The effects of heavy metal-rich sewage sludge on Collembola communities in grassland

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But mousie, thou art no thy lane
In proving foresight may be vain:
The best-laid schemes o mice an men
Gang aft agley,
An lea'e us nought but grief an pain,
For promis'd joy!

Robert Burns: To a Mouse

ABSTRACT

The purpose of this research was to investigate how the land application of sewage sludge (and particularly the heavy metals it contains) affects grassland Collembola. The effects of digested, undigested, copper-rich, zinc-rich and cadmium-rich sludges were investigated using a small plot trial established at SAC Auchincruive, South Ayrshire. An initial investigation was also conducted at a similar trial site established at Hartwood, Lanarkshire. The metal-rich sludges were derived from treatment works with naturally high inputs of the specific metal. Effects on euedaphic and hemiedaphic Collembola were examined in 1995 using litterbags as a sampling method, and effects on hemiedaphic and epigeal Collembola were investigated in 1995 and 1996 using pitfall trapping and suction sampling.

The euedaphic *Mesaphorura* spp. and *Neelus minimus* were adversely affected by the application of zinc-rich sludge, and *Mesaphorura* spp. were also adversely affected by copper-rich sludge. Both suction samples and pitfall traps indicated that *Lepidocyrtus cyaneus* and *Isotoma viridis* were adversely affected by cadmium-rich sludge, and the pitfall traps also found *Isotomurus maculatus* and the total collembolan abundance to be adversely affected. Furthermore, the abundance of *L. cyaneus, I. viridis* and *Heteromurus nitidis* in suction samples was inversely related to the concentration of cadmium in the soil.

The abundance of *Isotoma anglicana* in suction samples, on the other hand, tended to increase with soil cadmium concentration. This species was also more abundant in litterbags collected from the zinc-rich and copper-rich plots, hence suggesting that it was tolerant to all three metals investigated. *Isotomurus palustris* also appeared to be tolerant to heavy metals, and the abundance of this species (when sampled by suction) was highest in the zinc-rich and cadmium-rich plots.

On the Auchincruive trial site, reproduction of *Isotoma/Isotomurus* species was promoted by the addition of sludge irrespective of metal contamination. At the Hartwood site, however, the abundance of juveniles was lowest in the cadmium-rich plots. Reproduction of *Isotoma/Isotomurus* species was therefore adversely affected by

cadmium-rich sludge at Hartwood (but not at Auchincruive). The difference between sites was thought to be the result of differences in the composition of *Isotoma/Isotomurus* juveniles, with a larger proportion of the juveniles at Hartwood belonging to a metal sensitive species (e.g. *I. viridis*).

All three sampling methods indicated that the effects of season (and succession in the case of litterbags) were more pronounced than those of treatment. *Isotoma notabilis* was found to be an early coloniser of decomposing oak leaves, while *I. palustris* and *I. anglicana* were late colonisers. The species diversity was found to increase as decomposition progressed. Seasonal peaks in abundance were also found to be species specific, with some species reaching their maximum density in April (e.g. *Ceratophysella denticulata* and *Sminthurides malmgreni*), others in May (e.g. *Sminthurinus aureus* and *Isotoma/Isotomurus* juveniles), and others in August (e.g. *H. nitidis* and *L. cyaneus*). Effects of grass cutting were apparent and several species (e.g. *I. viridis*, *Sminthurides pumilis* and *Tomocerus longicornis*) were more abundant following cutting. Furthermore, the abundance of *I. viridis*, *S. pumilis* and *T. longicornis* was inversely related to grass height. This was thought to be the consequence of pitfalls and suction samplers being more efficient at sampling these species in shorter grass.

In addition to the contaminated sludge experiment, initial research was also conducted to investigate the effects of the land application of pharmaceutical wastes on euedaphic and hemiedaphic Collembola. Two wastes, sodium ammonium sulphate (SAS) and aerobically treated sludge (ATS), were investigated using small plot trials. One year after SAS application, the collembolan community was more diverse and the species *Folsomia candida* and *Mesaphorura* spp. were particularly favoured by SAS. As the effects of SAS were only studied one year after application, it is not known if the collembolan community was adversely affected by this waste immediately following application. Three months following the application of ATS waste, the collembolan density was found to be severely depleted in the ATS plots when compared to both the inorganically fertilised plots and unfertilised control plots. Adverse effects appeared to be non-specific and were thought to be a consequence of ATS containing low levels of penicillin which adversely affect the micro-organisms on which Collembola feed.

The findings suggest that the best sampling regime for Collembola would be achieved from combining methods which sample epigeal species (e.g. suction sampling and pitfall trapping) with methods which sample euedaphic species (e.g. litterbags). While suction sampling was more efficient at sampling epigeal species (e.g. *Sminthurus viridis*, *T. longicornis* and *Dicyrtoma* spp.), pitfall trapping caught more species that were strictly hemiedaphic (e.g. *C. denticulata*, *S. aureus* and *S. pumilis*). The best sampling regime for epigeal and hemiedaphic species should therefore include both pitfall trapping and suction sampling. A combination of both sampling methods also enables sub-lethal effects of chemicals on activity to be monitored, and in this study the cadmium sensitive species *L. cyaneus* was found to have a lower activity level in the cadmium-rich plots than the digested plots. Where sampling has to be limited to one method (e.g. on account of resource constraints), the choice of method should reflect its efficiency in sampling the type of species (euedaphic, hemiedaphic or epigeal) expected to be most susceptible to the management practice under investigation.

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Some commonly used abbreviations

ATS: Aerobically treated sludge
BOD: Biochemical oxygen demand
COD: Chemical oxygen demand
DCA: Detrended correspondence analysis
PTE's: Potential toxic elements (e.g. cadmium, copper, zinc)
SAS: Sodium ammonium sulphate
AN: Ammonium nitrate
NPK: Inorganic fertiliser (specifically ammonium nitrate, triple superphosphate and muriate of potash)
CEC: Council of the European Communities
NS: Non significant
df: Degrees freedom

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* Please note that Chapter 3 section 3.4 includes important amendments to the text following recommendations made prior to thesis binding.

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CHAPTER 1. Introduction

The Commission of the European Communities Directive concerning urban waste water treatment, stipulates that by 1998 sewage sludge will no longer be eligible for disposal at sea (CEC 1991). With an estimated 1.1 million tonnes of sewage sludge (dry solids) produced annually in the UK (Smith 1996), alternative disposal methods must be found. It has therefore been predicted that in the next decade the disposal of sewage sludge on agricultural land will become more widespread (Rund 1995). Probably the main drawback in using sludge as an agricultural fertiliser lies in the fact that it contains significant quantities of heavy metals such as cadmium, zinc, lead and copper (Petruzzelli *et al.* 1994).

As heavy metals are persistent, they can accumulate to high levels in areas of long-term sludge application (Aitken *et al.* 1994). Adverse effects of metals in sludge have been found for euedaphic Collembola, aphids and mites (Lübben 1989; Culliney & Pimentel 1986; Glockemann & Larink 1989: respectively); earthworms have also been found to have elevated metal burdens in areas where sludge has been applied (Helmke *et al.* 1979; Kruse & Barrett 1985). More information is therefore required to determine the impact of sewage sludge (and in particular the heavy metals it contains) on invertebrates and to ascertain if current legislation governing the safe metal loading will protect all invertebrate species adequately.

In 1994, small plot trials were established which aimed to examine the effects of heavy metals in sewage sludge on soil microbial activity, agricultural productivity and long-term soil fertility. The trials investigated sewage sludges that had been derived from treatment works with naturally high inputs of cadmium, copper or zinc, to enable effects of specific metals to be examined. As the effects of heavy metals in sewage sludge on insect communities has received little attention in contrast to the many studies on micro-organisms (Smith 1996), it was decided to utilise these trials to investigate the effects of metal-rich sludges on Collembola (commonly known as springtails).

Collembola are generally considered to be beneficial to agriculture as they regulate decomposition and mineralisation and hence promote soil fertility (Seastedt 1984). Furthermore, they are known to act as prey to polyphagous predators (e.g. beetles and spiders) and consequently help to maintain healthy populations of these useful predators (Frampton 1988). It is therefore important to be aware of the effects of agricultural practices on this essential group of arthropods.

Collembola have successfully been used as indicators of heavy metal pollution (Bengtsson & Rundgren 1988; Filser *et al.* 1995), and a previous study also found them to be sensitive to the application of metal-rich sludge (Lübben 1989). Collembola, as a consequence of their small size, provide useful monitors in small plot trials, where the use of larger invertebrates would be inappropriate. Chapter 2 provides a literature review on the effects of environmental disturbances (in particular agricultural practices and heavy metal pollution) on Collembola.

Chapter 3 introduces the two contaminated sludge trial sites where Collembola were monitored, namely SAC Auchincruive, South Ayrshire, and Hartwood, Lanarkshire. This chapter also describes the collembolan identification techniques used in this research.

In Chapter 4, an investigation into the effects of four different sewage sludges (specifically undigested, digested, copper-rich and zinc-rich) is reported. In this work litterbags were used to investigate the effects of these sludges on euedaphic and hemiedaphic Collembola and on the decomposition rate. The effects of season and succession on the collembolan population structure and diversity were also investigated using litterbags.

The effects of undigested, digested, copper-rich, zinc-rich and cadmium-rich sludges on hemiedaphic and epigeal Collembola are examined using suction in Chapter 5 and pitfalls in Chapters 6. The effects of season and grass cutting on Collembola and how grass height influences the sampling efficiency of various species are also considered.

While litterbags sample euedaphic and hemiedaphic Collembola, pitfall traps and suction samplers both sample hemiedaphic and epigeal species. A comparison between the species caught by these three sampling methods is made in Chapter 7. The number of individuals of any species sampled by suction is thought to be directly related to their density, whereas, the number caught by pitfall traps is related to both the density and the activity of a species. The pitfall trap and suction data is therefore combined to determine if sub-lethal effects of metals on activity occurred.

In Chapter 8, litterbags are used to determine the influences of two very different pharmaceutical wastes on euedaphic and hemiedaphic Collembola. Some pharmaceutical wastes, like sewage sludges, are waste products which contain essential plant nutrients and therefore have the potential to be used as agricultural fertilisers. Current legislation permits pharmaceutical wastes to be used as fertilisers if they benefit the land without causing pollution (Aitken 1994).

In Chapter 9, the risks of applying the metal-contaminated sludge to agricultural land are assessed and current safe metal loadings discussed on the basis of the findings. Recommendations for future studies are also given.

CHAPTER 2. Literature Review

2.1 Collembola

Taxonomy, physiology and distribution

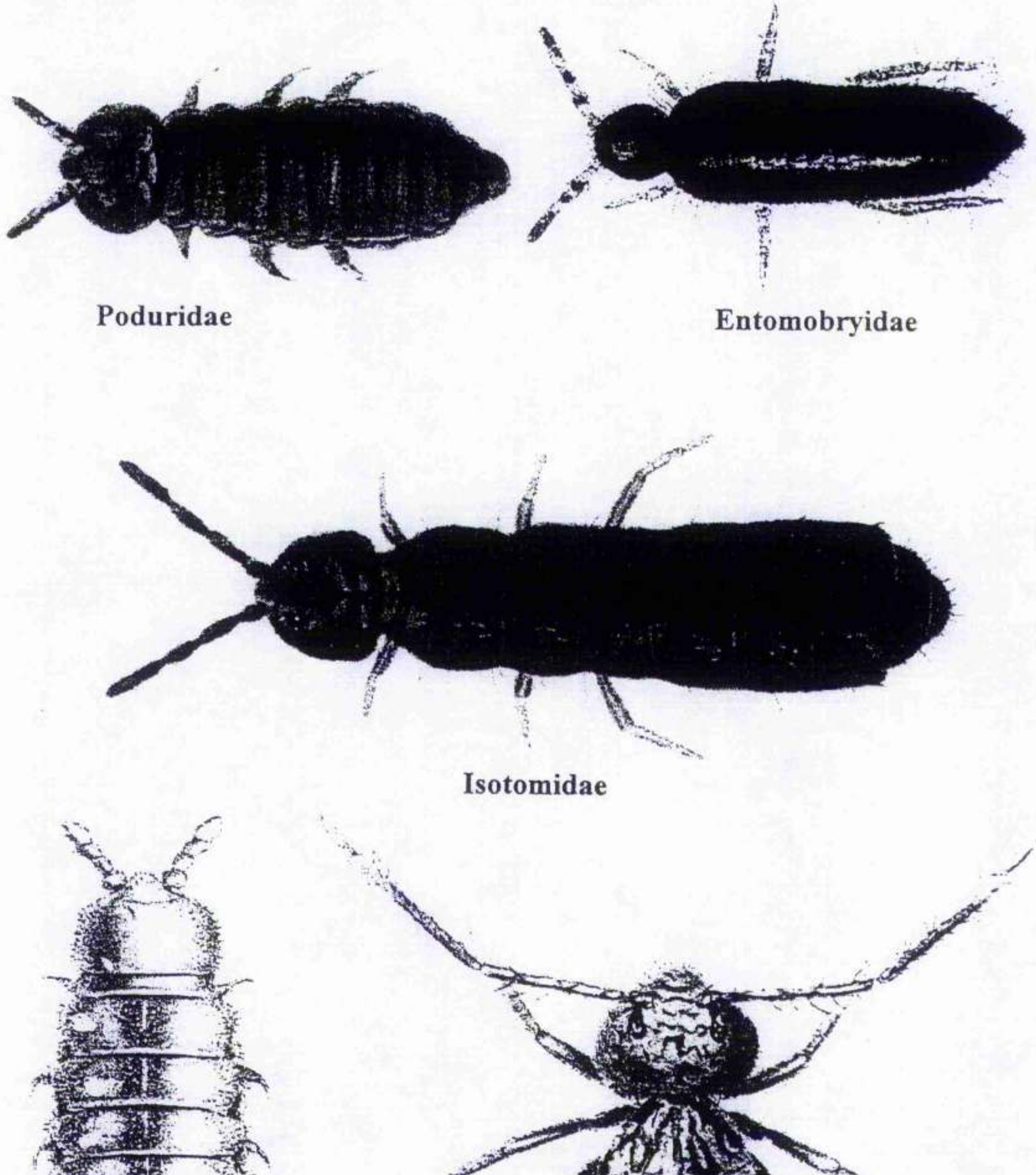
Collembola (commonly known as springtails) are small (ranging in length from 0.2 - 10 mm), primitive arthropods that lack complete metamorphosis and moult throughout their life-span (Gillot 1995). They were originally placed in the subclass Apterygota (meaning wingless) within the class Insecta, but since their mouthparts are different from all other insects, they are now frequently placed in the class Ellipura along with the Protura (Gillot 1995).

The European Collembola are generally divided into five families (Fjellberg 1980): the globular Sminthuridae; the Poduridae (generally with an obvious prothorax and a granular cuticle); the highly varied Isotomidae; the often large and scaly (or hairy) Entombrydae; and the white, blind Onychiuridae (see Figure 2.1).

Collembola occur throughout the world and are found in habitats as diverse as caves, snow fields, the intertidal zone, bird nests and termite mounds (Wallwork 1970). They are frequently the most abundant grassland arthropods, with typical densities of 22,800 m^{-2} (Sheals 1957), 27,000 m^{-2} (Dhillon & Gibson 1962) and 109,000 m^{-2} (Haarløv 1960) for Scotland, England and Denmark, respectively. Although about 6,500 species have been described to date, the actual number of existing species may be in excess of 50,000 (Hopkin 1997).

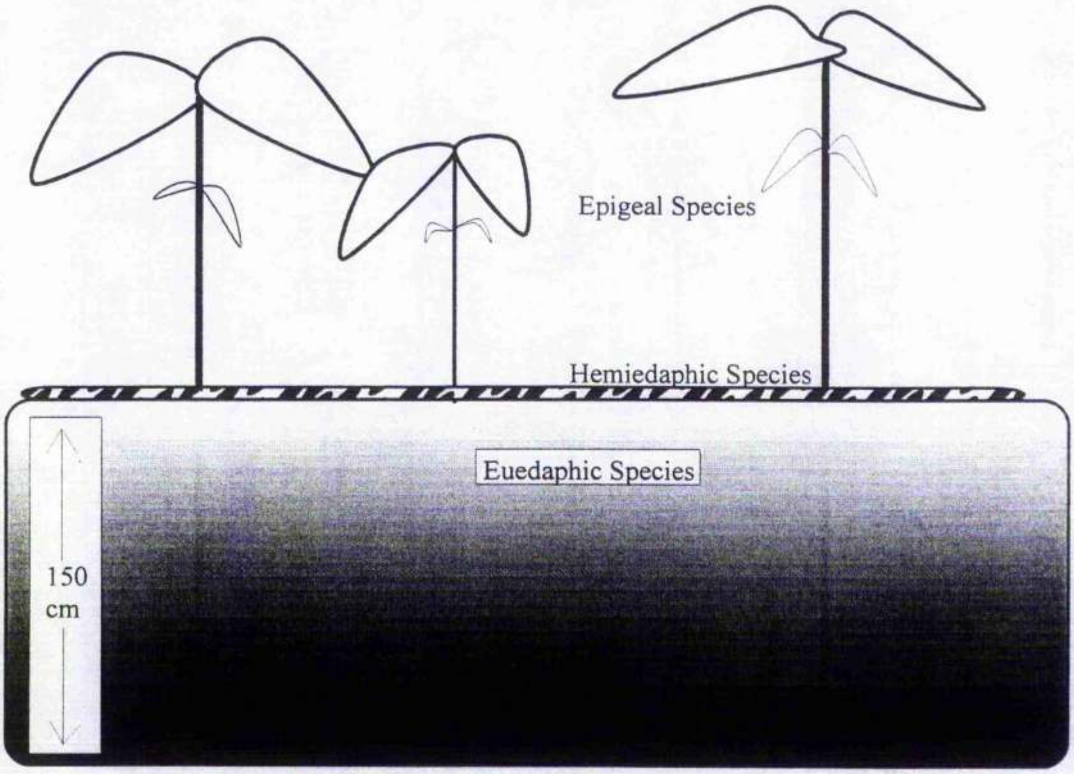
Collembola have a marked distribution pattern in the soil profile, and have been classified into three groups, principally using this distribution: the euedaphic species which live solely underground (e.g. *Folsomia candida* (Willem)); the hemiedaphic species that live principally in the litter layer (e.g. *Isotoma notabilis* Schäffer); and epigeal (or epedaphic) species that live above the ground and frequently climb the vegetation (e.g. *Sminthurus viridis* (Linné): Christiansen 1964). The morphology of these three groups is principally related to their vertical distribution (see Figure 2.2)

Figure 2.1 The five families of European Collembola



and these groups may therefore be termed ecomorphological groups. For example, the euedaphic species possess morphological adaptations to a subterranean existence including reduced appendages (e.g. legs, furca and antennae), the lack of dense scales or setae (both common in epigeal species), and the lack of coloured pigment and occelli (Wiles & Frampton 1996).

Figure 2.2 Vertical distribution of Collembola



Agricultural importance of Collembola

Only a few species of Collembola are considered detrimental to agriculture. For example, *S. viridis* frequently damages clover and peas, while *Protaphorura armata* (Tullberg) damages sugar beet seedlings (Sievers & Ulber 1990). The vast majority of collembolan species are, however, generally regarded as beneficial to agriculture. In particular, they are an important source of food for larger invertebrates such as lyniphiid spiders and Coleoptera (Sunderland 1975). The carabid beetle *Loricera pilicornis* is specially adapted to capture Collembola with its extremely hairy antennae (Hintzpeter & Bauer 1986). These polyphagous predators also consume small agricultural pests (such as aphids and mites), and therefore Collembola are indirectly profitable to agriculture through helping to maintain larger populations of these beneficial predators. There is also evidence that Collembola protect plants from root pathogens through the selective grazing of fungi (Curl & Harper 1990). Finally they are thought to play an important role in decomposition and mineralisation (see below).

The role of Collembola in decomposition

Although a few species of Collembola are phytophagous, saprophagous and even predacious, it is generally accepted the majority are mycophagous. It is therefore hardly surprising that although fungi and bacteria are directly responsible for most of the organic breakdown in soil, Collembola act as regulators to the decomposition process. For example, in litterbags where the litter arthropods were eliminated the rate of decomposition was reduced by, on average, 23% (Seastedt 1984). Collembola are thought to act as regulators through the following processes.

Interactions with fungi: Engelmann (1961) suggested that the removal of senescent fungal tissue by Collembola stimulates growth and remobilizes nutrients bound in fungal biomass in the form of collembolan faeces. As Collembola tend to aggregate together, it is likely that their grazing is of considerable local significance for nutrient mobilisation. Although the respiration rate of micro-flora is generally increased by grazing, it has been noticed that this rate may be decreased if overgrazing occurs (Hanlon & Anderson 1979). It has also been observed that while grazing by *F. candida* increased bacterial standing crops, it decreased fungal standing crops (Hanlon & Anderson 1979). Furthermore, grazing did not increase fungal respiration when nutrients were limiting but did increase it at higher nutrient levels.

Collembola may also promote fungal growth through the distribution of fungal spores (Hanlon & Anderson 1979), and through the stimulation of spores to germinate by passage through the gut (MacFadyen 1978). It would therefore appear that the effects of Collembola on fungi are dependent on several factors including nutrient availability, grazing intensity, spore distribution and spore germination.

Enhancing bacteria growth: The guts of Collembola may form favourable environments for bacterial growth (Hanlon & Anderson 1979), and enhanced microbial activity has been observed in the faeces of other soil animals (e.g. the millipede *Glomeris marginata* (Villers): Anderson & Bignell 1980). Digestive transit may also assure chemical and biological breakdown, and the mixing of mineral and organic elements with micro-organisms (Hanlon & Anderson 1979). Through the fragmentation of organic matter, Collembola expose new surfaces thus increasing the surface area for microbial attack (Bird & Chatarpaul 1986). Predacious Collembola (e.g. *Friesea mirabilis* (Tullberg)) may also have an indirect effect on bacterial growth through regulating the density of bactivorous nematodes (Blair & Crossley 1988).

Preferential grazing: Klironomos *et al.* (1992) discovered that through preferentially grazing dark pigmented fungi on decomposing leaf litter, *F. candida* increased the rate that the dark pigmented primary saprophytes (commonly demaiaceous fungi) were replaced by non-pigmented secondary saprophytes. Other studies have also indicated that Collembola prefer to feed on dark pigmented fungi (Mills & Sinha 1971; Aitchison 1983). This supports the hypothesis that through selective grazing fungal succession is accelerated by favouring the colonisation of secondary saprophytes.

Collembola are therefore regarded as important regulators in the decomposition and mineralisation of organic matter through their interactions with fungi, bactivorous nematodes and bacteria.

2.2 Fertilisers

Crop nutrition

In addition to carbon, hydrogen and oxygen (obtained from the air and water), plants require an adequate supply of other essential nutrients from the soil. The most important of these are nitrogen, phosphorus and potassium (N, P and K respectively). Soil levels of these nutrients are often the limiting factor to plant growth (Simpson

1986). By artificially increasing nutrient levels through fertilisation, the crop yield can be greatly increased. With today's increasing food demands it is becoming essential to obtain the optimum yield from the available agricultural land.

Two categories of fertilisers are generally recognised: organic manures (e.g. sewage sludge, seaweed and slurry), and inorganic fertilisers that are frequently artificially produced (e.g. potassium chloride). Artificially produced inorganic fertilisers can be designed to meet the exact requirements of a crop in a particular area. Organic fertilisers are generally the by-product of some other process and are therefore less expensive than artificial fertilisers. For example slurry (a by-product of livestock farming) and sludge from paper mills are both used as organic fertilisers (Smith 1996). Organic fertilisers also have the added bonus of enhancing organic matter content and hence the physical structure of the soil.

<u>Pharmaceutical waste</u>

Pharmaceutical companies produce large quantities of waste, the majority of which comes from the fermentation processes that produce steroids and antibiotics. This waste consists predominantly of fungi mycelium (from the fermentation micro-organism) and left over substrate, such as molasses (which has provided the organism with food during fermentation). Consequently it contains significant quantities of nitrogen, phosphorus and potassium (plant macro-nutrients), and lower concentrations of plant micro-nutrients such as zinc, magnesium and calcium (Larsen *et al.* 1991). It is therefore considered essentially non-toxic and is seldom polluted by heavy metals (Larsen *et al.* 1991). Despite this, disposal still provides a problem as a consequence of the high level of biologically degradable material which can cause eutrophication in aquatic environments (Larsen *et al.* 1991).

Pharmaceutical waste has the potential for use as an organic fertiliser since it contains the essential plant nutrients, possess little threat of pollution (at least in the long-term), and has been shown to increase crop yields (De Roo 1975; Volz & Heichel 1979). Under current legislation in the UK, the application of pharmaceutical waste to agricultural land is permitted if the material is applied "to fertilise or beneficially condition the land" and does not cause pollution (Aitken 1994). Furthermore, pharmaceutical companies are looking for alternative methods of disposal (Wright 1978), and would be prepared to make waste available at minimum costs (Larsen *et al.* 1991). If the use of pharmaceutical waste is to become more widespread, further studies are needed to determine the possibility of pollution (especially from antibiotics, salt and zinc) and any deleterious effects land application may have on the invertebrate fauna.

2.3 Sewage sludge

It is estimated that in the UK 1.1 million tonnes of sewage sludge (dry solids) is produced annually (Smith 1996). Forty-four percent of sludge produced in the UK is used as an agricultural fertiliser, while 30% is currently disposed of at sea. However, a Commission of the European Communities (CEC) Directive stipulates that by 1998 sludge will no longer be eligible for disposal at sea (CEC 1991). Since this is one of the primary methods of sludge disposal in the UK, alternative methods must be found. Recycling sludge by using it as an agricultural fertiliser not only provides a cheap source of fertiliser, but also a more efficient and environmentally friendly method of sludge disposal than alternative methods (e.g. incineration and landfill). In the next decade it is therefore likely that the use of sewage sludge as fertiliser will become more widespread. For example, the UK Department of Environment predicts that between 1991 and 2006 the amount of sludge used for agricultural purposes will almost double from 465,000 to 926,000 tonnes (Rund 1995).

Sewage sludge not only contains the essential plant nutrients N and P (estimated average content of N and P in sludge dry solids is 3.8% and 2.2% respectively: Smith 1996), but also has a high organic matter content (e.g. 50%: Smith 1996). The addition of organic matter can improve the physical structure and water retention capacity of the soil, especially in areas where the soil is impoverished and sandy. In addition, sludge is potentially a source of sulphur and plant micro-nutrients such as copper, magnesium and zinc (Smith 1996).

Potentially toxic elements in sewage sludge

Elements such as copper and zinc are not usually considered beneficial in sewage sludge because they are generally present naturally in the soil at levels sufficient to meet the crop's requirement. Indeed heavy metals such as copper, zinc, lead, cadmium and nickel are considered one of the main drawbacks in using sewage sludge as an agricultural fertiliser (Petruzzelli *et al.* 1994). These elements, collectively known as potentially toxic elements (PTEs), are persistent and will accumulate in the upper layers of the soil where they can reach levels that are potentially toxic to plants and animals (Posthuma *et al.* 1993; Van Straalen *et al.* 1987). High levels of cadmium have been found in the livers and kidneys of lamb's grazing on pasture fertilised with sewage sludge (Coghlan 1997).

Levels of metals derived from industry (e.g. cadmium, chromium and nickel) have, on the whole, decreased in sewage sludge principally as a result of more stringent controls. For example, cadmium levels have decreased by 30% in the past decade (Smith 1996). It is thought that the phasing-out of leaded fuel should also significantly reduce the levels of lead entering the sewage system (Comber & Gunn 1994). It is more difficult to control metals such as copper and zinc that are principally derived from domestic waste. It has been estimated that 62% of the copper and 64% of the zinc (Critchley & Agg 1986) entering a UK sewer system came from domestic sources. The principal source of contamination by these two metals is thought to be from the galvanisation and subsequent corrosion of domestic plumbing systems. Consequently these two metals generally limit the application of sewage sludge to agricultural land in the UK (Smith 1996). Levels of mercury have remained static over the past decade, while other metals (including zinc and copper) have decreased (Smith 1996). It has been proposed that mercury (thought to be principally derived from dental waste) has the potential to become the limiting element in the future (Smith 1996). However, as mercury is no longer used by dental practitioners, it is likely levels of mercury in sewage sludge will also drop. Since heavy metals are persistent, and can therefore accumulate in the soil to potentially toxic levels, the agricultural use of sewage sludge must be carefully regulated.

Legislation's involving PTE's and sewage sludge

The CEC's 1986 Directive on "The protection of the environment, and in particular the soil, when sewage sludge is used in agricultural land" addresses the problem of PTE's in sewage sludge (CEC 1986). As part of this Directive, tables containing sludge and soil limits of heavy metals have been devised based on the available scientific research (See Table 2.1). There is, however, little available data on the effects of PTE's in sewage sludge on invertebrates and more work is needed to ensure current legislation (as stated in the 1986 directive) will be sufficient to protect the invertebrate fauna.

Table 2.1 Maximum metal concentrations as set by CEC (1986)

	Cadmium	Zinc	Copper
Sludge	40 mg kg^{-1}	4,000 mg kg^{-1}	1,750 mg kg^{-1}
Soil	3 mg kg^{-1}	300 mg kg^{-1}	140 mg kg^{-1}
Maximum application rate	0.15 kg $ha^{-1} yr^{-1}$	30 kg $ha^{-1} yr^{-1}$	12 kg $ha^{-1} yr^{-1}$

Factors affecting the bioavailability of PTE's in sludge and soil

pH: Soil pH is considered the most important factor influencing the bioavailability of PTE's (Smith 1996). With the exception of selenium and molybdenum, the bioavailability of heavy metals decreases as soil pH increases (Chang *et al.* 1983). This is the result of a decrease in metal mobility as they form insoluble precipitations of hydroxides, carbonates and organic complexes in more alkaline soil (Kiekens 1984). Current UK legislation (CEC 1986) compensates for pH changes in bioavailability of zinc, copper and nickel by setting lower acceptable concentrations in acidic soils (pH $<$ 6) and prohibiting the application of sludge on land where the soil pH is less than 5. Likewise, higher permissible values of metals are set for soils with a pH greater than 7. Since heavy metals are persistent, remaining in bioavailable forms in excess of ten years (Benninger-Truax & Taylor 1993), long-term monitoring is essential even when sludge application has been terminated. It is particularly important to maintain the soil pH as any decrease in pH (for example from acid rain) would result in an increase in metal availability to potentially toxic levels.

Organic matter: Organic matter (in the soil and sludge) bonds with PTE's rendering them less available for uptake by plants (Petruzzelli *et al.* 1978). However, it has been suggested that as the organic matter in the sludge decomposes, the PTE's will be released hence becoming available. This has been termed the time "bomb effect" (Beckett & Davis 1979) as it predicts a large increase in metal availability on cessation of sludge application (as the organic matter decomposes). However, some studies contradict this theory and heavy metal uptake by crops has been found to remain constant once application has been terminated (McGrath 1987).

Sludge properties: The form of PTE's in sludge not only depends on the catchment area of the sewage works but also the sludge treatment process. Aluminium polychloride conditioning generally extracts less PTE's than conditioning by iron chloride and calcium hydroxide (Petruzzelli *et al.* 1994). This is likely to be a consequence of enhanced metal hydroxide precipitation in alkaline conditions. Metals in liquid sludge were found to be more available to plants compared with solid sludge (Carlton-Smith 1987). This was thought to be the result of the sludge (and hence metals) coming into closer contact with the root system.

2.4 The effects of fertilisers on Collembola

Inorganic fertilisers

Siepel & Van de Bund (1988) applied canonical correspondence analysis to microarthropod (Acarina and Collembola) data from a range of grassland sites in the Netherlands. They found that inorganic fertilisers had the greatest influence on microarthropod population structure, while grass cutting and grazing were of minor importance. Inorganic fertilisers generally increase the abundance of total Collembola and also change the population structure. *Isotoma notabilis, Lepidocyrtus cyaneus* (Tullberg), *Folsomia multiseta* Stach, *Sminthurinus aureus* (Lubbock), *Proisotoma minuta* (Tullberg) and *Isotoma viridis* (Bourlet) are favoured by fertilisation, while *Schoetella ununguiculata* (Tullberg) is not (Marshall 1977). Very high fertilisation rates (390 kg N ha^{-1} yr^{-1}), were found to adversely affect all collembolan species (Siepel & Van de Bund 1988). This may have been a consequence of the increased salt content (which may cause desiccation), or impurities such as copper or chlorine present in the fertiliser (Marshall 1977). Fertilisers generally cause changes in pH (some fertilisers are alkali e.g. calcium nitrate, while others are acidic e.g. sodium sulphate), changes in the carbon/nitrogen ratio, increases in available nutrients, and changes in the micro and macro-flora (Curry 1994). It is therefore difficult to say whether changes in the collembolan community were the result of the fertilisers or associated environmental changes (for example an increased food supply).

Organic fertiliser

Farmyard manure contains high levels of organic matter, and the increase in density of Collembola that regularly follows slurry application is thought to be principally the result of an increased food supply (Marshall 1977; Lübben 1989). Gusenleitner (1959), however, found a reduction in collembolan density one month after application of liquid-manure and adverse effects were still detectable over one year following application. This could have been the result of oxygen depletion (and the subsequent anaerobic conditions) that can follow application, heavy metal impurities, or the high levels of ammonia and soluble salts (e.g. earthworms are particularly sensitive to liquid-manure mainly as a consequence of the high ammonia and salt concentrations: Marshall 1977). Long-term use (22 years) of liquid-manure was found by Gunhold (1957) to adversely alter the collembolan community structure; eliminating all but manure-loving species (e.g. *Stenaphorura quadrispina* Börner, *L. cyaneus* and *Folsomia quadrioculata* (Tullberg)) and the water-loving *Isotomurus palustris* (Müller). Like inorganic fertilisers, organic fertilisers generally enhance the macro and micro-floral community. This in turn provides more food for Collembola and increased plant cover which generally dampens external temperature fluctuations making the micro-habitat more favourable. In addition, manure increases the organic matter content of the soil and therefore improves the soils physical structure and provides food directly (Marshall 1977).

Sewage sludge

Sewage sludge, like farmyard manure, has high organic matter levels and therefore has similar effects on Collembola through the same channels (e.g. direct and indirect increases in food abundance, improving soil structure and increasing plant cover). Sludge application has been found to increase the diversity and abundance of ground beetles (Larsen *et al.* 1996), the density of soil nematodes (Larink *et al.* 1990) and the density of Gasmidae mites (Glockemann & Larink 1989). Unsurprisingly, the addition of sewage sludge is also highly beneficial to Collembola (Höller-Land 1959; Lübben 1989), with the exception of *Isotomodes productus* (Axelson) which actually preferred control plots (Lübben 1989). However, the positive aspect of recycling nutrients may be counteracted by the contamination risk of heavy metals and organic compounds present in sewage sludge (Smith 1996).

Heavy metals are particularly troublesome because they are persistent. Heavy metal concentrations (cadmium, zinc, copper and lead) in soil, earthworms, spiders and plants have been found to be greater in sludge treated plots than control plots (Benninger-Truax & Taylor 1993). Furthermore, negative effects of heavy metals (cadmium, nickel, chromium, zinc and copper) in sewage sludge have been found for three mites species: namely *Hypoaspis aculeifer* (Canestrini), *Dendrolaelaps samsinaki* Hirschmann and *Pergamasus septentrionalis* Oudemans (Glockemann & Larink 1989). Beetles, however, appear to be unaffected by heavy metals in sewage sludge and this may be related to their efficient detoxification process (Larsen *et al.* 1996).

An experiment involving small plots (4.5m x 6.4m) was conducted in Braunschweig, Germany between 1980-1987, to investigate the effects of heavy metals in sewage sludge on euedaphic Collembola (Lübben 1989). Sludge was either of low metal content or was artificially contaminated with heavy metal salts (zinc, cadmium, copper, nickel, chromium and lead). Two different application rates were also examined; high sludge (300 $m^3ha^{-1}\ yr^{-1}$) and low sludge (100 $m^3ha^{-1}\ yr^{-1}$). On all plots receiving sludge the abundance of Collembola was higher than control plots, and heavy metals had no effect on overall collembolan abundance. However, at the species level differences were found: *I. notabilis*, *Cryptopygus thermophilus* (Axelson), *Willemia intermedia* Mills and *S. aureus* were all found in lower numbers on the contaminated sludge plots than on the uncontaminated plots. Other species, namely *Mesaphorura* spp., *F. candida*, *Bourletiella hortensis* Fitch and *Sphaeridia* spp. appeared to favour contaminated plots.

Experiments to date therefore suggest that the land application of sewage sludge to grassland enhances collembolan abundance and changes the population structure. Furthermore, although heavy metals in sludge do not adversely affect the total collembolan abundance, more subtle effects can be observed at the species level.

2.5 The effects of heavy metals on Collembola

Effects of heavy metals in the field

Collembola (as a group) are able to withstand high concentrations of heavy metals and individual growth rate tends to be affected long before an increase in mortality is observed (Bengtsson *et al.* 1983; Joosse & Verhoef 1983). This is likely to be the result of a low metal assimilation rate combined with an efficient excretory system (e.g. only 0.13% of ingested lead was accumulated permanently in the body: Hågvar & Abrahamsen 1990). It has been observed that Collembola were the least affected group of soil arthropods near a zinc smelter (Strojan 1978a). Furthermore, Collembola tend to accumulate much lower concentrations of heavy metals than for example isopods, earthworms and spiders (Janssen & Hogervorst 1993). However, lead concentrations were found to be higher in *Orchesella cincta* (Linné) than isopods, diplods and Coleoptera in a study by Rabitsch (1995).

