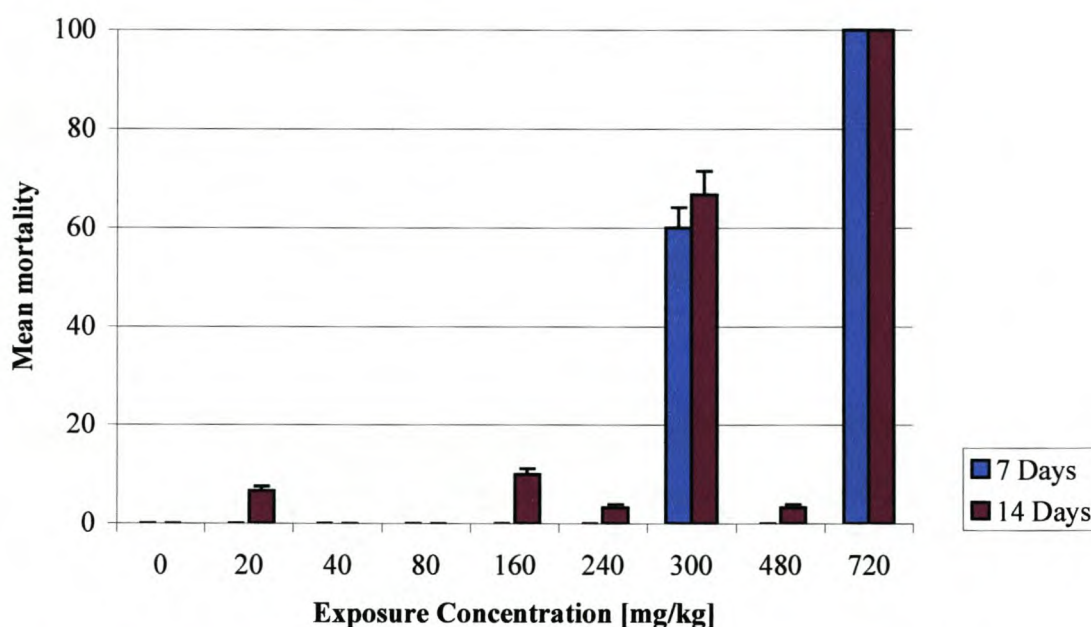
**Table 3.2.1.1:** Mean ( $\pm$ SD) percentage mortality and change in mass ( $\pm$ SD) of *E. fetida* after an exposure period of seven and 14 days to a range of mancozeb concentrations between 0 mg.kg<sup>-1</sup> and 6 000 mg.kg<sup>-1</sup>. Total percentage mass change was calculated using surviving earthworms only.

Concentration mg.kg <sup>-1</sup>	n	Mortality [%]		Mass Change [%]	
		7	14	7	14
0	30	0	0	+8.25 $\pm$ 0.8	-13.22 $\pm$ 0.7
187.50	30	0	0	-16.30 $\pm$ 0.6	-26.22 $\pm$ 0.8
375	30	0	0	-19.73 $\pm$ 0.7	-27.63 $\pm$ 0.7
750	30	10 $\pm$ 1.1	13.33 $\pm$ 1.3	-31.32 $\pm$ 1.4	-37.67 $\pm$ 1.5
1 500	30	60 $\pm$ 4.2	66.67 $\pm$ 4.8	-68.02 $\pm$ 4.3	-76.37 $\pm$ 4.9
3 000	30	10 $\pm$ 1.3	23.33 $\pm$ 1.9	-32.61 $\pm$ 1.5	-48.60 $\pm$ 2.0
6 000	30	76.67 $\pm$ 6.1	86.67 $\pm$ 8.6	-79.57 $\pm$ 0.6	-90.26 $\pm$ 8.8

### 3.2.2. Penconazole

#### 3.2.2.1. Mortality of *E. fetida* exposed to penconazole

100% mortality occurred after seven days in the highest exposure concentration. All the other exposure concentrations except the 300 mg.kg<sup>-1</sup> had no earthworm deaths after 7 days (Table 3.2.1.1). No significant ( $P>0.05$ ) difference was observed in percentage mortality between seven and 14 days of exposure (Fig. 3.2.1.1). A significantly ( $P<0.05$ ) higher percentage mortality occurred at 300 mg.kg<sup>-1</sup> than at 480 mg.kg<sup>-1</sup>, where only two individuals died in the latter after 14 days (Table 3.2.2.1). The 14-day LC<sub>50</sub> of penconazole was calculated as 379 mg.kg<sup>-1</sup>.



**Figure 3.2.2.1:** Percentage mortality ( $\pm$ SD) of *E. fetida* after seven and 14 days of exposure to a range of penconazole concentrations from 0 mg.kg<sup>-1</sup> to 720 mg.kg<sup>-1</sup>. n =30.

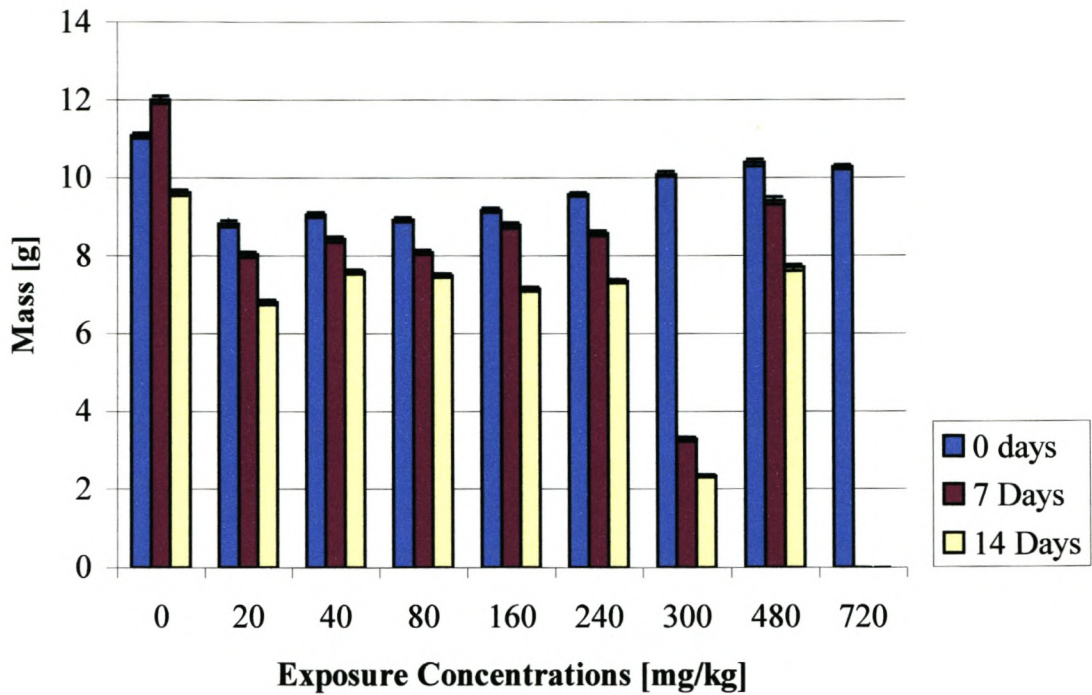
#### 3.2.2.2. Changes in mass of *E. fetida* exposed to penconazole

All exposure groups had a significantly ( $P<0.05$ ) lower total mass compared to the control (Fig. 3.2.2.2). After seven days of exposure to penconazole, no significant

( $P < 0.05$ ) difference in mass percentage could be observed between 20  $\text{mg.kg}^{-1}$ , 40  $\text{mg.kg}^{-1}$ , 80  $\text{mg.kg}^{-1}$ , 160  $\text{mg.kg}^{-1}$ , 240  $\text{mg.kg}^{-1}$  and 480  $\text{mg.kg}^{-1}$  (Table 3.2.2.1). After 14 days the control groups had a significant ( $P < 0.05$ ) decrease in total mass of worms, but when compared to the experimental groups it had a significantly ( $P < 0.05$ ) higher total mass (Fig. 3.2.2.2). Earthworms exposed to 300  $\text{mg.kg}^{-1}$  of penconazole, had the lowest total mass of all the exposure concentrations that and it was significantly ( $P < 0.05$ ) lower than all the other exposure groups.

**Table 3.2.2.1:** Mean ( $\pm$ SD) percentage mortality and change in total mass ( $\pm$ SD) of *E. fetida* after an exposure duration of seven to 14 days to a range of penconazole concentrations between 0  $\text{mg.kg}^{-1}$  and 720  $\text{mg.kg}^{-1}$ . Total percentage mass change was calculated using surviving earthworms only.

Concentration $\text{mg.kg}^{-1}$	n	Mortality [%]		Mass Change [%] *	
		7	14	7	14
0	30	0	0	+8.25 $\pm$ 0.8	-13.22 $\pm$ 0.7
20	30	0	6.67 $\pm$ 0.9	-9.05 $\pm$ 0.9	-22.91 $\pm$ 1.3
40	30	0	0	-6.89 $\pm$ 0.9	-16.13 $\pm$ 0.7
80	30	0	0	-9.35 $\pm$ 0.6	-16.07 $\pm$ 0.7
160	30	0	10.00 $\pm$ 1.1	-4.17 $\pm$ 0.8	-22.14 $\pm$ 1.5
240	30	0	3.33 $\pm$ 0.6	-10.44 $\pm$ 0.7	-23.31 $\pm$ 0.8
300	30	60.00 $\pm$ 4.2	66.67 $\pm$ 4.8	-67.5 $\pm$ 4.3	-76.47 $\pm$ 4.9
480	30	0	3.33 $\pm$ 0.6	-9.43 $\pm$ 1.0	-25.92 $\pm$ 1.2
720	30	100	-	-100	-



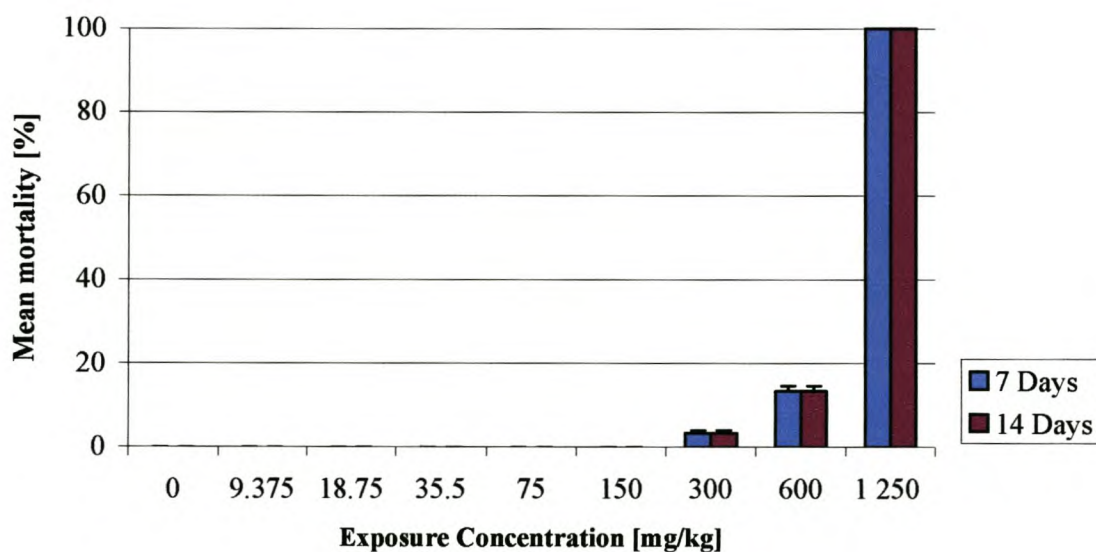
**Figure 3.2.2.2:** Changes in total mass of surviving *E. fetida* ( $\pm$ SD) after a seven and 14 day exposure period to penconazole concentrations ranging from 0 mg.kg<sup>-1</sup> to 720 mg.kg<sup>-1</sup>. n = 30.