Although Collembola (as a group) appear to be to be relatively unaffected by heavy metals, there appears to be species specific differences in sensitivity. Several field studies have found that while the overall collembolan density remains unaffected by metal pollution, more subtle effects on the population structure occur (Strojan 1978a; Tyler *et al.* 1984; Muskett & Jones 1980; Lübben 1989). Tranvik *et al.* (1993) found a decrease in abundance of *Isotomiella minor* (Schäffer), and an increase in *Folsomia fimetarioides* (Axelson) along a metal gradient towards a brass mill. *Isotomiella minor* was also described as a metal sensitive species (occurring only at low lead levels - <1g kg^{-1} of soil) by Hågvar & Abrahamsen (1990) who studied the fauna in a naturally lead-contaminated soil. *Folsomia sensibilis* Kseneman and *Mesaphorura yossi* Rusek were also described as sensitive while *F. quadrioculata*, *O. armatus* and *F. mirabilis* were described as tolerant (occurring abundantly at 3-5 g Pb kg^{-1}, 10 g Pb kg^{-1} and > 15 g Pb kg^{-1} of soil, respectively: Hågvar & Abrahamsen 1990). *Isotoma olivacea* Tullberg was actually more abundant in polluted areas and was rare in unpolluted areas (Hågvar & Abrahamsen 1990). Although the total collembolan density showed hardly any decrease at the more contaminated areas (10 g Pb kg^{-1} of soil), due to an increase in abundance of *I. olivacea* the diversity was severely reduced. Collembolan diversity may be reduced directly through toxic effects, or indirectly through a decrease in food resources, predators or change in vegetation structure (though the latter was discounted by Hågvar & Abrahamsen 1990).

Effects of heavy metals on growth, reproduction and energy demands

Several laboratory studies have been conducted on Collembola to examine the effects of heavy metals on life-history traits (Joosse & Verhoef 1983; Bengtsson *et al.* 1983, 1985a). *Onychiurus armatus* decreased their growth rate (Bengtsson *et al.* 1983; 1985a) and number of eggs produced (Bengtsson *et al.* 1983; Tranvik *et al.* 1993) when fed on fungi with high levels of lead and copper (150 and 300 ppm metal). As fertility in *O. armatus* is related to body size (Bengtsson *et al.* 1983), heavy metals not only affected fertility directly through a decrease in egg number, but also indirectly through the reduction in adult size attained. The reduction in growth rate was, however, alleviated when the Collembola had access to an abundance of nitrogen enriched fungi (Bengtsson *et al.* 1985a). It is therefore possible that the adverse effects of metals on *O. armatus* growth rate was a consequence of less energy being available for growth. This is likely the result of the additional energy demands of detoxification processes.

Posthuma *et al.* (1993) and Joosse & Verhoef (1983) found that growth rate and egg production in *O. cincta* decreased when they were fed on *Pleurococcus* algae contaminated with lead. This was not necessarily the result of the energetic demands of excretion as a decrease in metabolic rate was also found in lead contaminated Collembola. This decrease in metabolic rate could be caused by a decrease in food consumption (contaminated food may be less palatable), or an inhibition of respiratory enzymes which, in turn, could result in the decrease in growth and reproduction observed.

The metal concentrations used in these laboratory experiments are comparable to metal levels found in areas near metal smelters and mills (Bengtsson *et al.* 1983). Detrimental effects of heavy metals at contaminated sites may therefore be occurring through changes in growth rate and reproduction. Such changes would not be determined simply from sampling the polluted area to determine the species abundance and composition.

Effects of heavy metals on decomposition

A reduction in soil respiration rate and nitrification as a consequence of high heavy metal pollution has been found in the lab (Komulainen & Mikola 1995). Furthermore, in intensely polluted forest areas where the Collembola diversity was depleted, decomposition and mineralisation were found to be retarded (Strojan 1978b; Rühling & Tyler 1973; Hågvar & Abrahamsen 1990). These findings were likely to be the result of changes in invertebrate species composition and of an overall decrease in activity of the soil fauna and flora in general. It has been predicted that microarthropod species that digest fungal cell walls (where heavy metals generally accumulate) will be more susceptible to metal poisoning (Siepel 1994). It has been shown that such species have the greatest influence on decomposition (Siepel & Maaskamp 1994), and hence the observed decrease in decomposition rate may be expected in polluted areas.

2.6 Adaptations of Collembola to heavy metals

Heavy metals are considered to present a stable, permanent and often intense selection pressure since they are non-degradable substances that are toxic at high concentrations (Posthuma & Van Straalen 1993). Natural selection is therefore likely to favour resistant individuals in polluted areas. Selection may result in one or more of the following: behavioural adaptations, physiological adaptations and life-history adaptations.

Behavioural adaptations to heavy metals

The ability to avoid metal polluted food has been observed in isopods and Collembola (e.g. *O. cincta*: Joosse & Verhoef 1983). Tranvik & Eijsackers (1989) found that *F. fimetarioides* preferred metal tolerant fungi while *I. minor* preferred metal sensitive species. In addition to selecting metal tolerant fungal species, *F. fimetarioides* was found to be able to distinguish between metal contaminated food and clean food, an ability *I. minor* did not share. This suggested that *F. fimetarioides* had a competitive advantage over *I. minor* in polluted areas. Indeed an almost inverse relationship was found between *F. fimetarioides* and *I. minor* along a metal gradient at Gusum brass mill, Sweden, with *F. fimetarioides* dominating the most polluted areas (Bengtsson & Rundgren 1988). It must however, be noted that while *I. minor* specimens for this experiment were collected from an unpolluted area, *F. fimetarioides* were collected from a polluted area. It is therefore possible that the differences observed were not the result of species adaptation but of individual acclimatisation.

Bengtsson and Rundgren (1988) found an increase in Collembola at a depth of 2-10 cm in polluted areas and suggested that Collembola migrated down the soil profile to less polluted soil. Collembola are known to migrate deeper into the soil to avoid adverse weather conditions (Hale 1967; Hassall *et al.* 1986), and it is possible that they could do the same to avoid heavy metals, especially since metal concentrations are known to decrease with increased soil depth (Bengtsson & Rundgren 1988). Collembola from polluted areas could therefore have behavioural adaptations which enable them to avoid polluted micro-habitats and food.

The behavioural avoidance of polluted micro-habitats and food could explain why *O. armatus* fed on copper-enriched fungi in the laboratory had higher copper burdens than species collected in soils of a corresponding copper concentration (Bengtsson & Rundgren 1988). However, this may also have been the consequence of copper being present in less available forms in the field.

Physiological adaptations to heavy metals

Several species of invertebrates are known to have organs associated with the detoxification of heavy metals. Earthworms concentrate metals in metal binding cells known as chlorangocytes (Ireland & Richards 1977), while snails are known to concentrate lead in their midgut gland (Hopkin 1989). Studies of the gut wall of the Collembola *Tomocerus minor* (Lubbock) and *O. cincta* (Humbert 1978; Van Straalen *et al.* 1987: respectively) have found many granules that are thought to have been expelled into the gut lumen by exocytosis. It is thought that these granules have an affinity for heavy metals, forming strong chemical bonds. During moulting, the complete midgut epithelium (including the granules) is lost. High concentrations of lead have been found in the exfoliated gut of *O. cincta* fed a lead-enriched diet (Joosse & Buker 1979), and hence it is thought this is the primary method of excreting assimilated heavy metals in Collembola (Joosse & Verhoef 1983; Van Straalen *et al.* 1987). *Orchesella cincta* specimens collected from long-term or intensely polluted sites were found to be more efficient at excreting cadmium and lead than specimens from pristine habitats (Van Straalen *et al.* 1987). This excretion efficiency was not affected by the exposure level of the metals or exposure time. This suggests enhanced excretion was a genetic adaptation to heavy metals rather than physiological acclimatisation. However, this study did not investigate whether the increase in excretion efficiency was an adaptation to heavy metals, since the progeny of the individuals in the polluted area were not investigated and therefore it was not known whether they also showed a greater excretion of metals.

As a consequence of their ability to eliminate heavy metals, collembolan body burdens of lead, cadmium and zinc have been found to be lower than other soil invertebrates (Van Straalen & Van Wensem 1986). However, even in Collembola excretion efficiency seldom reaches 100% (Van Straalen *et al.* 1987) and, under continuous exposure, metals may accumulate to toxic levels. Detoxification mechanisms are also thought to be energetically costly, and in earthworms a decrease in glycogen reserves has been associated with lead sequestration (Richards & Ireland 1978). Posthuma *et al.* (1993) found *O. cincta* derived from polluted populations had a higher mortality rate than individuals derived from unpolluted populations when exposed to low metal concentrations in the laboratory. This may indicate a trade-off between tolerance development and longevity (possibly due to the faster growth rate of juveniles). Life-history traits such as growth rate and reproduction may therefore be affected indirectly through the decrease in available energy.

Life-history adaptations to heavy metals

Natural selection may also act on life-history traits directly. For example, if the density of individuals in a habitat is reduced below it's carrying capacity (e.g. if lots of individuals are killed by metal poisoning), competition will decrease and density dependant factors (e.g. food) will no longer be the main driving force of selection. Species and individuals that have rapid growth, earlier reproduction and increased allocation per clutch will be favoured in such an uncrowded environment (i.e. the population will become more r-selected than K-selected: Begon & Mortimer 1981). Schaffer (1974) proposed that when an environment has greater juvenile mortality K-selected individuals will be favoured, but when mortality is greater in adults r-selected individuals will be favoured As heavy metals are persistent, and excretion in Collembola is seldom 100% (Van Straalen *et al.* 1987), it is probable that the adverse effect of metals will increase with age (especially if juveniles grow quicker and hence moult more frequently). Again life-history theories predict that in a metal polluted environment evolution will favour smaller adults, earlier maturation and increased clutch size (i.e. r-selected individuals).

Several laboratory based experiments have been conducted with Collembola to test the validity of these predictions (Posthuma *et al.* 1993; Tranvik *et al.* 1993). *Orchesella cincta* (Posthuma *et al.* 1993) and *O. armatus* (Tranvik *et al.* 1993) derived from polluted populations were found to have increased juvenile growth rate hence reaching maturity earlier than Collembola derived from pristine habitats. Metal adapted *O. armatus* and *I. notabilis* were found to have an increased clutch allocation (Tranvik *et al.* 1993). Through increasing both their reproductive rate and through earlier maturation, metal-adapted Collembola become more r-selected as predicted by the theory of life-history evolution. As these differences were observed in the progeny of the Collembola they could be concluded to be inherited, not simply occurring through acclimatisation.

The increased reproductive rate (through earlier maturation and/or increased clutch allocation) cannot, however, be concluded to be an adaptation to heavy metals because polluted habitats often differ from pristine habitats with respect to food, predators and vegetation (Posthuma *et al.* 1993). Natural selection may have acted on any of the above environmental changes. However, the study by Tranvik *et al.* (1993) looked at Collembola along a metal gradient and suggested metals were a prime factor of importance.

The sensitivity of life-history characteristics to metals depends on the species and the life-history characteristics. For example in *O. cincta* the cadmium NOEC (no observed effect.concentration) for growth was 0.042 μmol g^{-1} food while that for clutch size was a much higher 0.5 μmol g^{-1} food (Posthuma *et al.* 1993). In *O. armatus*, on the other hand, the most sensitive life-history characteristic was egg production (Bengtsson *et al.* 1985b). It can be assumed that evolutionary modifications will occur first to life-history characteristics with low NOEC. This prediction was confirmed for *O. cincta* with growth rate increasing while clutch size remained unchanged in metal polluted Collembola (Posthuma *et al.* 1993). It would therefore be expected that populations (or species) with low genetic variation for life-history characteristics, and/or have low NOEC for life-history characteristics, are more prone to extinction (Posthuma *et al.* 1993).

Life-history theory has been taken one step further by Siepel (1995). He suggested that in a polluted environment asexual species will be preferred. These species are considered to produce more offspring and are therefore more r-selected. Siepel (1995) proposed that a chance mutation, resulting in a pollutant resistant clone, will be fixed more quickly in an asexual population, as it is not recombined every generation as a sexual mutation would be. He found a positive correlation between the number of parthenogenetic microarthropods and the concentration of heavy metals, supporting this hypothesis (Siepel 1995). It is therefore possibly that the percentage of parthenogenetic species may be a good indication of heavy metal pollution.

2.7 Effects of other agricultural practices on Collembola

Insecticides, fungicides and herbicides

Several studies have argued that as herbicides (with the exception of the triazine herbicides - e.g. atrazine) are applied to plants, they rarely come into contact with the soil and hence the soil fauna (Curry 1970; Fox 1964). Fox (1964) found the herbicide *Dalapon* (2,2 Dichloropropionic acid) to actually increase soil collembolan numbers. However, diversity was not measured, and the increased abundance may have been the result of an increase in a few tolerant species. Herbicides may have an indirect effect on soil Collembola because weed elimination may reduce food sources. Furthermore, epigeal Collembola may be affected directly by herbicides. Negative affects on mortality and fecundity of atrazine have been shown in the lab for *O. cincta* (Badejo & Van Straalen 1992), *Onychiurus* spp. (Mola *et al.* 1987), *F. candida* and *Tullbergia granulata* Mills (Subagja & Snider 1981). This suggests there is a possibility of herbicides affecting non-target invertebrates, either directly or indirectly (e.g. through a reduction in food availability).

Folsomia fimetaria Linné was found to be able to detect and avoid the organophosphorous insecticide, dimethoate, under laboratory conditions (Fábián & Petersen 1994). In the field, however, there was no evidence that dimethoate influenced the distribution of *F. fimetaria*. This suggests it could not escape from the insecticide in the three-dimensional soil system, and therefore succumbed to it's toxic effects (Fábián & Petersen 1994).

Krogh (1991) was able to classify Collembola into two groups with respect to the effect of the pesticide isofenphos: resilient species (euedaphic species - e.g. *Mesaphorura* spp.

and epigeal species e.g. - *L. cyaneus*) and sensitive species (hemiedaphic species - e.g. *Folsomia nana* (Gisin)). He attributed this to the vertical distribution of the insecticide, which peaks in the hemiedaphic zone.

Frampton (1988) looked at the effects of four foliar fungicides on the epigeal Collembola *S. aureus* in the laboratory, and the effects of the insecticide dimethoate and the fungicide pyrazophos in the field. He found all four fungicidal treatments were toxic to *S. aureus* in the lab. In the field pyrazophos and dimethoate decreased the numbers of *S. aureus, Sminthurinus elegans* (Fitch), *S. viridis* and *Jeannenotia stachi* (Jeannenot: Frampton 1988). The effects of dimethoate appeared to be more immediate than those of pyrazophos suggesting that the adverse effects of pyrazophos were indirect (e.g. by decreasing food availability). Krogh (1991) also suggested an indirect toxic effect of the fungicide benomyl to Collembola.

It would therefore appear that broad-spectrum insecticides (e.g. dimethoate) are directly toxic to Collembola, while fungicides (e.g. pyrazophos) are more likely to be indirectly toxic (e.g. through a decrease in food availability). Field and laboratory studies indicate that Collembola are particularly susceptible to insecticides (Wiles & Frampton 1996), and the above evidence suggests they are also indirectly susceptible to fungicides. Adverse effects on Collembola could, in turn, adversely affect nutrient cycling (and hence fertility). Pesticides may also indirectly decrease the abundance of important polyphagous predators as Collembola are often an important part of their diet (Sunderland 1975).

<u>Grazing and grass cutting</u>

A negative correlation has been found between sheep biomass (grazing pressure) and collembolan numbers in perennial ryegrass swards (Walsingham 1978). Purvis & Curry (1978) and Siepel *et al.* (1989) also found adverse effects of grazing on Collembola. Grazing generally decreases the surface litter layer, and therefore it would be expected that hemiedaphic species (living in the litter layer) would be particularly sensitive to grazing pressures. All collembolan species (including those living in the soil) may be affected through an increase in soil compaction or a decrease in vegetation cover. Decreasing the vegetation cover can drastically alter the soil micro-climate and the maximum soil temperature in intensely grazed fields was found to be 14°C higher than lightly grazed fields in New South Wales (Davidson *et al.* 1979).

The effects of grass cutting are similar to grazing with respect to decreasing the vegetation cover (and consequent changes in the soil micro-climate) and litter layer (as cuttings are generally removed from the site). The changes are, however, more sudden and less selective than grazing, and there is no return of organic matter through dung. The pressure of grass cutting on collembolan populations is, in general, less consistent than that of grazing and between cuts there can be significant recovery of populations (Curry & Tuohy 1978). While Curry & Tuohy (1978) found cutting to adversely affect Collembola, Purvis & Curry (1978) found effects to be varied and to depend on the time of cutting. Early cutting for silage decreased collembolan diversity, whereas, late silage cutting was actually found to increase diversity. It would therefore appear that grass cutting can be beneficial to Collembola when conducted late in the season.

Cultivation (mechanical manipulation of the soil)

The effects of cultivation on Collembola appear to be inconclusive. While some authors report cultivated land to have impoverished collembolan populations both in density and diversity (Curry 1994; Edwards & Lofty 1975), others report that cultivated plots have enhanced populations (Loring *et al.* 1981). For example, Mallow *et al.* (1985) found an immediate decrease in collembolan diversity after ploughing, whereas, Sheals (1957) observed a rapid recolonisation when cultivated land was resown. With respect to Collembola, cultivated land differs from permanent grassland because there is usually a period when the cultivated land is fallow (i.e. not supporting vegetation). The absence of vegetation is likely to result in a shortage of food, especially for epigeal species (Loring *et al.* 1981). Furthermore, the Collembola will be less protected from the external environment. This (in addition to soil compaction) is likely to cause the observed decline in collembolan numbers immediately after ploughing. However, in the long-term, ploughing can enhance the nutrient availability and physical structure of the soil and hence benefit Collembola (Burnett 1968).

2.8 Effects of management in general

Diversity

Although a moderate level of management can promote biological diversity, as the intensity of management increases, the complexity of the community structure and biodiversity usually decreases (Curry 1994; Harper 1969). Intensive management practices can convert a biologically diverse community into a less diverse community, where species tolerant to management disturbances dominate. Adverse effects of intensive management have been found for leafhoppers, grasshoppers (Andrzejewska 1979) and microarthropods (Curry 1994). Conversely, some species, such as earthworms, can benefit from the greater productivity intensely managed grassland provides.

Different management practices appear to affect Collembola in different ways, and when different practices are combined the affects on Collembola can be unexpected. For example, when sheep grazing was combined with fertilisation the negative effects of intensive grazing were no longer apparent (Bardgett *et al.* 1993). It is possible that the positive effects of fertilisation masked the negative effects of grazing.

Species composition

As there are relatively few studies on grasslands where Collembola are identified to the species level, it is difficult to identify species which are sensitive to particular management practices. However, Frampton (1994) has suggested epigeal Collembola (e.g. *I. viridis, L. cyaneus*, and *S. viridis*) have the potential to be used to monitor pesticide toxicity, while Filser *et al.* (1995) suggests *I. minor* and *F. quadrioculata* may be used as indicators of copper contamination. Siepel & Van de Bund (1988) found high fertilisation rates favoured hemiedaphic species and epigeal species over euedaphic species. Brussaard *et al.* (1990) found that in less intensely managed plots (decreased fertiliser, pesticides and ploughing) *O. armatus, S. quadrispina, Mesaphorura krausbaueri* Börner and *F. mirabilis* dominated, while in intensely managed plots *Ceratophysella denticulata* (Bagnall) and *F. candida* dominated.

2.9 Sampling Collembola in grasslands

Collembola have mainly been sampled in grasslands in four ways: pitfall traps, suction samples, litterbags and soil cores. The first two methods predominantly sample the epigeal and hemiedaphic species, whereas, the latter two methods predominantly sample the euedaphic and hemiedaphic species. They will therefore be considered separately.

Sampling of euedaphic and hemiedaphic Collembola

Soil cores: Originally soil corers were metal tubes sharpened at one end to enable them to be pushed through the soil (Southwood 1971). Such corers helped standardise the volume of soil sampled within and between experiments, and enabled the soil to be sampled with minimum disturbance. The problem with these simple corers was that when the soil sample was forced from the metal core some species were killed by compression (Southwood 1971). As the predominant methods for extracting Collembola from soil cores are behavioural extractors (see below), it is essential that the animals are living (Southwood 1971). The problem of compression was solved by O'Connor (1957) who devised the split corer. This corer could be split in two after collecting the soil sample, thus enabling the sample to be retrieved without compression.

Litterbags: Litterbags were devised by Crossley and Hoglund (1962) and are mesh bags (made from nylon or fibreglass) containing a known weight of leaves or pine needles. Litterbags are generally placed between the litter layer and mineral soil, to minimise fluctuations in moisture (Siepel 1990). After a predetermined period of time, such as one month, litterbags are removed and the Collembola having freely invaded the litterbags are extracted, primarily by behavioural methods. Litterbags can be used simply to obtain a sample of Collembola by acting as an underground baited trap, or to measure the rate of decomposition. Litterbags can be placed in the soil with minimum disturbance and this is not thought to have a significant effect on the Collembola. Furthermore, any effect is consistent between samples collected with litterbags.

Soil corers have the advantage over litterbags in that the core of soil can be divided into several sections corresponding to differing soil depth. This provides a soil profile which, in turn, can give accurate information about the vertical distribution of different species. Furthermore, samples can be retrieved immediately with the soil corer, whereas, litterbags have to be left in the soil for several weeks to enable the Collembola to colonise them before samples can be obtained. However, litterbags can provide information on the succession of Collembola in decomposing leaf-litter, and can also be used to measure the rate of decomposition (Seastedt 1984).

Extraction: The Collembola contained in the soil cores or litterbags must be extracted by flotation, sieving or behavioural extraction methods. The latter method is most commonly used as it is quicker, less messy and enables a large number of samples to be processed at one time (Southwood 1971). Behavioural extractors are predominantly various modifications of the Tullgren funnel (Tullgren 1918). The Tullgren funnel relies on the principle that soil organisms move downwards away from the negative stimuli (provided by a light bulb) of light, desiccation and heat, towards the cooler damper bottom. As the organisms reach the bottom, they fall through a sieve into a funnel, and eventually to a collecting jar. Naturally, only living and motile stages of the organisms will be extracted in this way, and therefore Collembola that are moulting (and hence immobile) will not be extracted.

<u>Sampling of hemiedaphic and epigeal Collembola</u>

Pitfall traps: Pitfall traps are commonly used to sample surface active arthropods, in particular Coleoptera and Araneida (Luff 1975), but have also been used for Collembola (Duffield & Aebischer 1994; Greenslade 1974). Pitfall traps basically consist of plastic, metal or glass containers sunk into the ground so that the container's mouth is level with the soil surface. They rely on the principle that when surface active invertebrates walk into the trap they are unable to escape. This is generally enhanced by liquid in the trap which kills and preserves the specimens.

Pitfall traps are influenced by many factors including vegetation, type of preservative, weather and even the material the pitfall is made from (Greenslade 1964; Joosse 1965). Perhaps the most important factor influencing the catches is the activity of the individual species (Greenslade 1964), with the most mobile species being caught most frequently. However, pitfall trapping provides a cheap method of sampling Collembola with minimum labour, and is particularly useful when sampling areas with similar vegetation.

Suction samples: A number of machines have been devised to sample by suction. The D-vac (Dietrick *et al.* 1959) suction sampler is considered to be a suitable sampling method for epigeal and hemiedaphic Collembola (Frampton 1988). However, the D-vac is heavy and can become uncomfortable with prolonged use (MacLeod *et al.* 1994). The Ryobi sweeper-vac (as modified by MacLeod *et al.* 1994) is a cheaper, lighter and less noisy alternative to original suction samplers (Harwood 1994). Furthermore, the Ryobi was found to be as, or more, efficient (depending on the species in question) at sampling Carabidae, Araneae, Staphylinidae, Isopoda and Aphididae (MacLeod *et al.* 1994; Harwood 1994).

Suction samplers provide better quantitative information on population densities than pitfall traps (Frampton 1994). Johnson *et al.* (1957) found a sampling efficiency of 99.3% for Arthropleona and 100% for Symphypleona for the Wolf Portable Electric Blower. However, pitfall traps have the advantage over suction samplers in that they monitor nocturnal species in addition to diurnal (Duffield & Aebischer 1994), and can be used to monitor sub-lethal effects on collembolan activity (Frampton 1994). It has therefore been proposed that a combination of suction samples and pitfall traps provides the most efficient sampling regime of epigeal and hemiedaphic Collembola (Berbiers *et al.* 1989; Frampton 1994)

2.10 Comparing collembolan communities

Differences in overall collembolan abundance, and the abundance of individual species, can be compared between treatments using conventional statistics such as Analysis of

Variance (ANOVA) or Chi-Squared tests (X^2). Conventional statistics test specific hypothesis (derived prior to analysis) to look for effects of, for example, treatment or date. Bardgett *et al.* (1993) used an ANOVA (followed by Tukey tests) to test for the effects of sampling date and grazing on the number of Collembola. They found that both treatment and sampling date significantly affected Collembola, with the heavily grazed plots having more Collembola than the light or moderately grazed plots, and the abundance of Collembola peaking in July. The problem with conventional statistics is that it is only possible to look at one species at a time. For example, you could either test for differences in total collembolan abundance or abundance of species x. It is therefore difficult to look at treatment effects on the community as a whole using conventional statistics.

It is, however, possible to obtain a more complete picture of a community by using diversity indices. The diversity of a community depends on the species richness (i.e. number of species present) and the evenness (i.e. how dominant each species is: Pielou 1977). A community with a high diversity has lots of different species all occurring in approximately equal proportions. There are many different indices that measure diversity and several reviews on diversity exist including Magurran (1988) and Pielou (1977). The following concentrates on the diversity indices used in this thesis and why these specific indices were chosen (equations for diversity indices can be found in Appendix I).

The simplest and most widely adopted measure of diversity is species richness (S) which is simply equal to the number of species present in a sample (Magurran 1988). This measure is of limited use because its calculation does not take into account the evenness of the community and it is highly dependant on the number of individuals present (Pielou 1975). Despite these drawbacks, species richness is still probably the easiest and most widely used measure of diversity. Furthermore, the species richness can be used in combination with information on the evenness of a community to obtain a fairly accurate picture the communities diversity.

For this study, the easily calculated Berger-Parker's dominance index (**d**) was used to measure the evenness of the community (Berger & Parker 1970). This index looks at the proportion of dominance for the dominant species. It is independent of the number of species present (**S**) but is dependant on the number of individuals present (**N**) (Magurran 1988). This index has been described as one of the most satisfactory diversity measures available (May 1975) and is an ideal measure of evenness to combine with species richness (Magurran 1988). Magurran (1988) states that a combination of Berger-Parker and species richness were not only simpler to calculate than the Shannon or the Simpson index, but were also more informative.

Two measures of diversity were chosen that measured both dominance and richness. The first, $\mathbf{N_2}$ (the reciprocal of Simpson's index: Hill 1973), relies on the principal that the lower the diversity and the greater the concentration of dominants are in a community, the greater is the probability that two randomly picked individuals will belong to the same species (Magurran 1988). This measure of diversity therefore incorporates information of both dominance and species richness into it's calculation. However, it does have the drawback of being strongly influenced by the number of individuals present (Taylor 1978). The second diversity index used was the log series parameter α (Fisher *et al.* 1943). This index is frequently used in entomological research as a parameter to measure the richness and dominance of a community (Magurran 1988). Problems of this index arise from the fact that it is calculated from the number of species and the total number of individuals, and therefore is not strongly influenced by the evenness of the community. However, it is not unduly influenced by sample size or dominant species and has good discriminant ability (Taylor 1978).

These four indices were chosen to give an indication of overall diversity (α and $\mathbf{N_2}$), species richness (**S**) and evenness (**d**). These indices are among the most widely used indices (Magurran 1988) and might replace the once popular Shannon index which is now subject to much criticism (Magurran 1988; May 1975; Goodman 1975). Magurran (1988) recommends combining such indices to obtain the best measure of diversity. Larsen *et al.* (1996) used a combination of diversity and dominance indices to study the effects of sewage sludge and fertiliser on carabid populations. They found that sludge increased the diversity and evenness while fertiliser decreased them. Bengtsson & Rundgren (1988) also used a combination of diversity and evenness indices to determine the effect of pollution from Gusum brass mill on Collembola.

As repeated measures of diversity are usually normally distributed (Magurran 1988), conventional statistics such as ANOVA can be used to test for significant differences in the diversity between sites (Magurran 1988; Kappelle *et al.* 1995). Diversity indices do not, however, differentiate between species. Two habitats with totally different species compositions could therefore have the same diversity, if the species richness and dominance were similar.

Multivariate analysis (e.g. ordination, direct gradient analysis and classification) can overcome this problem by looking at the species present in addition to the relative proportions of these species. Ordination is a multivariate technique, the end point of which is to present relationship between samples (or species) graphically (usually two dimensional: Gauch 1982). In the resultant ordination diagram, samples that are similar, with respect to species composition, are grouped together and samples that are different lie further apart (Gauch 1982). Ordination therefore helps to clarify the relationships among samples (or species) with respect to species (or sample) similarity.

Detrended correspondence analysis (DCA) is the most frequently used ordination technique for ecological data (Digby & Kempton 1987). This is primarily the result of the widespread availability of the computer program DECORANA (Hill 1979), which generates ordinations by DCA (Digby & Kempton 1987). DCA is especially useful for the large matrices of species and samples, with a large number of zero values, which are frequently collected in ecological experiments (Foster *et al.* 1990). McCracken & Foster (1993) used DCA to determine that the distribution of surface active invertebrates caught by pitfall trapping was primarily related to substratum variables (e.g. pH and surface moisture content). Bolger (1985) also used DCA to determine that succession was the main factor that influenced microarthropods in litterbags.

Probably the main drawback in using DCA is that no information is given on the factors that cause the division of samples along the axes, and it is therefore termed an indirect gradient analysis. It is up to the interpreter to determine which of the known environmental variables are most likely to account for the samples location in the ordination diagram. This can be achieved by correlating known environmental variables with axes scores. Furthermore, the location of species in the species ordination helps to determine which species are accounting for the division of the samples. This information is particularly valuable when ecological relationships between species are known and can be related to the division of samples. Above all, DCA provides a good pictorial summary on the similarity of samples with respect to species composition.

CHAPTER 3. An introduction to the contaminated sludge trial sites and to identification techniques

3.1 Trial sites and experimental design

The trial sites were part of a larger scale experiment that was set up to investigate the long-term effects of applying metal-rich sewage sludge to agricultural land. This larger experiment had eight trial sites throughout the UK, namely: Gleadthorpe, Woburn, Watlington, Pwllpeiran, Rosemaund, Bridgets, Hartwood and SAC Auchincruive. For the purpose of this thesis the SAC Auchincruive and Hartwood sites were investigated.

The SAC Auchincruive, South Ayrshire, study site was situated on a sandy clay loam (21% clay; 2.6% organic carbon). The Hartwood, Lanarkshire, study site was also situated on a sandy clay loam (20% clay; 4.7% organic carbon). At both sites sludge cake had been applied and incorporated into the soil using small plot equipment in August of each year since 1994. After incorporation all plots were sown with Italian ryegrass (*Lolium multiflorum*). The trial sites consisted of a total of 69 plots (each 6 m x 8 m) established across three blocks. Within each block, 23 sludge treatments were allocated randomly to plots. Sludge treatments included unsludged control, digested sludge, undigested sludge, zinc-rich sludge (digested), cadmium-rich sludge (digested) and copper-rich sludge (undigested: see Appendices II and X for chemical analysis of sludges). Each plot was surrounded by a 1.2 m permanent grass strip to prevent the transfer of soil between plots during cultivation. The organic matter input was standardised across sludge treatments and the pH was maintained at six. The grass was cut on 16 May and 17 July in 1995 and on 24 May and 26 July in 1996.

The following soil measurements were taken on all plots prior to sludge application: soil pH, particle size (i.e. clay, silt and sand content), conductivity, cation exchange capacity, biomass carbon (C), *rhizobia* count, soil respiration rate, organic nitrogen (N), total N, phosphorus (P), potassium (K), magnesium (M), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cadmium (Cd), nickel (Ni), chromium (Cr), lead (Pb) and mercury (Hg) (see Appendices VI & VII). Following sludge application the pH, biomass C, *rhizobia* count, soil respiration rate, total N, Zn, Cu, Cd were measured on selected plots (see Appendices III & XI for 1995 and 1996 analyses respectively). Grass yield (see

Appendix VIII) was measured and metal contents estimated on all plots following application. Grass heights were also measured on 30 April, 31 May, 22 July and 4 August 1996 to provide addition information on the plots (see Appendix VIII).

3.2 Identification techniques

All Collembola were initially mounted in Hoyer's medium and identified under a light microscope (magnification x 400-1,000). The mounted Collembola were manipulated by moving the cover slip to see critical identification features. After initial identification, it was found that with the exception of problematic specimens, some species could be accurately identified under a dissecting microscope and it was therefore unnecessary to mount all such specimens. Routine checks were, however, carried out to ensure that new species which under the dissecting microscope may appear similar to previously identified species were not occurring. This was achieved by mounting 20 specimens (in one of the five samples collected from each plot) in Hoyer's medium and identifying them under high magnification (x 400-1,000). Collembola were identified to species level principally following the keys of Fjellberg (1980) and Gisin (1960), but those of Stach (1954; 1956) and Gough (1977) were also used.

3.3 The Taxonomic status of some species

There is currently much disagreement on the taxonomic status of several collembolan species (e.g. *Isotoma anglicana*, *Sminthurinus aureus* and *Protaphorura subarmata* Gisin: Hopkins 1997). For the purpose of this research, species were generally identified following the nomenclature of Fjellberg (1980). Fjellberg may be described as a 'splitter', separating Collembola into species on the basis of very small differences in morphology (Hopkins 1997). By taking this approach the information derived from this study could be maximised.

There is currently some discussion on whether *I. anglicana* is a true biological species, or just a morphospecies (Hopkins 1997). For the purpose of this research, it was decided to consider *I. anglicana* a separate species to *I. viridis*. It was found that in addition to manubrial and labial differences between *I. viridis* and *I. anglicana*, differences were also found in colouration with *I. anglicana* juveniles appearing more blue than the somewhat greyish *I. viridis* juveniles. It was also decided to regard *S.*

elegans a nd *S. aureus* as separate species although, as with *I. anglicana*, the taxonomic status of *S. elegans* has been queried by some workers. For example, while Stach (1956) considered *S. elegans* to be a different coloured variation of *S. aureus*, Fjellberg (1980) gave *S. elegans* species status.

The species identified as *Isotomurus palustris* in 1995 (following Fjellberg 1980), was divided into the species *I. palustris* and *I. maculatus* in 1996. It was decided to divide this species following the research by Carapelli *et al.* (1996) who used DNA sequencing to support speculations that different colour variations of *I. palustris* should be given separate species status. In 1996, it was also decided to classify all *Isotoma* and *Isotomurus* spp. that were less than 0.7 mm as *Isotoma/Isotomurus* juveniles. This was done to minimise the time taken in mounting and identifying these juveniles which allowed a much larger number of samples to be processed than would otherwise have been possible.

3.4 Amendments and additions

Data transformation

Prior to all parametric statistical analyses the variance to mean ratios were checked to detect if species abundances were normally distributed. For most species it was found the variance and mean was about equal which indicated that the distribution was not normal, but was random (Sokal & Rohlf 1995). When utilising parametric statistical techniques, discrepancies from normality can decrease the test's reliability and it was therefore necessary to normalise the data by transformation. The random distribution indicated that for the majority of species the most effective transformation could be achieved by square rooting the data (Fowler & Cohen 1992). The effectiveness of the square root transformation was compared with the log transformation (which is more commonly used) by comparing the variance and mean ratios and the frequency distribution histograms of the transformed data. For the majority of species the square root transformation was found to be more effective than the log transformation at normalising the data. Because it is not appropriate to use different transformations for different species, it was necessary to use a standard transformation that worked for the majority of species. It was, however, recognised that after square root transformation the variances for some species still differed significantly between treatments and consequently it was decided to set the probability at 0.01 to avoid type one errors (see page 49).

Interaction effects

In Chapter 4 (see pages 49 & 50), Chapter 5 (see page 77 & 78) and Chapter 6 (see page 112) ANOVAs were performed without replication and the opportunity to test for significant interaction effects was therefore lost (Sokal & Rohlf 1995). When conducting ANOVAs without replication there is only one observation per subgroup and you are therefore unable to calculate the error SS (sum of squares). The interaction SS becomes the only source of error and is then known as the remainder SS. The possibility of interaction was, however, considered prior to all analyses. This was achieved by plotting species abundance against dates (for each treatment) to ensure that treatment effects were reproduced on all sampling dates. No interaction between date and treatment was evident for any of the species under investigation and was therefore not considered further in this study.

Null hypotheses

The null hypotheses for the statistical tests conducted in this thesis are as follows:

Chapter 4, Chapter 5 and Chapter 6

- There is no difference between the mean number of species 'x' in different sludge treatments (see pages 49, 77, 78 & 112)
- There is no difference between the mean number of species 'x' in the three blocks (see pages 49, 77, 78 & 112)
- There is no difference between the mean number of species 'x' on different sampling dates (see pages 49, 77 & 112)

Chapter 4 page 50

- There is no difference in the diversity when measured by diversity index 'x' in different sludge treatments
- There is no difference in the diversity when measured by diversity index 'x' on different sampling dates
- There is no difference in the diversity when measured by diversity index 'x' in the three blocks

Chapter 4 page 50

- There is no difference between the mean weight of organic matter lost from the litterbags in different sludge treatments
- There is no difference between the mean weight of organic matter lost from the litterbags in the three blocks
- There is no interaction effect between block and treatment that influences the mean weight of organic matter lost from the litterbags

Chapter 5 and Chapter 6

- There is no relationship between the abundance of species 'x' and grass height (see pages 78 & 113)

Chapter 5 page 78

- There is no relationship between the abundance of species 'x' and cadmium concentration

Chapter 7 page 141

- The median relative abundance of species 'x' caught by pitfall trapping is equal to the median relative abundance of species 'x' caught by suction sampling

Chapter 8 pages 151 & 161

- The observed frequencies of species 'x' are evenly distributed between treatments
- The relative frequencies of dominant species are evenly distributed between treatments
- The relative frequencies of asexual and sexual individuals are evenly distributed between treatments
- The relative frequency of euedaphic and hemiedaphic individuals are evenly distributed between treatments

Confounding of sampling efficiency and treatment effects

Some species when sampled by suction or pitfalls were found to be negatively correlated with grass height (see page 100 for suction samples and page 128 for pitfall traps). It is possible that the increased grass height decreased the sampling efficiency for these species and Greenslade (1964) found that the abundance of carabid beetles caught in pitfall traps was negatively correlated with vegetation cover. It was also found that grass height was affected by sludge treatment (see Appendix VIII) and it is therefore possible that treatment effects were merely due to changes in sampling efficiency. If this had been the case the plots with the shortest grass heights (namely the control plots) would be predicted to have the highest collembolan abundance. With the exception of *S. viridis* (which was not correlated with grass height) the opposite was true. Furthermore the DCA ordinations for 1996 (see page 97 for the suction DCA and page 127 for the pitfall DCA) indicated that treatment splits in long grass (April & June) were as clear as those in short grass (May and August). It would therefore appear that while sampling efficiency may have been confounded with treatment effects (due to differences in grass heights between treatments), in this study treatment effects were so strong that this problem did not appear to arise.

Pooling the samples within a plot

Collection of Collembola data on any particular sampling date involved taking a number of individual samples (i.e. five with the exception of the pharmaceutical trials described in Chapter 8) from within each plot. Prior to any analyses, the information gathered from each plot was pooled in order to give a more accurate indication of the collembolan community structure and composition for each plot. This is a standard sampling technique utilised in a wide variety of biological studies in order to overcome problems of spatial heterogeneity.

CHAPTER 4. Effects of sewage sludge on euedaphic and hemiedaphic Collembola sampled by litterbags

4.1 Introduction

From 1998, the disposal of sewage sludge at sea will no longer be permissible in Europe (CEC 1991). As this is currently one of the principal methods of disposal in the UK, alternative methods must be found (Smith 1996). In comparison to alternative disposal methods such as incineration and landfill, using sewage sludge as an agricultural fertiliser not only provides a cheap method of disposal, but also a more environmentally friendly method. It has therefore been predicted that in the next decade the agricultural recycling of sewage sludge will become more widespread (Rund 1995).

Sewage sludge is generally classed as digested or undigested (raw) depending on the treatment process from which it is derived (SAC 1997). Undigested sludge only undergoes primary treatment which involves the settlement of solids in the form of a sludge (Smith 1996). This raw sludge often has significant quantities of pathogens (e.g. viruses, parasitic worms and bacteria) and an offensive odour (Smith 1996). Undigested sludge must therefore be injected (or immediately incorporated) into the soil, and livestock grazing is prohibited for three weeks following application (CEC 1986). Undigested sludge may, however, be stabilised by a variety of processes including: anaerobic and aerobic digestion, composting and the addition of lime. Mesophilic anaerobic digestion is the primary method of stabilisation in the UK, and 44% of sludge (dry solids) spread on agricultural land is treated in this way (Smith 1996). Anaerobic digestion reduces the odour and level of pathogens through the action of micro-organisms under anaerobic conditions.

It has been well documented that the addition of sewage sludge to agricultural land favours invertebrates. For example, sludge has been found to increase the abundance of Collembola (Lübben 1989; Höller-land 1959), Carabidae (Larsen *et al.* 1996), earthworms (Cuendet & Ducommun 1990), soil nematodes (Larink *et al.* 1990) and soil mites (Glockemann & Larink 1989). The application of sludge can benefit invertebrate species in several ways. For instance, it can increase the food availability both directly and indirectly (through increased plant growth), increase the vegetation height (hence making the micro-climate more stable), and improve the physical structure of the soil (Pimentel & Warneke 1989; Marshall 1977; Curry 1994). However, sewage sludge often has the drawback of containing heavy metals which can accumulate in areas of long-term sludge application (Aitken *et al.* 1994).

Heavy metals such as copper, zinc, cadmium and lead, enter sewage works from domestic waste, industrial waste and road run-off (Smith 1996). The levels of metals entering sewage works from industrial sources has declined over the past thirty years. This decline is predominately a consequence of more stringent controls on effluent metal loadings, and the subsequent adoption of cleaner manufacturing technologies (Smith 1996). Domestic waste now accounts for the largest proportion of certain metals including copper and zinc (Critchely & Agg 1986). Domestic sources include medicated shampoo (which contains zinc) and washing up liquid (which contains nickel: Smith 1996). However, the principal contamination source of domestic effluent is thought to be the corrosion of plumbing systems (Comber & Gunn 1994; Critchley & Agg 1986). Metal inputs from domestic sources are much harder to control than industrial inputs, and it is likely that they will continue to be problematic.

As heavy metals are persistent, long-term sludge application can lead to their accumulation in the soil to levels that may potentially be toxic to livestock and invertebrates. At present, sludge application is controlled by regulations governing the safe metal loading of soils (CEC 1986; CEC 1991), however, more research is required to substantiate that maximum metal levels are safe for all invertebrate species.

Larsen *et al.* (1996) found no adverse effects of heavy metals in sewage sludge for the density and diversity of Carabidae. However, Carabidae appear to have an efficient excretory system for heavy metals, and several studies found their body burdens of metals to be lower than in other invertebrates in polluted areas (Janssen & Hogervorst 1993; Van Straalen & Van Wensem 1986). Land application of heavy metal contaminated sewage sludge has been found to result in increased body burdens of metals in earthworms and spiders (Benninger-Truax & Taylor 1993), suggesting that the metals are present in bioavailable forms. Furthermore, green peach aphids (*Appelia schwartzi* Börner) feeding on plants grown on contaminated sludge were found to have a lower fitness (decreased fecundity and longevity) than those feeding on plants grown on uncontaminated sludge (Pimentel & Warneke 1989).

Field studies indicate that heavy metal contaminated sludge does not affect the abundance of gasmid mites (Glockemann & Larink 1989) or Collembola (Lübben 1989), but can affect their population structure. Some species (e.g. *F. candida* and *B. hortensis*) were found to prefer contaminated plots, while other species (e.g. *I. notabilis* and *S. aureus*) preferred uncontaminated plots. It therefore appears that species specific differences in metal susceptibility exist. *Sminthurinus aureus*, being detritivorous (Curry 1994), may ingest high levels of metals through directly consuming sewage sludge. Species that can digest fungal cell walls, where heavy metals are frequently concentrated (Gast *et al.* 1988), are also thought to be more susceptible to heavy metal poisoning (Siepel 1994).

Siepel (1995) suggested that while asexual species may be favoured in areas of long-term metal contamination, they may be adversely affected by the application of fertilisers. The application of metal-rich sewage sludge may therefore favour sexual species in the short-term, but favour asexual species in the long-term (once the metals have accumulated through repeated application). It would therefore appear, at least for Collembola and Acarina, that the effects of heavy metals in sewage sludge are subtle, altering the species composition rather than the overall abundance.

Changes in the collembolan community structure are likely to affect the rate of decomposition. Retarded decomposition and mineralisation rates have been observed in heavily polluted sites where the collembolan diversity was depleted (Strojan 1978b; Rühling & Tyler 1973; Hågvar & Abrahamsen 1990). As organic matter decomposes, the microarthropod community often progresses through a series of successional phases (Anderson 1975). It is possible that through retarding decomposition, high metal levels will also slow down the succession of microarthropods within the decomposing material.

This chapter aims to look at the effects that different types of sewage sludges have on: the abundance and diversity of euedaphic and hemiedaphic Collembola; the abundance of sexual and asexual species; and the rate of decomposition and succession.

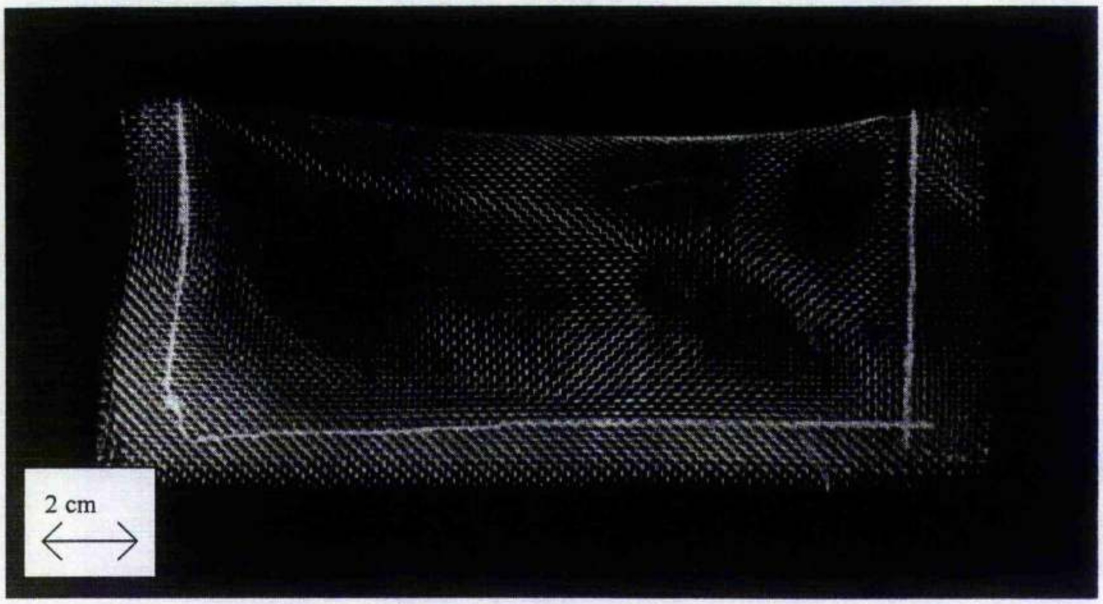
4.2 Sampling euedaphic and hemiedaphic Collembola

In previous studies, soil Collembola have mainly been sampled in two ways. Soil corers were the first method to be widely used (e.g. Milne 1962; Lübben 1989;) while the second, more recent method, is the litterbag (Bolger 1985; Siepel 1990). When buried in the soil litterbags act as underground baited traps which attract Collembola. For the purpose of this research, litterbags were chosen to monitor the euedaphic/hemiedaphic Collembola for two main reasons: they do not involve removing soil from the experimental area (and as the study sites were part of larger scale experiments, minimal disruption was necessary) and behavioural extraction methods yielded a higher percentage of total Collembola (and in particular Onychiuridae spp.) from litterbags than soil cores.

Onychiuridae have been previously shown to have a relatively poor behavioural extraction efficiency as a consequence of their low mobility (Marshall 1972). The lower extraction efficiency of Collembola in soil cores may have been the result of the soil at the experimental sites having a relatively high organic content. It has been observed that in soils with high organic contents Collembola get trapped within the soil cores during behavioural extraction (Southwood 1971). In agreement with Crossley & Hoglund (1962), extraction appears more efficient for litterbags, with both immature individuals and poorly mobile Onychiuridae being well represented.

Litterbags were made from 15 cm x 15 cm squares of nylon mesh with a mesh size of 1 mm (see Figure 4.1 below). These were then filled with freshly fallen sycamore (*Acer pseudoplatanus*) or oak (*Quercus robur*) leaves collected at SAC Auchincruive. Leaves were washed to remove soil particles and dried in a drying cabinet at 60°C for 24 hours (or until no further weight loss was observed). Into each bag 2.50 grams of dried leaves were inserted. The litterbags were then rehydrated for an hour before they were buried at a depth of 2-5 cm. After a predetermined period of time (e.g. one month) the bags were removed and the Collembola extracted (see below).

Figure 4.1 The litterbag



Problems of sampling Collembola using litterbags

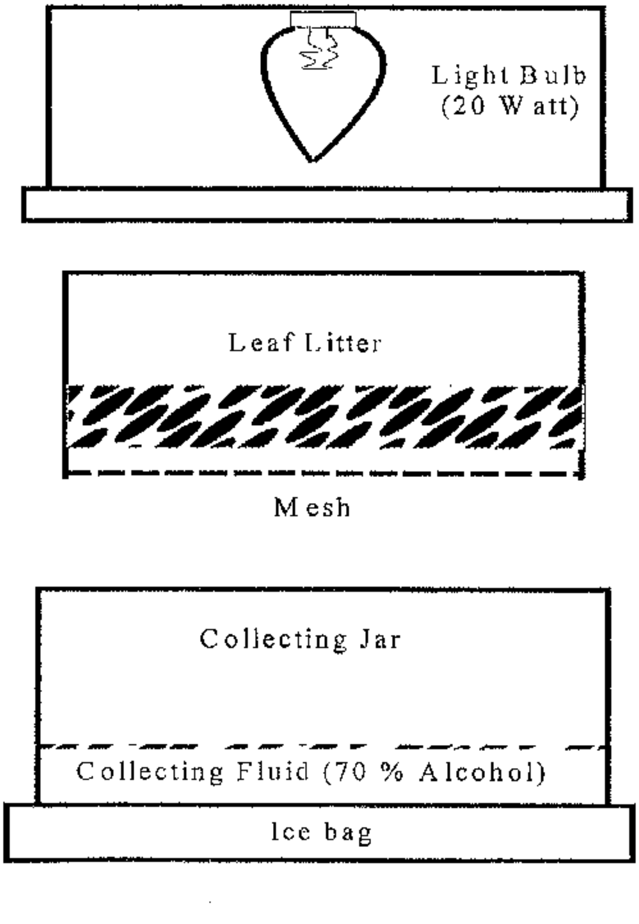
Within a given area, differences have been found between samples of microarthropods derived from litterbags and those derived from soil cores. It is consequently thought that litterbags do not accurately reflect the true soil population (Crossley & Hoglund 1962; Siepel 1990). Collembola in litterbags are influenced by several factors the most important of which is probably the successional stage of the leaf litter. Litterbags may also provide a more stable micro-climate and have a higher moisture content than the surrounding soil (Witkamp & Olson 1963; Crossely & Hoglund 1962). The artificial confinement of the litter can alter the fungal community (Seastedt 1984) and exclude predators too large to enter the bags. All these factors may influence the collembolan community within the litterbag.

Comparison between samples derived from litterbags and soil cores have found macrophytophagous (e.g. algae eating species) to be under represented in the litterbags, while fungivorous species were over represented (Siepel 1990). Extraction efficiency may, however, be greater in litterbags than soil cores especially in soil with a high organic content (Crossley & Hoglund 1962) and additional information on microarthropod succession may be obtained. Litterbags were therefore considered to be the most efficient method of sampling Collembola in our experimental plots.

Extraction

Collembola were extracted using modified Tullgren funnels (see Figure 4.2) similar to MacFadyen's high gradient cylinder extractor (MacFadyen 1961). Haarløv (1947) found that during extraction animals can become trapped in condensed water on the sides of the funnels. In this study, this problem was eliminated by omitting the funnel. MacFadyen (1961) used a water bath to maximise the extraction gradient, but in this study extraction was improved by placing the collection vessel on an ice bag (which was renewed daily). This is likely to have increased the extraction efficiency not only by increasing the temperature gradient, but also by preventing the alcohol from evaporating. It has been found that alcohol can repel animals, and hence reduce the extraction efficiency of behavioural methods in confined spaces (MacFadyen 1961).

Figure 4.2 The modified Tullgren funnel



Treatment of Collembola following extraction

Collembola were collected in 70% alcohol and immediately after collection they were heated in the alcohol on a hot plate, for about four hours, until they ceased to float. This prevented the Collembola from dehydrating which makes them more difficult to identify (Fjellberg 1980). Onychiuridae were boiled in alcohol for a further four hours before mounting to dissolve their high lipid content. This makes the pseudocelli (one of the main identification features) easier to see (Fjellberg personal communication). Collembola were identified to species level primarily following Fjellberg (1980) and Gisin (1960). Further information on the identification of Collembola can be found in Chapter 3.

4.3 Methods

Trial site and treatments

The euedaphic and hemiedaphic Collembola were investigated at the SAC Auchincruive site (see Chapter 3). For the purpose of this study, four different sludge treatments were investigated, together with a no sludge control. The four sludge treatments were digested, zinc-rich (digested), undigested and copper-rich (undigested: see Table 4.1 & Appendix II for sludge properties). The metal-rich sludges were obtained from sewage treatment works which had high inputs of the respective metals.

Table 4.1 Treatments

Treatment no.	Treatment	Mean metal concentration	Sewage treatment works
1	Control	Background levels (see Appendix III)	
2A	Digested uncontaminated sludge	Low levels (see Appendices II & III)	Banbury
3	Undigested uncontaminated sludge	Low levels (see Appendices II & III)	Carterton
7	Zinc-rich digested sludge	5,238 mg Zn kg^{-1} sludge 180 mg Zn kg^{-1} soil 1995 288.5 kg Zn ha^{-1} $year^{-1}$	Coleshill
11	Copper-rich undigested sludge	4,331 mg Cu kg^{-1} sludge 69 mg Cu kg^{-1} soil 1995 67.9 kg Cu ha^{-1} $year^{-1}$	Selkirk

For this experiment, the highest metal loadings were investigated for the zinc-rich and copper-rich sludge. The five treatments studied were located randomly within each of three blocks and are marked with an asterix on Figure 4.3.

Sampling euedaphic/hemiedaphic Collembola

15 litterbags (see page 44), of 15 cm x 15 cm squares (mesh size 1 mm), were buried at a depth of 2-5 cm in the selected plots on 6 December 1994. Each bag contained 2.50 g of oven dried oak leaves which were re-hydrated before burial. Five litterbags from each plot were lifted after 10, 20 and 30 weeks. A further five litterbags were buried in each plot on 16 May 1995, and these were lifted after 10 weeks (see Figure 4.4). The experiment was designed to provide information on succession (by comparing the 10, 20 and 30 week litterbags) and season (by comparing the two sets of litterbags lifted after 10 weeks). Collembola were extracted with modified Tullgren funnels over a period of six days (see page 45). Collembola were extracted in alcohol, prepared and mounted (see page 46 & Chapter 3).

Figure 4.4 Burial and lifting times for litterbags

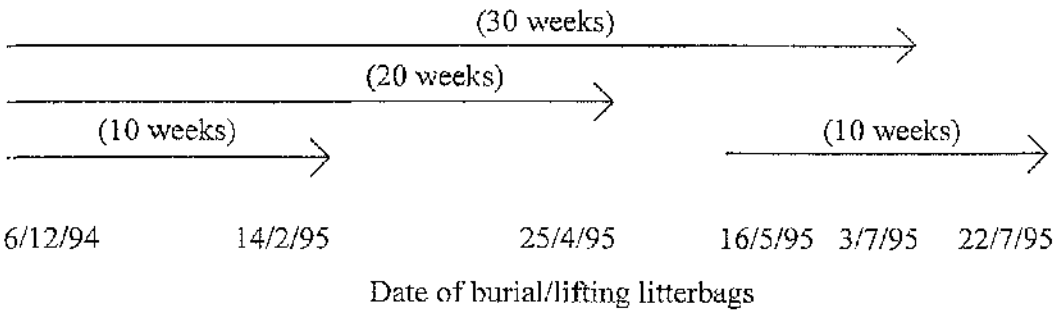
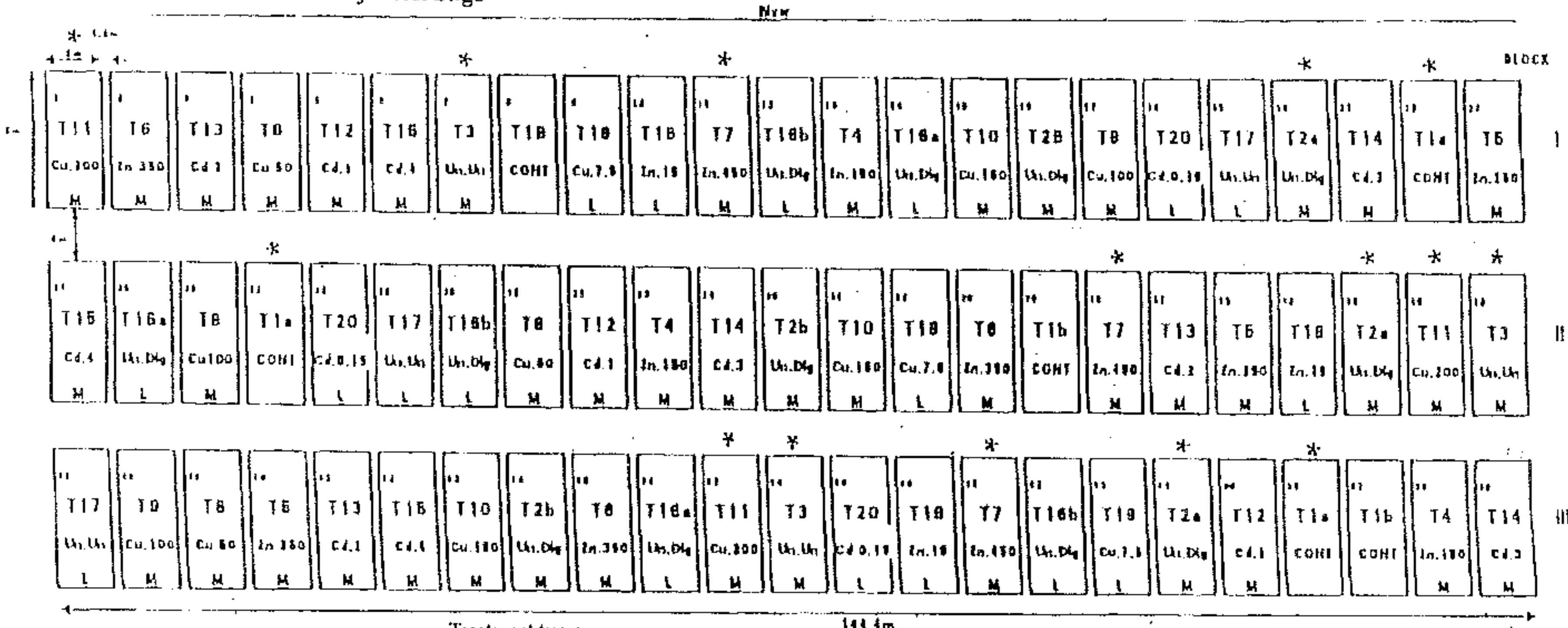


Figure 4.4 The above figure shows the dates litterbags were buried/lifted, with the number of weeks the litterbags were buried for in brackets.

Measuring the rate of decomposition

Decomposition rates were assessed using litterbags identical to those used to sample Collembola. The 1 mm mesh excluded the macrofauna (earthworms, Diploda, Isopoda etc.) and therefore gave an estimate of decomposition by the mesofauna (Acari, Collembola, echytracid worms etc.) and the soil microbes (Anderson 1975). To study decomposition, sycamore leaves were used instead of oak leaves because they

Figure 4.3 SAC Auchincruive contaminated sludge plan showing plots sampled by litterbags



Codes:
1 - 69 Plot numbers
T1 - T20 Treatment numbers
I - III Block numbers

Build-up rate:
M Moderate build-up
L Long-term build-up

Treatment type:

CONT Control
Un.Dig Uncontaminated digested
Un.Un Uncontaminated undigested

Zn.150 High Zn (target soil metal concentration 150 mg kg^{-1})
Zn.250 High Zn (target soil metal concentration 250 mg kg^{-1})
Zn.350 High Zn (target soil metal concentration 350 mg kg^{-1})
Zn.450 High Zn (target soil metal concentration 450 mg kg^{-1})
Zn.15 High Zn (15 kg ha^{-1} Zn annually)

Cu.50 High Cu (target soil metal concentration 50/mg kg^{-1})
Cu.100 High Cu (target soil metal concentration 100/mg kg^{-1})
Cu.150 High Cu (target soil metal concentration 150/mg kg^{-1})
Cu.200 High Cu (target soil metal concentration 200/mg kg^{-1})
Cu.7.5 High Cu (7.5 kg ha [illegible] annually)

Cd.1 High Cd (target soil metal concentration 1 mg kg^{-1})
Cd.2 High Cd (target soil metal concentration 2 mg kg^{-1})
Cd.3 High Cd (target soil metal concentration 3 mg kg^{-1})
Cd.4 High Cd (target soil metal concentration 4 mg kg^{-1})
Cd.0.15 High Cd (0.15 kg ha^{-1} Cd annually)

decomposed faster than oak leaves and would therefore maximise any differences in the decomposition rates between treatments in the limited time available. In accordance with other studies, the litterbags were not rehydrated before burial as this can influence the decomposition rate (Suffling & Smith 1974). Thirteen litterbags were buried randomly on the selected plots (see Figure 4.3) on 24 May 1995, and were lifted after ten weeks on 2 August 1995.

After collection, all the leaves were carefully removed from the litterbags and oven dried at 60°C for about 24 hours (or until no further weight loss was observed) to obtain the dry weight. The dry weight included both the inorganic (infiltrated soil) and organic fractions (leaves) of the litterbags. The dried material was therefore ground and ignited at 500°C overnight to determine the ash-free dry weight (i.e. weight of soil). The organic matter lost from the litterbags could then be calculated from the following equation (where 2.50 g is the initial dry weight of sycamore leaves per litterbag).

Organic matter lost = 2.50 g - (dry weight - ash weight)

4.4 Statistical analysis

The five samples collected from a plot on a particular sampling date were pooled to obtain an indication of collembolan community structure for each plot on each sampling date. The pooled data were utilised in the following statistical analyses.

Species composition

The pooled data were square-root transformed to normalise the distribution and to homogenise the variances. Three-way analyses of variance (ANOVAs) were performed to test for date/succession, block and treatment effects. ANOVAs were performed on the number of: total Collembola; the most frequently occurring species (i.e. those occurring in over 50% of samples); euedaphic individuals; hemiedaphic individuals; sexual individuals and parthenogenetic individuals. The Tukey multiple comparison test was applied to locate significant differences found by ANOVA (Zar 1984). It was found that although transformation normalised the data for most species, variances still differed significantly between treatments for some species (F-test). When utilising ANOVAs and Tukey tests, heterogeneous variances can increase the likelihood of a type one error (i.e. wrongly rejecting the null hypothesis) and therefore can increase the probability of finding significant effects when they are not present (Day & Quinn 1989). To counteract this, the significance level was set at 0.01 (rather than 0.05: Day & Quinn 1989).

Detrended Correspondence Analysis

Detrended Correspondence Analyses (DCA's) were performed on the species relative abundance (without downweighting of rare species) using the software DECORANA (Hill 1979). The samples and species scores for axes 1 and 2 were plotted to obtain sample and species ordinations respectively. DCA was chosen as it provided a method of looking simultaneously at the effects of treatment, succession and season on the overall collembolan community structure.

Diversity

The following four measures of diversity were calculated: **S** (number of species) to measure species richness, **d** (Berger-Parker's dominance index: Berger & Parker 1970) to measure evenness and $\mathbf{N_2}$ (the reciprocal of Simpson's index: Hill 1973) and α (Fisher's log series parameter: Fisher *et al.* 1943) to measure overall diversity. A combination of diversity indices was used to provide the most accurate information on the diversity of the community (Magurran 1988). See pages 30-32 for information on diversity indices and Appendix I for equations for diversity indices.

Repeated measures of diversity are usually normally distributed (Magurran 1988), and therefore conventional statistics can be used to test for significant differences in diversity (Magurran 1988; Kappelle *et al.* 1995). This was not, however, the case in this study and the square root transformation was required to normalise the data (see page 36). Three-way ANOVAs (followed by Tukey multiple comparison tests) were therefore conducted to test for effects of treatment, block and date, on the diversity indices.

Decomposition

Decomposition rates were compared between plots using a 2-way ANOVA (followed by the Tukey multiple comparison test) to test for treatment, block and interaction effects. As the data were normally distributed and the variances homogenous (F-test), transformation was not necessary.

4.5 Results

General information

A total of 14,829 individuals, from 26 species, were collected in the litterbags between 14 February and 22 July 1995 (a species list can be found in Appendix IV).

Species composition and date

Both *L. cyaneus* and *Heteromurus nitidis* (Templeton) appeared to show a seasonal effect and increased as the summer progressed (see Figure 4.5 & Table 4.2). For example, both species were significantly more abundant in 3 and 22 July samples than February or April ones, and they were also more abundant in 22 July samples than 3 July samples. Changes in the abundance of these two species were more likely a consequence of season rather than succession, since the two sets of litterbags buried for 10 weeks (specifically February and 22 July) differed most with respect to the abundance of these species. Both *L. cyaneus* and *H. nitidis* are sexually reproducing species, and it is therefore not surprising that the number of sexual individuals followed a similar pattern (see Figure 4.6 & Table 4.2). The asexual *Mesaphorura* spp. (consisting of *M. macrochaeta* Rusek and *M. hylophila* Rusek) also followed a similar pattern to *L. cyaneus* and *H. nitidis*, with the highest abundance found in litterbags collected on 22 July (see Figure 4.5 & Table 4.2).

Some species do not, however, show a seasonal pattern but instead appear to be dependant on the successional stage of the leaf litter (i.e. the length of time the litterbags were buried). *Isotomurus palustris* was significantly more abundant in the litterbags from 25 April and 3 July (buried for 20 and 30 weeks, respectively) than those from 14 February or 22 July (both buried 10 weeks: see Figure 4.7 & Table 4.2). The lower abundance of *I. palustris* in 22 July litterbags compared to 3 July litterbags, indicates that the abundance of *I. palustris* was primarily dependant on succession rather

Table 4.2 Effect of treatment and sampling date on species composition

	Date			Treatment		
Species	**F-value**	**Probability df = 3, 24 n = 15**	**Location of difference df = 24, $k = 4$**	**F-value**	**Probability df = 4, 24 n = 12**	**Location of difference df = 24, $k = 5$**
Total	10.49	< 0.001	22 July & February > 3 July 22 July > April	1.87	N.S.	-
Eudaphic	12.73	< 0.001	22 July > February, April & 3 July	2.52	N.S.	-
Hemiedaphic	8.65	< 0.001	22 July > April & 3 July	1.54	N.S.	-
Sexual	35.81	< 0.001	22 July > February, April & 3 July 3 July > April	1.51	N.S.	-
Parthenogenetic	6.94	< 0.001	February & 22 July > 3 July	1.65	N.S.	-
I. notabilis	6.34	0.001	February & 22 July > 3 July	1.23	N.S.	-
I. anglicana	19.40	< 0.001	3 July > 22 July > April > February	2.53	* 0.05	Zn > All, Cu > U, D & C
I. palustris	7.86	< 0.001	3 July > April > February & 22 July	0.25	N.S.	-
F. fimetarioides	2.56	N.S.	-	0.31	N.S.	-
F. fimetaria	1.37	N.S.	-	1.76	N.S.	-
L. cyaneus	7.73	< 0.001	22 July > 3 July > February & April	0.74	N.S.	-
H. nitidis	140.51	< 0.001	22 July > 3 July > February & April	1.78	N.S.	-
Mesaphorura spp.	11.64	< 0.001	22 July > 3 July & April > February	2.68	* < 0.05	Zn & Cu < C, D, U
N. minimus	2.44	N.S.	-	2.75	* < 0.05	C > D, Zn < All, Cu > D & U
C. denticulata	6.83	< 0.001	February & 22 July > April	1.96	N.S.	-

Table 4.2 The above table shows the results of the 3-way ANOVAs followed by Tukey tests (probability for Tukey tests set at 0.01 and k = the number of means being compared). As no block effect was found for any of the species tested (df = 2, 24), this information is omitted from the table. The codes for the treatments are as follows: C = control, D = digested sludge, U = undigested sludge, Zn = zinc-rich sludge and Cu = copper-rich sludge.

* Although the probability level for the ANOVAs was set at < 0.01, it was decided to include ANOVAs with P ≤ 0.05 when the Tukey test showed a difference at < 0.01.

than season. The abundance of total Collembola, *I. notabilis* and asexual individuals (principally *I. notabilis*) followed the opposite pattern (see Figures 4.6 & 4.8 & Table 4.2), and were more abundant in litterbags buried for 10 weeks (February and 22 July) than in litterbags buried for 30 weeks (3 July).

Figure 4.5 Effect of date on abundance of *H. nitidis, L. cyaneus* and *Mesaphorura* spp. irrespective of treatment

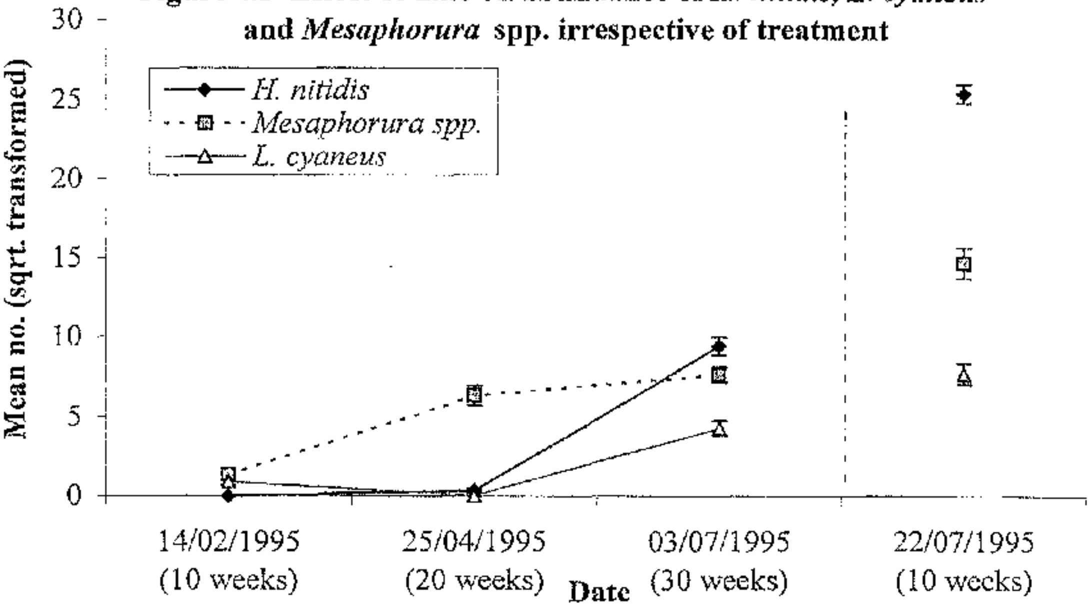


Figure 4.6 Effect of date on abundance of sexual & asexual individuals irrespective of treatment

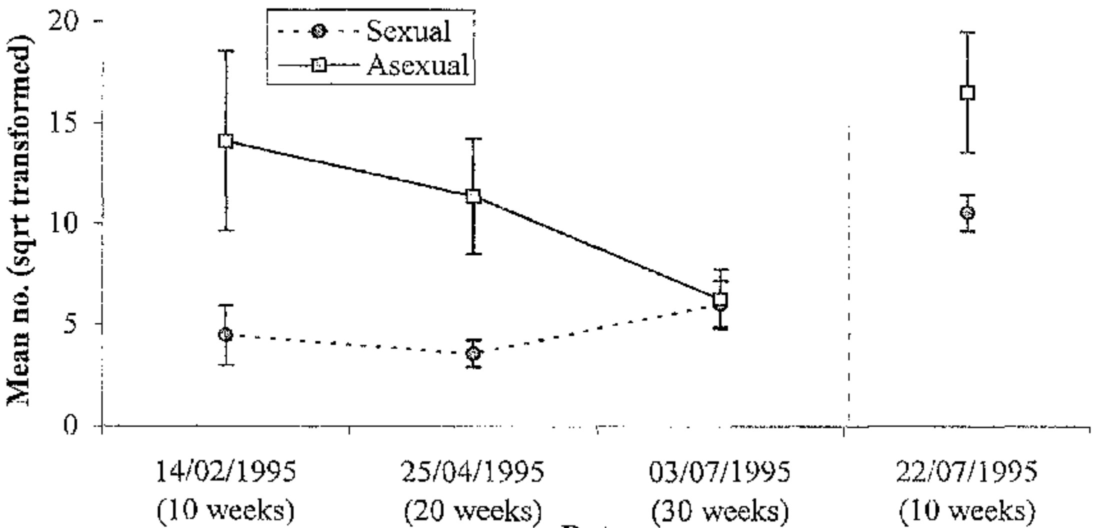


Figure 4.5 & 4.6 The above graphs show the effects of date/burial time on the species composition and number of sexual and asexual individuals in litterbags. Error bars show standard deviations and burial time is given in brackets below date.

The abundance of *I. anglicana* appeared to be dependant on both season and succession. This species had a higher abundance in April litterbags (20 weeks) than February litterbags (10 weeks), and also had a higher abundance in 3 July litterbags (30 weeks) than April litterbags (20 weeks: see Figure 4.7 & Table 4.2). Like *I. palustris*, this species was less abundant in litterbags from 22 July (10 weeks) than 3 July (30 weeks), but unlike *I. palustris*, the abundance on 22 July was greater than in February or April, suggesting that changes in the abundance of this species were not just the consequence of succession but also of season.