### 3.2.3. Trifloxystrobin

#### 3.2.3.1. Mortality of *E. fetida* exposed to trifloxystrobin

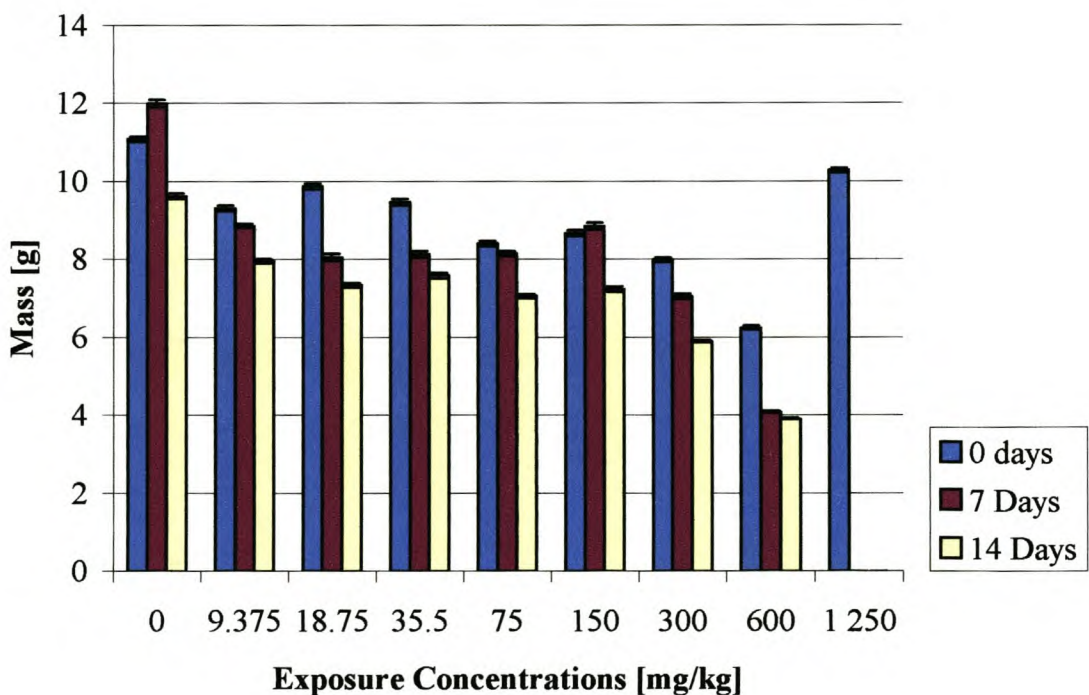
100% mortality occurred at the highest exposure concentration of 1 250 mg.kg<sup>-1</sup> and no mortalities occurred in exposure concentrations 9.375 mg.kg<sup>-1</sup>, 18.75 mg.kg<sup>-1</sup>, 35.5 mg.kg<sup>-1</sup>, 75 mg.kg<sup>-1</sup> and 150 mg.kg<sup>-1</sup> after both seven and 14 days of exposure (Fig. 3.2.3.1). No significant ( $P>0.05$ ) difference in percentage mortality occurred over seven and 14 days (Fig. 3.2.3.1). There was not a significant ( $P>0.05$ ) difference in percentage mortality between exposure groups and the control group, except for the highest concentration of 1 250 mg.kg<sup>-1</sup> (Table 3.2.3.1). The 14-day LC<sub>50</sub> for trifloxystrobin was calculated as 770 mg.kg<sup>-1</sup>.



**Figure 3.2.3.1:** Percentage mortality ( $\pm$ SD) of *E. fetida* after seven and 14 days of exposure to a range of trifloxystrobin concentrations from 0 mg.kg<sup>-1</sup> to 1 250 mg.kg<sup>-1</sup>. n =30.

### 3.2.3.2. Changes in mass of *E. fetida* exposed to trifloxystrobin

The control group showed a significantly ( $P < 0.05$ ) higher total mass when compared to all the exposure groups (Fig. 3.2.3.2). No significant ( $P > 0.05$ ) difference in total mass of earthworms could be observed in the 18.75 mg.kg<sup>-1</sup>, 35.5 mg.kg<sup>-1</sup> and 75 mg.kg<sup>-1</sup> exposure groups after seven days. All the other exposure groups showed significantly ( $P < 0.05$ ) different total mass from one another after seven days. After 14 days, the exposure groups of concentrations 18.75 mg.kg<sup>-1</sup>, 35.5 mg.kg<sup>-1</sup>, 75 mg.kg<sup>-1</sup> and 150 mg.kg<sup>-1</sup> did not have a significant ( $P > 0.05$ ) difference in total mass (Fig. 3.2.3.2).



**Figure 3.2.3.2:** Changes in total mass of surviving *E. fetida* ( $\pm$ SD) after a seven and 14 day exposure period to trifloxystrobin concentrations ranging from 0 mg.kg<sup>-1</sup> to 1 250 mg.kg<sup>-1</sup>. n =30.

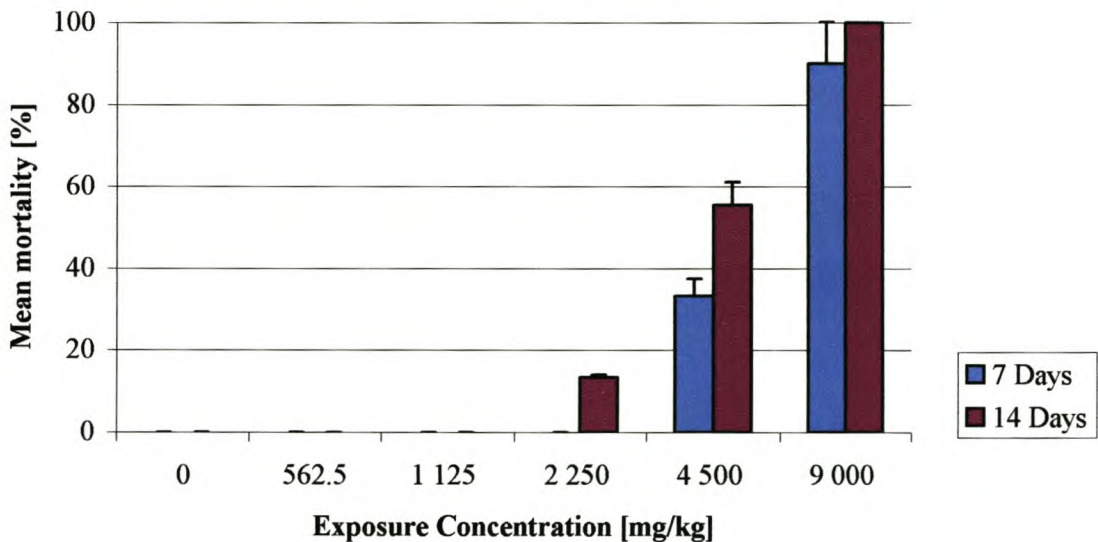
**Table 3.2.3.1:** Mean ( $\pm$ SD) percentage mortality and change in mass ( $\pm$ SD) of *E. fetida* after an exposure period of seven to 14 days to a range of trifloxystrobin concentrations between 0 mg.kg<sup>-1</sup> and 1 250mg.kg<sup>-1</sup>. Total percentage mass change was calculated using surviving earthworms only.

Concentration mg.kg <sup>-1</sup>	n	Mortality [%]		Mass Change [%] *	
		7	14	7	14
0	30	0	0	+8.25 $\pm$ 0.8	-13.22 $\pm$ 0.7
9.38	30	0	0	-4.84 $\pm$ 0.9	-14.55 $\pm$ 1.3
18.75	30	0	0	-15.18 $\pm$ 0.9	-22.84 $\pm$ 0.7
35.50	30	0	0	-14.16 $\pm$ 0.6	-19.92 $\pm$ 0.7
75	30	0	0	+3.27 $\pm$ 0.8	-16.12 $\pm$ 1.5
150	30	0	0	+2.20 $\pm$ 0.7	-16.59 $\pm$ 0.8
300	30	3.33 $\pm$ 0.6	3.33 $\pm$ 0.6	-11.78 $\pm$ 4.3	-26.19 $\pm$ 4.9
600	30	13.33 $\pm$ 1.3	13.33 $\pm$ 1.3	-34.59 $\pm$ 1.0	-37.34 $\pm$ 1.2
1 250	30	100	-	-100	-

### 3.2.4. Glyphosate

#### 3.2.4.1. Mortality of *E. fetida* exposed to glyphosate

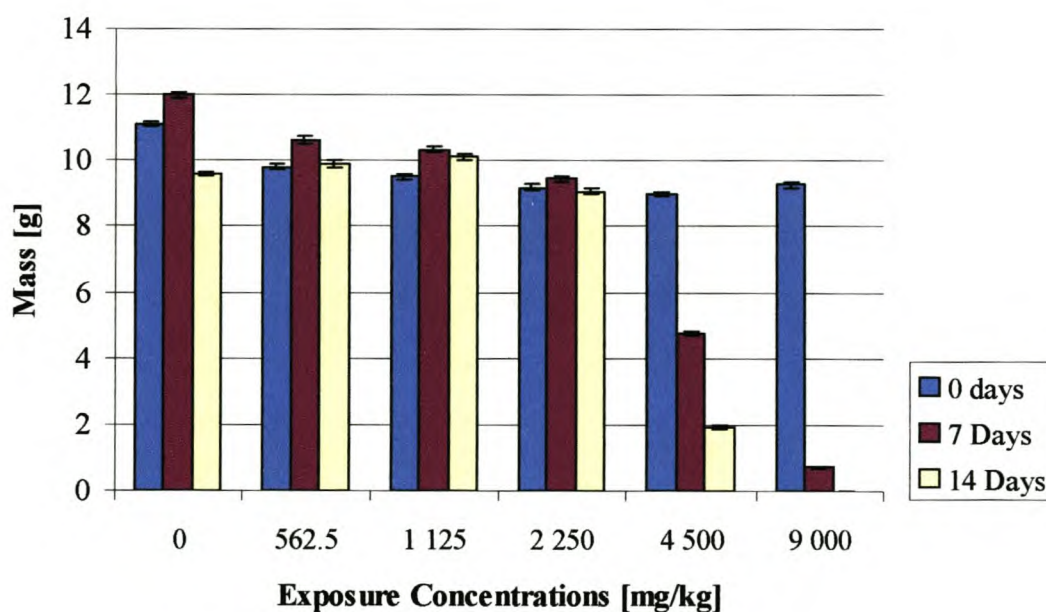
A significant ( $P < 0.05$ ) increase in mean percentage mortality was observed in the exposure groups (Fig. 3.2.4.1). In all exposure groups, but the 4 500 mg.kg<sup>-1</sup>, and the control no significant ( $P > 0.05$ ) difference could be observed in mean percentage mortality after seven and 14 days of exposure (Table 3.2.4.1). The exposure groups of 562.5 mg.kg<sup>-1</sup>, 1 125 mg.kg<sup>-1</sup> and 2250 mg.kg<sup>-1</sup> did not differ significantly ( $P > 0.05$ ) in mean percentage mortality from the control over 14 days of the exposure period (Fig. 3.2.4.1). After 14 days of exposure, all earthworms succumbed in the 9 000 mg.kg<sup>-1</sup> exposure group. The 14-day LC<sub>50</sub> of glyphosate was calculated at 3 740.40 mg.kg<sup>-1</sup>.



**Figure 3.2.4.1:** Mortality percentage ( $\pm$ SD) of *E. fetida* after seven and 14 days of exposure to a range of glyphosate concentrations from 0 mg.kg<sup>-1</sup> to 9 000 mg.kg<sup>-1</sup>. n = 30.

### 3.2.4.2. Changes in mass of *E. fetida* exposed to glyphosate

After seven days, all exposure groups had a significantly ( $P < 0.05$ ) lower total mass compared to the control group (Fig. 3.2.4.2). Exposure groups  $562.5 \text{ mg.kg}^{-1}$  and  $1\ 125 \text{ mg.kg}^{-1}$ , had a similar mass increase as the control at the start of the experiment and at the end had a significantly ( $P < 0.05$ ) higher total mass after 14 days (Fig. 3.2.4.2). There was no significant ( $P > 0.05$ ) difference in percentage mass between exposure groups,  $562.5 \text{ mg.kg}^{-1}$  and  $1\ 125 \text{ mg.kg}^{-1}$ , at the start of the experiment and also not after seven days (Table 3.2.4.1). After 14 days no significant ( $P > 0.05$ ) difference in mean mass percentage was observed between the  $562.5 \text{ mg.kg}^{-1}$ ,  $1\ 125 \text{ mg.kg}^{-1}$ ,  $2\ 250 \text{ mg.kg}^{-1}$  and the control (Table 3.2.4.1). A significant ( $P < 0.05$ ) difference in total mass did occur in the exposure groups,  $4\ 500 \text{ mg.kg}^{-1}$  and  $9\ 000 \text{ mg.kg}^{-1}$ , and the control group (Fig. 3.2.4.2).



**Figure 3.2.4.2:** Changes in total mass of surviving *E. fetida* ( $\pm$ SD) after a seven and 14 day exposure period to glyphosate concentrations ranging from  $0 \text{ mg.kg}^{-1}$  to  $9\ 000 \text{ mg.kg}^{-1}$ .  $n = 30$ .

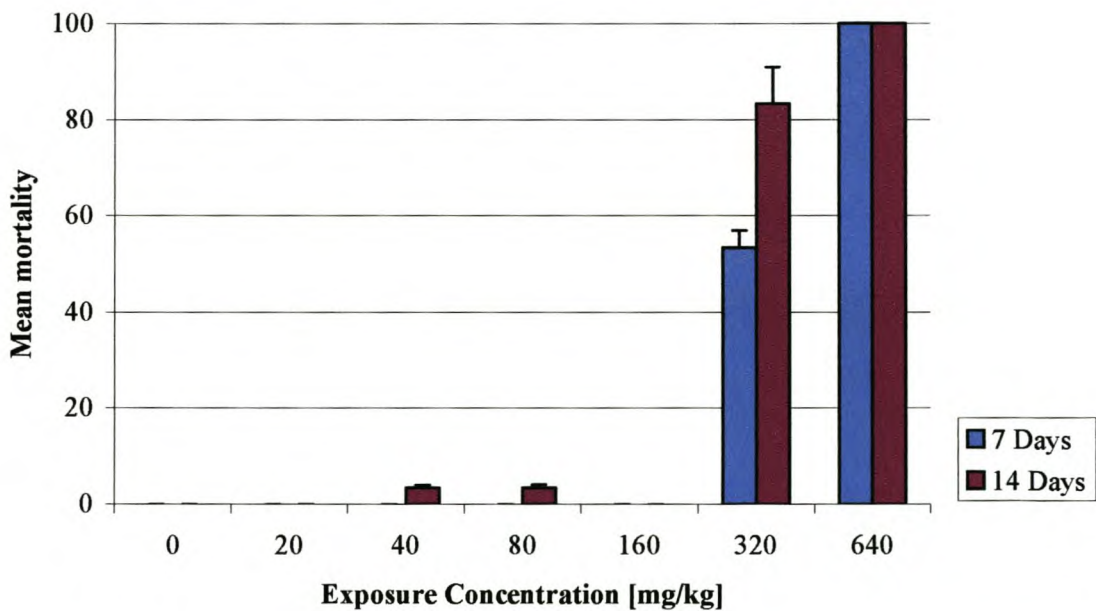
**Table 3.2.4.1:** Mean ( $\pm$ SD) percentage mortality and change in mass ( $\pm$ SD) of *E. fetida* after an exposure period of seven to 14 days to a range of glyphosate concentrations between 0 mg.kg<sup>-1</sup> and 1 250mg.kg<sup>-1</sup>. Total percentage mass change was calculated using surviving earthworms only.

Concentration mg.kg <sup>-1</sup>	n	Mortality [%]		Mass Change [%] *	
		7	14	7	14
0	30	0	0	+8.25 $\pm$ 0.8	-13.22 $\pm$ 0.7
562.5	30	0	0	+8.50 $\pm$ 1.0	+0.96 $\pm$ 1.2
1 125	30	0	0	+8.71 $\pm$ 1.0	+6.32 $\pm$ 1.0
2 250	30	0	13.33 $\pm$ 0.7	+2.79 $\pm$ 0.9	-1.31 $\pm$ 1.0
4 500	30	33.33 $\pm$ 4.2	55.56 $\pm$ 5.6	-46.90 $\pm$ 1.5	-78.60 $\pm$ 3.5
9 000	30	90.00 $\pm$ 10.2	100	-92.47 $\pm$ 10.4	-100

### 3.2.5. N-acetyl salicylic acid

#### 3.2.5.1. Mortality of *E. fetida* exposed to N-acetyl salicylic acid

No significant ( $P>0.05$ ) difference in mean percentage mortality was observed between exposure groups of N-acetyl salicylic acid and the control group, except in 320 mg.kg<sup>-1</sup> (Fig. 3.2.5.1). The highest exposure concentration group of 640 mg.kg<sup>-1</sup> had 100% mortality after less than seven days (Table 3.2.5.1). In the 320 mg.kg<sup>-1</sup> exposure group there was a significant ( $P<0.05$ ) difference in mean mortality of *E. fetida* between seven and 14 days. After 14 days of exposure, the 320 mg.kg<sup>-1</sup> exposure group had significantly ( $P<0.05$ ) more mortalities than the control group (Fig. 3.2.5.1). The 14-day LC<sub>50</sub> was calculated at 312 mg.kg<sup>-1</sup>.



**Figure 3.2.5.1:** Percentage mortality ( $\pm$ SD) of *E. fetida* after seven and 14 days of exposure to a range of N-acetyl salicylic acid concentrations from 0 mg.kg<sup>-1</sup> to 640 mg.kg<sup>-1</sup>. n= 30.

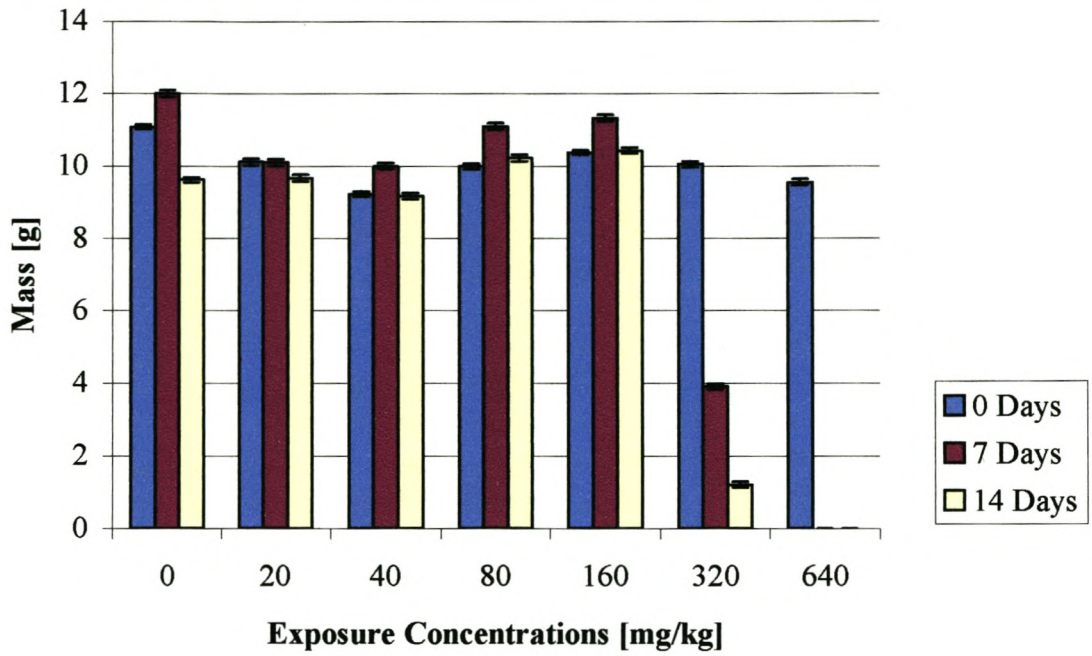
### 3.2.5.2. Changes in mass of *E. fetida* exposed to *N*-acetyl salicylic acid

All exposure concentrations had a significantly ( $P < 0.05$ ) lower total earthworm mass from the control after seven days. All the earthworms in the highest concentration of  $640 \text{ mg.kg}^{-1}$  died before the first seven days of exposure (Table 3.2.5.1). After 14 days, exposure groups  $20 \text{ mg.kg}^{-1}$ ,  $40 \text{ mg.kg}^{-1}$ ,  $80 \text{ mg.kg}^{-1}$  and  $160 \text{ mg.kg}^{-1}$ , did not have a significant ( $P > 0.05$ ) change in mass when compared to the control group (Table 3.2.5.1). A significantly ( $P < 0.05$ ) lower total mass was observed between the  $320 \text{ mg.kg}^{-1}$  exposure group and the control group (Fig. 3.2.5.2).

**Table 3.2.5.1:** Mean ( $\pm$ SD) percentage mortality and change in mass ( $\pm$ SD) of *E. fetida* after an exposure duration of seven to 14 days to a range of *N*-acetyl salicylic acid concentrations between  $0 \text{ mg.kg}^{-1}$  and  $640 \text{ mg.kg}^{-1}$ . Total percentage mass change was calculated using surviving earthworms only.

Concentration $\text{mg.kg}^{-1}$	n	Mortality [%]		Mass Change [%] *	
		7	14	7	14
0	30	0	0	+8.25 $\pm$ 0.8	-13.22 $\pm$ 0.7
20	30	0	0	-0.16 $\pm$ 0.9	-4.37 $\pm$ 1.0
40	30	0	3.33 $\pm$ 0.6	+8.33 $\pm$ 0.8	-0.56 $\pm$ 1.1
80	30	0	3.33 $\pm$ 0.7	+11.12 $\pm$ 0.9	+2.35 $\pm$ 1.1
160	30	0	0	+9.17 $\pm$ 0.8	+0.50 $\pm$ 0.9
320	30	53.33 $\pm$ 3.6	83.33 $\pm$ 7.6	-61.05 $\pm$ 3.8	-87.92 $\pm$ 7.9
640	30	100	-	-100	-





**Figure 3.2.5.2:** Changes in total mass of *E. fetida* ( $\pm$ SD) after a seven and 14 day exposure period to N-acetyl salicylic acid concentrations ranging from 0 mg.kg<sup>-1</sup> to 640 mg.kg<sup>-1</sup>. n = 30.

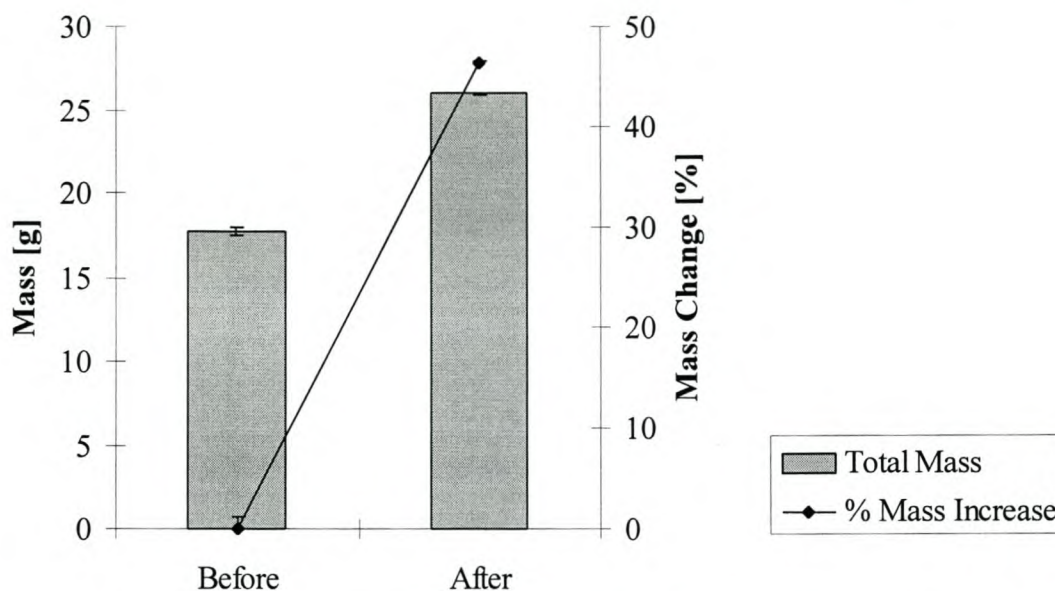
### 3.3. Preference Behaviour Tests

#### 3.3.1. Preference and mass change of *E. fetida* exposed to Copper oxychloride

Earthworms avoided the substrate with the highest concentration (882.78 mg.kg<sup>-1</sup>) of copper oxychloride (Fig. 3.3.1). In the cubicles with substrate that did have earthworms in them, no significant ( $\chi^2 = 5.04$ , 7d.f,  $P > 0.50$ ) difference in distribution of earthworms occurred for uncontaminated substrate and substrate contaminated with copper oxychloride (Table 3.1.1). A significant ( $P < 0.05$ ) increase of  $46.45 \pm 0.1$  % was observed in the mean mass of the earthworms after seven days of exposure.

**Table 3.3.1:** The overall distribution of the 50 earthworms (*E. fetida*) in the different sections of the test container after seven days of exposure to a range of copper oxychloride concentrations at the LC<sub>50</sub> –value and lower.

	A	B	C
1	17.66 mg.kg <sup>-1</sup> 4	0 mg.kg <sup>-1</sup> 5	88.28 mg.kg <sup>-1</sup> 9
2	0 mg.kg <sup>-1</sup> 6	882.78 mg.kg <sup>-1</sup> 0	0 mg.kg <sup>-1</sup> 8
3	176.56 mg.kg <sup>-1</sup> 3	0 mg.kg <sup>-1</sup> 7	8.83 mg.kg <sup>-1</sup> 8



**Figure 3.3.1:** The mean ( $\pm$ SD) mass (g) and mean ( $\pm$ SD) increase (%) in mass of the earthworms (*E. fetida*) in the test container before and after an exposure period of seven days to a range of copper oxychloride concentrations to assess avoidance behaviour.  $n = 50$ .