Figure 4.7 Effect of date on abundance of *I. anglicana* and *I. palustris* irrespective of treatment

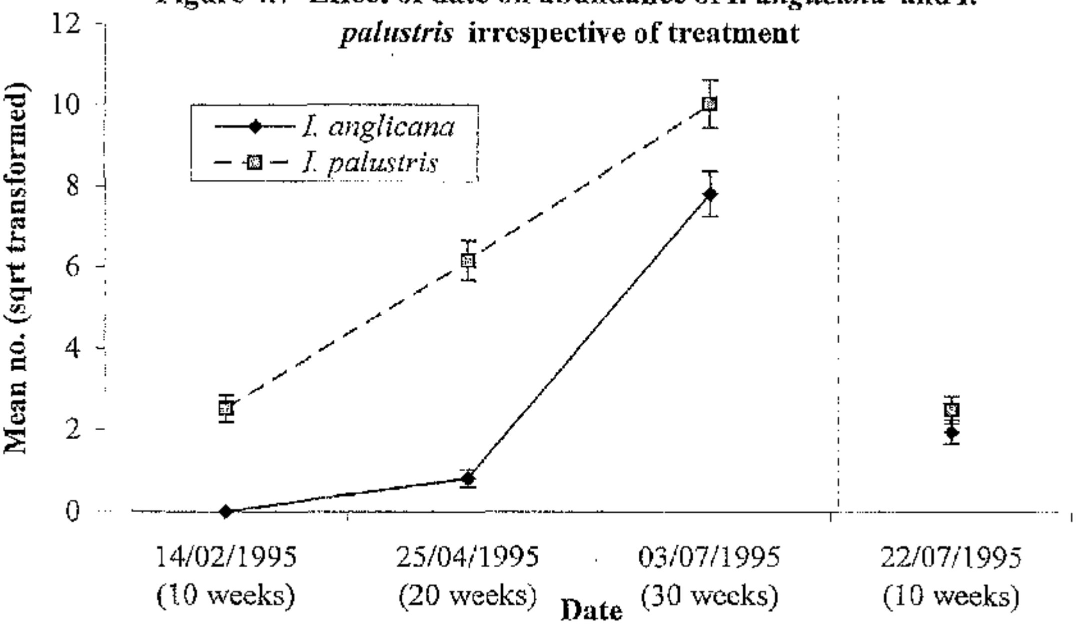


Figure 4.7 The above graph shows the effects of date/burial time on the species composition in litterbags. Error bars show standard deviations and burial time is given in brackets below date.

The number of hemiedaphic species appeared to follow a successional pattern with a significantly greater abundance in the 22 July litterbags (10 weeks) than April (20 weeks) or 3 July litterbags (30 weeks: see Figure 4.9 & Table 4.2). The number of euedaphic species, however, appeared to follow a seasonal pattern with the biggest difference in abundance occurring between the February and 22 July litterbags, both of which were buried for 10 weeks (see Figure 4.9 & Table 4.2). *Ceratophysella denticulata* did not follow either a successional or seasonal pattern but instead, with the exception of the April litterbags having a significantly lower abundance than the February or 22 July litterbags, the population remained relatively constant (see Figure 4.8 & Table 4.2).

Figure 4.8 Effect of date on abundance of total Collembola, *I. notabilis* and *C. denticulata* irrespective of treatment

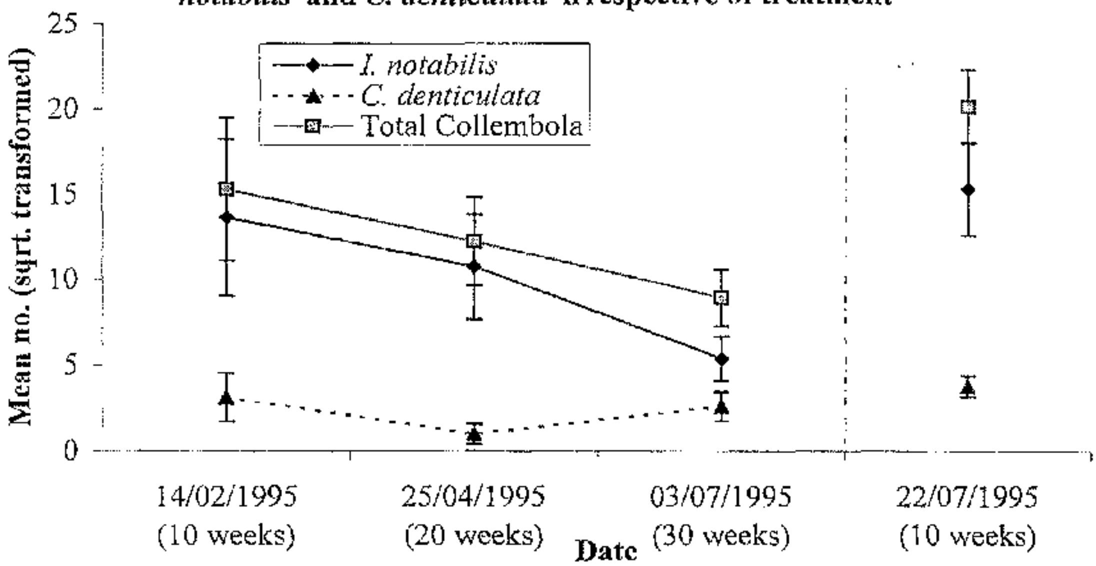


Figure 4.9 Effect of date on ecomorphology irrespective of treatment

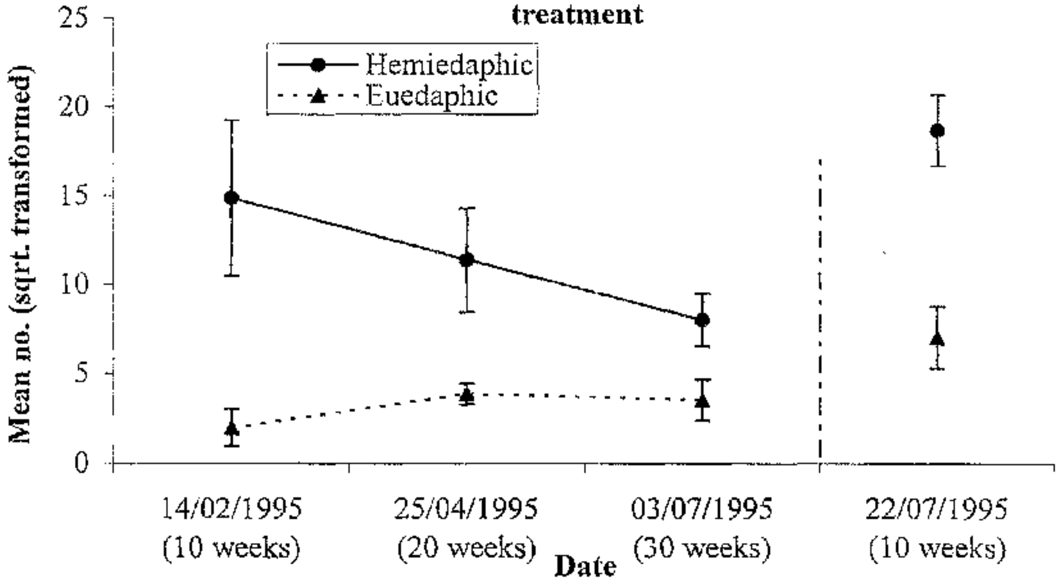


Figure 4.8 & 4.9 The above graphs show the effects of date/burial time on species composition and ecomorphology. Error bars show standard deviations and burial time is given in brackets below date.

Species composition and treatment

No treatment effect was found for the number of total Collembola, however, a treatment effect was found for *Neelus minimus, Mesaphorura* spp. and *I. anglicana* (see Table 4.2 and Figure 4.10). *Isotoma anglicana* was more abundant in the zinc and copper-rich plots than the undigested, digested or control plots. *Mesaphorura* spp. showed the opposite trend, being less abundant in both the zinc and copper-rich plots than the undigested, digested or control plots (see Figure 4.10 & Table 4.2). *Neelus minimus* had a higher abundance in the copper-rich plots but a lower abundance in the zinc-rich plots, when compared to the undigested and digested plots (see Figure 4.10 & Table 4.2). The abundance of *N. minimus* was also lower in the digested sludge plots than the control suggesting that this species was adversely affected by the addition of digested sludge (see Figure 4.10 & Table 4.2).

These results must, however, be treated with caution as the variances were heterogeneous, which can increase the probability of finding an effect of treatment when none is present (Day & Quinn 1989). However, although the probability was only 0.05 for the ANOVAs, differences were found to be significant to 0.01 in the Tukey tests, hence suggesting that treatment effects were real.

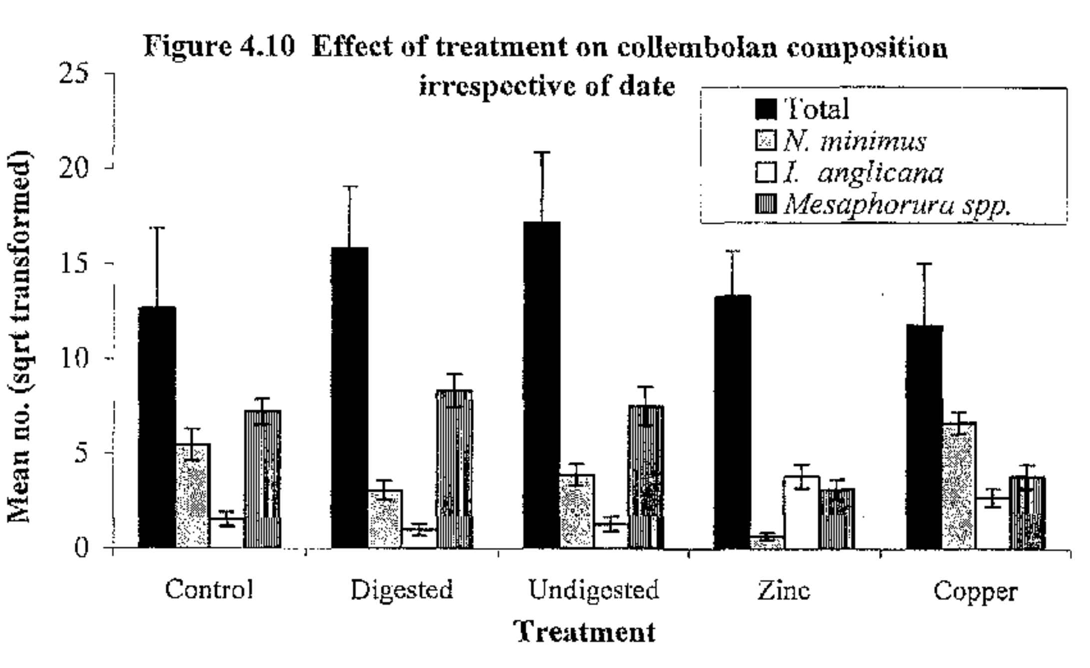


Figure 4.10 The above graph shows the effects of treatment on species composition of litterbags. Error bars show standard deviations.

Diversity

Four measures of diversity were calculated for each plot on the four sampling dates. These were **S** to measure richness, **d** to measure evenness, and α and $\mathbf{N_2}$ to measure diversity (see pages 30-32 for information on diversity indices). The calculated diversity values can be seen in Appendix V. Three-way ANOVAs were conducted on these indices (square root transformed) to test for effects of treatment, block and date. No treatment effect was found for any of the four diversity indices. A date effect was, however, found for all diversity measurements (see Table 4.3) and a block effect was found for species richness (see Table 4.4).

Species richness (**S**) tended to show a seasonal effect, with the summer samples (7 and 22 July) having significantly more species than the February or April samples (see Table 4.3). This is more likely to be the result of season rather than succession because the largest difference in species richness occurred between 14 February and 22 July samples, both of which were buried for 10 weeks. The other measures of diversity did not, however, follow the same pattern. Both overall diversity measures (α and $\mathbf{N_2}$) were significantly higher in litterbags from 3 July (30 weeks) than those from February (10 weeks), April (20 weeks) or 22 July (10 weeks), suggesting a successional effect rather than a seasonal one (see Table 4.3). This was probably the consequence of the dominance (here measured by **d**) being significantly less (indicating a more even community) on 3 July (30 weeks) than in February (10 weeks) or April (20 weeks: see Table 4.3). There was also an effect of season, with the 22 July samples having a higher overall diversity than the February samples (see Table 4.3). It would therefore appear that the evenness and overall diversity of Collembola in the litterbags was primarily dependant season and succession, whereas, the species richness was primarily dependant only on season.

An effect of block was apparent for species richness (**S**), with block one having a significantly lower richness than block three (see Table 4.4). It was found that previous to sludge application, block three had the highest clay and organic content and the lowest sand content (see Appendix VI). Since the water holding capacity of a soil is positively correlated to its clay content (Smith 1996), it is possible that block three also had the highest moisture content.

Table 4.3 Effect of date on collembolan diversity

Diversity Index	F-value	Probability $df = 3, 24$ $n = 15$	February	April	3 July	22 July	Location of difference $df = 24, k = 4$
Species Richness (S)	20.90	< 0.001	2.28 *±0.41*	2.60 *±0.43*	3.04 *±0.32*	3.25 *±0.23*	3 & 22 July > April & February
Berger-Parker (d)	15.85	< 0.001	0.89 *±0.12*	0.88 *±0.12*	0.65 *±0.08*	0.77 *±0.11*	February & April > 3 July
Hill's (N_2)	18.40	< 0.001	1.26 *±0.28*	1.31 *±0.35*	1.99 *±0.28*	1.58 *±0.29*	3 July > February, April & 22 July 22 July > February
Fisher's (α)	18.05	< 0.001	1.07 *±0.32*	1.26 *±0.24*	1.75 *±0.32*	1.42 *±0.15*	3 July > February, April & 22 July 22 July > February

<u>Table 4.3</u> This Table shows the results of the 3-way ANOVAs performed on the following four measures of diversity: d, S, N_2 and α. Block effects are given in Table 4.4. The mean value (square root transformed) for each diversity index is given for each date (with the standard deviations given below). It also shows the location of significant differences (determined by Tukey multiple comparison tests). As no treatment effect was found this information is omitted from the table.

Table 4.4 Effect of block on collembolan diversity

Diversity Index	F-value	Probability df = 2, 24 n = 20	Block 1	Block 2	Block 3	Location of difference df = 24 $k = 3$
Species Richness (**S**)	8.11	< 0.01	2.59 *±0.51*	2.77 *±0.53*	3.00 *±0.44*	Row 3 > Row 1
Berger-Parker (**d**)	0.46	N.S.	0.78 *±0.14*	0.81 *±0.15*	0.80 *±0.13*	-
Hill's (**N_2**)	0.12	N.S.	1.54 *±0.37*	1.51 *±0.46*	1.55 *±0.43*	-
Fisher's (α)	1.20	N.S.	1.30 *±0.31*	1.40 *±0.44*	1.42 *±0.32*	-

Table 4.4 This Table shows the results of the 3-way ANOVAs performed on the following four measures of diversity: d, S, N_2 and α. Effects of date can be found in Table 4.3. It also shows the mean diversity value (square root transformed) found for each block (± standard deviations shown below) and the results of the Tukey test ($P < 0.05$).

Detrended Correspondence Analysis (DCA)

The initial DCA indicated that plot 11 in block one had a consistently different collembolan composition from the other plots. When the species composition of this plot was examined it was found that *I. notabilis* was under represented. *Isotoma notabilis* was either absent (February, April and 3 July) or represented by only one individual (22 July) in this plot, whereas, in all other plots *I. notabilis* was one of the dominant species.

It is unknown what caused the elimination of this species in plot 11 block one and an investigation of the initial soil analysis (prior to sludge application) found that *Rhizobia* numbers, biomass carbon and soil respiration rates were of the same magnitude as other plots (see Appendix VII). Furthermore, the grass yields were also of the same magnitude (see Appendix VIII). The available data on this plot did not therefore show any clear difference between this plot and the other plots. A possible explanation may be that the plot had more emigration and immigration with the soil surrounding the experimental area than the other plots as a consequence of it being at the edge of the experimental trial.

The only way of coping with such outliers in DCA is to remove them from the analysis (Hill & Gauch 1980). It was therefore decided to omit this plot from the data set and repeat the DCA analysis (without the downweighting of rare species). The resultant sample ordination can be seen in Figure 4.11 below, and the species ordination is presented in Figure 4.12. The DCA produced eigenvalues of 0.356, 0.266, 0.196 and 0.097 for axes 1-4 respectively. These values indicate that the amount of between-plot variation in collembolan assemblages accounted for by axes 1-4 was 39%, 29%, 21% and 11% respectively. As axes 3 and 4 collectively accounted for less than 40% of this variation, they were not considered further.

From Figure 4.11 it can be seen that the main factor separating samples along axis 1 is season, with the summer samples (3 and 22 July) being well separated in the middle of axis one, while the winter/spring samples (14 February and 25 April) are clumped and to the left. There is also a separation between the two summer sampling dates along axes 1 and 2. This is probably a consequence of successional and/or seasonal changes in species composition.

Figure 4.11 Sample DCA ordination for litterbags (relative abundance)

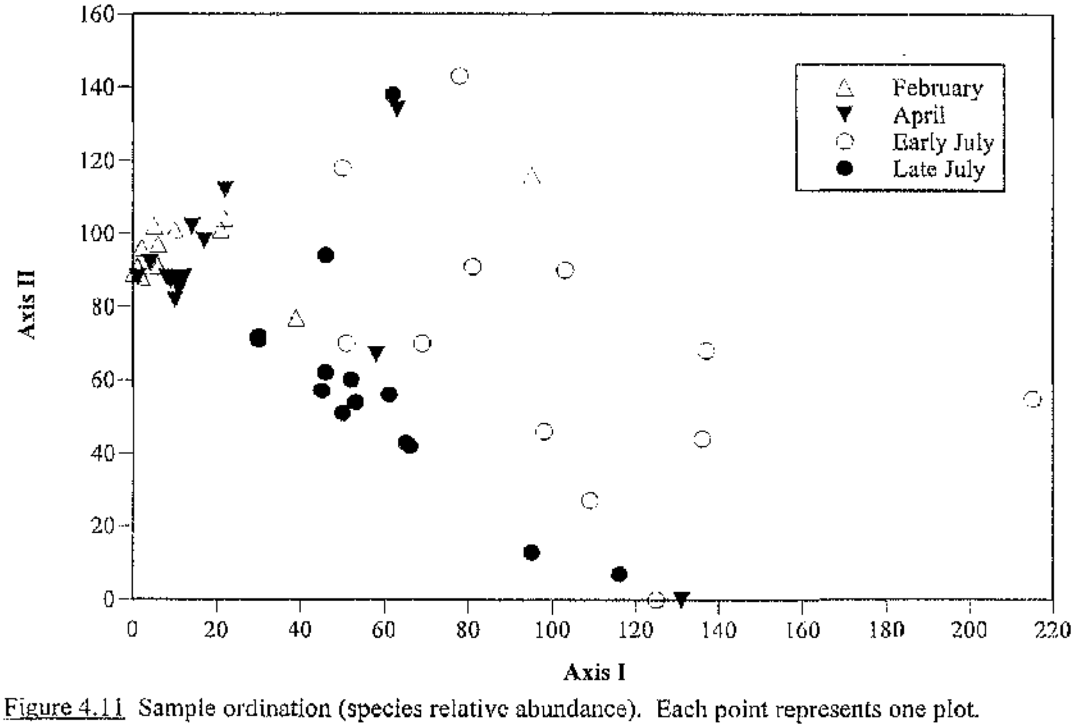


Figure 4.11 Sample ordination (species relative abundance). Each point represents one plot.

From the species ordination (Figure 4.12) it can be seen that species found to be early colonisers of leaf litter, namely *I. notabilis* (Bolger 1985 and above) and *Ceratophysella* spp. (Siepel 1990), lie to the left of axis 1, whereas, species that colonise later (*I. palustris* and *I. anglicana*: see above) lie to the right. The species ordination also suggests that there is a seasonal effect, with species that appear late in the summer occurring to the bottom right hand corner of the axes (*L. cyaneus*, *H. nitidis* and *Mesaphorura* spp: see above). It would therefore appear that the main driving forces separating samples along axes 1 and 2 were succession and season.

Figure 4.12 Species DCA ordination for litterbags (relative abundance)

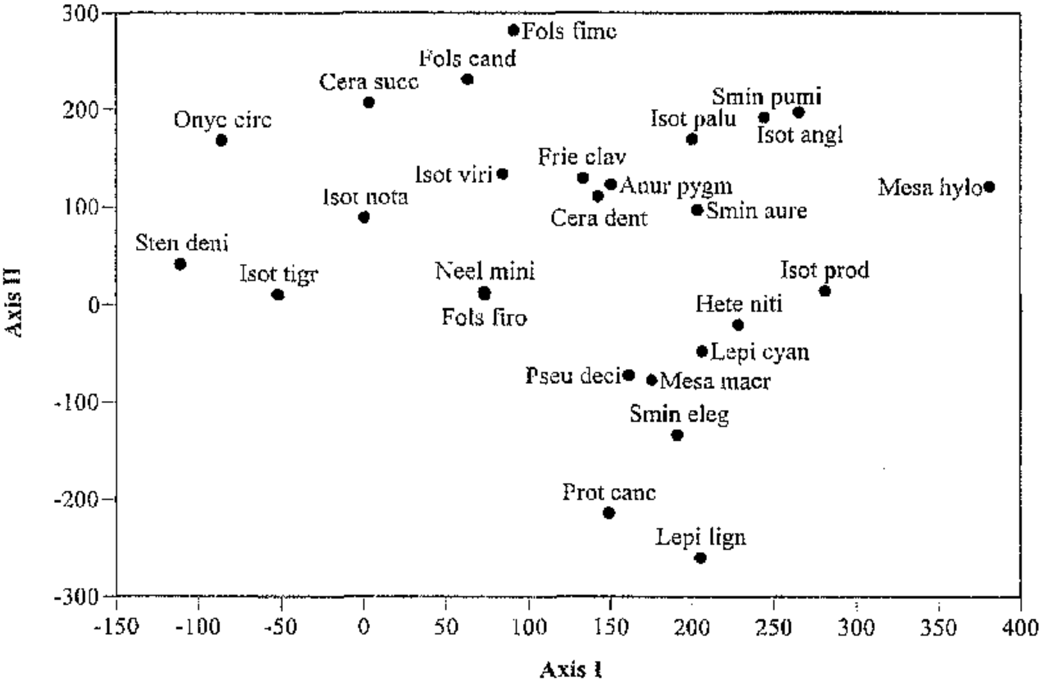


Figure 4.12 Species ordination (relative abundance). Species codes can be found in Appendix IX.

The axes scores for the summer samples were re-drawn showing the 3 and 22 July samples separately. This was done to enable treatment effects to be observed more easily (see Figures 4.13 & 4.14 for 3 and 22 July, respectively). From Figures 4.13 and 4.14 it can be seen that although the majority of the variation in collembolan community structure can be accounted for by season/succession, there is also some variation between treatments. In particular, for both dates in July the zinc plots lie distinct from the digested sludge plots and the control plots. Copper plots are also separate from the control plots on both sampling dates.

Figure 4.13 DCA ordination for 3/7/95 showing treatment split.

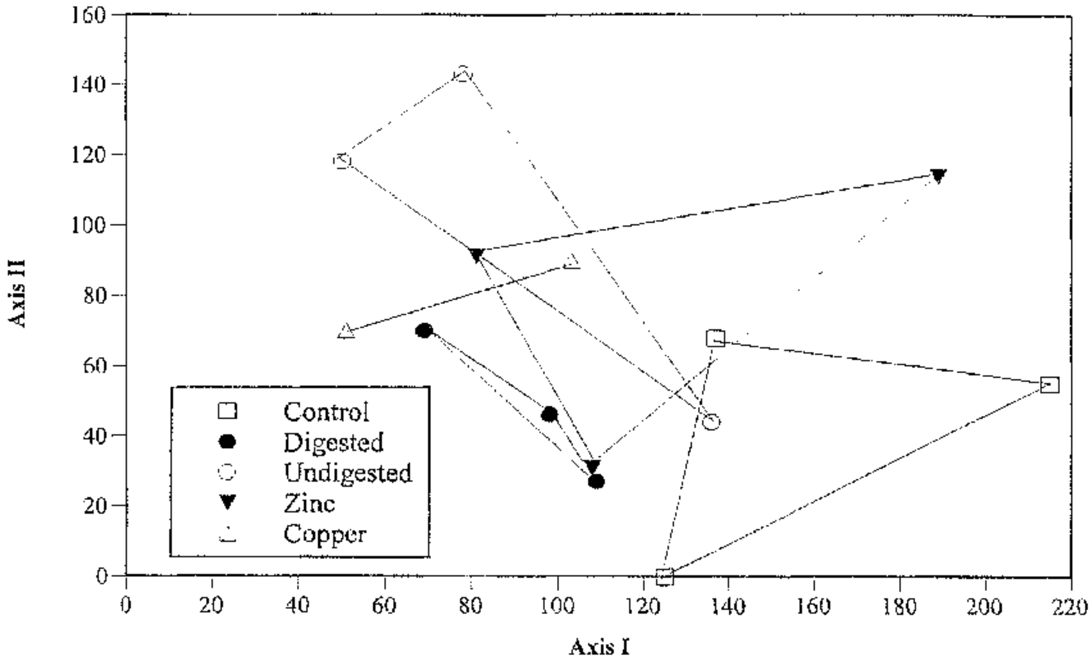
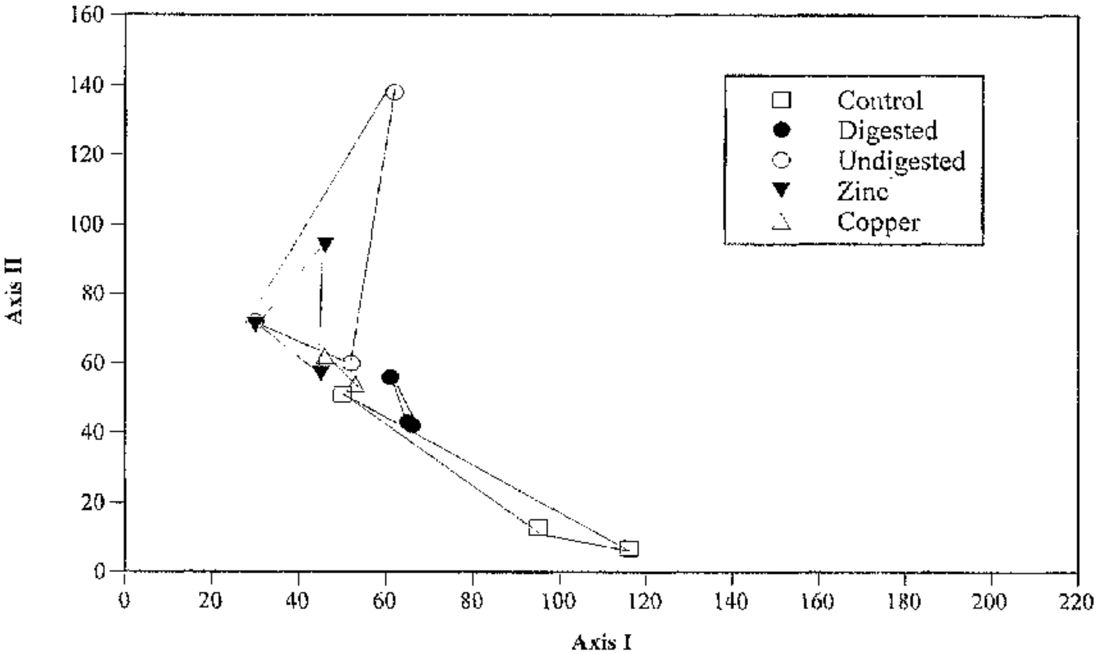


Figure 4.14 DCA ordination for 22/7/95 showing treatment split.



Figures 4.13 & 4.14 DCA ordination for Auchincruive 3 and 22 July 1995 respectively. Each point represents one plot.

The separation of treatments is clearest on 22 July, however, even on this date there are large differences between replicas of a treatment. Closer examination of the ordinations found that block two differed most between the three replicas of a treatment. The DCA sample ordination therefore indicates that although block did not affect the abundance of any dominant species (see above), it affected the community as a whole. The pH and conductivity of block two was found to be lower than the other blocks prior to sludge application and this may have contributed to the observed separation of block two (see Appendix VI).

The species ordination indicates that moisture may be involved in the division of samples along axis 2 (see Figure 4.12). While *I. viridis* and *I. palustris* (Joosse 1970) prefer damp conditions and occur towards the top of the ordination, *L. cyaneus* and *Isotomodes productus* (Axelson) prefer dry conditions (Joose1970; Fjellberg 1980) and occur towards the bottom of the ordination. However, there is insufficient information on the humidity preferences of the species present to draw definite conclusions.

Decomposition

Decomposition rate was estimated from the loss of organic matter from the litterbags. Treatment and block effects were tested for using a 2-way ANOVA (on untransformed data), followed by the Tukey multiple comparison test. Results of these tests can be seen in Table 4.5 below.

Table 4.5 The effect of treatment and block on decomposition

Treatment	**Mean O.M. lost (g)**						
Control	0.29 *(± 0.08)*						
Digested	0.31 *(±0.10)*						
Undigested	0.32 *(±0.06)*	**Treatment**			**Block**		
Zinc	0.29 *(±0.09)*	**F-value**	**Prob df = 4, 180 n = 39**	**Where difference lies df = 180 *k = 5***	**F-value**	**Prob df =2, 180 n = 65**	**Where difference lies df = 180 *k = 3***
Copper	0.36 *(±0.07)*	5.09	< 0.001	Cu > C & Zn	5.11	< 0.01	Block 3 > Block 1

Table 4.5 This table shows the mean decomposition rate for each of the five treatments (standard deviations are given below in brackets). It also shows the F and probability values (for treatment and block) obtained by 2-way ANOVA and the results of the Tukey test ($P<0.05$). The following codes are used O.M. - organic matter, Cu - copper-rich sludge, C - control and Zn - zinc-rich sludge.

No interaction was found between block and treatment effect ($F=2.13$ at df 8, 180). The addition of digested, zinc-rich and undigested sludge did not significantly affect the decomposition rate. However, plots receiving the copper-rich sludge had significantly higher decomposition rates than the control plots. It would therefore appear that copper-rich sludge was indirectly beneficial to soil fertility through increasing the decomposition rate. A block effect was also apparent, with block one having a significantly lower decomposition rate than block three. A similar block effect was found for species richness which increased from block one to three. It is therefore possible that the greater number of species in block three increased the decomposition rate or that a third, unknown factor, affected both decomposition and species richness.

4.6 Discussion

Effect of date on species composition

Date and succession were the main factors that determined the collembolan abundance and species composition in the litterbags, while treatment was less important. It would appear that with respect to sampling date, collembolan species fall into three main categories: species that are dependant on season (e.g. *L. cyaneus, H. nitidis* and *Mesaphorura* spp.); species that are dependant on the successional stage of the leaf litter (e.g. *I. notabilis* and *I. palustris*); and species that are unaffected by season and succession (e.g. *F. fimetaroides, F. fimentaria* and *N. minimus*). Other litterbag studies have also found both seasonal and successional effects on populations of Acari and Collembola (Anderson 1975; Bolger 1985).

Seasonal effects on collembolan abundance are dependant on species, year and geographical location (Christiansen 1964). In the UK there is a tendency for population peaks to occur in winter and summer (Christiansen 1964; Bardgett *et al.* 1993). Niijima (1975) found that Entomobryidae (the family in which both *H. nitidis* and *L. cyaneus* belong) peaked in abundance during summer in a temperate forest in Japan, and Brussaard *et al.* (1990) found *Mesaphorura krausbaueri* increased from April to July in agricultural land in the Netherlands. Joosse (1969) found that *L. cyaneus* was more abundant in the Netherlands during summer. Through examining the size classes of *L. cyaneus*, Joosse (1969) concluded that this species over-winters as half-grown individuals emerging in spring and laying eggs shortly afterwards. In the present study, *L. cyaneus* may therefore have been present in February, but as they were immobile they were not sampled by the litterbags until emerging sometime between 24 April and 3 July 1995. The similar seasonal pattern in *H. nitidis* suggests that it had a similar life-history.

Isotoma notabilis was the dominant collembolan species in the litterbags during the early stages of decomposition, and became less abundant as the leaf litter decomposed. This species was also dominant in the early successional stages of litterbags in a study by Bolger (1985). Odum (1969; 1985) suggested that r-selected species are more likely to be early colonisers, and as *I. notabilis* is asexual it can be considered to be relatively r-selected. *Isotoma notabilis* may also prefer to feed on primary saprophytes abundant in the early stages of decomposition. Both *I. palustris* and *I. anglicana* colonised the bags in the latter stages of succession suggesting that they may prefer to feed on secondary saprophytes. More work is therefore required to examine if feeding preferences play a role in the succession of these collembolan species in leaf litter.

Care must, however, be taken in interpreting these results as it is possible that other environmental changes occurred between 3 and 22 July that could have resulted in the observed change in species composition. For example, the grass was cut on 17 July and consequently the grass height was much lower on 22 July than 3 July. The decrease in grass height would result in changes in the micro-climate of the soil, with fluctuations in temperature and humidity being more pronounced. However, as the grass height was not measured, no conclusion can be drawn concerning its effect on the collembolan community.

<u>Effect of sewage sludge on Collembola</u>

The addition of sewage sludge has generally been found to increase the abundance of Collembola (Lübben 1989; Höller-Land 1959), and this is thought to be primarily the result of increases in food (Pimentel & Warneke 1989). In this study, both the digested and undigested sewage sludge had higher numbers of Collembola than the control or metal-rich sludges, however, as a consequence of the large variation between replicas within treatments, this increase was not significant. It is also notable that the sludge treatments did not affect the collembolan diversity, evenness or species richness. Diversity may have been expected to be reduced by the addition of metal-rich sludge, as heavy metals have previously been found to adversely affect diversity (Bengtsson *et al.* 1985a).

Neelus minimus and *Mesaphorura* spp. were adversely affected by zinc-rich sludge and *Mesaphorura* spp. were also adversely affected by copper-rich sludge. The susceptibility of *Mesaphorura* spp. to metal-rich sludge is surprising as *M. krausbaueri* was found to be favoured in areas where copper levels are high as a result of the intensive use of copper containing fungicides (Filser *et al.* 1995). Furthermore, *Mesaphorura* spp. occurred in their highest abundance in plots treated with metal-rich sludge (spiked with metal salts) in a study by Lübben (1989). The discrepancies between these results and the Auchincruive results may be the consequence of species specific differences in metal susceptibility existing within the genus *Mesaphorura*. It is well documented that species differ in their susceptibility to metal pollution (Filser *et al.* 1995; Tranvik & Eijsackers 1989; Posthuma & Van Straalen 1993).

Metals can be directly toxic to Collembola through decreasing their growth rate, reproduction and/or survival (Bengtsson *et al.* 1983; Joosse & Verhoef 1983; Posthuma *et al.* 1993). Metals may also be indirectly toxic through their action on fungi and habitat structure (Tranvik & Eijsackers 1989; Bengtsson *et al.* 1985a; Posthuma *et al.* 1993). For example, Tranvik & Eijsackers (1989) found that the metal tolerant species *F. fimetarioides* had a higher preference for metal tolerant fungi than the metal sensitive species *I. minor*.

As heavy metals decrease with soil depth (Bengtsson & Rundgren 1988), it is possible that *Mesaphorura* spp. and *N. minimus* did not actually decrease in abundance, but instead migrated down the soil profile to avoid the metals. There is evidence that euedaphic species migrate to greater soil depths (up to a depth of 10 cm) in metal polluted areas (Bengtsson & Rundgren 1988), and the migration of soil Collembola down the soil profile to avoid adverse weather conditions is well documented (Hale 1967; Marshall 1974). However, as the sewage sludges (and therefore the heavy metals) were incorporated into the soil to a depth of up to 25 cm, it is unlikely that the Collembola would be able to follow a concentration gradient to less polluted areas.

Isotoma anglicana was promoted by the addition of copper-rich and zinc-rich sludge, while *N. minimus* was promoted by the addition of copper-rich sludge. Other studies have also found some species of Collembola to be promoted by metals while others are adversely affected (Filser *et al.* 1995; Tranvik & Eijsackers 1989; Lübben 1989). Zinc and copper are essential micro-nutrients (Hopkin 1989) which are required in trace quantities by Collembola (Bengtsson *et al.* 1983). It is possible that *I. anglicana* and *N. minimus* were deficient in these metals prior to sludge application and were therefore favoured when metal levels increased following application. Low levels of copper have been found to increase the growth rate of *O. armatus* in the laboratory (Bengtsson *et al.* 1983), and the collembolan density in the vicinity of Gusum brass mill was higher at low levels of copper and zinc pollution than at unpolluted areas (Bengtsson & Rundgren 1988). The increase in abundance at low metal levels may also be the consequence of these species possessing metal-sensitive gut parasites (Bengtsson & Rundgren 1988), or of predators and/or competitors being more susceptible to zinc and copper. *Isotoma viridis*, which is closely related to *I. anglicana*, has previously been found to be sensitive to the presence of zinc-rich sewage sludge (Bruce *et al.* 1997).