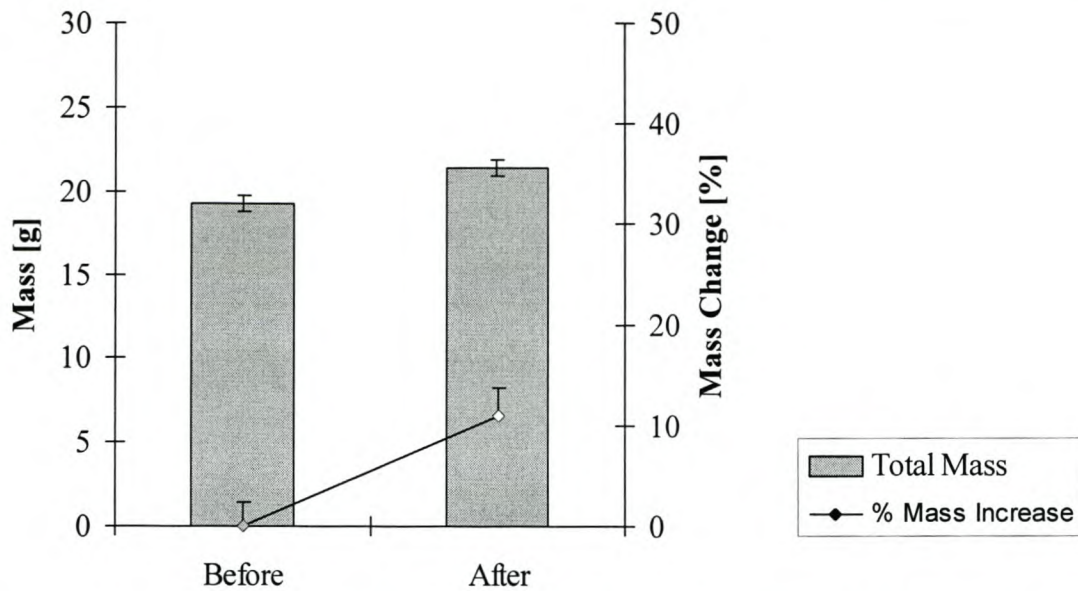
### 3.3.2. Preference and mass change of *E. fetida* exposed to Mancozeb

No earthworms were found in the highest ( $2\ 332.50\ \text{mg.kg}^{-1}$ ) and second highest ( $466.50\ \text{mg.kg}^{-1}$ ) concentrations of mancozeb (Table 3.3.2). One earthworm died in this experiment. Earthworms significantly ( $\chi^2 = 33.86$ , 8d.f,  $P < 0.001$ ) preferred substrates with less mancozeb or uncontaminated substrates to the higher concentrations of mancozeb. Comparing the cubicles in which earthworms were present (A1, A2, B1, B3, C1, C2 and C3), results showed that a significant ( $\chi^2 = 15.43$ , 6d.f,  $P < 0.02$ ) difference in the distribution of earthworms occurred. By omitting the data from the cubicle with the least amount of earthworms (C1), it was

shown that no significant ( $\chi^2 = 8.25$ , 5d.f,  $P > 0.10$ ) difference in distribution of earthworms occurred between the rest of the cubicles (A1, A2, B1, B3, C2 and C3) (Table 3.3.2). This meant that earthworms avoided the substrates of the three highest concentrations (2 332.50 mg.kg<sup>-1</sup>, 466.50 mg.kg<sup>-1</sup> and 233.25 mg.kg<sup>-1</sup>) of mancozeb. A significant ( $P < 0.05$ ) increase of  $11.05 \pm 2.6$  % was observed in the mean mass of the worms after seven days of exposure (Fig. 3.3.2). One earthworm died during the exposure period.

**Table 3.3.2:** The overall distribution of the 49 earthworms (*E. fetida*) in the different sections of the test container after seven days of exposure to a range of mancozeb concentrations at the LC<sub>50</sub> -value and lower.

	<b>A</b>	<b>B</b>	<b>C</b>
<b>1</b>	46.65 mg.kg <sup>-1</sup> <b>12</b>	0 mg.kg <sup>-1</sup> <b>3</b>	233.25 mg.kg <sup>-1</sup> <b>1</b>
<b>2</b>	0 mg.kg <sup>-1</sup> <b>8</b>	2 332.50 mg.kg <sup>-1</sup> <b>0</b>	0 mg.kg <sup>-1</sup> <b>8</b>
<b>3</b>	466.50 mg.kg <sup>-1</sup> <b>0</b>	0 mg.kg <sup>-1</sup> <b>5</b>	23.33 mg.kg <sup>-1</sup> <b>12</b>



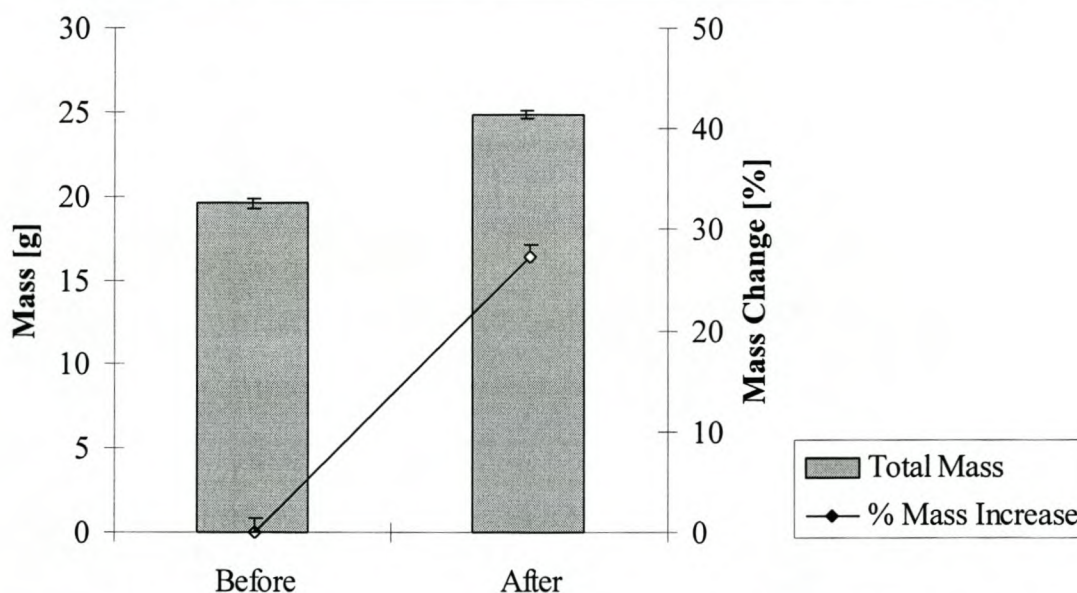
**Figure 3.3.2:** The mean ( $\pm$ SD) mass (g) and mean ( $\pm$ SD) increase (%) in mass of the earthworms (*E. fetida*) in the test container before and after an exposure period of seven days to a range of mancozeb concentrations to assess avoidance behaviour. n = 49.

### 3.3.3. Preference and mass change of *E. fetida* exposed to Penconazole

Earthworms avoided the substrate with the highest ( $379 \text{ mg.kg}^{-1}$ ) concentration of penconazole (Table 3.3.3). There was a significant ( $\chi^2 = 19.10$ , 8d.f,  $P < 0.02$ ) difference in the distribution for the different concentrations of penconazole. By excluding the cubicle with no earthworms (B2), no significant ( $\chi^2 = 11.44$ , 7d.f,  $P > 0.10$ ) difference in distribution of the earthworms occurred for the substrates with less penconazole or the uncontaminated substrates. A significant ( $P < 0.05$ ) increase of  $27.23 \pm 1.3$  % was observed in the mean mass of the worms after seven days of exposure (Fig. 3.3.3).

**Table 3.3.3:** The overall distribution of the 50 earthworms (*E. fetida*) in the different sections of the test container after seven days of exposure to a range of penconazole concentrations at the  $LC_{50}$ -value and lower.

	A	B	C
1	7.58 mg.kg <sup>-1</sup> 4	0 mg.kg <sup>-1</sup> 10	37.90 mg.kg <sup>-1</sup> 3
2	0 mg.kg <sup>-1</sup> 11	379.00 mg.kg <sup>-1</sup> 0	0 mg.kg <sup>-1</sup> 7
3	75.80 mg.kg <sup>-1</sup> 2	0 mg.kg <sup>-1</sup> 6	3.79 mg.kg <sup>-1</sup> 7

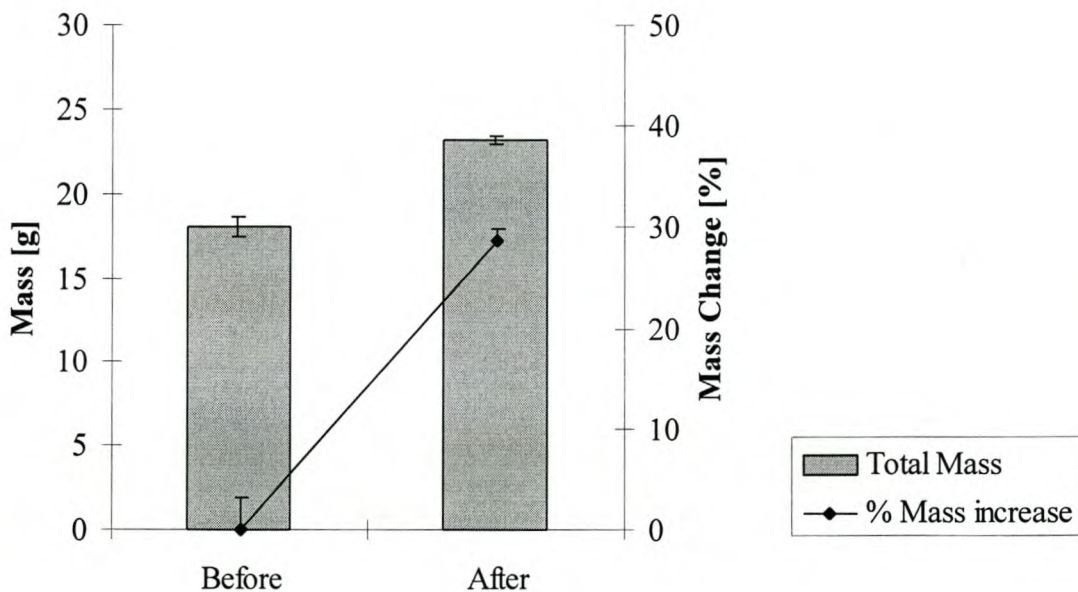


**Figure 3.3.3:** The mean ( $\pm$ SD) mass (g) and mean ( $\pm$ SD) increase (%) in mass of the earthworms (*E. fetida*) in the test container before and after an exposure period of seven days to a range of penconazole concentrations to assess avoidance behaviour.

n = 50.

### 3.3.4. Preference and mass change of *E. fetida* exposed to Trifloxystrobin

Earthworms showed a significant ( $\chi^2 = 19.46$ , 8d.f,  $P < 0.02$ ) difference in distribution. This was the only pesticide where an earthworm was found in the substrate with the highest concentration ( $770.00 \text{ mg.kg}^{-1}$ ) of pesticide (Table 3.3.4). Because this was the cubicle where the least earthworms were present, omitting it showed that the earthworms significantly ( $\chi^2 = 13.60$ , 7d.f,  $P > 0.05$ ) avoided the substrate with the highest concentration of trifloxystrobin. A significant ( $P < 0.05$ ) increase of  $28.63 \pm 1.3 \%$  was observed in the mean mass of the worms after seven days of exposure (Fig. 3.3.4).



**Figure 3.3.4:** The mean ( $\pm$ SD) mass (g) and mean ( $\pm$ SD) increase (%) in mass of the earthworms (*E. fetida*) in the test container before and after an exposure period of seven days to a range of trifloxystrobin concentrations to assess avoidance behaviour.  $n = 50$ .

**Table 3.3.4:** The overall distribution of the 50 earthworms (*E. fetida*) in the different sections of the test container after seven days of exposure to a range of trifloxystrobin concentrations at the LC<sub>50</sub> -value and lower.