It is important to note that as a consequence of the sludges arising from different sewage treatment works, other differences between sludges existed that may have affected the collembolan community. For example, the copper-rich sludge and the zinc-rich sludge had higher levels of nickel, lead, chromium and iron than the uncontaminated sludges (see Appendix II). It is also possible that the metal-rich sludges, being derived from more industrial areas, had higher levels of organic pollutants such as polynuclear aromatic hydrocarbons (PAH's) and polychlorinated biphenyls (PCB's). To determine which component or components were responsible for the observed changes in species composition it would be necessary to use a wider range of sludges which would be very time consuming. Alternatively, by artificially contaminating sludge with metal salts the effects of individual metals could be studied. Although this method is less time consuming, it is likely that the metal availability of artificially contaminated sewage sludge would differ from sludges which have been derived from treatment works with high metal inputs. Artificially contaminated sludge would not necessarily accurately reflect the influences of naturally contaminated sludge.

Diversity and succession

Goulden (1969) proposed that as leaf material decomposes, the organisms involved in decomposition progress through a series of developmental phases. In the first phase, the colonisation phase, species diversity increases as species rapidly immigrate into the newly established habitat. In the second phase, the common species become less common and the rare species less rare (the evenness of the community increases). In the third phase, rare species continue to colonise the habitat until it is fully saturated, and finally, in the fourth phase the diversity is degraded to a few abundant species adapted to the more unfavourable environment of the humus. Field studies using litterbags have, in general, supported Goulden's theory (Anderson 1975; Bolger 1985; Siepel 1990).

In this study, Collembola rapidly colonised litterbags within the first ten weeks of burial and this rapid colonisation was as predicted from phase one of Goulden's (1969) developmental phases. There was also a clear change in the evenness of the community with respect to succession. As the succession of leaf litter in the litterbags progressed, the evenness of the community increased which is indicative of Goulden's phase two of succession. However, as the species richness was predominately determined by the season, it was difficult to determine if developmental phase three had occurred and there is no evidence of phase four being reached.

Decomposition

Decomposition was measured as the organic matter lost from the litterbag and was therefore defined in its broadest sense for in addition to losses due to respiration and leaching, losses due to fragmentation, faeces production, faunal death and emigration were also included (Seastedt 1984). It was found that the addition of copper-rich sludge elevated the decomposition rate above that of the control. Chaney *et al.* (1978) found that low concentrations of the metal cadmium increased the activity of decomposers (measured by CO_2 efflux). This was thought to be a consequence of cadmium-sensitive species being replaced by more tolerant species which respired at a faster rate (Hopkin 1989). The change in species composition in the copper-rich plots may have similarly altered the decomposition rate, especially if *I. anglicana* and *N. minimus* respire faster than *Mesaphorura* spp. A faster respiration rate of *I. anglicana* than *Mesaphorura* spp. may be expected as hemiedaphic species are thought to have a higher level of activity and metabolic rate than euedaphic species (Van Straalen 1989). This can be supported by the fact that *I. anglicana* has longer legs than *Mesaphorura* spp. and well developed furca (jumping organ), suggesting it has a more active life.

Cadmium is generally considered as a non-essential element in invertebrates, whereas, copper is a micro-nutrient that is required in small concentrations (Hopkin 1989; Pokarzhevskii & Van Straalen 1996). It is therefore possible that previous to its addition copper was the limiting element to decomposition, and through adding copper this deficiency was eliminated and the rate of decomposition was increased. The essential levels of micro-nutrients in invertebrates is relatively understudied (Hopkin 1989), and it is therefore difficult to determine if the original soil concentration was limiting. However, current evidence indicates that copper deficiency in livestock may be widespread in Scotland (Smith 1996).

There are several drawbacks in using litterbags to study the decomposition rate. Firstly, the artificial confinement of leaf-litter may adversely affect the fungal productivity and hence would also affect the contribution of Collembola to decomposition (Seastedt 1984). Confinement has been found to underestimate the true decomposition rate (Witkamp & Olson 1963). Despite the adverse effects of artificial confinement, the ability to exclude macrofauna without the use of chemicals and in near normal conditions in the field makes litterbags the most popular method of studying decomposition (Seastedt 1984).

4.7 Main findings

1: Date and season were the main factors influencing the collembolan community with effects of treatment being less pronounced.

2: *Mesaphorura* spp., *H. nitidis* and *L. cyaneus* increased as the summer progressed as did the species richness.

3: *Isotoma anglicana* and *I. palustris* were late colonisers of decomposing oak leaves, while *I. notabilis* was an early coloniser. The evenness of the community increased as succession progressed and this resulted in an increase in overall diversity.

4: *Isotoma anglicana* was favoured by the addition of copper-rich and zinc-rich sludge, while *Mesaphorura* spp. were adversely affected. *Neelus minimus* was favoured by the addition of copper-rich sludge, but adversely affected by the addition of zinc-rich sludge.

5: The decomposition rate was highest in the copper-rich plots. This may be the consequence of copper previously being the limiting element to decomposition, or it may be the result of changes in the community structure of decomposers.

6: Block effects were apparent for the species richness and decomposition rate, with block three having a higher richness and decomposition rate than block one. It is possible that the higher decomposition rate was the consequence of the higher species richness. Alternatively, it is possible that a third unknown factor (e.g. soil moisture) affected both the decomposition rate and the species richness.

CHAPTER 5. Effects of sludge on hemiedaphic and epigeal Collembola as indicated by suction sampling

5.1 Introduction

Epigeal Collembola are an important food source for predators such as staphylinid beetles, carabid beetles and harvestmen (Hopkin 1997). As a consequence of this, they help to maintain healthy populations of polyphagous predators which are important in the control of agricultural pests. Furthermore, because they are larger than euedaphic Collembola, they are easier to work with and to identify. The majority of studies that look at the effects of agricultural practices on Collembola use soil cores as a method of sampling. As soil cores are inefficient at sampling epigeal Collembola (trapping on average less than one epigeal individual per soil core: Frampton *et al.* 1992), these species are frequently left unstudied.

The suction sampler provides a non-destructive method of sampling arthropods and is particularly useful for sampling small arthropods, such as Collembola, which cannot realistically be collected by the laborious and inefficient method of ground searching (Duffey 1974). Suction sampling is a more accurate sampling method than sweeping which is liable to large personnel errors (Johnson *et al.* 1957). It also provides a more accurate measurement of population densities than pitfall traps which are dependant not only on the abundance of species present, but also on the activity level of each species.

Suction sampling provides good quantitative data on collembolan population densities and Johnson *et al.* (1957) found a sampling efficiency of 99.3 % for Arthropleona and 100 % for Symphypleona with the Wolf Portable Electric Blower. Suction samplers have successfully been used to monitor the effects of insecticides (Frampton 1988), herbicides (Sotherton *et al.* 1987), fungicides (Frampton 1988), grazing (Purvis & Curry 1978), vegetation type (Curry & Cunningham 1978) and management intensity (Moreby *et al.* 1994) on epigeal and hemiedaphic Collembola.

Suction samplers do, however, have some limitations. For examples they are only applicable for sampling invertebrates on the surface or in the more accessible crevices of plants and the soil (Johnston *et al.* 1957). Furthermore, the sampling of small arthropods may be inefficient in damp habitats or wet weather because they can get stuck to the inside of the flexible hose in some suction samplers (Johnston *et al.* 1957). A further problem of suction sampling arises from the fact that animals in their natural habitats are commonly patchily distributed. This is problematic because suction sampling generally samples only a small area at one time (Southwood 1971). However, suction sampling is more effective than sweeping and as sweeping only samples epigeal specimens, suction is also a more complete method (Berbiers *et al.* 1989)

Samples derived from suctioning often contain leaves, grass and other debris and, as a consequence of this, hand sorting is laborious and time consuming (Marshall *et al.* 1994). Hand sorting is also prone to personnel error, especially when dealing with small arthropods (Johnston *et al.* 1957). This problem can, however, be overcome by floatation to extract inorganic soil followed by sieving to remove the larger debris (Frampton 1988).

The Dietrick vacuum insect sampler (Dietrick *et al.* 1959) and related designs (e.g. the Thornhill vacuum sampler) have become standard sampling techniques in agricultural research (MacLeod *et al.* 1994). However, these suction samplers are heavy and can become uncomfortable with prolonged use (MacLeod *et al.* 1994). The Ryobi RSV3100E sweeper-vac (as modified by MacLeod *et al.* 1994) is a cheaper, lighter and less noisy alternative to original suction samplers (Harwood 1994). Furthermore, the Ryobi sweeper vac has been found to be more efficient than conventional samplers in sampling several insect species (MacLeod *et al.* 1994). Since the insects are taken straight into the collecting bag, the problem of insects getting trapped inside the suction hose is also eliminated.

In this Chapter Ryobi suction samplers are used to determine the effects of different sewage sludges on the epigeal and hemiedaphic collembolan populations, while in Chapter 6 pitfall trapping will be used. A comparison will then be made between these two methods in Chapter 7.

5.2 Methods

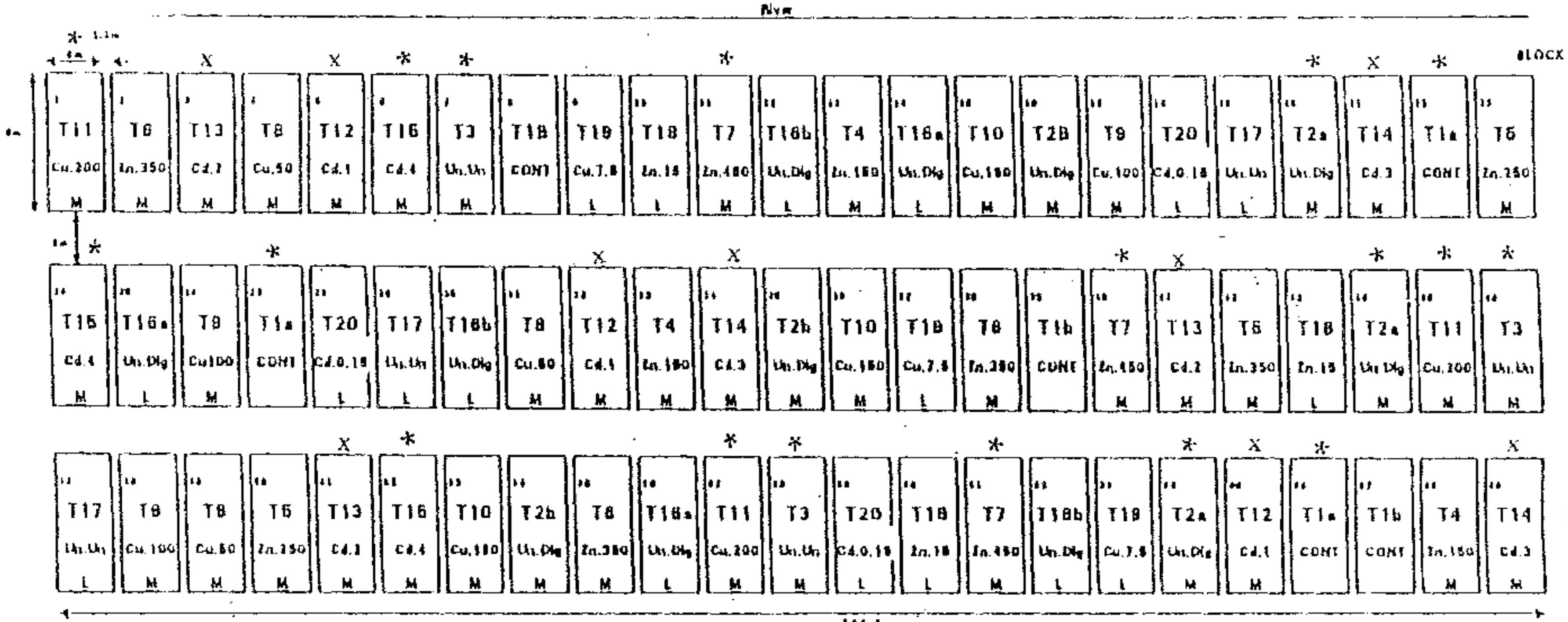
Trial sites and treatments

The epigeal and hemiedaphic Collembola were sampled in 1995 and 1996 at the SAC Auchincruive site, and in 1996 at the Hartwood site (both trial sites are described in Chapter 3). The sludge treatments investigated can be seen in Table 5.1, together with the year in which they were studied. A detailed analysis of the sludges is given in Appendix II for the 1994 sludge application and Appendix X for the 1995 sludge application. The plots studied are marked with an asterix in Figure 5.1 (Auchincruive) and Figure 5.2 (Hartwood). The effects of six different sludge treatments were studied in 1995 and four were studied in 1996. In 1996, the undigested uncontaminated and the undigested copper-rich treatments were omitted to enable a more detailed investigation of the remaining four treatments. The digested treatments represent a more common situation because digested sludge is used more frequently as an agricultural fertiliser. Furthermore, the digested treatments provided information on an essential micro-nutrient (zinc) and non-essential metal (cadmium).

Table 5.1 Treatments

Treatment number	Treatment	Mean metal concentration/application rate	Sewage treatment works	Year sampled
1	Control	Background levels (see Appendix XI)	-	1995 & 1996
2A	Digested uncontaminated sludge	Low levels (see Appendices II, III, X & XI)	Banbury	1995 & 1996
3	Undigested uncontaminated sludge	Low levels (see Appendices II, III)	Carterton	1995
7	Zinc-rich digested sludge	7,036 mg Zn kg^{-1} sludge 288.5 kg Zn ha^{-1} $year^{-1}$ 180 mg Zn kg^{-1} soil 1995 270 mg Zn kg^{-1} soil 1996	Coleshill	1995 & 1996
11	Copper-rich undigested sludge	3,394 mg Cu kg^{-1} sludge 67.9 kg Cu ha^{-1} $year^{-1}$ 69 mg Cu kg^{-1} soil 1995	Selkirk	1995
15	Cadmium-rich digested sludge	48.9 mg Cd kg^{-1} sludge 4.3 kg Cd ha^{-1} $year^{-1}$ 1.2 mg Cd kg^{-1} soil 1995 2.2 mgCd kg^{-1} soil 1996	Perry Oaks	1995 & 1996

Figure 5.1 SAC Auchincruive contaminated sludge plan showing plots sampled by suction



Codes: 1 - 69 Plot numbers
T1 - T20 Treatment numbers
I - III Block numbers

Build-up rate: M Moderate build-up
L Long-term build-up

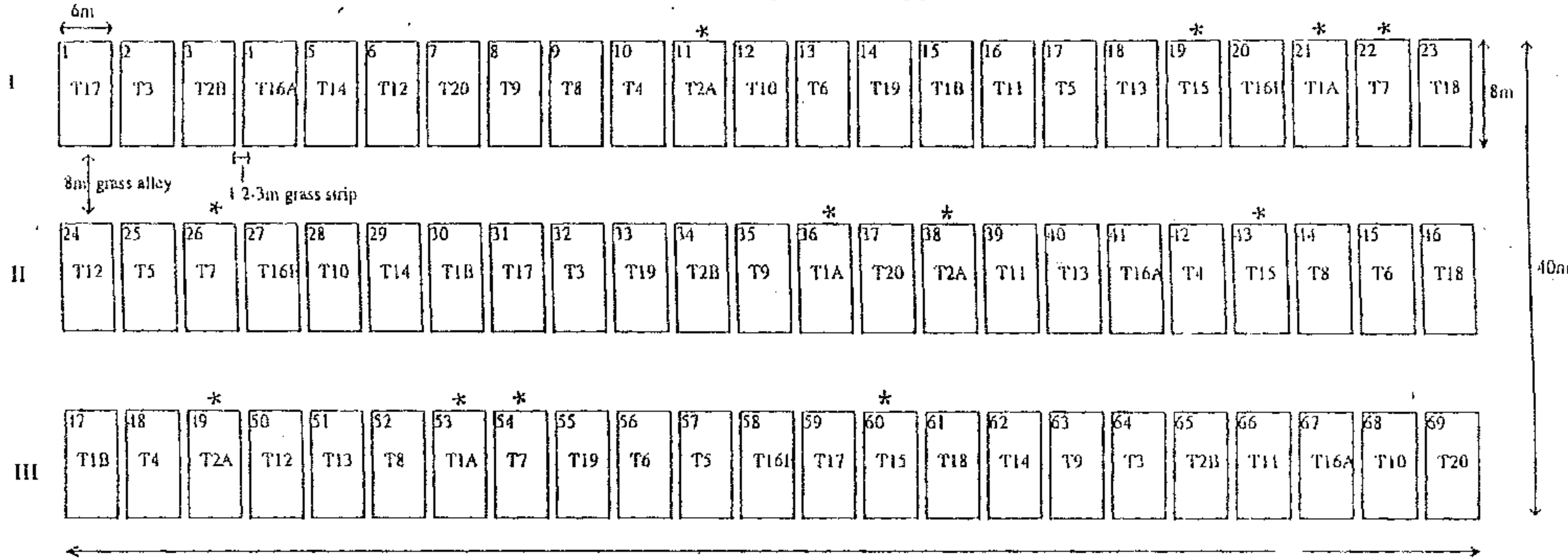
Treatment type:
CONT Control
Un.Dig Uncontaminated digested
Un.Un Uncontaminated undigested

Zn.150 High Zn (target soil metal concentration 150 mg kg^{-1})
Zn.250 High Zn (target soil metal concentration 250 mg kg^{-1})
Zn.350 High Zn (target soil metal concentration 350 mg kg^{-1})
Zn.450 High Zn (target soil metal concentration 450 mg kg^{-1})
Zn.15 High Zn (15 kg ha^{-1} Zn annually)

Cu.50 High Cu (target soil metal concentration 50/mg kg^{-1})
Cu.100 High Cu (target soil metal concentration 100/mg kg^{-1})
Cu.150 High Cu (target soil metal concentration 150/mg kg^{-1})
Cu.200 High Cu (target soil metal concentration 200/mg kg^{-1})
Cu.7.5 High Cu (7.5 kg ha^{-1} annually)

Cd.1 High Cd (target soil metal concentration 1 mg kg^{-1})
Cd.2 High Cd (target soil metal concentration 2 mg kg^{-1})
Cd.3 High Cd (target soil metal concentration 3 mg kg^{-1})
Cd.4 High Cd (target soil metal concentration 4 mg kg^{-1})
Cd.0.15 High Cd (0.15 kg ha^{-1} Cd annually)

Figure 5.2 Hartwood contaminated sludge plan showing plots sampled by suction



<u>Codes:</u> 1 - 69 Plot numbers
T1 - T 20 Treatment numbers
I - III Block numbers

<u>Build-up rate:</u> M Moderate build-up
L Long-term build-up

<u>Treatment type:</u>

CONT	Control
Un.Dig	Uncontaminated digested
Un.Un	Uncontaminated undigested
Zn.150	High Zn (target soil metal concentration 150 mg kg^{-1})
Zn.250	High Zn (target soil metal concentration 250 mg kg^{-1})
Zn.350	High Zn (target soil metal concentration 350 mg kg^{-1})
Zn.450	High Zn (target soil metal concentration 450 mg kg^{-1})
Zn.15	High Zn (15 kg ha^{-1} Zn annually)
Cu.50	High Cu (target soil metal concentration 50/mg kg^{-1})
Cu.100	High Cu (target soil metal concentration 100/mg kg^{-1})
Cu.150	High Cu (target soil metal concentration 150/mg kg^{-1})
Cu.200	High Cu (target soil metal concentration 200/mg kg^{-1})
Cu.7.5	High Cu (7.5 kg ha^{-1} annually)
Cd.1	High Cd (target soil metal concentration 1 mg kg^{-1})
Cd.2	High Cd (target soil metal concentration 2 mg kg^{-1})
Cd.3	High Cd (target soil metal concentration 3 mg kg^{-1})
Cd.4	High Cd (target soil metal concentration 4 mg kg^{-1})
Cd.0.15	High Cd (0.15 kg ha^{-1} Cd annually)

Initial findings from 1995 suggested that cadmium was particularly toxic to some species. It was therefore decided to sample all four cadmium concentrations on 4 August 1996 to investigate the effects of a range of concentrations on the epigeal and hemiedaphic Collembola. The plots sampled only on 4 August are marked with a cross on Figure 5.1. The concentrations of cadmium in the sludge and plots can be seen in Table 5.2. The ranges of cadmium concentrations were obtained by mixing the Perry Oaks cadmium-rich sludge with the Banbury digested sludge. Mean weight of the Banbury and Perry Oaks sludge applied to each plot can be seen in Table 5.2. This enabled the cadmium concentration to be altered without changing the organic input.

Table 5.2 Cadmium treatments sampled on 4 August 1996

Treatment number and treatment	Mean metal concentration 1996	Mean weight of Perry Oaks sludge applied	Mean weight of Banbury sludge applied
12 Low cadmium	14.7 mg Cd kg^{-1} sludge 1.0 kg Cd ha^{-1} $year^{-1}$ 0.7 Cd kg^{-1} soil	19 t ds ha^{-1}	68 t ds ha^{-1}
13 Medium cadmium	25.3 mg Cd kg^{-1} sludge 2.1 kg Cd ha^{-1} $year^{-1}$ 1.2 mg Cd kg^{-1} soil	42 t ds ha^{-1}	45 t ds ha^{-1}
14 High cadmium	35.8 mg Cd kg^{-1} sludge 3.2 kg Cd ha^{-1} $year^{-1}$ 1.7 mg Cd kg^{-1} soil	65 t ds ha^{-1}	22 t ds ha^{-1}
15 Very high cadmium	48.9 mg Cd kg^{-1} sludge 4.3 kg Cd ha^{-1} $year^{-1}$ 2.2 mg Cd kg^{-1} soil	87 t ds ha^{-1}	0

Sampling method

The Ryobi RSV3100E sweeper-vac (as modified by MacLeod *et al.* 1994) was used to sample Collembola. The Ryobi, which was originally designed to collect leaf litter and light-weight garden debris, is readily available from retail outlets throughout the UK for about £200. This machine is powered by a 31 cc air cooled engine operating at 6,800 - 7,000 rpm (manufacturer's data) and this generates a mean air speed of 16 m s^{-1} (MacLeod *et al.* 1994). Fine weave (< 0.5 mm) nylon/cotton mix voile bags were used to collect the samples and the bags were renewed after each sample was collected.

During 1995, suction samples were taken at the Auchincruive site on 12 and 25 July. In 1996, suction samples were taken at the Auchincruive site on 30 April, 31 May, 22 June and 4 August and at the Hartwood site on 12 August.

Each suction sample covered an area of 706 cm^2 which was sucked for 60 seconds. On each sampling date, five such samples were taken at random from each plot. No samples were taken within a distance of one metre from the edge of the plot to eliminate any edge effect (Gill 1969). To standardise across sampling dates, all suction samples were taken on sunny days when the grass was dry and between 10.00 - 17.00 hours.

Immediately after return to the laboratory, suction samples were placed in a freezer and left until a later date when they could be processed fully. The organic matter was extracted from the inorganic soil particles by repeated flotation in a saturated salt solution. The resultant organic matter (including Collembola) was then passed through two graded sieves (15 mm and 45 μm). These separated the debris (mainly blades of grass) from the Collembola. Collembola were then heated in alcohol to prevent desiccation and identified primarily following the keys of Fjellberg (1980) and Gisin (1960). See Chapter 3 for further information on identifying Collembola.

5.3 Statistical analysis

The data from the five samples collected from a plot on a particular sampling date were pooled to obtain an indication of collembolan community structure for each plot on each sampling date. The pooled data were utilised in statistical analyses. In all analyses, the 1995 and the 1996 data sets were investigated separately.

Species composition Auchincruive

Three-way analyses of variance (ANOVAs) were performed (on the square root transformed data) to test for date, block and treatment effects. ANOVAs were performed on the number of total Collembola and of the most frequently occurring species (i.e. occurring in over 50% of samples). To find the location of differences detected by ANOVA, Tukey multiple comparison tests were applied (Zar 1984). Although the square-root transformation normalised the data, in some species the variances still differed considerably between replicas of a treatment (F-test). To prevent wrongly rejecting the null hypothesis the significance level was set at 0.01 (rather than 0.05: Day & Quinn 1989).

Species composition Hartwood

As suction samples were only taken on one occasion at Hartwood, it was not possible to look for an effect of date. Two-way ANOVAs were therefore performed on the square-root transformed values to test for block and treatment effects. Tukey multiple comparison tests were then applied to find the location of differences found by ANOVA. As the variances were heterogeneous the probability value was set at 0.01.

Detrended Correspondence Analysis

DCA's were performed on the species relative abundance (without downweighting of rare species) using DECORANA (Hill 1979). DCA's were performed separately for the 1995 and 1996 data sets, and for the Hartwood and Auchincruive data sets. The species and sample scores for axes one and two were plotted to obtain species and sample ordinations respectively. DCA provides a method of looking simultaneously at the effects of treatment, plot and season on the overall collembolan structure.

Correlation analyses

A product moment correlation was calculated to investigate the relationship between grass height and the abundance of the most frequently occurring species at Auchincruive in 1996. The total number of each species (square root transformed) on a particular plot and sampling date was correlated against the mean grass height on that plot and sampling date. This correlation was performed on all plots sampled in 1996 (with the exception of the cadmium plots sampled only on 4 August) and all sampling dates.

In order to investigate the relationship between the abundance of the most frequently occurring species and the concentration of cadmium, a Spearman's rank correlation was calculated for the cadmium plots sampled at Auchincruive on 4 August 1996 (see Table 5.2). The digested plots were also sampled on this date to give a background cadmium level. It was decided to use the Spearman's instead of the product moment correlation because of the low number of observations and, as the cadmium concentration was not directly measured in all plots, an ordinal scale was used for cadmium concentrations.

5.4 Results

General information

At Auchincruive a total of 17,765 individuals from 15 species were identified in 1995, and 122,323 individuals from 22 species were identified in 1996 (see Appendix XII for species lists). In 1996 almost all samples taken at Auchincruive had a higher number of Collembola present than in 1995. At Hartwood a total of 22,082 individuals from 17 species were identified (see Appendix XIII for species list).

Results of ANOVAs

At Auchincruive the effects of date, block and treatment were investigated using 3-way ANOVAs on 1995 and 1996 data separately. Effects of treatment and block were investigated at Hartwood using a 2-way ANOVA.

Species composition and block

Although differences in soil parameters were found between blocks (see Appendix VI), no block effect was shown for any of the species investigated at either site. It would therefore appear that while date and treatment influenced species composition, block did not.

Species composition and date: Auchincruive

In 1995, the number of total Collembola, *L. cyaneus*, *S. elegans*, *S. aureus*, *H. nitidis*, *I. palustris*, *I. anglicana* and *I. notabilis* were more abundant in suction samples taken on 25 July than 12 July (see Table 5.3). This may have been the consequence of environmental conditions (e.g. humidity and temperature) being more favourable for these species on 25 July. As the grass was cut on 17 July, the higher abundance of these species on 25 July could also have been the result of an increase in their sampling efficiency in the shorter grass. Only one species was found to be more abundant on 12

Table 5.3 Effect of date and treatment on species sampled by suction: Auchincruive 1995

	Date					Treatment		
Species	F-value	Probability df = 1, 10 n = 18	12 July	25 July	Location of difference df = 10, k = 2	F-value	Probability df = 5, 10 n = 6	Location of difference df = 10, k = 6
Total	34.54	< 0.001	15.9 *±4.7*	26.0 *±6.2*	25 July > 12 July	2.29	N.S.	-
I. viridis	0.42	N.S.	5.6 *±3.3*	6.3 *±3.0*	-	2.49	N.S.	-
I. anglicana	111.95	< 0.001	4.6 *±2.7*	15.1 *±4.3*	25 July > 12 July	3.71	0.01	U, Cu & Cd > C Cd > D
I. notabilis	27.27	< 0.001	1.3 *±1.1*	3.3 *±1.5*	25 July > 12 July	3.62	0.01	U > All
I. palustris	30.99	< 0.001	8.0 *±4.9*	16.7 *±5.6*	25 July > 12 July	3.23	< 0.05	Cd > C & Zn
L. cyaneus	9.23	< 0.01	1.0 *±1.2*	3.3 *±3.1*	25 July > 12 July	2.32	N.S.	-
H. nitidis	13.47	0.01	0.8 *±0.9*	2.2 *±1.1*	25 July > 12 July	0.40	N.S.	-
S. viridis.	38.10	< 0.001	10.1 *±1.6*	7.1 *±1.2*	25 July < 12 July	0.62	N.S.	-
S. aureus	49.13	< 0.001	0.8 *±1.1*	3.5 *±1.1*	25 July > 12 July	0.91	N.S.	-
S. elegans	20.90	< 0.001	0.3 *±0.6*	1.7 *±1.1*	25 July > 12 July	1.05	N.S.	-
C. denticulata	0.10	N.S.	1.5 *±1.5*	1.3 *±0.9*	-	0.95	N.S.	-

Table 5.3 The above table shows the results of the 3-way ANOVAs followed by Tukey tests (probability for Tukey tests set at 0.01 and k = number of means compared). The mean values (square root transformed) for the 2 sampling dates is also given (with the standard deviations given below). As no block effect was found, this information is omitted from the table (df = 2, 10). The following codes are used for treatments: C - Control, D - digested; U - undigested, Zn - zinc-rich, Cu - copper- rich and Cd - cadmium rich.

* Although the probability level for the ANOVAs was set at < 0.01, it was decided to include ANOVAs with P ≤ 0.05 when the Tukey test showed a difference at < 0.01.

July than 25 July, namely *S. viridis* (see Table 5.3). It is possible that environmental conditions were less favourable for this species on 25 July, or that it was adversely affected by grass cutting.

In 1996, the abundance of total Collembola, *Isotomurus/Isotoma* juveniles, *I. palustris*, *S. aureus* and *I. maculatus* increased from April to May, reaching their highest abundance in the May samples and then decreased again in the August samples (see Table 5.4 and Figures 5.3-5.4). This indicates that environmental conditions were more favourable for these species in May. In addition, as the grass was cut in May, these species could also have been sampled more efficiently in the shorter grass. The decrease in abundance of these species from June to August was therefore unlikely to be a consequence of grass cutting (especially since the abundance of total Collembola, *S. aureus* and *I. palustris* increased following grass cutting in 1995) and was therefore probably the consequence of environmental conditions being less favourable in August.

Figure 5.3 Effect of date on abundance of total Collembola and *I. palustris* (1996) irrespective of treatment

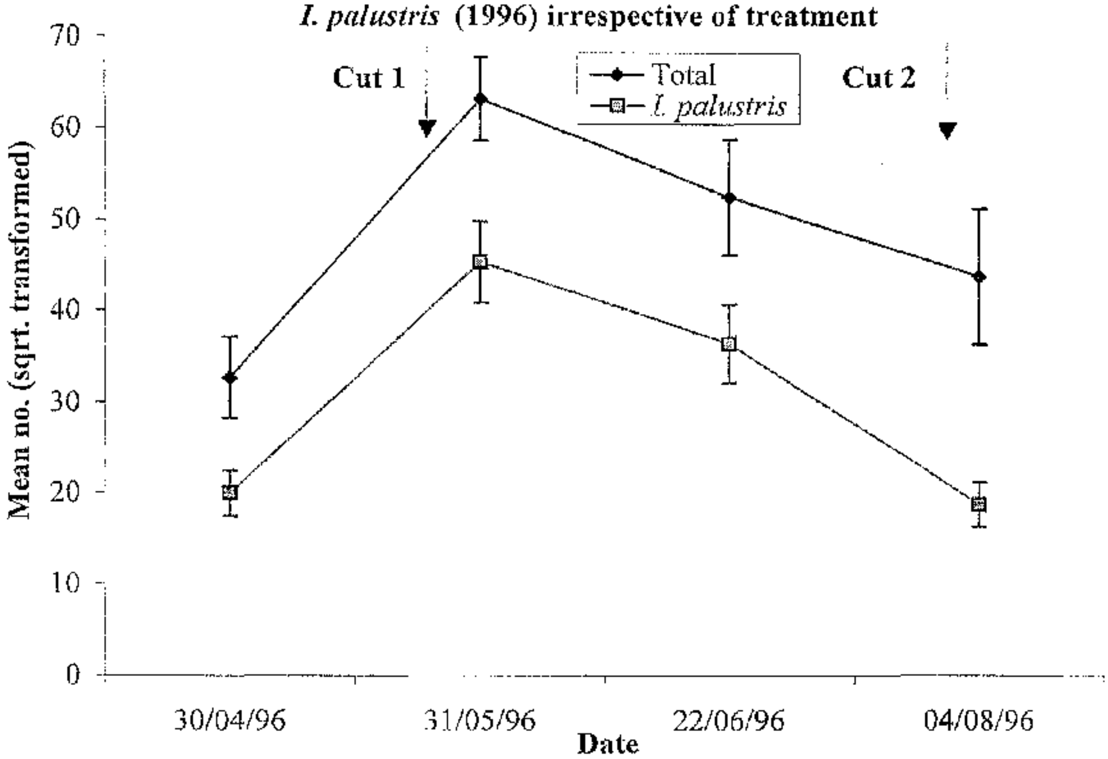


Figure 5.3 The above graph shows the effect of date on species abundance caught by suction sampling at Auchincruive in 1996. Error bars show standard deviations.

Table 5.4 Effect of date and treatment on species sampled by suction: Auchincruive 1996

	Date			Treatment		
Species	F-value	Probability df = 3 ,18 n = 12	Location of difference df = 18, *k* = 4	F-value	Probability df = 3 ,18 n = 12	Location of difference df = 18, *k* = 4
Total	20.04	< 0.001	May & June > April May > August	5.17	< 0.01	D > C
I. viridis	20.91	< 0.001	August > All	16.75	< 0.001	All > Cd
I. notabilis	7.29	< 0.001	May > June	2.12	N.S.	-
I. anglicana	129.75	< 0.001	May > April & August > June	24.14	< 0.001	All > C
I. palustris	86.70	< 0.001	May > June > August & April	12.51	< 0.001	Zn & Cd > C
I. maculatus	24.80	< 0.001	May > April & June > August	4.76	< 0.01	D > C
I. juvenile	56.36	< 0.001	May > June & April > August	2.66	N.S.	-
L. cyaneus	5.01	< 0.01	August > April	15.77	< 0.001	All > Cd
H. nitidis	8.12	< 0.001	August > All	8.12	< 0.001	D > C
T. longicornis	13.34	< 0.001	August > April & May June > April	1.34	N.S.	-
S. viridis.	4.60	< 0.01	June > April	23.15	< 0.001	C > All Cd > D & Zn
S. aureus	27.22	< 0.001	May & June > April > August	2.48	N.S.	-
S. elegans	1.58	N.S.	-	1.12	N.S.	-
S. malmgreni	13.91	< 0.001	April > All	1.89	N.S.	-
C. denticulata	31.76	< 0.001	April & May > June & August	6.08	< 0.01	Zn > C

Table 5.4 The above table shows the results of the 3-way ANOVAs followed by Tukey tests (probability for Tukey tests set at 0.01). As no block effect was found for any of the species tested (2, 18), this information is omitted from the table. Codes for the treatments: C - control, D - digested, Zn - zinc-rich and Cd - cadmium-rich.

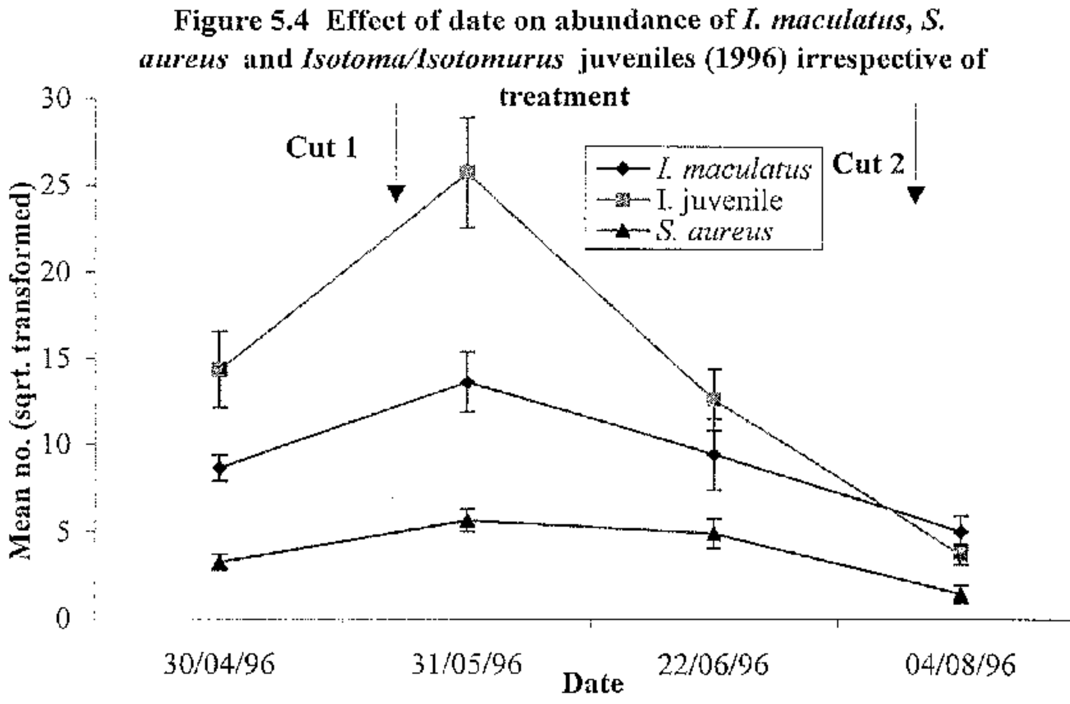


Figure 5.4 The above graph shows the effect of date on species abundance caught by suction sampling at Auchincruive in 1996. Error bars show standard deviations.

The abundance of *I. anglicana* also peaked in May 1996 and decreased again in June but, unlike the above species, this species was more abundant in August than June and occurred in its lowest abundance in June (Table 5.4 & Figure 5.5). *Isotoma notabilis* was also present in its lowest abundance in June when the grass was longest (Table 5.4, Figure 5.5 & Appendix VIII). Both these species increased after grass cutting in 1995 also. This suggests that they were either favoured in short grass, or more efficiently sampled in short grass. The abundance of *S. viridis*, on the other hand, was highest in June when the grass was longest (see Table 5.4, Figure 5.5 & Appendix VIII). This is in agreement with the 1995 samples where *S. viridis* was found to be more abundant before grass cutting. It would therefore appear that *S. viridis* was either favoured in long grass or adversely affected by grass cutting.

In 1996, several species tended to increase as the summer progressed and were found in their highest abundance in August (see Table 5.4 & Figure 5.6). For example, *T. longicornis* was more abundant in August than in April or May; *L. cyaneus* was more abundant in August than April; and *H. nitidis* and *I. viridis* were more abundant in August than on any other sampling date (see Table 5.4 & Figure 5.6). This suggests that conditions in August were more favourable for these species. Their increased abundance in August could also partly be a consequence of the suction sampler being more efficient at sampling these species in the shorter grass. In 1995, *L. cyaneus*, *I. viridis* and *H. nitidis* increased in late July following grass cutting. This further suggests that conditions became more favourable for these species as the summer progressed and that sampling was more efficient in shorter grass.

Figure 5.5 Effect of date on abundance of *I. anglicana, I. notabilis* and *S. viridis* (1996) irrespective of treatment

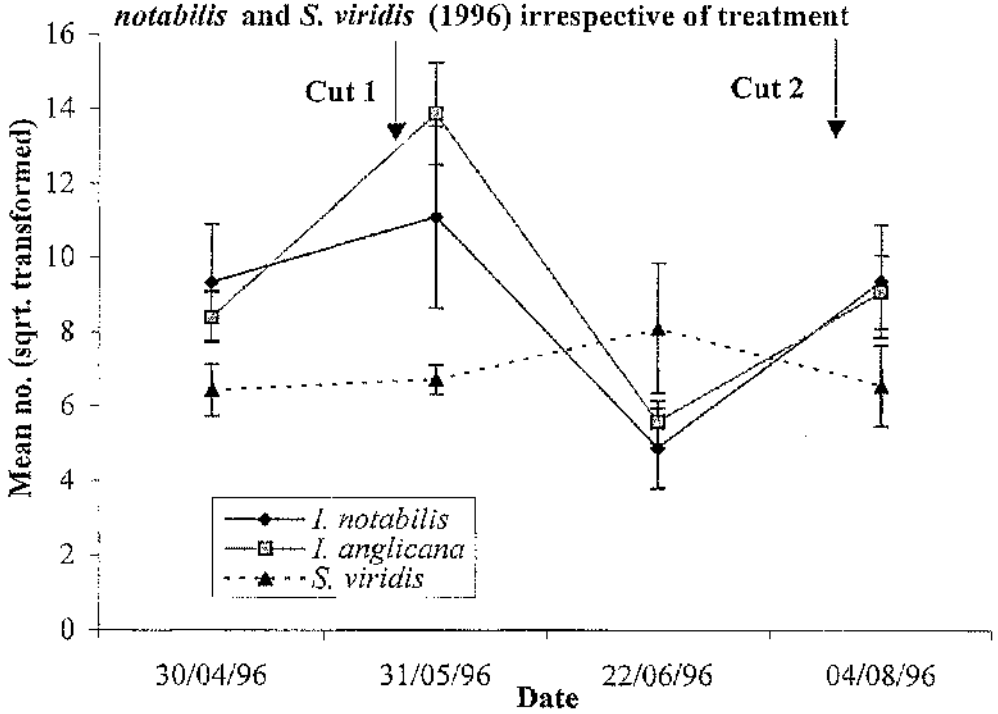
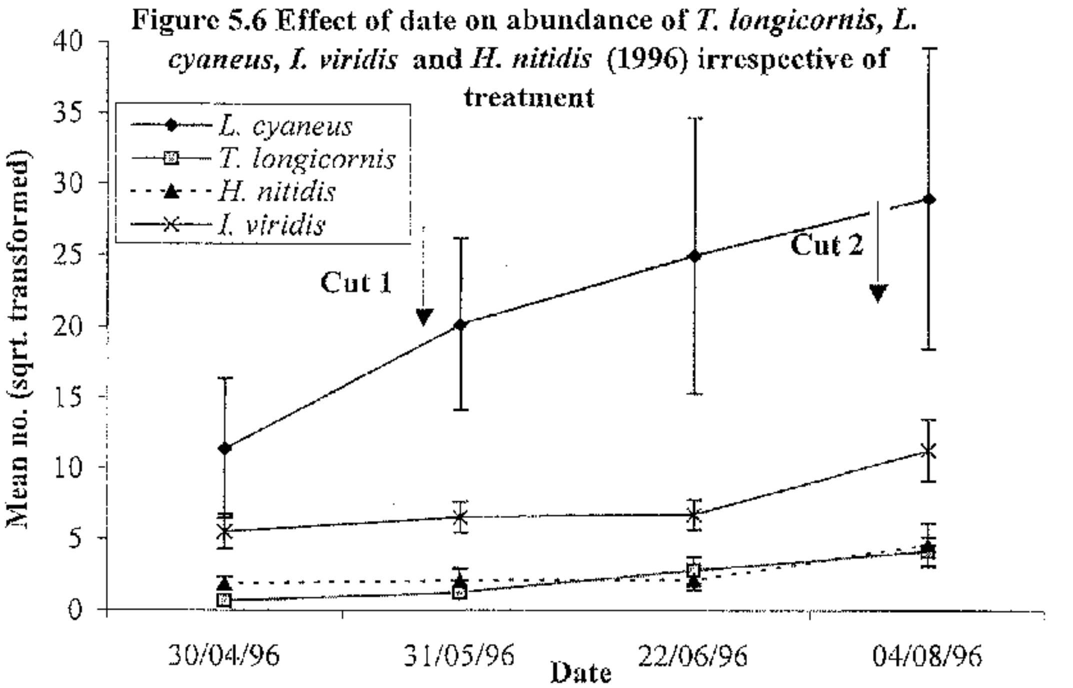


Figure 5.5 The above graph shows the effect of date on species abundance caught by suction sampling at Auchincruive in 1996. Error bars show standard deviations.

Ceratophysella denticulata was more abundant in April and May than in June or August and *S. malmgreni* occurred almost exclusively in the April samples (see Table 5.4 & Figure 5.7). It would therefore appear that these species were favoured by the cooler damper conditions in spring.



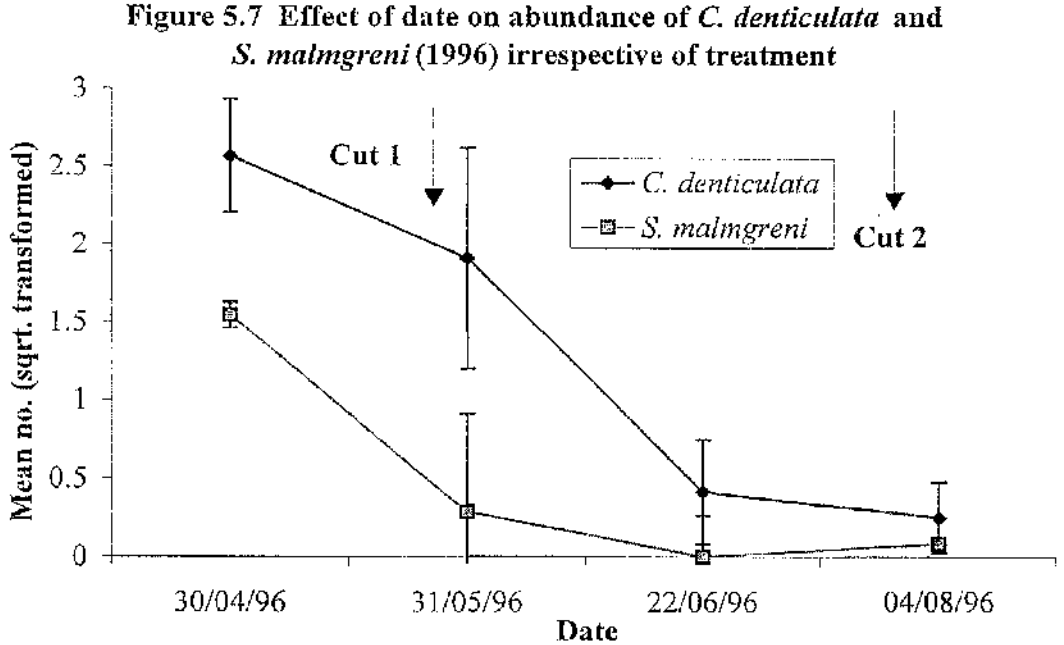


Figure 5.6 & 5.7 The above graphs show the effect of date on species abundance caught by suction sampling at Auchincruive in 1996. Error bars show standard deviations.

Species composition and treatment at Auchincruive

In 1995, the abundance of total Collembola was unaffected by the sludge treatments (see Table 5.3). Treatment effects were, however, apparent at the species level. *Isotoma notabilis* was more abundant in the undigested plots than in any other plots (see Table 5.3 & Figure 5.8). *Isotoma anglicana* was more abundant in the copper-rich, cadmium-rich and undigested plots than the control plots, and was also more abundant in the cadmium-rich plots than the digested plots (see Table 5.3 & Figure 5.8). *Isotoma anglicana* therefore appeared to be promoted by the addition of cadmium-rich, copper-rich and undigested sludge, but not by digested or zinc-rich sludge. *Isotomurus palustris* also showed a tendency to be more abundant on cadmium-rich than control plots, suggesting that this species was favoured by cadmium-rich sludge but not by any of the other sludge treatments (see Table 5.3 & Figure 5.8).

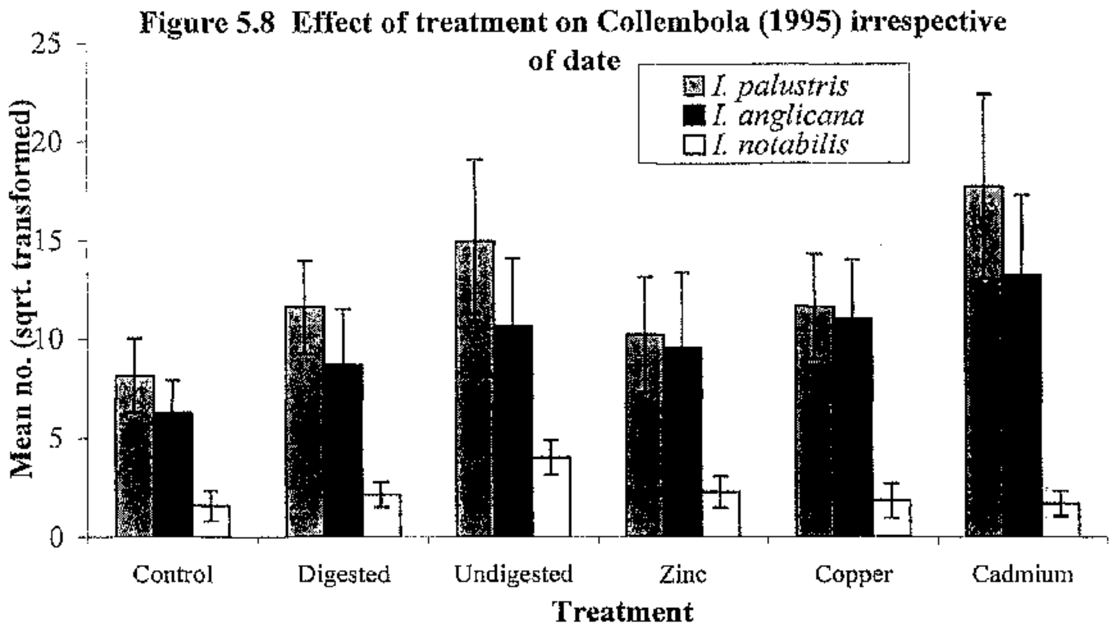


Figure 5.8 The above graph shows the effect of treatment on the species abundance caught by suction in 1995 at Auchincruive. Error bars show standard deviations.

The probability that the cadmium-rich and control plots were different with respect to the abundance of *I. palustris*, was found to be < 0.05 (see Table 5.3). As the variances were heterogeneous (F-test), the likelihood of a Type one error was increased (Day & Quinn 1989) and the results should therefore be treated with caution. The Tukey test, however, found that the probability of the cadmium-rich and control plots differing was < 0.01, hence suggesting that effects were real.

In 1996, the addition of digested sludge increased the abundance of *H. nitidis, I. maculatus* and total Collembola above that of the control plots (see Table 5.4 & Figure 5.9). When the sludge was contaminated with zinc or cadmium these species did not significantly increase, hence suggesting adverse effects of these metals.

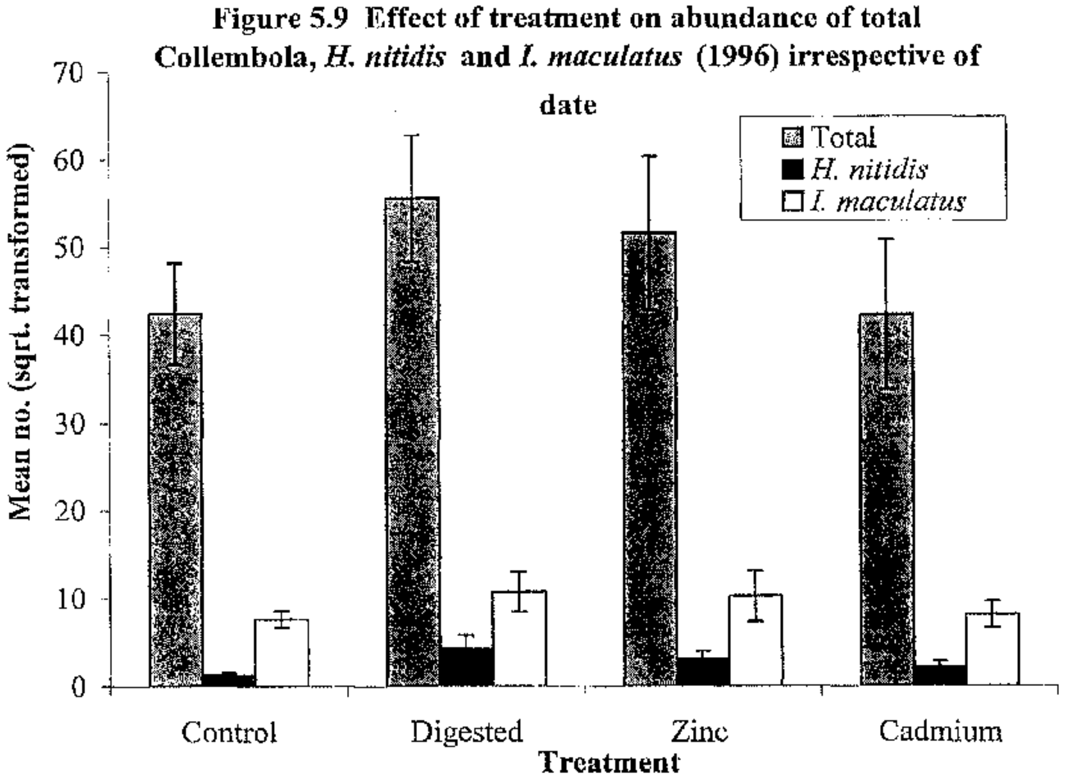


Figure 5.9 The above graph shows the effect of treatment on the species abundance caught by suction in 1996 at Auchincruive. Error bars show standard deviations.

The species *L. cyaneus* and *I. viridis* were clearly shown to be adversely affected by the addition of cadmium-rich sludge, with the abundance of these species in the cadmium-rich plots being lower than the abundance in all other plots (see Table 5.4 & Figure 5.10). *Isotomurus palustris*, on the other hand, was actually favoured by the addition of metal contaminated sludge, and the abundance of this species on the zinc and cadmium-rich plots (but not the digested plots) was significantly higher than the control plots (see Figure 5.11 and Table 5.4). The abundance of *I. palustris* in the cadmium-rich plots was higher than the control plots in 1995 also (see Table 5.3). This further indicates that

I. palustris was favoured by the addition of metal-rich sludge. *Ceratophysella denticulata* was significantly more abundant in the zinc-rich plots than the control plots (see Figure 5.11 and Table 5.4), suggesting that this species was favoured by zinc-rich sludge.

Figure 5.10 Effect of treatment on abundance of *I. viridis* and *L. cyaneus* (1996) irrespective of date

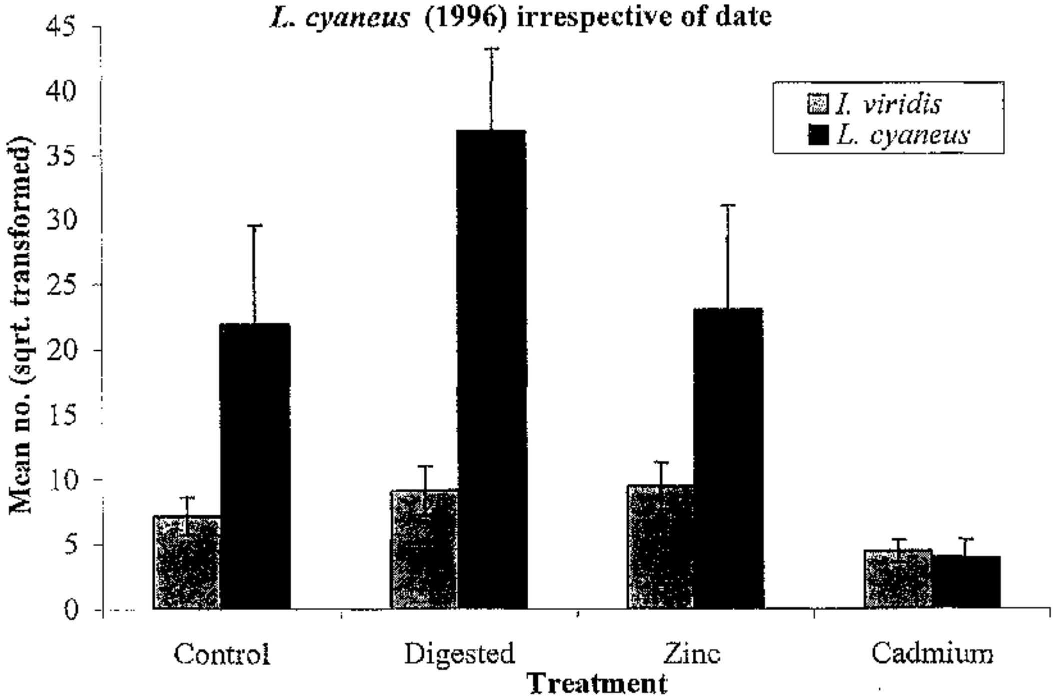
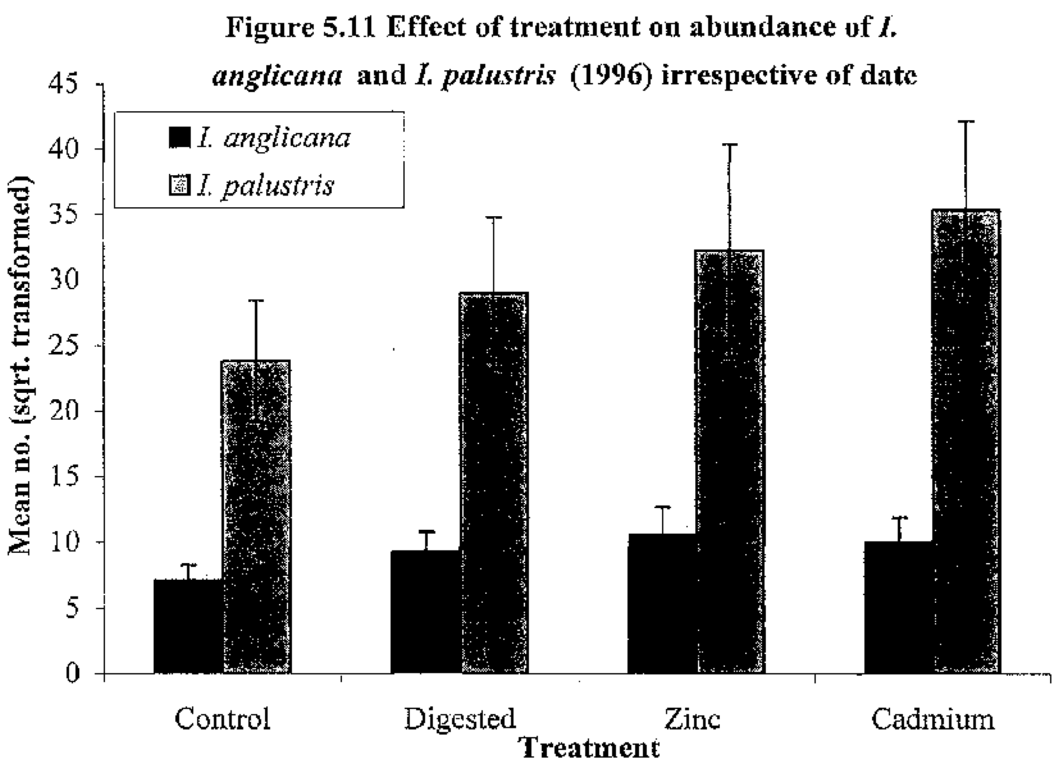


Figure 5.10 The above graph shows the effect of treatment on the species abundance caught by suction in 1996 at Auchincruive. Error bars show standard deviations.

Isotoma anglicana appeared to be tolerant to the presence of heavy metals in sewage sludge. The abundance of this species was higher in all plots receiving sludge, irrespective of metal contamination (see Figure 5.11 & Table 5.4). In 1995, the abundance of *I. anglicana* was higher on the cadmium-rich plots than control plots (see Table 5.3), suggesting that this species may be particularly favoured by cadmium-rich sludge.

Sminthurus viridis was the only species to occur in its highest abundance on the control plots, and was also more abundant on the cadmium-rich plots than the digested or zinc-rich plots (see Table 5.4 & Figure 5.12). This may be a result of this species being favoured by the cadmium-rich sludge, or it may be a consequence of higher sampling efficiency in the shorter grass of the control and cadmium-rich plots (see Appendix VIII).



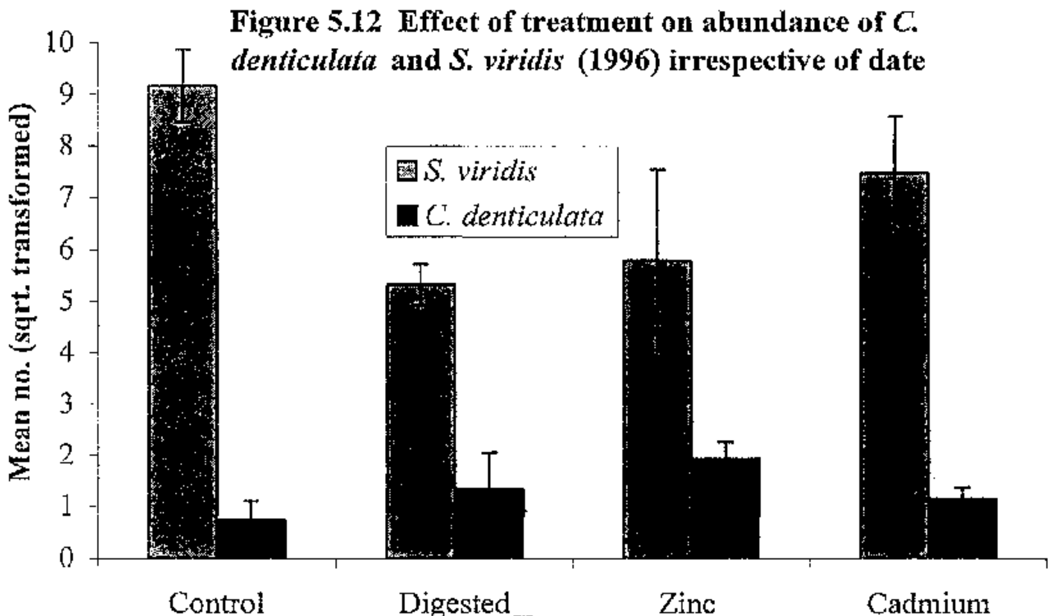


Figure 5.11 & 5.12 The above graphs show the effect of treatment on the species abundance caught by suction in 1996 at Auchincruive. Error bars show standard deviations.

Species composition and treatment at Hartwood

At Hartwood only *S. viridis* and *Isotoma/Isotomurus* juveniles were influenced by treatment (see Figure 5.13). The abundance of *S. viridis* was significantly higher in control plots than zinc-rich or digested plots ($F=14.44$ at $df = 3, 6$: $P < 0.01$: see figure 5.13). The abundance of *Isotoma/Isotomurus* juveniles was significantly lower in the cadmium-rich plots than in the other plots, suggesting that reproduction was adversely affected by cadmium-rich sludge ($F=13.34$ at $df = 3, 6$: $P < 0.01$: see Figure 5.13). The abundance of juveniles was also higher in the control and zinc-rich plots than the digested plots, suggesting that the addition of digested sludge (but not zinc-rich sludge) adversely affected reproduction of *Isotoma/Isotomurus* species (see Figure 5.13).

Figure 5.13 Effect of treatment on species composition at Hartwood irrespective of date

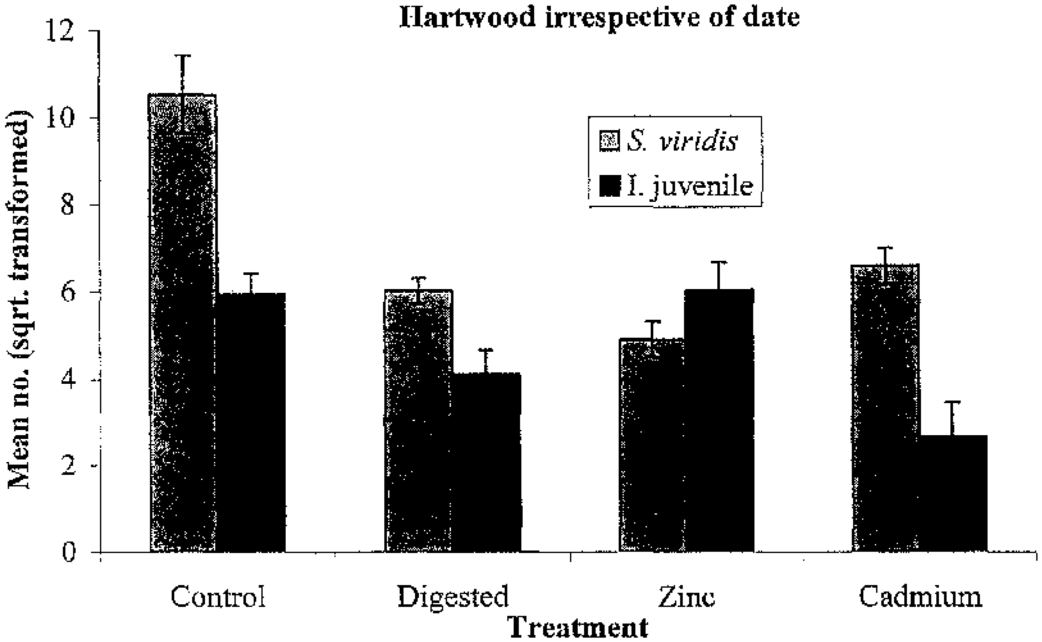


Figure 5.13 The above graph shows the effect of treatment on the species abundance caught by suction in 1996 at Hartwood. Error bars show standard deviations.

DCA Auchincruive 1995

DCA's were performed on the species relative abundance (without downweighting of rare species) for the Auchincruive and Hartwood data sets separately, and for the 1995 and 1996 data sets separately. The 1995 DCA produced eigenvalues of 0.336, 0.084, 0.023 and 0.016 for axes 1-4 respectively. These values indicate that the amount of between-plot variation in collembolan assemblages accounted for by axes 1-4 was 73.2%, 18.3%, 5.0% and 3.5% respectively. As axes 3 and 4 accounted for so little of this variation they were not considered further. The samples and species scores were plotted to obtain sample and species ordinations respectively (see Figures 5.14 and 5.15).

Figure 5.14 DCA sample ordination for suction samples: Auchincruive 1995

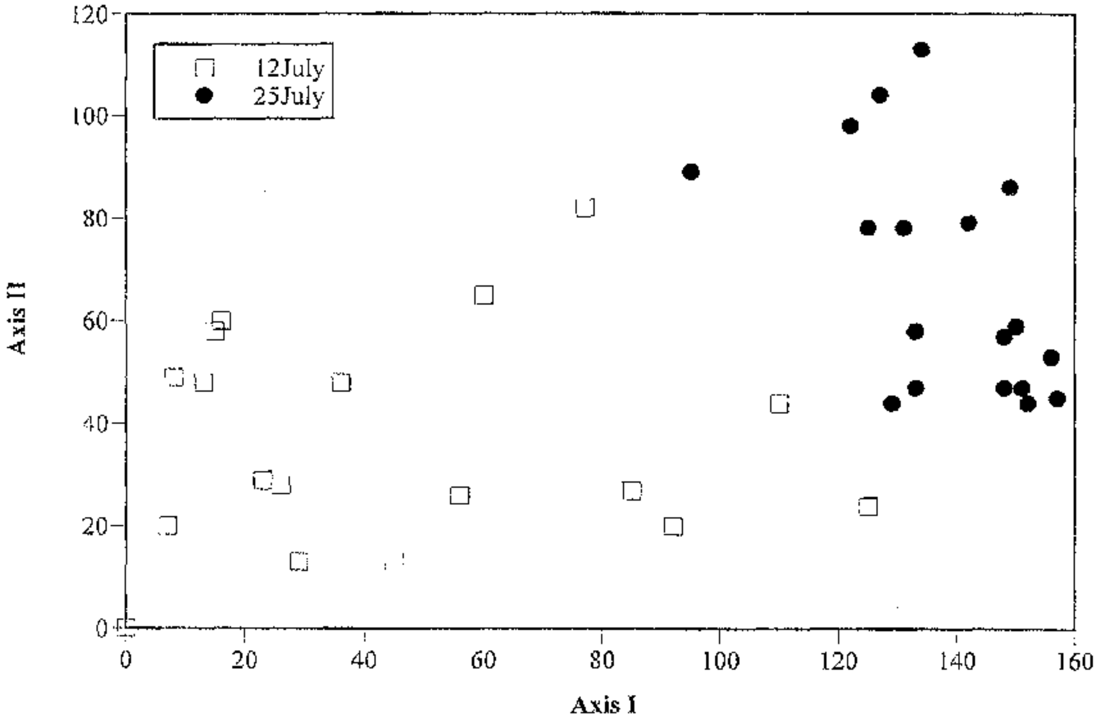


Figure 5.14 DCA sample ordination for the 1995 data set (based on species relative abundance). Each point represents one plot.

From Figure 5.14 it can be seen that sampling date was the main factor dividing samples along axes one, with 25 July samples lying to the right of 12 July samples. The species ordination indicates that the samples taken on 25 July had a higher occurrence of *I. anglicana*, *S. aureus* and *L. cyaneus* than those taken on 12 July. This is supported by the ANOVAs (see above). The species ordination therefore also suggests that date was the main factor influencing the division of samples along axes one.

The axes scores derived from the ordination were re-drawn showing digested and undigested treatments separately. This was done to enable treatment effects to be observed more easily. The digested treatment ordination indicates that on both sampling dates in 1995, the digested, zinc-rich and control plots lie distinct from the cadmium-rich plots. This suggests that the cadmium-rich plots had a different collembolan species composition from the other plots (see Figure 5.16). On 25 July, all digested treatments lie distinct (see Figure 5.16), hence suggesting that differences in collembolan composition between treatments were more pronounced in late July following grass cutting.

Figure 5.15 DCA species ordination for suction samples: Auchincruive 1995

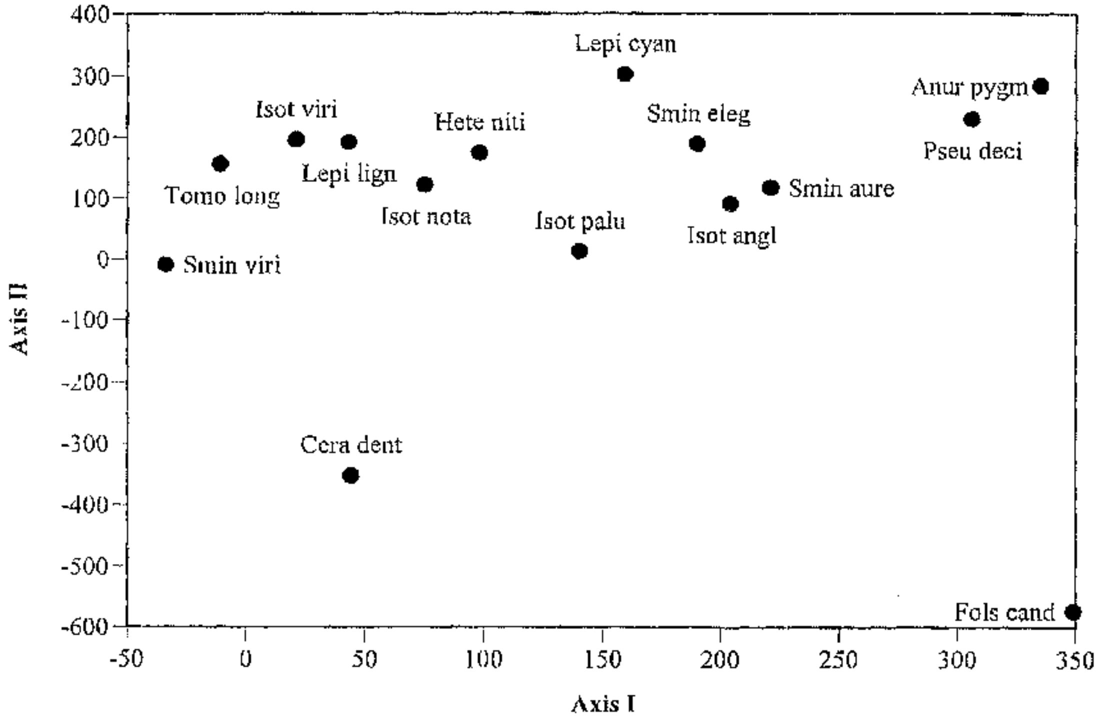


Figure 5.15 DCA species ordination for the 1995 data set (based on species relative abundance). Species codes can be seen in Appendix IX.

The undigested treatment ordination shows that on both sampling dates, the control and copper-rich plots differed most with respect to collembolan composition (see Figure 5.17). As was found for the digested treatments, differences between undigested treatments were more pronounced on 25 July (see Figure 5.17).