	<b>A</b>	<b>B</b>	<b>C</b>
<b>1</b>	15.90 mg.kg <sup>-1</sup> <b>11</b>	0 mg.kg <sup>-1</sup> <b>4</b>	77.00 mg.kg <sup>-1</sup> <b>4</b>
<b>2</b>	0 mg.kg <sup>-1</sup> <b>3</b>	770.00 mg.kg <sup>-1</sup> <b>1</b>	0 mg.kg <sup>-1</sup> <b>7</b>
<b>3</b>	154.00 mg.kg <sup>-1</sup> <b>7</b>	0 mg.kg <sup>-1</sup> <b>11</b>	7.70 mg.kg <sup>-1</sup> <b>2</b>

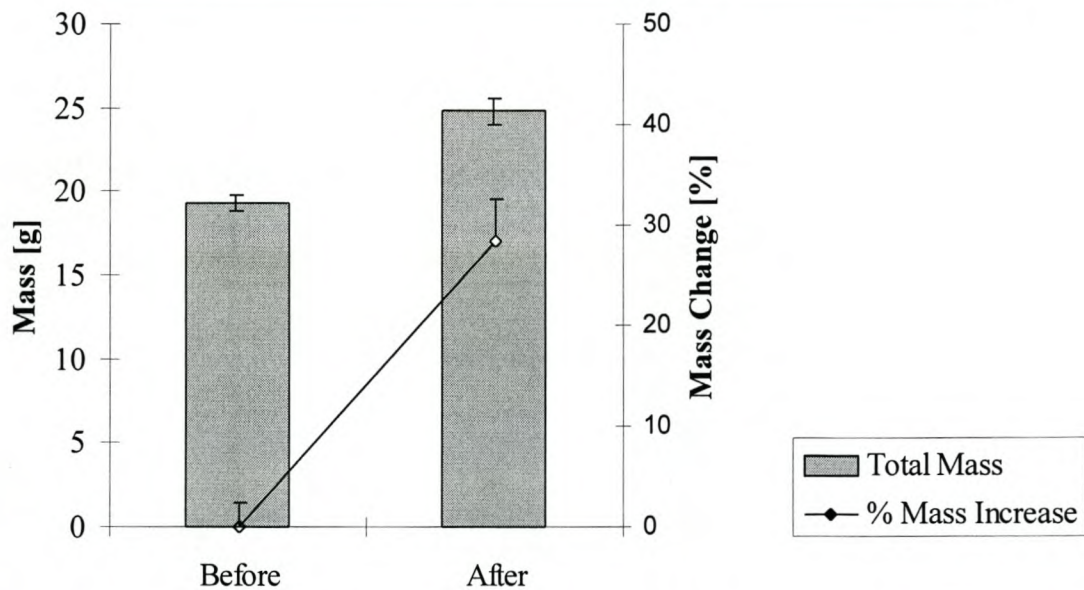
### 3.3.5. *Preference and mass change of E. fetida exposed to Glyphosate*

A significant ( $\chi^2 = 20.90$ , 8d.f,  $P < 0.01$ ) difference in distribution of earthworms occurred. No earthworms were present in the highest (3 737 mg.kg<sup>-1</sup>) glyphosate concentration (Table 3.3.5). After omitting cubicle B2, no significant ( $\chi^2 = 13.04$ , 8d.f,  $P > 0.05$ ) difference in distribution occurred in earthworm numbers for the different cubicles with contaminated and uncontaminated substrates. A significant ( $P < 0.05$ ) increase of  $28.41 \pm 4.2$  % was observed in the mean mass of the worms after seven days of exposure (Fig. 3.3.5).



**Table 3.3.5:** The overall distribution of the 50 earthworms (*E. fetida*) in the different sections of the test container after seven days of exposure to a range of glyphosate concentrations at the  $LC_{50}$  -value and lower.

	A	B	C
1	74.75 mg.kg <sup>-1</sup> 9	0 mg.kg <sup>-1</sup> 1	373.74 mg.kg <sup>-1</sup> 2
2	0 mg.kg <sup>-1</sup> 7	3 737.41 mg.kg <sup>-1</sup> 0	0 mg.kg <sup>-1</sup> 7
3	747.48 mg.kg <sup>-1</sup> 5	0 mg.kg <sup>-1</sup> 8	37.37 mg.kg <sup>-1</sup> 11



**Figure 3.3.5:** The mean ( $\pm$ SD) mass (g) and mean ( $\pm$ SD) increase (%) in mass of the earthworms (*E. fetida*) in the test container before and after an exposure period of seven days to a range of glyphosate concentrations to assess avoidance behaviour.

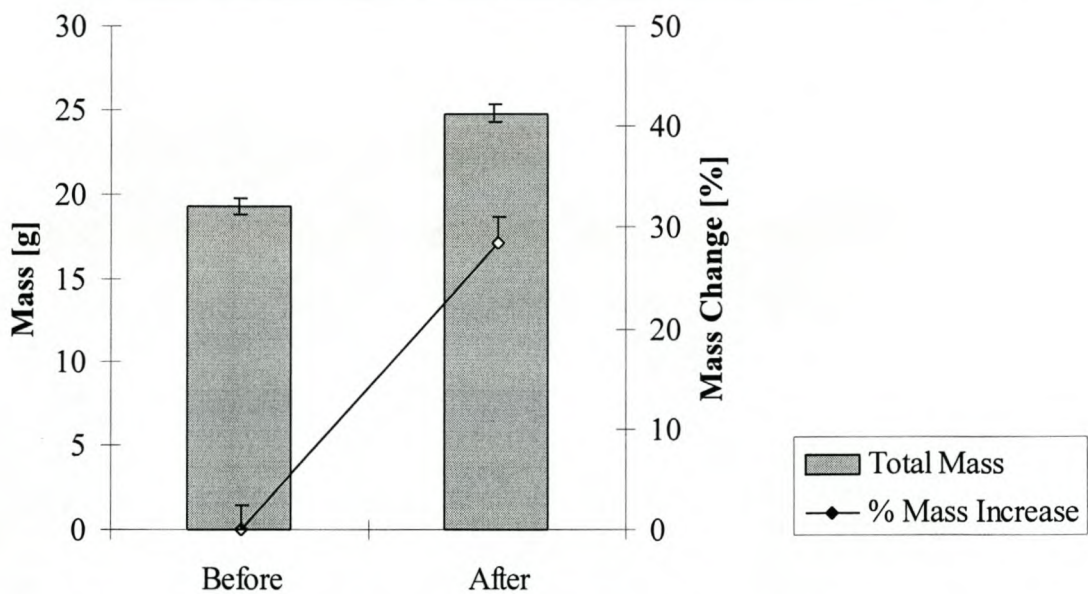
n = 50.

### 3.3.6. *Preference and mass change of E. fetida exposed to N-acetyl salicylic acid*

A significant ( $\chi^2 = 45.00$ , 8d.f,  $P < 0.0001$ ) difference in distribution of earthworms occurred when comparing all the cubicles with one another. No earthworms were present in the three highest concentrations (312.7 mg.kg<sup>-1</sup>, 62.54 mg.kg<sup>-1</sup> and 31.27 mg.kg<sup>-1</sup>) of N-acetyl salicylic acid (Table 3.3.6). In the contaminated substrates with the lowest of N-acetyl salicylic acid concentrations (6.25 mg.kg<sup>-1</sup> and 3.13 mg.kg<sup>-1</sup>) and uncontaminated substrates a significant ( $\chi^2 = 13.37$ , 5d.f,  $P < 0.02$ ) difference in distribution of earthworms occurred. Seventeen earthworms out of 50 were present in the substrate contaminated with 3.13 mg.kg<sup>-1</sup> (C3) of N-acetyl salicylic acid. By omitting the data from the cubicle with the highest number of earthworms (C3), no significant ( $\chi^2 = 2.55$ , 4d.f,  $P > 0.50$ ) difference in distribution occurred between the uncontaminated substrate and the cubicle with 6.25 mg.kg<sup>-1</sup> (A1) of N-acetyl salicylic acid. A significant ( $P < 0.05$ ) increase of  $28.47 \pm 2.6$  % was observed in the mean mass of the worms after seven days of exposure.

**Table 3.3.6:** The overall distribution of the 50 earthworms (*E. fetida*) in the different sections of the test container after seven days of exposure to a range of N-acetyl salicylic acid concentrations at the LC<sub>50</sub> –value and lower.

	A	B	C
<b>1</b>	6.25 mg.kg <sup>-1</sup> 7	0 mg.kg <sup>-1</sup> 3	31.27 mg.kg <sup>-1</sup> 0
<b>2</b>	0 mg.kg <sup>-1</sup> 6	312.70 mg.kg <sup>-1</sup> 0	0 mg.kg <sup>-1</sup> 9
<b>3</b>	62.54 mg.kg <sup>-1</sup> 0	0 mg.kg <sup>-1</sup> 8	3.13 mg.kg <sup>-1</sup> 17



**Figure 3.3.6:** The mean ( $\pm$ SD) mass (g) and mean ( $\pm$ SD) increase (%) in mass of the earthworms (*E. fetida*) in the test container before and after an exposure period of seven days to a range of N-acetyl salicylic acid concentrations to assess avoidance behaviour. n = 50.

## 4. Discussion

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### 4.1. Bait-lamina Tests

#### 4.1.1. *Field Trials (Bait-lamina sets & Soil Moisture)*

The feeding activity of the soil fauna in T1 of the organically managed vineyard block decreased when soil moisture content was low. The soil faunal activity did not increase in Dec. 2000 and Apr. 2001, when soil moisture was higher. A similar effect was observed in T1 of the conventionally managed vineyard block. It is possible that the soil faunal activity was confined to the deeper soil layers, not being assessed by the bait-laminae.

The feeding activity of the soil fauna from T2 of the organically managed vineyard block coincided with the fluctuations of the soil moisture content. When the soil moisture was low, a low feeding activity occurred. This is an indication that the moisture in the soil is one of the most important factors influencing the biological activity in the soil. The feeding activity of the soil fauna in T2 of the conventional vineyard block was at a constant height for all the sampling periods. The fact that the soil moisture did not affect the feeding activity of the soil fauna significantly is an indication that it is not always the soil moisture that can affect the soil fauna, but other physico-chemical properties also. The biological activity of the soil fauna in the IPM managed plots of the conventionally managed vineyard block remained unchanged before, during and after the treatment started. The feeding activity of the soil fauna decreased significantly with reduced soil moisture in the organically managed vineyard block. The integrated pest management system could have benefited the soil fauna from the conventionally managed vineyard block.

The soil faunal feeding activity of T3 from the organically managed vineyard block decreased with a decline in soil moisture, but did increase again after the first rain in Apr. 2001. The soil management, in particular the mulch covering of the soil, preserved the moisture of the soil and also benefited the biological activity of the soil. The months in which a lower feeding activity occurred could be due to the hot, above soil conditions. T3 of the conventionally managed vineyard block had a feeding activity, the extent of which, coincided with the decrease and increase in soil moisture. The importance of the water in soil is again indicated by this case. A similar result was observed in T4 of the organically managed vineyard as in T3 of the same vineyard block. T4 of the conventionally managed vineyard block also had similar feeding activity to that of T3 from the same vineyard block.

A high feeding activity was observed in T5 of the organically managed vineyard block during all the sampling periods. The moisture content also did not differ significantly before, during and after the treatment started. This is an indication that the moisture content and the treatment (addition of EM) together might have been responsible for the constant biological activity. The feeding activity of T5 from the conventionally managed vineyard block was low during all the sampling periods, despite an increase in moisture during Apr. 2001. This might have been due to an external factor that is not easily explainable. This is an example of how complicated the soil environment is. One explanation could be that the soil fauna moved to deeper soil layers and the bait-laminae could not assess the activity.

The extent of feeding activity of the soil fauna of T6 from the organically managed vineyard block coincided with the increases and declines of soil moisture. A similar effect in feeding activity was observed for T6 of the conventionally managed vineyard. The moisture content fluctuations played a big part in the amount of

biological activity in the soil. The feeding activity of T6 of the conventionally managed vineyard block showed a similar effect, but the recovery after the first rain in Apr. 2001 was not as big as in the organically managed vineyard block.

The moisture content of the soil is important because it is directly responsible for providing a medium for the soil's "aquatic" organisms, which include nematodes and protozoa (Swift *et al.* 1979). They utilise the moisture that is hygroscopically bound to the soil particles to move and to perform their biodegradation of organic material. The soil moisture is not the only factor influencing the activity of soil fauna. Larink & Kratz (1994) found that the factors influencing the feeding activity of the soil fauna as measured with the bait-lamina method, is the bait material used, the exposure period, the soil temperature, soil moisture and rainfall during the exposure period. According to these authors soil moisture is the most important factor influencing the feeding activity of the soil fauna. From the present results it is clear that it is not always the soil moisture that influenced the activity of the soil fauna, because the feeding activity did not always coincide with the fluctuations in soil moisture. It was, however, one of the most important factors that influenced the feeding activity of the soil biota. Summer in the Western Cape of South Africa is predominantly the growing season for grapes. After the spring rains when dry conditions set in, water loss through evaporation may be largely responsible for the depression in the activity of soil organisms in the top few centimetres of the soil. Once the soil forms a hard, dry crust at the soil surface, the loss of water is prevented within the deeper layers of the soil (Swift *et al.* 1979). This protective cover results in a fairly constant moisture content. The only water-reducing factor would be the plants.