With the exception of the cadmium plots on 25 July, there were considerable differences between replicas within a treatment. Even on 25 July, when all treatments were separated, the variation between replicas within a treatment was as great, if not greater than, the variation between treatments. Closer examination of the ordinations found that

Figure 5.16 DCA ordination for suction samples 1995: digested treatments

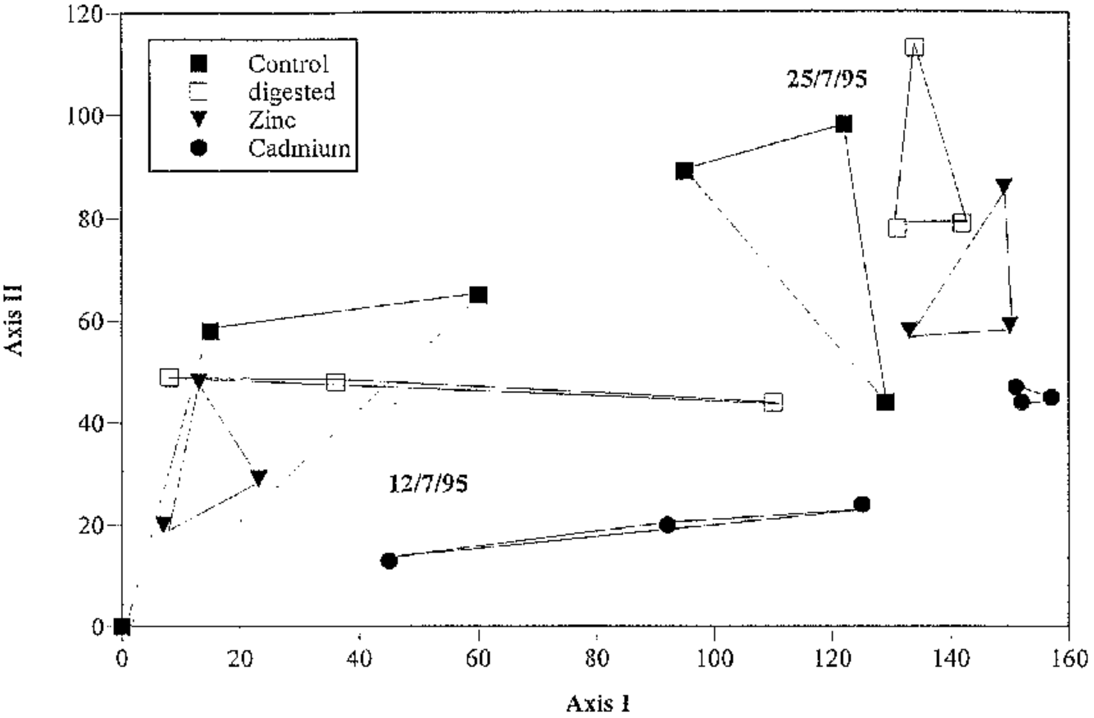


Figure 5.17 DCA ordination for suction samples 1995: undigested treatments

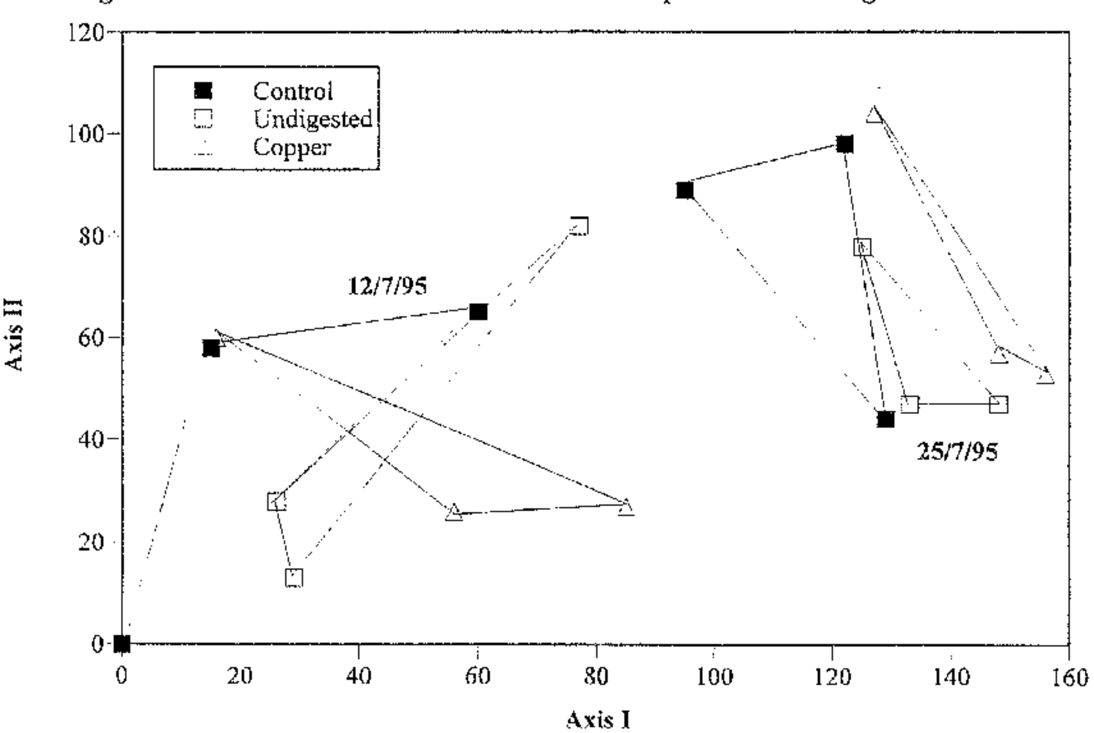


Figure 5.16 & 5.17 DCA ordination for the digested and undigested treatments at Auchincruive respectively (based on species relative abundance). Each point represents one plot.

for most treatments (on both sampling dates) block two differed most between the three replicas. The litterbag ordination also found that block two differed most between the three replicas in 1995 (see Chapter 4). It would therefore appear that while no individual species was affected by block (see above), the collembolan composition as a whole was.

The available data on soil properties indicated that pH and conductivity of block two was lower than the other blocks prior to sludge application (see Appendix VI). Several studies have found that Collembola show species specific preferences to pH (Hågvar 1990; Hågvar & Abrahamsen 1980; Hågvar & Kjøndal, 1981). The species ordination places the acidophilic species *A. pygmea* to the right of the species ordination and the calciophilic species *I. notabilis* to the left (see Figure 5.15: Hågvar & Abrahamsen 1980). It would therefore appear that soil pH may have influenced the species composition which could have contributed to the observed separation of block two.

<u>DCA Auchincruive 1996</u>

The 1996 DCA produced eigenvalues of 0.351, 0.088, 0.054 and 0.014 for axes 1-4 respectively. The between-plot variation in collembolan community structure accounted for by axes 1-4 was therefore 69.2%, 17.4%, 10.7% and 2.8% respectively. As axes 3 and 4 accounted for so little of the observed variation, they were not considered further. The species and sample scores for axes 1 and 2 were plotted to obtain species and sample ordinations respectively (see Figures 5.18 & 5.19).

In 1996, the August samples tended to lie above and to the left of the other sampling dates (see Figure 5.18). The species ordination (see Figure 5.19) indicates that the samples taken in August had the highest occurrence of *T. longicornis, H. nitidis* and *I. viridis* and this is supported by the ANOVAs (see above). Date did not, however, solely account for the division of samples, and there was considerable variation between samples collected on the same date (see Figure 5.18).

Figure 5.18 DCA sample ordination for Auchincruive suction samples 1996

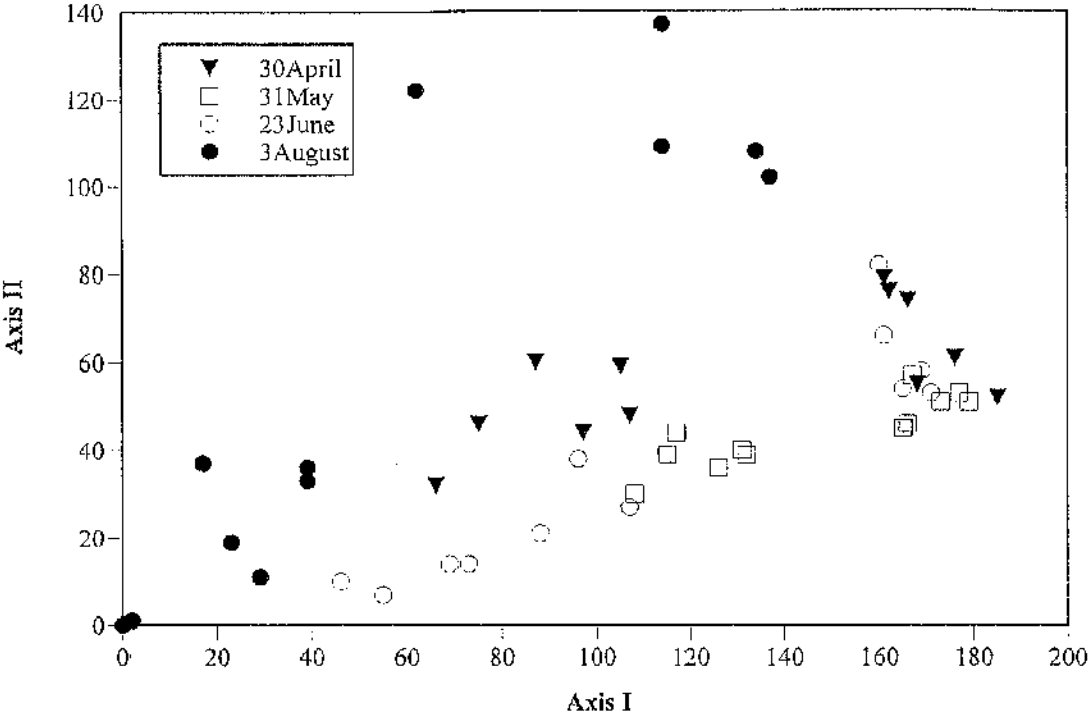
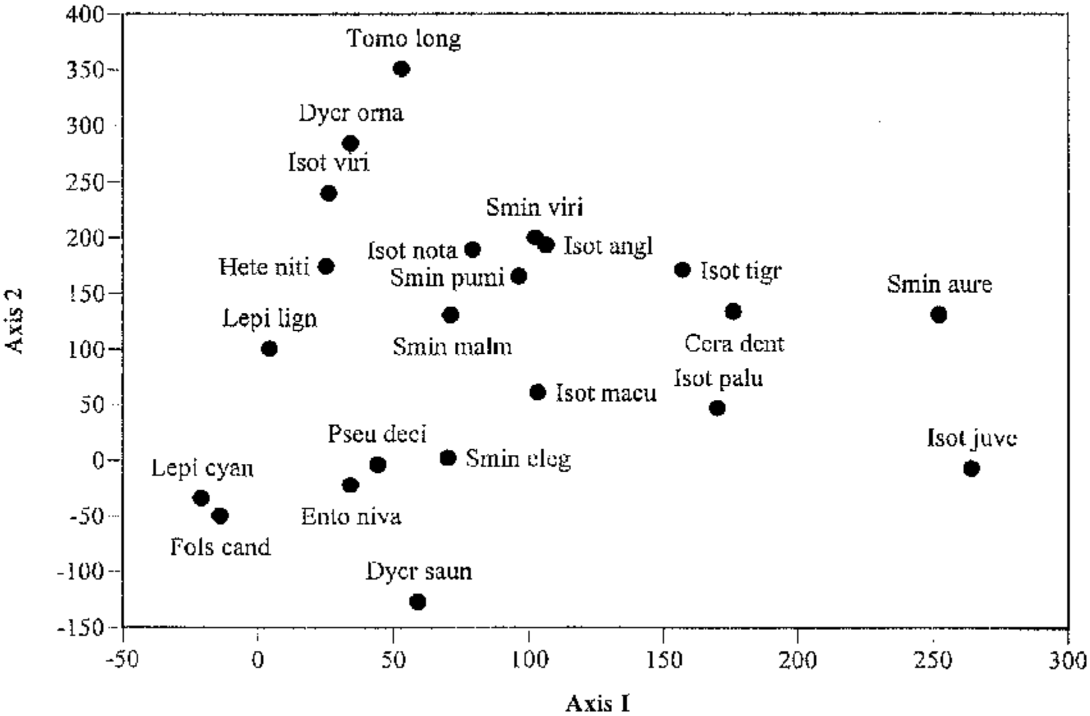


Figure 5.19 DCA species ordination for Auchincruive suction samples 1996



Figures 5.18 & 5.19 DCA sample and species ordination respectively (based on species relative abundance). Each point represents one plot. Species codes can be seen in Appendix IX.

To enable treatment effects to be observed more easily, the axes scores derived from the ordination were re-drawn showing each sampling date separately (see Figures 5.20-5.23). For all sampling dates, the biggest difference in collembolan community structure was found between the cadmium-rich and the digested plots, with the cadmium-rich plots consistently lying to the right of the digested (see Figures 5.20-5.23). The species ordination also indicates that treatment was important in separating the samples, with cadmium-sensitive species (e.g. *L. cyaneus* and *I. viridis*: see above) lying to the left of the species ordination, while tolerant species (e.g. *I. palustris* and *I. anglicana*: see above) lie to the right (see Figure 5.19). On all sampling dates, the separation between the cadmium-rich and digested treatments was substantially larger than differences between the replicas of these treatments (see Figures 5.20-5.23).

Large differences between replicas, however, occurred for the zinc-rich and the control treatments. In the case of the control treatments, the block two replica was consistently separated to the right of the other replicas for all sampling dates. This block was also separated from the other blocks in 1995. In 1996, however, block two did not differ greatly from the other replicas in the cadmium-rich or digested plots. It would therefore appear that in 1996 the separation of block two in the control treatment was the result of differences in this individual plot rather than the entire block. Examination of the available soil data for this plot in 1996 indicated that biomass carbon was higher, while pH and grass yield were lower in this replica than the other two control replicas (see Appendices VIII & XI).

For the zinc-rich plots, block two occurred towards the left of the ordination and block one towards the top. In the April and May ordinations, block two differed most between the three replicas of the zinc-rich plots, while in June and August block one differed most. Examination of the soil data did not show any variable that differed consistently between these plots (see Appendices VII, VIII & XI). However, as the three blocks appeared in the same position relative to each other (i.e. block two always to the left of blocks one and three) on different sampling dates, it is likely that the separation of blocks was a true effect.

Figure 5.20 DCA ordination 30 April (1996)

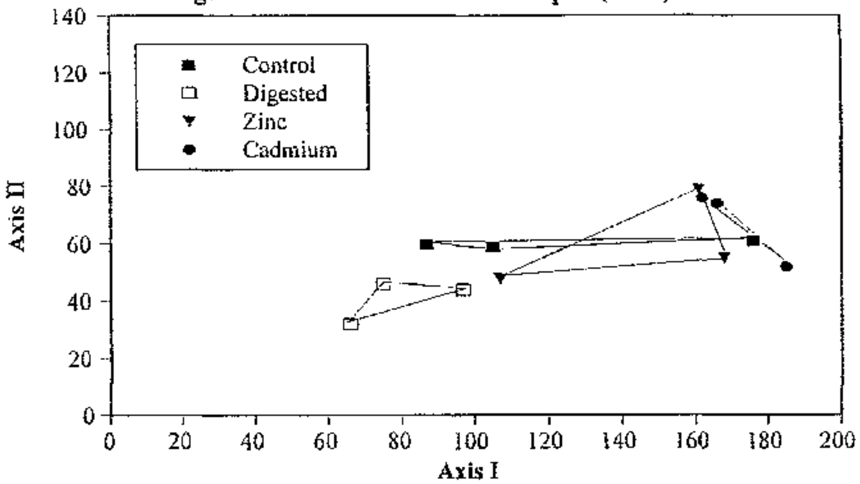


Figure 5.21 DCA ordination 31 May (1996)

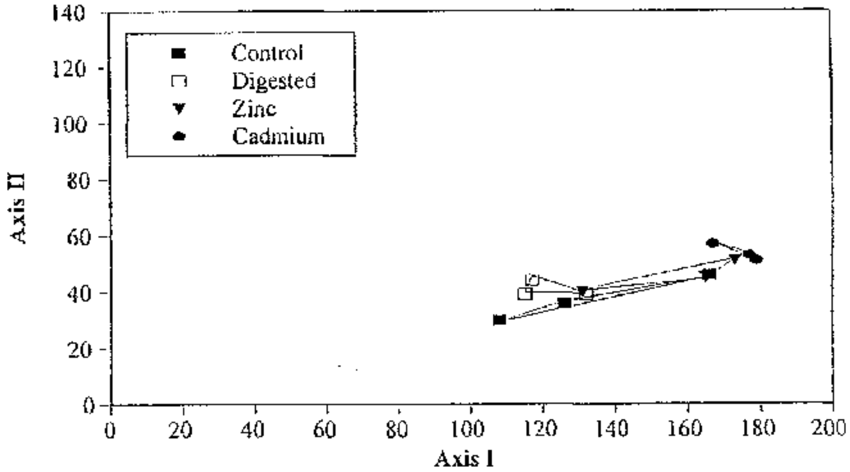


Figure 5.22 DCA ordination 22 June (1996)

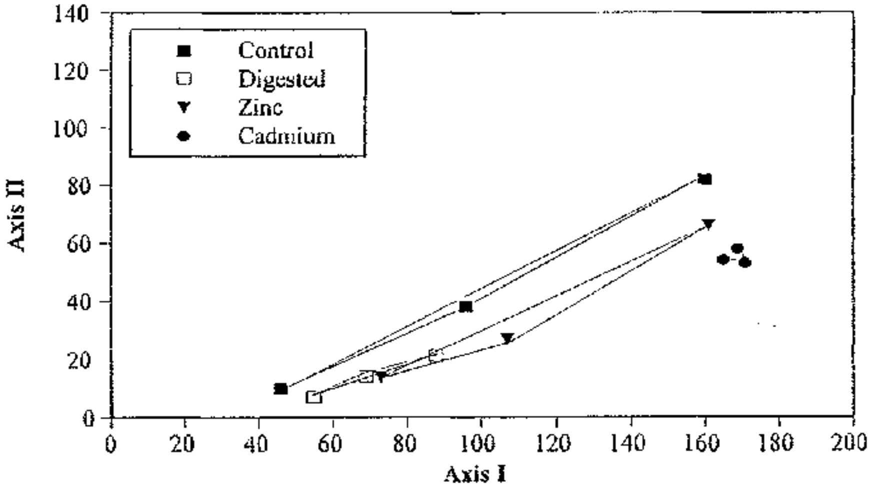
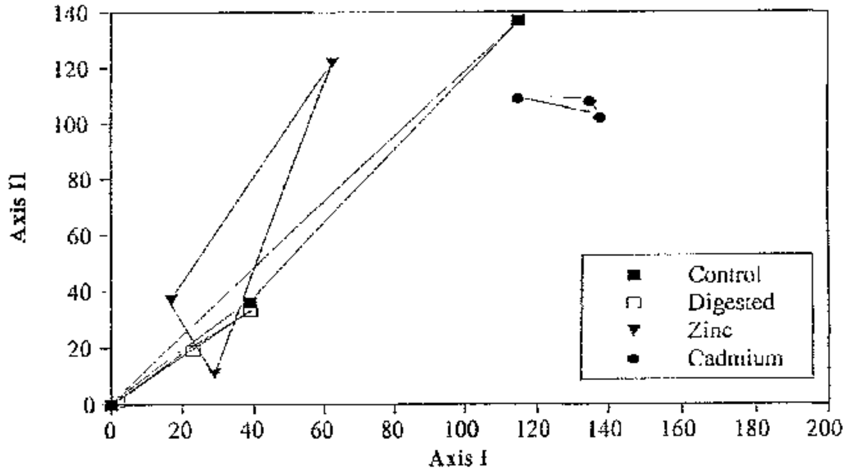


Figure 5.23 DCA ordination 4 August (1996)



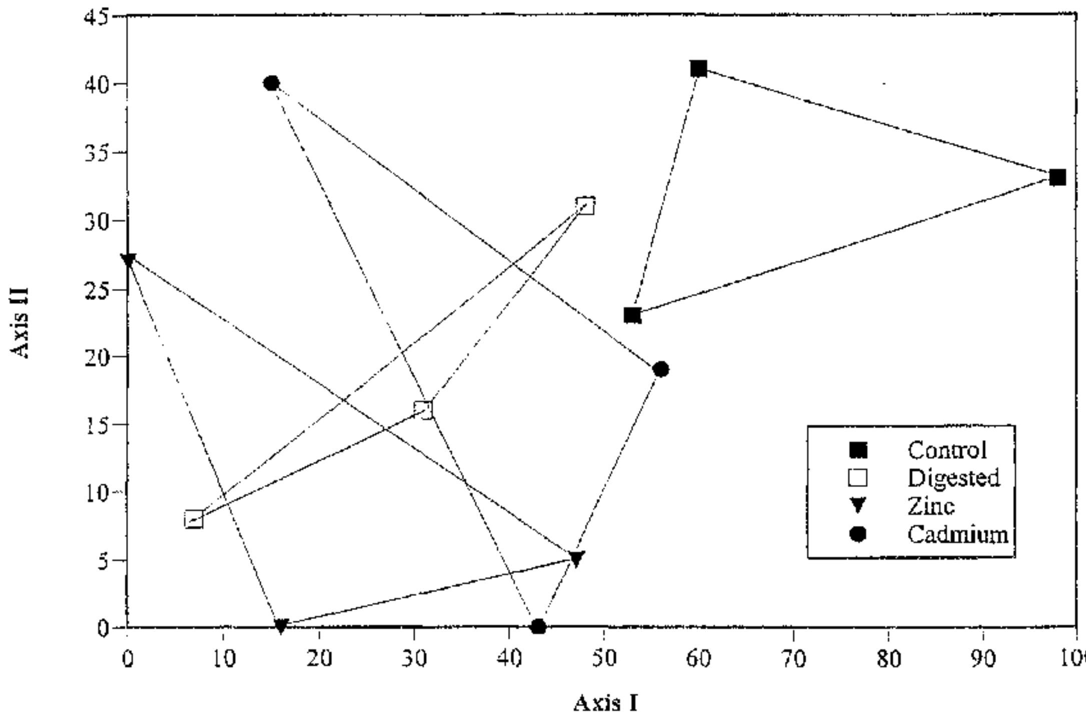
Figures 5.20-5.23 DCA for Auchincruive for April, May, June and August data respectively (1996). Each point represents one plot.

For both the zinc-rich and control plots, replica differences appeared to be consistent between dates, indicating that this variation was not simply a sampling error, but a real difference between the plots. It would therefore appear that date, treatment and other unknown soil parameters (e.g. pH and soil moisture) influenced the collembolan species composition in 1995 and 1996.

DCA Hartwood 1996

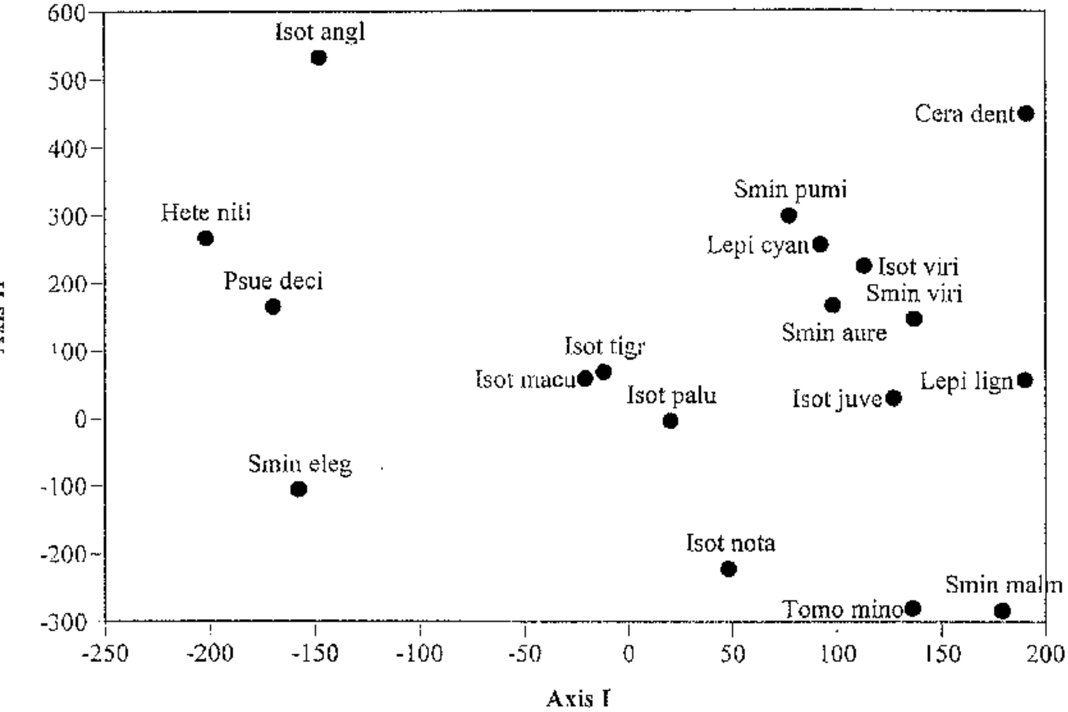
The Hartwood DCA produced eigenvalues of 0.115, 0.02, 0.006 and 0.003 for axes 1-4 respectively. This indicates that the amount of between-plot variation in collembolan community structure accounted for by axes 1-4 was 79.9%, 13.9%, 4.2% and 2.1% respectively. It can be seen that the control treatments lie distinct from all three sludge treatments, hence indicating the collembolan composition differed most between the control and sludge treatments (see Figure 5.24). The Hartwood DCA indicates that differences between replicas within a treatment were generally as large as difference between treatments.

Figure 5.24 DCA sample ordination for Hartwood 1996 (relative abundance)



Figures 5.24 DCA sample ordination (relative abundance). Each point represents one plot.

Figure 5.25 DCA species ordination for Hartwood 1996 (relative abundance)



Figures 5.25 DCA species ordination respectively (relative abundance). Species codes can be found in Appendix IX.

There was evidence of a block effect, with the block one replicas tending to lie to the right of the block two and three replicas. This may be a result of block one being situated close to a row of trees, or of other unknown parameters (e.g. soil pH or moisture content). The Hartwood species ordination suggests that humidity may have influenced the division of samples; species which prefer damp conditions occurred towards the bottom of the ordination (e.g. *I. palustris*, *S. malmgreni* and *T. minor*: Joosse 1970; Fjellberg 1980), while those which prefer drier conditions occurred towards the top (*L. cyaneus*: Joosse 1970: see Figure 5.25).

Relationship between grass height and species abundance at Auchincruive

A product moment correlation was calculated to investigate the relationship between grass height and the abundance of frequently occurring species (i.e. the species shown in Table 5.4). Species which were found to be correlated with grass height are presented in Table 5.5. The abundance of *I. viridis* and *I. anglicana* showed a negative correlation with grass height thus indicating their abundance decreased as grass height increased or they were sampled more efficiently in short grass. The abundance of *I. anglicana* and *I. viridis* was also found to increase in August 1996 after grass cutting (see Table 5.4), and this further suggests that these species may be sampled more efficiently in shorter grass. It is, however, difficult to draw any definite conclusions as grass height may have been confounded with sampling date.

Table 5.5 Relationship between grass height and species abundance

Species	Product moment correlation coefficient	t-value	Probability df = 46, n = 48
I. anglicana	-0.50	-3.93	< 0.01
I. viridis	-0.30	-2.14	< 0.05

Relationship between cadmium concentration and species abundance at Auchincruive

A Spearman's rank correlation was carried out to investigate the relationship between the abundance of frequently occurring species (caught by suction on 4 August) and cadmium concentration. The species *I. viridis*, *L. cyaneus* and *H. nitidis* were negatively correlated with cadmium concentration, and therefore decreased in abundance as the cadmium concentration increased (see Table 5.6). *Lepidocyrtus cyaneus* and *I. viridis* were less abundant in plots receiving cadmium-rich sludge than in other plots, further suggesting that they were adversely affected by cadmium (see Table 5.3 & Table 5.4).

Table 5.6 Relationship between cadmium concentration and species abundance

Species	Spearman's rank correlation coefficient	Probability n = 15
I. palustris	0.14	N.S.
I. maculatus	0.14	N.S.
I. notabilis	0.03	N.S.
I. juvenile	0.33	N.S.
I. viridis	-0.70	< 0.01
I. anglicana	0.43	0.1
T. longicornis	0.12	N.S.
H. nitidis	-0.68	< 0.01
L. cyaneus	-0.52	0.05
S. viridis	0.29	N.S.

The species *I. anglicana* tended to show a positive relationship with cadmium concentration, increasing in abundance with cadmium concentration (see Table 5.6). This is in agreement with the 1995 Auchincruive results, which found *I. anglicana* to be more abundant in cadmium-rich plots than digested or control plots (see Table 5.3).

5.5 Discussion

Effect of date on collembolan composition at Auchincruive

In 1996, almost all samples taken at Auchincruive had a higher number of Collembola present than samples taken in 1995. This was thought to be primarily a consequence of the 1995 summer being particularly dry. Purvis & Curry (1978) also found that excessively dry summers adversely affected Collembola, and they concluded that this was the result of a reduction in microbial activity and hence food for Collembola.

The DCA ordinations indicated that in 1995 date was the main factor influencing collembolan composition, while in 1996 both date and treatment had large influences. Some species reached their maximum population density in April (e.g. *C. denticulata* and *S. malmgreni*), others May (e.g. *I. palustris, I. maculatus* and *Isotoma/Isotomurus* juveniles), others in June (e.g. *S. viridis*) and others in August (e.g. *H. nitidis, T. longicornis*, *I. viridis* and *L. cyaneus*). Other authors have also found that different collembolan species respond to seasonal changes in different ways (Joosse 1969; Brussaard *et al.* 1990). By only presenting seasonal fluctuations for the abundance of total Collembola, and omitting data on individual species, much information is therefore lost (Joosse 1969; Christiansen 1964).

Joosse (1969) found that species with maximum population densities in spring were more hydrophilic than species which had population maximums in summer. *Sminthurides malmgreni* and *I. palustris* which peaked in April and May respectively, are known to prefer humid habitats (Fjellberg 1980; Joosse 1970), while *L. cyaneus* which peaked in abundance in late summer, is known to prefer drier areas (Joosse 1970). However, the species *I. viridis* and *T. longicornis*, which were found in their highest abundance in late summer, prefer humid habitats (Joosse 1970; Fjellberg 1980). It would therefore appear that while the seasonal abundance of some species can be related to their humidity preference, this does not follow for all species.

Both Hale (1965) and Joosse (1969) found a maximum oviposition rate in April/May for several species of Collembola including *I. viridis* and *I. palustris*. In agreement with these authors, the number of *Isotoma/Isotomurus* juveniles was higher in May at Auchincruive therefore indicating that oviposition primarily occurred in April/May. It is also interesting to note that the abundance of juveniles sharply decreased from May to June. As this decrease did not correspond to an increase in *Isotoma/Isotomurus* adults, it is suggested that juvenile mortality was high. This is in agreement with Van Straalen (1985) who found that predation pressure was higher in juvenile Collembola than for adults or eggs. The decrease in abundance of juveniles in June may, however, simply be the result of a lower sampling efficiency in long grass. The abundance of *I. anglicana*, *I. palustris*, *I. notabilis* and *I. maculatus* decreased from May to June. This may be a consequence of a decrease in sampling efficiency in long grass, or alternatively it may be the result of an increase in adult mortality following egg laying. Egg-laying has previously been found to increase adult mortality in the species *I. viridis* (Joosse 1969).

The abundance of *I. anglicana*, *I. notabilis*, *I. palustris* and *I. maculatus* peaked in May, while that of *L. cyaneus*, *I. viridis*, *T. longicornis* and *H. nitidis* peaked in August. Both these sampling dates followed grass cutting. This would appear to contradict the results of Curry & Tuohy (1978) who found cutting to be detrimental to epigeal and hemiedaphic Collembola. However, these authors used a different sampling method and it is possible that suction sampling is more efficient in short grass (Harwood 1994). Furthermore, the abundances of *I. viridis* and *I. anglicana* were negatively correlated with grass height, further indicating an increased sampling efficiency in shorter grass.

The epigeal species *S. viridis*, appeared to be adversely affected by grass cutting in 1995 and 1996. As *S. viridis* climbs grass stems (Frampton 1994), it was probably as efficiently sampled in long grass as short grass. The lower numbers of *S. viridis* after grass cutting was therefore considered to be a real effect, and was thought to be related to its habit of climbing stems making it more susceptible to cutting. By sampling immediately before and after grass cutting, it would be possible to determine if this species was adversely affected by the cut.

Effect of treatment on species composition

It has been well documented that the addition of sewage sludge can promote Collembola (Lübben 1989; Pimentel & Warneke 1989; Höller-Land 1959), and at Auchincruive the addition of digested sludge increased the abundance of Collembola in 1996. Although the abundance in digested plots was greater than control plots in 1995 at Auchincruive and in 1996 at Hartwood, this difference was not significant as a result of the large variation between replicas. It is likely that if sampling had occurred over a longer period of time, significant effects would have become apparent.

At the species level, it was found that while some species appeared to be adversely affected by the cadmium-rich sludge (e.g. *I. viridis*, *H. nitidis* and *L. cyaneus*), others were not (e.g. *I. palustris*, and *I. anglicana*). Species specific differences in metal susceptibility are well documented (Bengtsson & Rundgren 1988; Hagvar & Abrahamsen 1990; Posthuma & Van Straalen 1993; Filser *et al.* 1995), and this again exemplifies the importance of taking the individual species approach rather than looking at the group Collembola as a whole.

Cadmium may be directly toxic to Collembola through decreasing their growth rate, reproduction or survival (Bengtsson *et al.* 1983 Tranvik *et al.* 1993; Posthuma *et al.* 1993; Joosse & Verhoef 1983), and there was evidence of a decrease in *Isotoma/Isotomurus* juveniles in the cadmium-rich plots at Hartwood. This was not, however, the case at Auchincruive. Differences between sites were probably the result of a bigger proportion of the juveniles at Hartwood consisting of metal sensitive species (e.g. *I. viridis*) than at Auchincruive.

The cadmium-rich sludge may have also affected Collembola indirectly through affecting their habitat structure and the soil microbial community (Posthuma *et al.* 1993). It was found that the grass height in the cadmium-rich plots was lower than the digested sludge plots (see Appendix VIII), and this decrease in plant cover may have resulted in a less favourable microclimate (Pimentel & Warneke 1989). None of the species adversely affected by the cadmium-rich sludge showed a positive correlation with grass height, hence suggesting that the decrease in grass height did not adversely affect any species. Furthermore, *I. viridis* showed a negative correlation suggesting that it was actually favoured shorter grass. It is therefore unlikely that the lower abundance in the cadmium-rich plots was a consequence of grass height.

Collembola may be predisposed to poisoning if their preferred fungal species are sensitive to cadmium, or if they are unable to avoid polluted food. Furthermore, if these species are able to digest fungal cell walls, where heavy metals are frequently accumulated, they will be exposed to metal concentrations far in excess of those found in the soil (Siepel 1994). *Lepidocyrtus cyaneus* was found to have a higher metal concentration than *O. cincta* in a study by Van Straalen & Van Wensem (1986). This suggests that metals were more available, or that excretion was less efficient, in *L. cyaneus* than *O. cincta*. However, as no data is available on the accumulation of metals in the other species found at Hartwood and Auchincruive, no conclusions can be drawn concerning differences in metal accumulation.

Despite levels of zinc being higher than cadmium, toxic effects of zinc were not found. The threshold concentration at which deleterious effects occur is usually higher for essential micro-nutrients (e.g. copper, zinc and iron) than for nonessential ecological impurities (e.g. cadmium and mercury: Pokarzhevskii & Van Straalen 1996; Hopkin 1989). Essential elements are also regulated more efficiently than nonessential elements (Janssen & Hogervorst 1993). A less toxic effect of zinc may therefore be expected, as it is a micro-nutrient which is required in trace quantities while cadmium has no known positive function. Furthermore, cadmium is a very toxic element with a higher concentration factor (Hopkin 1989; Janssen & Hogervorst 1993), and lower solubility than zinc (Rühling & Tyler 1973).

Isotomurus palustris and *I. anglicana* were promoted by the addition of contaminated sludge. These species may have a more efficient detoxification mechanism than metal sensitive species such as *L. cyaneus*. The detoxification of metals in Collembola is thought to be primarily under the control of metal binding granules in the gut wall which are lost when the gut is exfoliated (Humbert 1978; Van Straalen *et al.* 1987). *Isotomurus palustris* would be more efficient at excreting heavy metals if it possessed these granules, while metal sensitive species did not. The abundance of this species is not, however, greater in the metal-contaminated plots than the digested plots. This suggests that although it was able to maintain high populations in the metal-rich plots, it did not benefit from the decrease in abundance of sensitive species.

Sminthurinus aureus has been found to be less abundant on metal enriched sludge plots than on uncontaminated sludge plots (Lübben 1989), and therefore appears to be susceptible to metal poisoning. *Sminthurinus aureus* may consume sewage sludge directly and would therefore be particularly susceptible to poisoning from metal contaminated sludge (Curry 1994). In this study, no adverse affects of metal rich sludge were found for this species. While the sludge used by Lübben (1989) was artificially contaminated with metal salts, the sludges in this investigation were derived from sewage treatment works that had naturally high inputs of specific metals. It is possible that the metals were less available in the naturally rich sludges used in the present study, or that other metals were present in Lübben's sludge (e.g. nickel, chromium or lead) that were more toxic to this species.

At both the Hartwood and Auchincruive sites the abundance of *S. viridis* was higher in control plots than sludged plots, suggesting adverse effects of sludge. The abundance of *S. viridis* was also higher in cadmium-rich plots than digested or zinc-rich plots at Auchincruive. It is possible that this species was adversely affected by the higher grass yield in the zinc-rich and digested plots. However, as the abundance of this species showed no correlation with grass height, and it was found in its highest abundance when the grass was longest, this is unlikely to be the case. It is therefore suggested that *S. viridis*, being phytophageous, did not benefit from the increase in micro-organisms occurring in the sludged plots. *Sminthurus viridis* may actually have been adversely affected by the addition of sludge if the abundance of predators had increased. The abundance of carabid beetles has been found to increase following sludge application (Larsen *et al.* 1996). As this species consumes living plant tissue, which generally has lower concentrations of heavy metals than fungi (Curry 1994), it may also have had a competitive advantage over mycophagous Collembola in the cadmium-rich plots.

It was found that Collembola were heterogeneously distributed within the study areas, and consequently there were large differences between the five suction samples in a plot. Pooling the five samples helped to overcome the problem of spatial heterogeneity and square root transformation normalised the data for most species (see Chapter 3). However, as the variances for some species were still heterogeneous, it was necessary to set the probability value at 0.01 to avoid Type one errors (Day & Quinn 1989). In this study, each suction sample covered an area of 706 cm^2. The problem of spatial heterogeneity may have been reduced if the area of a suction sample had been decreased, and the number of samples taken increased.

5.6 Main findings

1: The lower abundance of Collembola found in 1995 when compared to 1996, was thought to be primarily due to the summer of 1995 being exceptionally dry.

2: Species specific differences were found with respect to season which could only partly be attributed to humidity preferences.

3: The *Isotoma/Isotomurus* oviposition rate peaked in May, and this was followed by an increased mortality of adults.

4: Suction sampling of some species (e.g. *I. anglicana* and *I. viridis*) appeared to be more efficient in shorter grass. The species *S. viridis*, on the other hand, was found to be less abundant following cutting, and this was thought to be a consequence of this species being particularly sensitive to cutting.

5: The addition of sludge increased the abundance of total Collembola and of several species (e.g. *I. maculatus* and *I. palustris*).