The long dry conditions that prevail during the growing season of grapes in the Western Cape, could cause water reserves (in the form of moisture trapped below the soil crust) to become depleted due to the transpiration of plants. Most soil meso- (e.g. Collembola, Acari, Diptera larvae and smaller Coleoptera) and macro-fauna (e.g. millipedes, isopods, insects, molluscs, and earthworms) occupy the soil pores of the soil and they possess the ability to migrate over large distances horizontally or vertically (Swift *et al.* 1979). Microfauna (e.g. protozoa, nematodes and the smallest Collembola and Acari) live in the moisture films that are hygroscopically bound to soil particles (Swift *et al.* 1979). Microfauna is able to move within the water films, but it is obviously confined to the extent of the water films that are hygroscopically bound to the soil particles. As the soil dries out, these water films shrink and the microfauna are trapped. Microfauna has various adaptations enabling them to survive desiccation. These adaptations include encystment, anabiosis and the production of desiccation-resistant eggs (Swift *et al.* 1979). Some of the other major factors that influence the feeding activity of soil fauna according to Larink & Kratz (1994) like soil temperature and rainfall, indirectly influence the water available to the soil organisms, that in turn affect their biological activity in the soil.

The preservation of soil moisture seems to be very important to the activity of the soil fauna. Not only does moisture create a medium for chemical processes in the soil to take place, but it also creates a favourable medium for soil fauna to move in and perform their processes in organic matter breakdown. The drying out of the soil under dry summer conditions presumably initiate the migration of soil fauna. In such instances the recorded feeding activity of the bait-laminae might be a misrepresentation, because the biological activity is just moved to deeper soil layers. In a vineyard where no irrigation takes place during summer, like the one at Plaisir de

Merle, the roots of the vines would be expected to be deeper than in vineyards where irrigation does take place during summer. The vertical migration of soil fauna may then still be beneficial for the vines because the soil fauna would still be in contact with the roots and the nutrients released from their processes would still be available to the plants. These are just a few reasons for the limitations of the bait-lamina method. Although a low feeding activity is recorded during the dry months of the year, it does not necessarily mean that there is no biological activity in the soil.

The breakdown of organic matter in the soil is a combination of a number of different and complicated processes. The organisms responsible for the breakdown of organic matter in the soil form complex foodwebs within the soil. The biochemical transformation of the organic matter depends on the biological activity of the soil organisms. In the organically managed vineyard block, the organically managed treatment plots had a higher feeding activity of the soil fauna than the conventionally managed treatment plots (Fig. 3.1.1 a-f). The organic management of the soil largely benefited the organically managed plots (T3, T4 & T5), but not the conventionally managed plots (T1, T2 & T6) of the organically managed vineyard block. In the conventionally managed vineyard block, other than in the organically managed block, there was not always such a big difference in the feeding activity of the soil fauna, if any, between the organically and conventionally managed soil. In fact, the feeding activity of the conventionally managed treatment plots of T1 and T2 of the conventionally managed vineyard block was higher than that of the organically managed plots (Fig. 3.1.1a&b). There could be argued that when farming organically, the soil organisms would have a greater impact on the soil and the breakdown of organic matter. When farming conventionally, organic practices would not



necessarily be beneficial to soil organisms. The conventional farming methods are also not always detrimental to soil organisms.

#### **4.1.2. *Limitations of the bait-lamina assessment tests***

The bait-lamina test does not distinguish which taxa feed on the bait substance or in what proportion (Helling *et al.* 1998). Since only a limited number of parameters could be measured, the influence of additional parameters on biodiversity and feeding activity of the soil fauna can only be presumed. Thus, the interpretation of the data has to be done carefully and with due consideration of the limitations of the bait-lamina technique in mind.

#### **4.1.3. *Microcosm Trials***

The bait-laminae in the microcosm gave a reflection of the recovery of the soil faunal populations from the dry conditions in the field, because the addition of water to the soil created more favourable conditions for the soil fauna. The results of the bait-lamina sets of Apr. 2001 could be seen as a similar recovery of the soil faunal populations in the field because that trial took place after the first rain of the year. Because the feeding activity of the soil fauna reflects the soil biological activity (Helling *et al.* 1998), the amount of bait eaten at different times during the trials would be a reflection of the difference in soil biological activity. Moisture content of the soil plays an important part in the recovery of soil biological activity (Swift *et al.* 1979). By adding water to the soil in the microcosms and incubating it, the soil biological activity could recover. When compared to the recovery in the field there is a distinctly lower biological activity in the microcosms, except for T3 of the organically managed vineyard block. A possible explanation for this phenomenon could be the fact that soil organisms have the ability to migrate through the soil. Because the bait-laminae only assess the feeding activity of the first 8-10 cm of soil,

the dry weather conditions may have caused the soil fauna to move deeper into the soil as a vertical moisture gradient developed when dryer conditions during the summer months occurred. In the microcosms, where the soil moisture is constant, the soil fauna is not affected in a similar way as in the field. When the topsoil dries out, it forms a dry, hard and almost impenetrable layer, but the deeper soil layers remain moist. Under very dry weather conditions the protective layer can become thicker and drive soil fauna even deeper into the soil. Deeper than the 8-10 cm of the soil that is assessed by the bait-laminae. In T3 of the organically managed vineyard block, the chance that the soil fauna would migrate away from the topsoil was not as great as in the other management treatment plots, because the soil moisture is preserved (Table 3.1.2). This could be the reason why the microcosms of T3 from the organically managed vineyard had such high feeding activities compared to T1. The results of the microcosms compliment the findings of the field data from this study.

## 4.2. Acute Toxicity Tests

### 4.2.1. Mancozeb

Mancozeb had a  $LC_{50}$  of 2 332.60  $mg.kg^{-1}$  (Results 3.2.1.1). No earthworms died at concentrations lower than 750  $mg.kg^{-1}$ . Although this was the real  $LC_{50}$  value (calculated using the whole range of exposure concentrations), another  $LC_{50}$  value was determined for exposure concentrations ranging from 185.50  $mg.kg^{-1}$  to 1 500  $mg.kg^{-1}$ . The  $LC_{50}$  for Mancozeb, only considering exposure concentrations of 187.50  $mg.kg^{-1}$  to 1 500  $mg.kg^{-1}$ , was calculated at 1 207.50  $mg.kg^{-1}$ . This is very similar to the  $LC_{50}$  calculated for mancozeb by Vermeulen *et al.* (2001) of 1 262  $mg.kg^{-1}$  in similar acute toxicity tests with *E. fetida*, with exposure concentrations ranging from 400  $mg.kg^{-1}$  to 2 000  $mg.kg^{-1}$ . The second mortality peak occurred at a concentration of 6 000  $mg.kg^{-1}$ , with a mortality rate slightly higher

than 20% after 14 days between the two mortality peaks at 3 000 mg.kg<sup>-1</sup> (Fig. 3.2.1.1). The low mortality percentage at 3 000 mg.kg<sup>-1</sup> could have been a result of the earthworms not burrowing into the substrate. The earthworms were not exposed continuously to the entire concentration of pesticide. Kokta (1992) stated that if a pesticide's LC<sub>50</sub> exceeds an exposure concentration of 1 000 mg.kg<sup>-1</sup>, it can be considered as non-toxic to earthworms. Therefore, mancozeb should be considered non-toxic to *E. fetida*. This agrees with the literature review on mancozeb by Edwards & Bohlen (1992) who concluded that mancozeb is not considered to be harmful to earthworms.

The control group of earthworms showed an increase in mean mass percentage after seven days, but lost more than 13% of their initial mean body mass when compared after two weeks of exposure. Römbke *et al.* (1992) suggested that the control earthworms should not lose more than 10% of the initial mass after 14 days to guard against misinterpretation of the data of the toxicity test as a result of starvation of the earthworms. The decrease in mass of exposed earthworms can therefore be attributed to the effect of the mancozeb and not to starvation. The negative effect of mancozeb on the earthworms' mass is in agreement with the findings of Vermeulen *et al.* (2001).

#### **4.2.2. Penconazole**

The LC<sub>50</sub> for penconazole was calculated as 379 mg.kg<sup>-1</sup>. This is much lower than the LC<sub>50</sub> for earthworms from data in the LIAISON Toxicity Database (2000) calculated at >1 000 mg.kg<sup>-1</sup> in soil. Taking the suggestion of Kokta (1992) into account, that states that a LC<sub>50</sub> that exceeds 1 000 mg.kg<sup>-1</sup> is not harmful to earthworms, results from the present study shows that penconazole should be considered toxic to *E. fetida*.

The mass of the earthworms was negatively affected in all exposure concentrations when compared to the control group. This decrease in body mass of the earthworms could not be attributed to starvation of the earthworms, because it was the effect of penconazole that was responsible for the loss of body mass. Exposure concentrations of 20 mg.kg<sup>-1</sup> to 240 mg.kg<sup>-1</sup> as well as the 480 mg.kg<sup>-1</sup> had similar body mass decreases. The decrease in mean body mass of earthworms exposed to 300 mg.kg<sup>-1</sup> was significantly lower than that of the lower concentrations (20 mg.kg<sup>-1</sup> to 240 mg.kg<sup>-1</sup>). This could mean that penconazole affected the earthworms in the same way for any exposure concentration between 20 mg.kg<sup>-1</sup> and 240 mg.kg<sup>-1</sup>, but a threshold concentration is reached where penconazole becomes more toxic to the earthworms. The earthworms in the 480 mg.kg<sup>-1</sup> exposure group were not exposed to the entire amount of the pesticide because they did not burrow into the contaminated substrate. The loss in mean body mass is possibly due to the starvation of the earthworms.

#### 4.2.3. *Trifloxystrobin*

All individuals exposed to 1 250 mg.kg<sup>-1</sup> of trifloxystrobin died within seven days of exposure. Earthworm mortality did not occur in the exposure concentrations of 9.4 mg.kg<sup>-1</sup> to 150 mg.kg<sup>-1</sup>. The mortalities in the 300 mg.kg<sup>-1</sup> and 600 mg.kg<sup>-1</sup> exposure groups occurred within seven days in both cases (Table 3.2.3.1). With a LC<sub>50</sub> of 770 mg.kg<sup>-1</sup>, well below the 1 000 mg.kg<sup>-1</sup> value that Kokta (1992) considers to be not harmful to earthworms, trifloxystrobin should be considered harmful to *E. fetida*.

The body mass changes of the earthworms were similar for the control and for the lowest exposure concentration of 9.4 mg.kg<sup>-1</sup> (Table 3.2.3.1). Because the biomass decrease was not very different to that of the control group for the exposure

concentrations ranging from 9.4 mg.kg<sup>-1</sup> to 150 mg.kg<sup>-1</sup>, these changes cannot be considered solely to be the result of the effect of trifloxystrobin on the earthworms. Römbke *et al.* (1992) suggested that the control group's body mass should not decrease by more than 10% after 14 days to that of the initial mass. A significantly ( $P < 0.05$ ) lower earthworm body mass occurred in the 300 mg.kg<sup>-1</sup> and 600 mg.kg<sup>-1</sup> exposure groups when comparing it to the control. This decrease in total body mass of the earthworms could be attributed to the effect of trifloxystrobin on *E. fetida*. Trifloxystrobin exposure resulted in negative effects to earthworms at exposure concentrations higher than 300 mg.kg<sup>-1</sup>.

#### 4.2.4. *Glyphosate*

Glyphosate had the highest LC<sub>50</sub> (3 740.40 mg.kg<sup>-1</sup>) of all the pesticides tested in this study. The LC<sub>50</sub> is more than 3.5 times greater than the LC<sub>50</sub> value Kokta (1992) considers not to be harmful to earthworms. The amount of glyphosate applied to T1 in this study was 2 250 mg.kg<sup>-1</sup> and only one application was performed. Considering this recommended application concentration which is much less than the LC<sub>50</sub>, glyphosate should be considered to be of little or no harm to earthworms.

The body mass change of *E. fetida* in the exposure groups of 562.50 mg.kg<sup>-1</sup> to 2 250 mg.kg<sup>-1</sup> did not differ much from that of the control group. The decrease in total body mass of the earthworms occurred in the exposure groups of 4 500 mg.kg<sup>-1</sup> and 9 000 mg.kg<sup>-1</sup>. Earthworms were affected negatively at concentrations that exceeded the amount of pesticide applied to the treatment plots.

#### 4.2.5. *N-acetyl salicylic acid*

The LC<sub>50</sub> of 312 mg.kg<sup>-1</sup> for N-acetyl salicylic acid is well within the 1 000 mg.kg<sup>-1</sup> range that Kokta (1992) suggested to be harmful to earthworms. This

suggests that it would render N-salicylic acid harmful to earthworms in the field. But when the recommended application concentration ( $80 \text{ mg.kg}^{-1}$ ) is applied, earthworm mortality may not be expected as a result of N-acetyl salicylic acid.

The body mass of earthworms was not adversely affected at N-acetyl salicylic acid concentrations lower than  $160 \text{ mg.kg}^{-1}$ . The decrease in mass of the earthworms coincided with the mortality of the earthworms at  $320 \text{ mg.kg}^{-1}$ . Earthworm biomass was not affected at exposure concentrations of  $80 \text{ mg.kg}^{-1}$ .

#### 4.2.6. *Ecotoxicological Relevance of Acute Toxicity Tests*

The ecotoxicological relevance of the acute toxicity tests conducted in this study, should be investigated further. One important reason is that none of the pesticides used in this study was applied more than twice during the year. This would have a definite effect on the accumulation, degradation and distribution of the pesticide within the soil. Kokta (1992) formulated the estimated environmental concentration (EEC) of a pesticide in soil with a mean bulk density of  $1.5 \text{ g.cm}^{-3}$ . This EEC would only apply for the upper 2.5 cm of soil, in which Kokta (1992) believes the pesticide would be uniformly distributed. The EEC is calculated by taking into account the amount of pesticides applied during the season. The EEC is determined by adding the full amount of the first application (100%) of a pesticide, with 50% of subsequent applications of the same pesticide (Kokta, 1992). Vermeulen *et al.* (2001) found that mancozeb has little or no detrimental effects on earthworm populations at single dosages of  $8 \text{ mg.kg}^{-1}$  or an EEC of  $44 \text{ mg.kg}^{-1}$ .

In the light of the acute toxicity data for pesticides resulting from this study, it is very difficult to determine whether the pesticides could have hazardous effects on the earthworms in natural and agro-ecosystems. The recommended application concentrations of the pesticides had little or no effect on *E. fetida* mortality (Table

4.1). Three out of the five pesticides tested (mancozeb, penconazole and trifloxystrobin) were harmful (decrease in biomass) to *E. fetida* at recommended application concentrations. The persistence of the pesticides and the species naturally inhabiting the agricultural soil is a major factor when trying to extrapolate from laboratory conditions to field situations. Edwards and Coulson (1992) suggested that an adjustment factor of 10 would put *E. fetida* on the same sensitivity level of the more sensitive earthworm species. Maboeta (2000), evaluating the effects of copper oxychloride on earthworms, agreed that a safety factor of 10 would be adequate to bring *E. fetida* in line with more sensitive earthworm species and that it should be taken into consideration when doing risk assessment studies. Contrary to the findings of Edwards and Coulson (1992), who found that *Apporectodea caliginosa* was the most sensitive earthworm species that they studied, Ma and Bodt (1993) concluded that *A. caliginosa* may be less sensitive to agrochemical contamination than other earthworms species. The aim of the present acute toxicity tests was not to extrapolate from laboratory conditions to field situations, but to assess to what extent the pesticides used would affect a “standard” soil organism (*E. fetida*) in a standardised toxicity test (OECD 1984). This objective was largely achieved, but in order to put the effects that pesticides have on soil organisms into an agro-ecosystem perspective, one also needs to include laboratory-to-field comparisons.

From Table 4.1 it is clear that the two pesticides (glyphosate and N-acetyl salicylic acid) used in the organically managed vineyard block had little or no negative effects on *E. fetida* health at the recommended application concentrations. The  $LC_{50}$  of glyphosate was  $\pm 1.5$  times greater than the recommended application concentration. The  $LC_{50}$  for N-acetyl salicylic acid was  $\pm 4$  times greater than the single application concentration. Because pesticides become bound to organic matter

in the soil affecting their bioavailability (Hodge *et al.* 2000; Ash & Lee 1980; Ma 1988), soil organisms are less prone to suffer detrimental effects of the aforementioned pesticides.

The three pesticides that did cause negative effects by lowering earthworm body mass are mancozeb, penconazole and trifloxystrobin. The recommended application concentration of 750 mg.kg<sup>-1</sup> for mancozeb is  $\pm 3$  times smaller than the LC<sub>50</sub> value (Table 4.1). The LC<sub>50</sub> of penconazole was  $\pm 4.5$  times greater than the recommended application concentration of 80 mg.kg<sup>-1</sup>. It was not clear whether the decrease in body mass after 14 days can be attributed to the effect of the penconazole or to the earthworms' starvation. The LC<sub>50</sub> of trifloxystrobin was calculated as being  $\pm 21.5$  times greater than the recommended application concentration of 35.50 mg.kg<sup>-1</sup>. Trifloxystrobin caused negative effects by lowering the total body mass of *E. fetida* significantly lower than that of the control. Although no mortality occurred at 35.50 mg.kg<sup>-1</sup>, the mass of *E. fetida* was negatively affected. If a safety factor of 10 is applied to the LC<sub>50</sub> calculated for trifloxystrobin (770 mg.kg<sup>-1</sup>), the predicted "safe" concentration would be 77.00 mg.kg<sup>-1</sup>. This predicted concentration would have been two times greater than the actual concentration of 35.50 that did cause negative effects to *E. fetida*.

Further investigation into the adjustment factor for acute toxicity tests for this situation needs to be done. Modern pesticide spraying programmes and application techniques have done a lot to lower the risk of pesticide contamination to NTO's. In this study a range of pesticides was applied at recommended application concentrations to the experimental vineyard, but none of the pesticides were applied more than twice within the study period. This creates other possible problems like



mixture toxicity, which can complicate matters further when trying to predict how “safe” agro-chemical pesticides are.

**Table 4.1:** A summary of the mean ( $\pm$ SD) percentage mortality and change in mean mass ( $\pm$ SD) of *E. fetida* after an exposure period of seven to 14 days to the recommended application (label) concentrations of pesticides used in this study. The recommended application concentrations are given as the single application concentrations. The 14-day  $LC_{50}$  (from Results 3.2), calculated using a range of pesticide concentrations, is also given for easy comparison with the amount of pesticide actually applied at a time.

Recommended Application Concentration	n	Mortality [%]		Mass Change [%]		14-Day $LC_{50}$ [mg.kg <sup>-1</sup> ]
		7	14	7	14	
<b>Control</b> 0 mg.kg <sup>-1</sup>	30	0	0	+8.25 $\pm$ 0.8	-13.22 $\pm$ 0.7	-
<b>Mancozeb</b> 750 mg.kg <sup>-1</sup>	30	0	0	-19.73 $\pm$ 0.7	-27.63 $\pm$ 0.7	2 332.50
<b>Penconazole</b> 80 mg.kg <sup>-1</sup>	30	0	0	-9.35 $\pm$ 0.6	-16.07 $\pm$ 0.7	379.00
<b>Trifloxystrobin</b> 35.50 mg.kg <sup>-1</sup>	30	0	0	-14.16 $\pm$ 0.6	-19.92 $\pm$ 0.7	770.00
<b>Glyphosate</b> 2 250 mg.kg <sup>-1</sup>	30	0	13.33 $\pm$ 0.7	+2.79 $\pm$ 0.9	-1.31 $\pm$ 1.0	3 740.40
<b>N-acetyl salicylic acid</b> 80 mg.kg <sup>-1</sup>	30	0	3.33 $\pm$ 0.7	+11.12 $\pm$ 0.9	+2.35 $\pm$ 1.1	321.00

### 4.3. Preference Behaviour Tests

*E. fetida* avoided the highest ( $LC_{50}$ -value) concentration of all the pesticides, except for trifloxystrobin, used in this preference behaviour experiments (Fig. 3.3.1 to Fig. 3.3.6). This indicates that the earthworms can detect and avoid these pesticides successfully, when it occurs in high concentrations. Slimak (1997) found that earthworms could avoid a range of pesticides applied at label concentrations.

Only the substrate with the highest concentration ( $882.78 \text{ mg.kg}^{-1}$ ) of copper oxychloride did not contain any earthworms after seven days (Fig.3.3.1). The earthworms could detect and avoid the highest concentration of the pesticide. It could be possible that the earthworms detected the heavy metal (copper) part of the pesticide. The mechanisms by which earthworms detect metals in soil are of importance to the organism, but not clearly understood yet. Because not all heavy metals are essential to earthworms, they should be able to detect the essential metals that are of importance to the animal's physiology. *Lumbricus terrestris* can distinguish between uncontaminated leaves and leaves that are contaminated with copper and zinc (Depta *et al.* 1999). Copper and zinc are both essential metals that are required in small amounts for the normal functioning of the earthworm's physiology, but can cause various acute and sublethal (Ma 1984) effects when earthworms are exposed to high concentrations of the metals. The earthworm mass increased more than  $\pm 45\%$  during seven days in copper oxychloride (Fig. 3.3.1). The earthworms were not affected in a negative way at the concentrations applied to the cubicles with test substrate. Helling *et al.* (2000) found, however, that copper oxychloride could have negative affects on the growth and reproduction *E. fetida* at very low concentrations. Theses authors also found that cocoon production and growth rate of juveniles of *E. fetida* was affected at concentrations as low as

8.92 mg.kg<sup>-1</sup>. A possible explanation for the increase in biomass of earthworms during the present study can be the fact that the earthworms could migrate freely between uncontaminated and contaminated substrates and were therefore not exposed to the pesticide continuously. Furthermore, adult earthworms were used in our study and juveniles, as in the study by Helling *et al.* (2000) may be more sensitive to the metal.

One can thus expect that in natural and agro-ecosystems earthworms would migrate freely between less and more metal polluted soils if not limited by ambient factors. Maboeta (2000) found that the copper concentrations in vineyard soils of the Western cape vary between 15 mg.kg<sup>-1</sup> and 50 mg.kg<sup>-1</sup> and that at these two concentrations, the burrowing activity of earthworms (*A. caliginosa*) was inhibited. The effective copper concentrations in the substrates used in the present study, where the distribution of *E. fetida* was not significantly ( $P < 0.05$ ) different, varied between 4.5 mg.kg<sup>-1</sup> and 88.28 mg.kg<sup>-1</sup>. The gain in biomass of *E. fetida* was possibly due to the fact that the copper in the organic substrate had a low bioavailability to the earthworms, because (Ma 1982) found that Cu<sup>+2</sup> ions strongly binds with organic matter that in turn limits its bioavailability.

*E. fetida* detected and avoided the three substrate cubicles with the highest mancozeb concentrations (Table 3.3.2). Mancozeb contains two physiologically essential heavy metals (manganese and zinc). The absorption of essential metals into the body is not governed by the requirements of the organism, but by the quantity and availability of the metal in the soil (Beeby 1991). Similar to copper, the bioavailability of these two metals is also determined by the amount of organic matter in the soil. The condition of the earthworms did not seem to be affected negatively

and a significant ( $p < 0.05$ ) total biomass increase of  $\pm 11\%$  occurred over the test period of seven days (Fig. 3.3.2).

Kokta (1992) suggested that mancozeb is not persistent in soil, but frequent application of the pesticide would increase the amount of the metal constituent of the pesticide remarkably. This increase would cause earthworms to migrate from the area. The application of mancozeb to the conventional vineyard block in this study was done only twice and at a concentration of  $750 \text{ mg.kg}^{-1}$ . It seems that the short-lived persistence of the mancozeb together with the regulatory ability of earthworms, mancozeb would not pose a hazard in this situation. Avoidance behaviour using *E. fetida* would thus be a poor indicator of acute or sub-acute toxicity for mancozeb when applied less frequently, because of the low toxicity of mancozeb to *E. fetida*. Although *E. fetida* seems not to be sensitive enough to low concentrations of mancozeb, the metal constituent of the pesticide formulation can accumulate in soils at concentrations high enough for earthworms to detect and avoid. *E. fetida* avoid the metal constituents of the pesticide and not the whole pesticide formulation. This complies with the findings of Reinecke *et al.* (2002) who concluded that avoidance behaviour of *E. fetida* would be a poor bioindicator of toxicity to mancozeb.

Earthworms avoided the highest concentration of penconazole in this experiment (Table 3.3.3). The recommended field application rate of  $22.5 \text{ mg.kg}^{-1}$  was applied to the conventional vineyard block. This concentration is almost 17 times smaller than the  $LC_{50}$  of  $379 \text{ mg.kg}^{-1}$  calculated for *E. fetida*. Earthworms did not avoid a concentration of  $75.80 \text{ mg.kg}^{-1}$  that is 3 times greater than the recommended application concentration. Kokta (1992) considers pesticides with a  $LC_{50}$  greater than  $1\,000 \text{ mg.kg}^{-1}$  not to be harmful to earthworms. Although the determined  $LC_{50}$  is below  $1\,000 \text{ mg.kg}^{-1}$ , considering the amount applied to the vineyard ( $22.5 \text{ mg.kg}^{-1}$ ),

earthworms may not be negatively affected by penconazole. The increase of  $\pm 27\%$  (Fig. 3.3.3) in biomass of the earthworms during the test period of seven days, indicated that penconazole did not adversely affect the condition of adult *E. fetida* after exposure to a range of concentrations of penconazole in comparison with an uncontaminated substrate.

Earthworms responded in a similar way to trifloxystrobin as they did to penconazole. *E. fetida* numbers showed a significant ( $P < 0.02$ ) avoidance response for the highest trifloxystrobin concentration of  $770.50 \text{ mg.kg}^{-1}$  (Table 3.3.4). Trifloxystrobin exhibits a combination of chemodynamic properties in and on the leaves of plants to interfere with the respiration of the fungi (Bayer AG 2001) and up to 50% of the applied amount penetrates the plant's waxy layer. Most of the pesticide would remain on the leaves of the vines and not reach the soil. Adverse health effects to soil organisms is minimal as trifloxystrobin has low mobility and rapid photolysis (New York State Department of Environmental Conservation 2000). This fungicide inhibits fungal spore germination and early life stages of pathogen development (Bayer AG 2001). This means that most of the non-fungal NTO's are not negatively affected if the recommended application methods and concentrations are applied. The mass of the adult earthworms was not affected negatively and an increase of  $\pm 28\%$  occurred after seven days of exposure to trifloxystrobin (Fig. 3.3.4). This finding compliments the findings found by the New York State Department of Environmental Conservation (2000), that trifloxystrobin does not cause adverse effects to wildlife in ground based applications.

Earthworms only avoided the highest concentration ( $3\ 737.41 \text{ mg.kg}^{-1}$ ) of glyphosate, an organophosphate herbicide. Hodge *et al.* (2000) found non-avoidance behaviour of *A. caliginosa* for certain organophosphate insecticides at recommended

label concentrations. The recommended application concentration ( $2\ 250\ \text{mg.kg}^{-1}$ ) is three times greater than the highest concentration of glyphosate ( $747.48\ \text{mg.kg}^{-1}$ ) where avoidance responses were not detected. The results are inconclusive regarding the ability of *E. fetida* to avoid a single treatment of glyphosate at the recommended application concentration, since the concentration where avoidance response was observed was  $\pm 66\%$  higher than that of the recommended application concentration. Earthworms showed an increase of  $\pm 28\%$  in total mass after the test period of seven days (Fig. 3.3.5), showing that the condition of earthworms may not have been affected negatively by the lower concentrations of glyphosate. This compliments the findings by Sassman *et al.* (1984) that glyphosate does not cause detrimental effects in most soil invertebrates.

Earthworms avoided three concentrations of N-acetyl salicylic acid used in this experiment (Table 3.3.6). The exposure concentrations that were avoided by the earthworms ranged from 31.27 to 312.7  $\text{mg.kg}^{-1}$ . The recommended application rate for N-acetyl salicylic acid ( $80\ \text{mg.kg}^{-1}$ ) falls within this range. Earthworms showed a significant ( $P < 0.02$ ) preference for the substrate with the lowest concentration of N-acetyl salicylic acid (Table 3.3.6). Salicylic acid is a phenolic acid found naturally in plants and insects, where it enhances the plant's natural ability to defend itself against pathogens (EPA 1998). It is possible that N-acetyl salicylic acid affected the earthworms favourably at low concentrations, which in turn resulted in the earthworms preferring the substrate with the smallest amount of N-acetyl salicylic acid to that of the uncontaminated substrate or the higher N-acetyl salicylic acid concentrations (Fig. 3.3.6). It is not clear what affected the earthworms to prefer the lowest concentration of the pesticide, but it could have something to do with their metabolism or the quality of the substrate. The important fact is that *E. fetida* could

detect the different concentrations of N-acetyl salicylic acid and choose at what concentration to live in. This could make avoidance behaviour of *E. fetida* a sensitive bioindicator for N-acetyl salicylic acid, because it seems as though *E. fetida* can detect N-acetyl salicylic acid at a relatively low concentration. Earthworms can therefore respond to unfavourably high N-acetyl salicylic acid concentrations long before adverse effects can occur. N-acetyl salicylic acid is used as a commercially available organic fungicide that is registered at SGS of South Africa (SGS 2001). In the substrate cubicles occupied by the earthworms, the two lowest exposure concentrations (6.25 mg.kg<sup>-1</sup>, 3.13 mg.kg<sup>-1</sup>) of N-acetyl salicylic acid and the uncontaminated substrate, earthworms had an increase of  $\pm 28\%$  in mass for the duration (seven days) of the experiment (Fig. 3.3.6). This indicates that the condition of earthworms was not adversely affected.

The results of these behaviour tests show that *E. fetida* could detect and avoid all the pesticides tested, depending on the concentration levels. For certain pesticides *E. fetida* can be a sensitive bioindicator and for other pesticides a poor bioindicator of toxicity in acute and/or sub-acute lethal toxicity tests. The value of *E. fetida* as a bioindicator for a specific pesticide would depend on the sensitivity it has for that particular pesticide. Avoidance response cannot account for or predict the long-term effect of pesticides, but it can be used as a quick and inexpensive preliminary toxicity test for other more definitive ecotoxicological test procedures. As could be seen from these tests, the ecotoxicological relevance of behaviour of earthworms as bioindicator varies for the different pesticides or pesticide groups. The extrapolation from laboratory to field situations is often very complicated, because the chemical composition of pesticides and their interaction with the soil plays an important role in determining how the pesticides affect exposed animals (Vermeulen *et al.* 2001).

Bioavailability of active substances also governs whether and to what extent pesticides and mixtures of pesticides influence the organisms. Species differences and variation in response to different chemical stimuli must also be taken into account when considering using the avoidance behaviour of *E. fetida* as a tool for assessing environmental contamination by pesticides.



## 5. Conclusions

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1. It was concluded that the use of the bait-lamina technique in the field trials did provide sufficient information as to whether organic management differed from conventional management practices in terms of soil biological activity as measured with the bait-laminae. It is recommended that further investigation is done, partly because the results of the present study only represent one growing season and also because of the various limitations of the bait-lamina technique.
2. The bait-lamina technique is a reliable method to measure soil biological activity, both in the field and especially in the laboratory. This technique provides quick results and the data is easy to interpret. Although some results were different from what was expected, Larink (1994b) stressed that in many cases results can contradict each other. Therefore, to apply the bait-lamina test as successful as possible, the physico-chemical information of the soil such as soil moisture, soil temperatures and rainfall, should be taken, preferably for the time that the bait-laminae are in the soil.
3. Soil moisture proved to be the most important factor that affects the biological activity of the soil fauna. In the microcosm trials, where temperature and moisture were controlled, the bait-laminae results in the soil that was organically managed showed a higher feeding activity than the conventionally managed soil.
4. The acute toxicity tests provided valuable information about the short-term effects of the pesticides used in viticulture in the Western Cape. The negative effects on earthworms included loss of biomass and the preference of not burrowing into the contaminated substrate. In behavioural experiments *E. fetida* always avoided the highest pesticide concentration ( $LC_{50}$ -value) of all the pesticides used.

5. It is recommended that when assessing the soil environment ecotoxicologically, the use of acute toxicity tests should be combined with behavioural tests to provide more insight into the situation. Laboratory studies should be done in combination with field trials to support the field data.
  
6. The goals of this study were met, but in order to better understand what happens to the soil fauna in vineyards under different management practices, further investigation is needed. Questions that remained unanswered, such as mixture toxicity, soil faunal population structure and seasonal changes of the soil fauna biodiversity need to be addressed.

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\* Original not seen.

## Appendix A

Environmental Protection Agency toxicity classification.

<b>Acute Toxicity to Rat</b>					
<b>Class</b>	<b>Oral LD<sub>50</sub> (mg.kg<sup>-1</sup>)</b>	<b>Dermal LD<sub>50</sub> (mg.kg<sup>-1</sup>)</b>	<b>Inhalation</b>	<b>Eye Effects</b>	<b>Skin Effects</b>
<b>I</b>	≤50	≤200	≤0.2	Corrosive; corneal opacity not reversible within 7 days	Corrosive
<b>II</b>	50-500	200-2 000	0.2-2.0	Corrosive; corneal opacity reversible within 7 days; irritation persisting for 7 days.	Severe irritation at 72 hours
<b>III</b>	500-5 000	2 000-20 000	2.0-20	No corneal opacity; irritation reversible within 7 days.	Moderate irritation at 72 hours.
<b>IV</b>	≥5 000	≥20 000	≥20	No irritation	Mild or slight irritation at 72 hours.

## Appendix B

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[The following detailed explanation of the Treatments is a Revised Version of Addendum A of Project WW18/26. Project Leader: P. J. Raath from the ARC-Infruitec/Nietvootbij.]

### **Treatment 1 (T1) – Full surface chemical weed control.**

#### Tillage and weed control

- Kill winter weeds full surface with Sting/Roundup
- Do pre-emergence weed control using Simazine (3 litres per ha)
- Evaluate whether an additional 3 litres per ha of Simazine is necessary and apply if necessary

#### Fertilisation

Fertilisation of grapevines will be done based on the results of the soil samples. If the pH, K and P levels are too low they will be adjusted. Nitrogen fertilisation will be done according to production and on account of vegetative growth. The following programme is suggested as a guideline:

- Apply 50 kg/ha LAN (Limestone Ammonium Nitrate, a growth stimulator) four weeks after bud break.
- Apply KCl and double Superphosphate [ $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ] according to needs as determined by soil analysis.
- Apply 50 kg/ha LAN post-harvest.



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**Treatment 2 (T2)- Soil managed according to IPM (Integrated Pest Management) guidelines.**

Tillage and weed control

- Sow vetch (Cover crop).
- Determine weed population and decide if post-emergence chemical control is necessary.
- Evaluate weed population and apply post-emergence herbicide if necessary.
- Evaluate weed population herbicide and if needed apply post-emergence herbicide

Fertilisation

Fertilisation of cover crops will entail 80 kg/ha LAN about six weeks after sowing of grains; this is not applicable to N-binders.

Phosphate and K might have to be applied during seedbed preparation.

Fertilisation of grapevines will be done based on the results of the soil samples. If the pH, K and P levels are too low it will be adjusted. Nitrogen fertilisation will be done according to production and on account of vegetative growth. The following programme is suggested as a guideline:

- Apply 50 kg/ha four weeks after bud break.
- Apply KCL and double Superphosphate [ $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ] according to needs as determined by soil analysis.
- Apply 50 kg/ha LAN post-harvest.

**Treatment 3 (T3) Organic management based on Self-sufficiency**

Tillage and weed control

- Sow vetch in working row.
- Slash cover crop immediately before flowering.

- Evaluate growth of cover crop and slash/plough it in (disc harrow) if necessary.
- Use compost as a weed control in vine row.

### Fertilisation

Fertilisation of cover crops will entail application of compost to soil before sowing of seed, according to soil and compost analysis.

Fertilisation of grapevines will be done based on the results of the soil samples. If the pH, K and P levels are too low it will be adjusted. Nitrogen fertilisation will be done according to production and on account of vegetative growth. The following programme is suggested as a guideline:

- Apply 100-150 kg/ha N in the form of compost in three instalments during the season, on the vine row, taking vegetative growth into account.

### **Treatment 4 (T4)- Organic management based on the use of commercial organic products.**

#### Tillage and weed control

- The same procedure will be followed as for T3

#### Fertilisation

Fertilisation of cover crops will entail application of commercial organic fertiliser, instead of compost, to soil before sowing of seed, according to soil analyses. If proper commercial products are not available, compost will be used.

Fertilisation of grapevines will be done based on the results of the soil samples. If the pH, K and P levels are too low it will be adjusted. Nitrogen fertilisation will be done according to production and on account of vegetative growth. The following programme is suggested as a guideline:

- Apply 100-150 kg/ha N in the form of compost in three instalments during the season, on the vine row, taking vegetative growth into account.
- Growth stimulators like Biocult and Penac will also be applied according to their prescriptions.

**Treatment 5 (T5)- T3 with additional use of EM (Effective Micro-organism)-technology.**

Tillage and weed control

- Sow vetch in working row.
- Slash cover crop immediately before flowering.
- Evaluate growth of cover crop and slash/plough it in (disc harrow) if necessary.
- Spray EM on soil to stimulate weed emergence, plough it in if population are high.
- Spray the soil and cover crop with EM every three weeks from bloom until after harvest.
- Use compost as a weed control on vine row.

Fertilisation

Fertilisation of cover crops will entail application of compost to soil before sowing of seed, according to soil and compost analysis.

Fertilisation of grapevines will be done based on the results of the soil samples. If the pH, K and P levels are too low it will be adjusted. Nitrogen fertilisation will be done according to production and on account of vegetative growth. The following programme is suggested as a guideline:

- Apply 100-150 kg/ha N in the form of compost in three instalments during the season, on the vine row, taking vegetative growth into account.
- EM will be used to prepare compost

- EM will be sprayed on the vine row every two weeks, starting in August, to stimulate microbial breakdown of compost.

**Treatment 6 (T6)- Treatment 2 with additional use of EM-technology.**

The same procedures will be followed as for T2, but EM will be used in the following ways:

Spray cover crop with EM every three weeks from the time it is sown until the end of the growing season, i.e. three weeks after harvest.

Spray the vine row with EM every three weeks.