6: Only *S. viridis* was adversely affected by sludge application. This was thought to be a consequence of this species being phytophageous and therefore was not able to benefit from the increase in micro-organisms which accompanied sludge application.

7: Species specific differences were found with respect to cadmium sensitivity. Sensitive species included *L. cyaneus, I. viridis* and *H. nitidis*, while tolerant species included *I. anglicana* and *I. palustris*.

8: Cadmium was more toxic than zinc, and this was thought to be related to zinc being more efficiently regulated than cadmium.

CHAPTER 6. Effects of sewage sludge on hemiedaphic and epigeal Collembola sampled by pitfall trapping

6.1 Introduction

Pitfall traps provide a cheap method of sampling surface active invertebrates (Luff 1975) and Collembola are often the most frequently caught arthropods in the traps (Boyd 1995; Duffield & Aebischer 1994). Although pitfalls have seldom been used to sample Collembola in the past (Joosse 1965, Berbiers *et al.* 1989), they are now becoming more popular (Duffield & Aebischer 1994; Mebes & Filser 1997; Badejo & Olaifa 1996; Winklehner *et al.* 1997).

Pitfall traps have a number of advantages over suction sampling. For example, the labour involved in placing and collecting pitfall traps (once they are established) is less than that involved in suction sampling. In addition, nocturnal species (such as *O. cincta* and *T. minor*: Joosse 1965) tend to be under-represented in suction samples (since they are almost always taken during the day), while pitfall traps efficiently sample both nocturnal and diurnal species. Pitfall trapping may also help overcome problems of spatial heterogeneity that arise from Collembola aggregating in their natural habitat. A further advantage pitfall trapping has over suction sampling is that it gives an indication of the activity of the individuals, and therefore pitfall traps have the potential to be used to measure sub-lethal effects of chemicals (Frampton 1994).

Pitfall traps are, however, influenced by several factors the most important of which is the activity of the individual species, with the most mobile species being consistently over-represented in catches (Greenslade 1964). Other factors that can influence the efficiency of pitfalls include the vegetation, weather, type of preservative and even the material the pitfall is made from (Greenslade 1964; Joosse 1965; Berbiers *et al.* 1989). Joosse (1965) found that the abundance of *Entombrya nivalis* (Linné) in pitfall traps was negatively correlated with temperature, while that of *O. cincta* was positively correlated with temperature. Rainfall was found to lower the abundance of *E. nivalis* in pitfall catches, while it actually increased the abundance of *L. cyaneus* (Joosse 1965).

It has been found for some collembolan species (e.g. *Tomocerus minor* (Lubbock) and *I. viridis*) that a higher proportion of individuals are caught within the first two days of placing pitfall traps than on subsequent days (Joosse & Kapteijn 1968). This has been termed the 'digging in' effect (Joosse 1965). The digging in effect is unlikely to be caused by any mechanical disturbance that occurs when placing the traps (i.e. walking in the field) as such disturbances last for less than an hour (Joosse & Kapteijn 1968). The increased abundance may instead be caused by the increased CO_2 production that occurs when soil is disturbed (Joosse & Kapteijn 1968). CO_2 has been found to attract and stimulate collembolan activity in the laboratory (Joosse & Kapteijn 1968; Ruppel 1953). The proportionally higher catches of Collembola in the first two days may therefore be the consequence of Collembola being attracted to, or stimulated by, the elevated CO_2 that results from soil disturbance when inserting the pitfall traps.

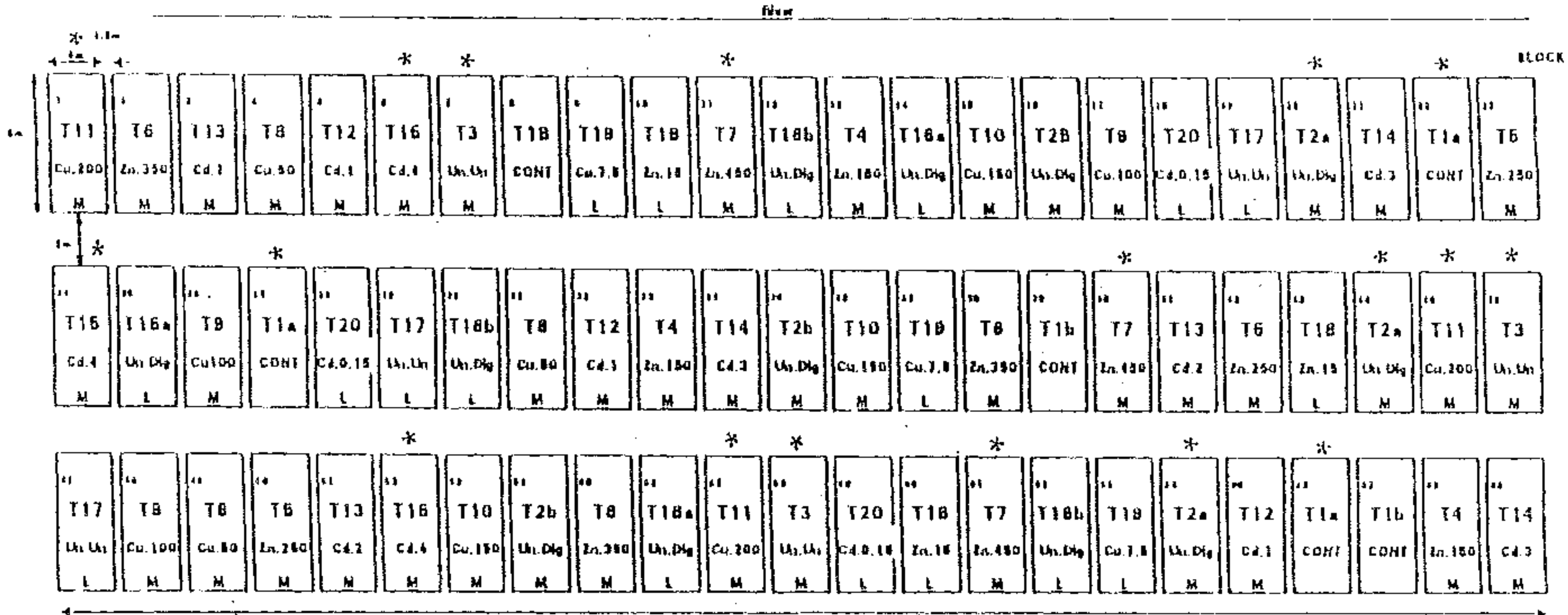
It would therefore appear that both suction samples and pitfall traps have their downfalls and strong points, and a comparison between these two sampling methodologies will be made in Chapter 7. It is likely that the most effective sampling regime would included both these sampling methods (Frampton 1994; Berbiers *et al.* 1989). In this chapter, pitfall traps were used to investigate the effects of different types of sewage sludge on surface active Collembola.

6.2 Methods

Trial site and treatments

The Auchincruive trial site (described in Chapter 3) was used for this experiment. The following six treatments were investigated: control, digested, undigested, zinc-rich, copper-rich and cadmium-rich. The treatments are presented in Table 6.1 alongside the year in which they were studied (plots studied are marked with an asterix in Figure 6.1). The chemical properties of the five sludges are presented in Appendices II and X.

Figure 6.1 SAC contaminated sludge plan showing plots sampled by pitfalls



Codes: I - 69 Plot numbers
T1 - T 20 Treatment numbers
I - III Block numbers

Build-up rate: M Moderate build-up
L Long-term build-up

Treatment type:

CONT	Control
Un.Dig	Uncontaminated digested
Un.Un	Uncontaminated undigested
Zn.150	High Zn (target soil metal concentration 150 mg kg^{-1})
Zn.250	High Zn (target soil metal concentration 250 mg kg^{-1})
Zn.350	High Zn (target soil metal concentration 350 mg kg^{-1})
Zn.450	High Zn (target soil metal concentration 450 mg kg^{-1})
Zn.15	High Zn (15 kg ha^{-1} Zn annually)
Cu.50	High Cu (target soil metal concentration 50/mg kg^{-1})
Cu.100	High Cu (target soil metal concentration 100/mg kg^{-1})
Cu.150	High Cu (target soil metal concentration 150/mg kg^{-1})
Cu.200	High Cu (target soil metal concentration 200/mg kg^{-1})
Cu.7.5	High Cu (7.5 kg ha^{-1} annually)
Cd.1	High Cd (target soil metal concentration 1 mg kg^{-1})
Cd.2	High Cd (target soil metal concentration 2 mg kg^{-1})
Cd.3	High Cd (target soil metal concentration 3 mg kg^{-1})
Cd.4	High Cd (target soil metal concentration 4 mg kg^{-1})
Cd.0.15	High Cd (0.15 kg ha^{-1} Cd annually)

Table 6.1 Treatments

Treatment number	Treatment	Mean metal concentration	Sewage treatment works	Year sampled
1	Control	Background levels (see Appendices III & XI)		1995 & 1996
2A	Digested uncontaminated sludge	Low levels (see Appendices II, III, X & XI)	Banbury	1995 & 1996
3	Undigested uncontaminated sludge	Low levels (see Appendices II & III)	Carterton	1995
7	Zinc-rich digested sludge	7,036 mg Zn kg^{-1} sludge 288.5 kg Zn ha^{-1} $year^{-1}$ 180 mg Zn kg^{-1} soil 1995 270 mg Zn kg^{-1} soil 1996	Coleshill	1995 & 1996
11	Copper-rich undigested sludge	3,394 mg Cu kg^{-1} sludge 67.9 kg Cu ha^{-1} $year^{-1}$ 69 mg Cu kg^{-1} soil 1995	Selkirk	1995
15	Cadmium-rich digested sludge	48.9 mg Cd kg^{-1} sludge 4.3 kg Cd ha^{-1} $year^{-1}$ 1.2 mg Cd kg^{-1} soil 1995 2.2 mg Cd kg^{-1} soil 1996	Perry Oaks	1995 & 1996

<u>Sampling by pitfall traps</u>

Pitfall traps consisted of plastic beakers of 150 ml volume, and a diameter of 6 cm. These were buried in the ground so that their rims were flush with the soil surface. The mouth of the traps were covered by a wide mesh gauze (mesh size 1.5 cm) which was secured by a metal staple. This gauze prevented small mammals and amphibians from entering the traps. Pitfall traps contained 20 ml of ethylene glycol which acted as a preservative. On each sampling date (see below) five pitfall traps were inserted randomly in each plot, and retained *in situ* for a period of seven days. Pitfall traps were not established within 1 m of the edge of each plot to avoid any edge effect (Gill 1969).

As with the suction samples, pitfalls were also studied over a period of two years (1995 - 1996). During 1995, pitfalls were inserted in all six treatments described in Table 6.1 (total of 18 plots) on 26 June and again on 24 July. This provided initial information on these treatments. It was then decided (as with the suction samples) to concentrate on the digested sludge treatments in 1996, namely the control, digested, zinc-rich and cadmium-rich sludges. During 1996, pitfalls were inserted on 24 April, 27 May, 20

June and 1 August. For each year, on subsequent sampling dates, pitfalls were inserted into the same holes as the first pitfalls (26 June 1995 and 24 April 1996) to avoid the 'digging in' effect on subsequent dates. Pitfall traps were therefore established in the same location for all sampling dates in 1995 and 1996.

After collection, the pitfall samples were rinsed with alcohol to remove the preservative and heated in alcohol until the Collembola ceased to float (to prevent dehydration). Samples were then stored in a cold room (5°C) until they could be sorted and the Collembola identified (see Chapter 3).

Body-lengths

Measurements were taken for the body lengths of all specimens of *I. palustris* collected from all pitfall traps established during the week commencing 1 August 1996. These measurements were taken to investigate sub-lethal effects on growth and reproduction. Body lengths (minus the head) were measured under the dissecting microscope using a stage mounted graticule (accurate to 0.1 mm).

6.3 Statistical analysis

As in Chapters 4 and 5, the five samples collected from a plot on a particular sampling date were pooled to obtain an indication of collembolan community structure for each plot. The pooled data were utilised in statistical analyses. For all analyses, the 1995 and the 1996 data sets were investigated separately.

Species composition

Three-way analyses of variance (ANOVAs) were performed (on the square root transformed data) to test for date, block and treatment effects. The Tukey multiple comparison test was applied to locate differences found by ANOVA (Zar 1984). ANOVAs were performed on the number of total Collembola and the most frequently occurring species. Although the square-root transformation normalised the data for most species, in some species the variances still differed considerably between replicas of a treatment (F-test). To prevent wrongly rejecting the null hypothesis the significance level was set at 0.01 (rather than 0.05: Day & Quinn 1989).

Detrended Correspondence Analysis

DCA's were performed on the species relative abundance (without downweighting of rare species) using DECORANA (Hill 1979). DCA was chosen as it provided a method of looking simultaneously at the effects of treatment, block and season on the overall collembolan population structure.

Correlation analyses

To investigate the relationship between grass height and species abundance, a product moment correlation was calculated. The total number of each species caught by pitfall trapping (square root transformed) on a particular plot and sampling date was correlated against the mean grass height on that plot and sampling date. This correlation was performed on all plots sampled in 1996 and all sampling dates.

6.4 Results

General information

A total of 11,153 individuals from 16 species were identified in 1995, while in 1996 44,745 individuals from 20 species were identified (see Appendix XIV for species lists). As was found in the suction samples (see Chapter 5), more Collembola were collected in almost all samples in 1996 compared to 1995.

Results of ANOVAs

To test for effects of date, block and treatment, 3-way ANOVAs were performed on the 1995 and 1996 data separately. The results of these tests can be seen in Table 6.2 (for the 1995 data) and Table 6.3 (for the 1996 data) and Figures 6.2-6.9 below.

Species composition and date

In 1995, the numbers of total Collembola and the species *I. viridis*, *I. anglicana*, *S. aureus* and *C. denticulata* were more abundant on 24 July than 26 June (see Table 6.2). This may indicate an increase in abundance or activity as the summer progressed. However, as the grass was cut on 17 July, pitfall catches of the above species may be greater on 24 July as a consequence of an increased sampling efficiency in shorter grass.

Table 6.2 Effect of date and treatment on species sampled by pitfall traps: 1995

	Date					Treatment		
Species	F-value	Probability df = 1, 10 n = 18	June	July	Location of difference df =10 , k = 2	F-value	Probability df = 5, 10 n = 6	Location of difference df = 10, k = 6
Total	25.10	< 0.001	14.7 *± 3.6*	19.6 *± 2.8*	July > June	2.42	N.S.	-
I. viridis	8.20	< 0.01	1.2 *± 1.0*	2.3 *± 1.5*	July > June	3.30	< 0.05	Cd < D
I. anglicana	104.63	< 0.001	2.9 *± 0.9*	7.2 *± 1.5*	July > June	1.48	N.S.	
I. palustris	0.04	N.S.	10.9 *± 3.4*	11.1 *± 2.2*	-	0.87	N.S.	-
L. cyaneus	2.84	N.S.	1.4 *± 1.6*	2.6 *± 3.1*	-	3.36	0.01	Cd & Cu < D
S. viridis.	0.02	N.S.	4.7 *± 1.0*	4.7 *± 1.0*	-	0.87	N.S.	-
S. aureus	67.85	< 0.001	5.6 *± 1.5*	9.0 *± 0.9*	July > June	1.13	N.S.	-
S. elegans	4.08	N.S.	2.4 *± 1.8*	4.0 *± 2.7*	-	1.84	N.S.	-
C. denticulata	27.32	< 0.001	3.1 *± 1.8*	6.3 *± 2.1*	July > June	2.85	N.S.	-

Table 6.2 The above table shows the results of the 3-way ANOVAs followed by Tukey tests (probability for Tukey tests set at 0.01 and k = number of means compared). The mean values (square root transformed) for the June and July samples are also show with the standard deviations given below in italics. As no block effect was found for any of the species tested (df = 2, 10), this information is omitted from the table. Codes for the treatments are : D - digested, Cu - copper-rich and Cd - cadmium-rich.

* Although the probability level for the ANOVAs was set at < 0.01, it was decided to include ANOVAs with P ≤ 0.05 when the Tukey test showed a difference at < 0.01.

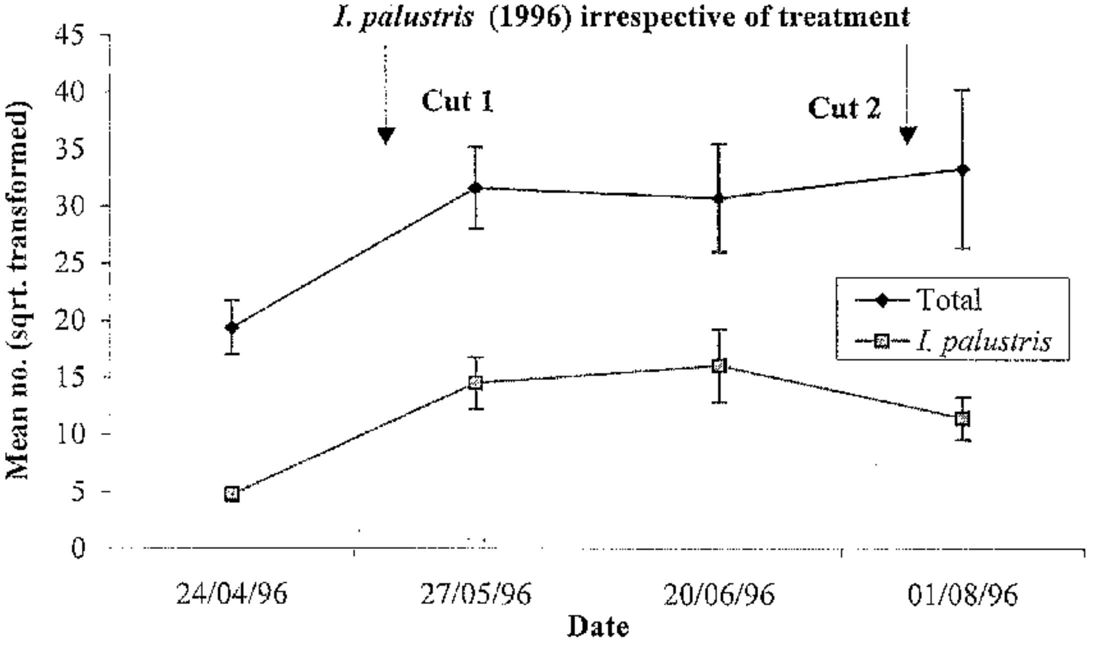
Table 6.3 Effect of date and treatment on species sampled by pitfall traps: 1996

	Date			Treatment		
Species	F-value	Probability df = 3, 18 n = 12	Location of difference df =18, k = 4	F-value	Probability df = 3, 18 n = 12	Location of difference df = 18, k = 4
Total	7.58	< 0.001	All > April	6.14	< 0.01	D > Cd
I. tigrina	1.02	N.S.	-	1.32	N.S.	-
I. viridis	12.14	< 0.001	August > All	8.97	< 0.001	D > Cd
I. notabilis	7.85	< 0.001	April > All	4.51	< 0.01	D > C
I. anglicana	62.93	< 0.001	May > June & August > April	3.47	N.S.	-
I. palustris	25.28	< 0.001	All > April	6.32	< 0.01	All > C
I. maculatus	17.87	< 0.001	May & April > August May > June	8.76	< 0.001	D > C & Cd
I. juvenile	31.12	< 0.001	All > August May > April	12.61	< 0.001	All > C
L. cyaneus	5.83	< 0.01	August > April & May	10.60	< 0.001	C & D > Cd
H. nitidis	7.65	< 0.001	August > April & May	4.21	0.01	D > C
S. viridis.	0.39	N.S.	-	3.99	N.S.	-
S. aureus	23.72	< 0.001	May & June > April June > August	1.39	N.S.	-
S. elegans	0.97	N.S.	-	1.40	N.S.	-
S. pumilis	17.95	< 0.001	August > All	0.78	N.S.	-
C. denticulata	1.21	N.S.	-	4.56	< 0.01	Zn > Cd

Table 6.3 The above table shows the results of the 3-way ANOVAs followed by Tukey tests (probability for Tukey tests set at 0.01). As no block effect was found for any of the species tested (df = 2, 18), this information is omitted from the table. The codes for the treatments are: C - control, D - digested, Zn - zinc-rich and Cd - cadmium-rich.

In 1996, the abundance of total Collembola and of *I. palustris* in pitfall traps was significantly lower in April than on any other sampling date (see Table 6.3 and Figure 6.2). This suggests that Collembola in general, and the species *I. palustris,* were less abundant, or active, in April than on any other sampling date. *Isotoma viridis, S. pumilis, H. nitidis* and *L. cyaneus*, on the other hand, occurred in their highest abundance in August pitfalls (see Table 6.3 & Figure 6.3). *Isotoma viridis* was also found in its highest abundance in late summer 1995 after grass cutting. These species may be more abundant, or active, in late summer or, as the grass was cut prior to the August sampling, they may be sampled more efficiently in short grass.

Figure 6.2 Effect of date on abundance of total Collembola and *I. palustris* (1996) irrespective of treatment



Figures 6.2 The above graph shows the effect of date on the abundance of species/total Collembola caught by pitfall trapping at Auchincruive in 1996. Error bars show standard deviations.

Sminthurinus aureus increased in pitfalls from April to May/June suggesting it increased, or became more active, as the summer progressed (see Table 6.3 & Figure 6.4). It then significantly decreased from June to August. This was unlikely to be the consequence of *S. aureus* being susceptible to grass cutting, as its abundance increased following the May cut, and also after cutting in 1995. It is therefore suggested that environmental conditions were unsuitable for this species in August 1996.

Isotoma/Isotomurus juveniles peaked in abundance in May pitfalls, before declining to reach their lowest abundance in August pitfalls (see Table 6.3 & Figure 6.4). This suggests that *Isotoma/Isotomurus* juveniles predominantly hatch in May, and may also indicate an increased sampling efficiency in shorter grass. *Isotoma anglicana* also showed a peak in abundance in May pitfalls, but for this species the lowest abundance was found in April pitfalls (see Table 6.3 & Figure 6.4). It is therefore suggested that conditions were more favourable for this species in May, and that it may also be sampled more efficiently in shorter grass.

Figure 6.3 Effect of date on abundance of *H. nitidis, I. viridis, S. pumilis* and *L. cyaneus* (1996) irrespective of treatment

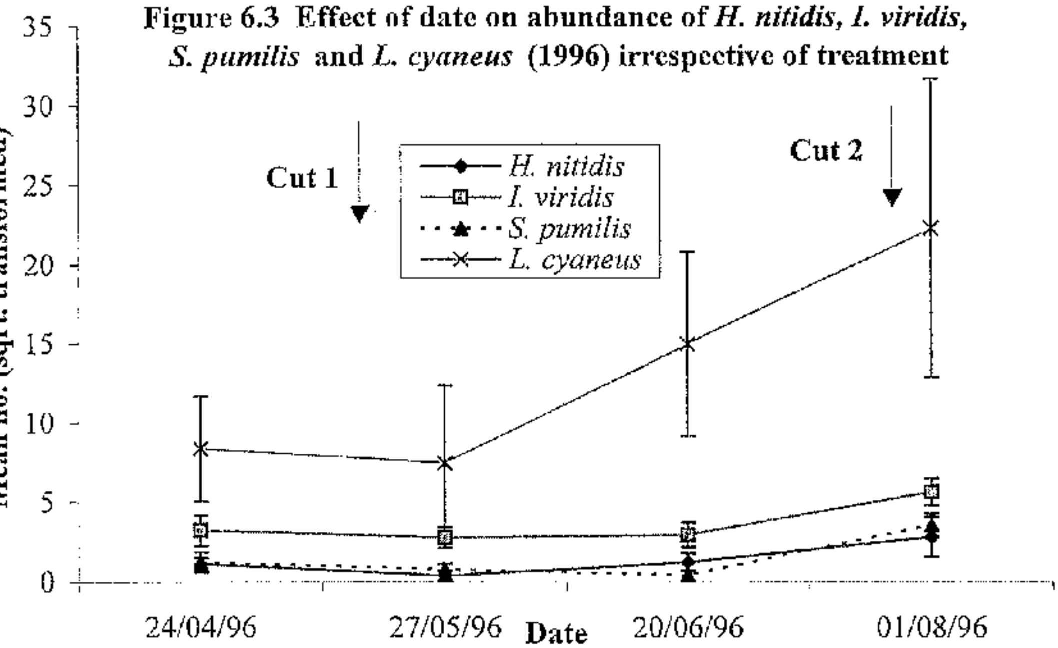
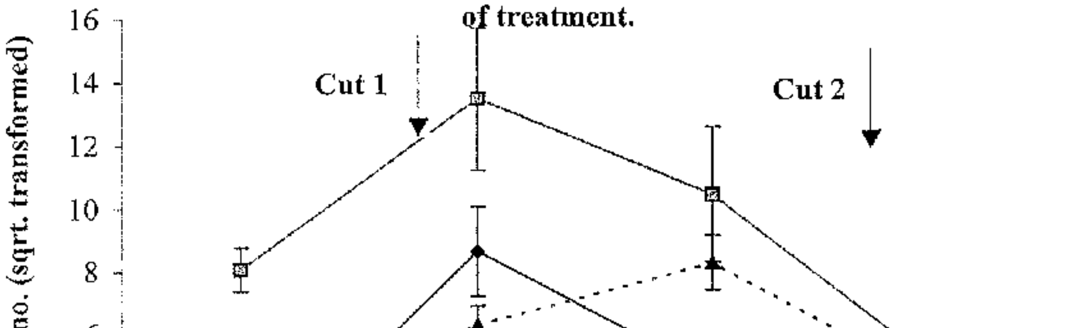


Figure 6.4 Effect of date on abundance of *I. anglicana, Isotoma/Isotomurus* juveniles and *S. aureus* (1996) irrespective of treatment.



Figures 6.3 & 6.4 The above graphs show the effects of date on the collembolan composition caught by pitfall trapping at Auchincruive in 1996. Error bars show standard deviations.

Isotoma notabilis was significantly more abundant in April than on any other sampling date (see Table 6.3 & Figure 6.5). *Isotoma maculatus* also tended to decline as the season progressed (see Table 6.3 & Figure 6.5). The abundance of this species was significantly higher in May than in June or August, and was also more abundant in April than August. It would therefore appear that these two species were favoured by the cooler conditions in April and May.

Figure 6.5 Effect of date on abundance of *I. notabilis* and *I. maculatus* (1996) irrespective of treatment

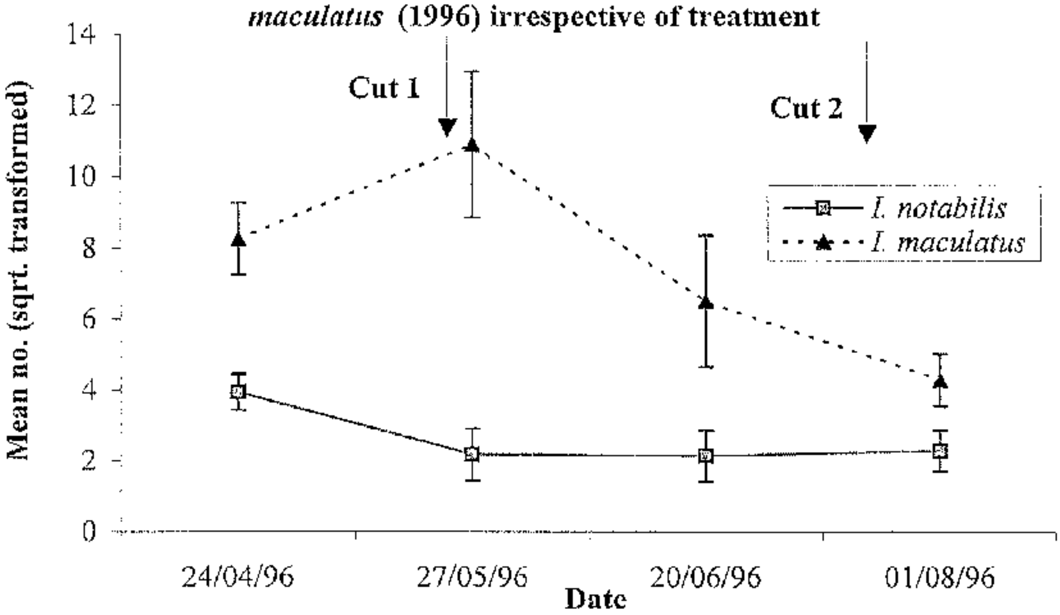


Figure 6.5 The above graph shows the effects of date on the collembolan composition caught by pitfall trapping at Auchincruive in 1996. Error bars show standard deviations.

Species composition and treatment

In 1995, two species were found to be affected by treatment, namely *L. cyaneus* and *I. viridis* (see Table 6.2). Both *L. cyaneus* and *I. viridis* were adversely affected by the addition of cadmium-rich sludge, and the abundance of these species in pitfall traps was significantly less in cadmium-rich plots than digested plots (see Table 6.2 & Figure 6.6). The probability that the cadmium-rich and the digested plots were different with respect to the abundance of *I. viridis*, was significant at a probability of < 0.05 (see Table 6.2). As the variances were heterogeneous (F-test), the likelihood of a Type one error was increased (Day & Quinn 1989), and this result should therefore be treated with caution. The Tukey test, however, found that the probability of the cadmium-rich and digested plots differing was < 0.01, suggesting that effects were real.

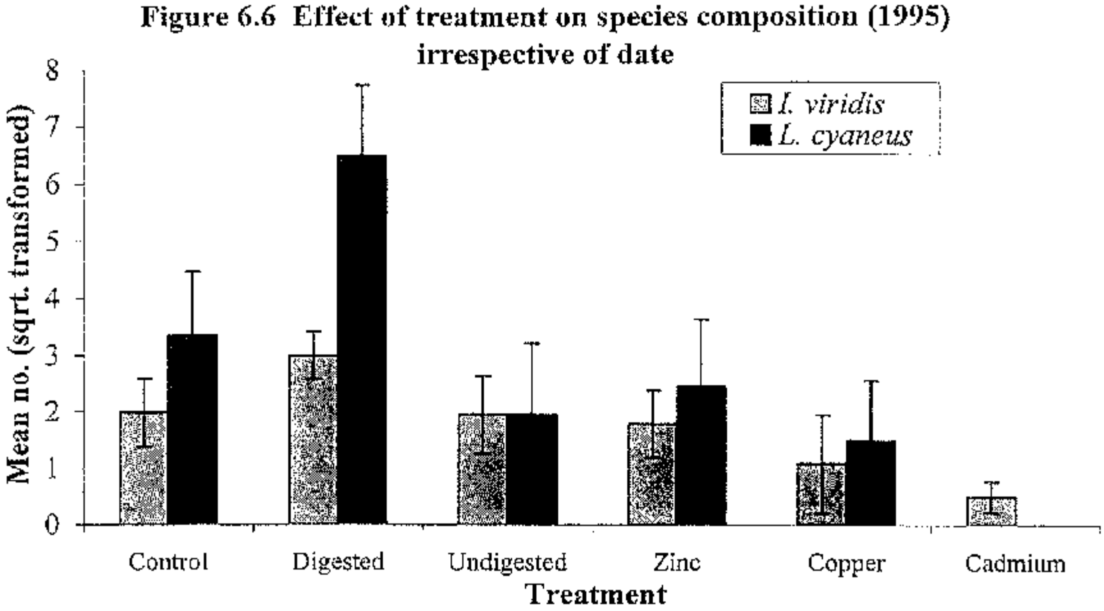


Figure 6.6 The above graph shows the effects of treatment (Figure 6.6) on the abundance of species caught by pitfall trapping at Auchincruive in 1995. Error bars show standard deviations.

The cadmium-rich sludge adversely affected *I. viridis* and *L. cyaneus* in 1996 also (see Table 6.3 & Figure 6.7). The numbers of *I. viridis* and *L. cyaneus* caught by pitfall traps in the digested plots was higher than in cadmium-rich plots. Furthermore, the abundance of *L. cyaneus* in the cadmium-rich plots was also less than the control plots. In this year, the total collembolan abundance was found to be adversely affected by the application of cadmium-rich sludge, with the total abundance being lower in pitfalls in the cadmium plots than those in digested plots (see Table 6.3 & Figure 6.7).

The abundance of *I. maculatus, H. nitidis* and *I. notabilis* was greater in the digested sludge plots than the control plots (see Table 6.3 & Figure 6.8). The abundance of these species in the zinc and cadmium-rich plots was not significantly different from the control plots. This suggests that the presence of zinc and cadmium in sludge prevented these species from reaching the abundance found in the digested plots. Furthermore the abundance of *I. maculatus* in the cadmium-rich plots was actually significantly lower than the digested plots. *Isotoma/ Isotomurus* juveniles and *I. palustris* had a significantly higher abundance in all plots receiving sludge than control plots (see Table 6.3 & Figure 6.9). It would therefore appear that they were not adversely affected by the presence of cadmium or zinc in sludge. *Ceratophysella denticulata* was actually more abundant in the zinc-rich sludge plots than the cadmium-rich sludge plots (see Table 6.3 & Figure 6.9).

Figure 6.7 Effect of treatment on abundance of total Collembola, *I. viridis* and *L. cyaneus* (1996) irrespective of date

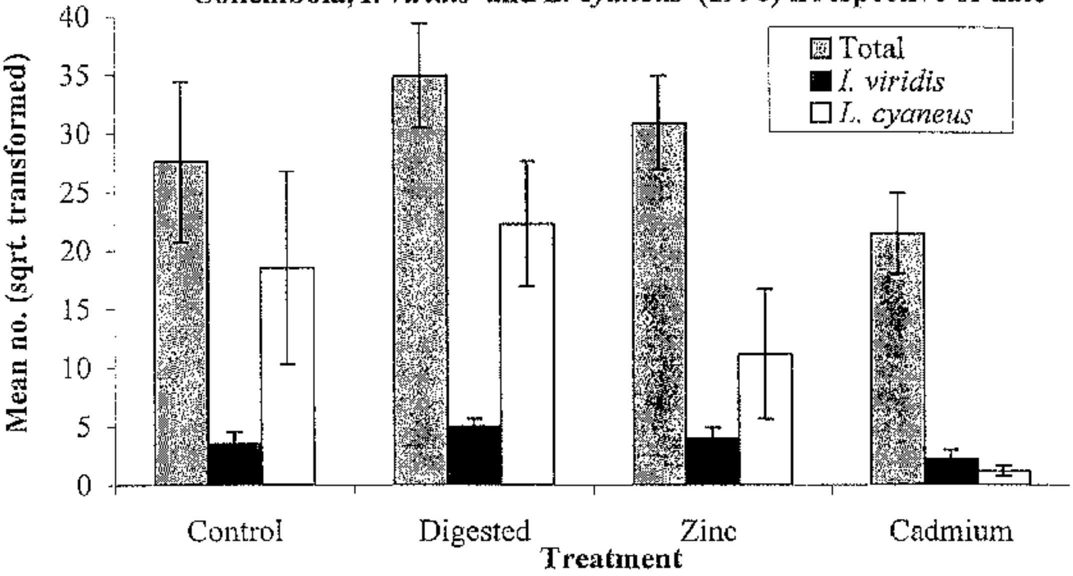
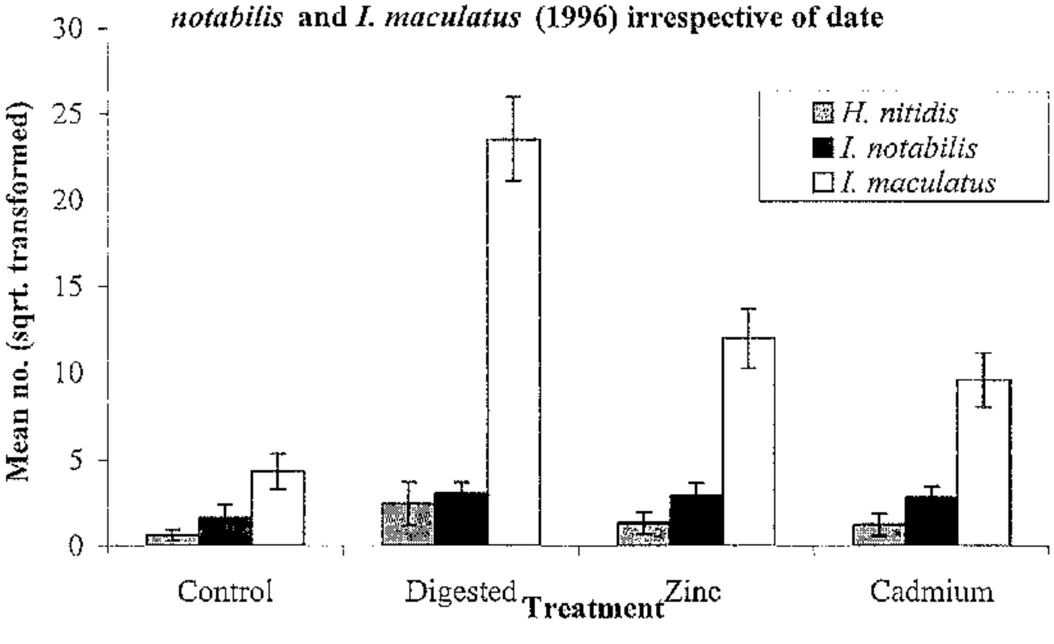


Figure 6.8 Effect of treatment on abundance of *H. nitidis, I. notabilis* and *I. maculatus* (1996) irrespective of date



Figures 6.7 & 6.8 The above graphs show the effects of treatment on the abundance of total Collembola and species composition caught by pitfall trapping at Auchincruive in 1996. Error bars show standard deviations.

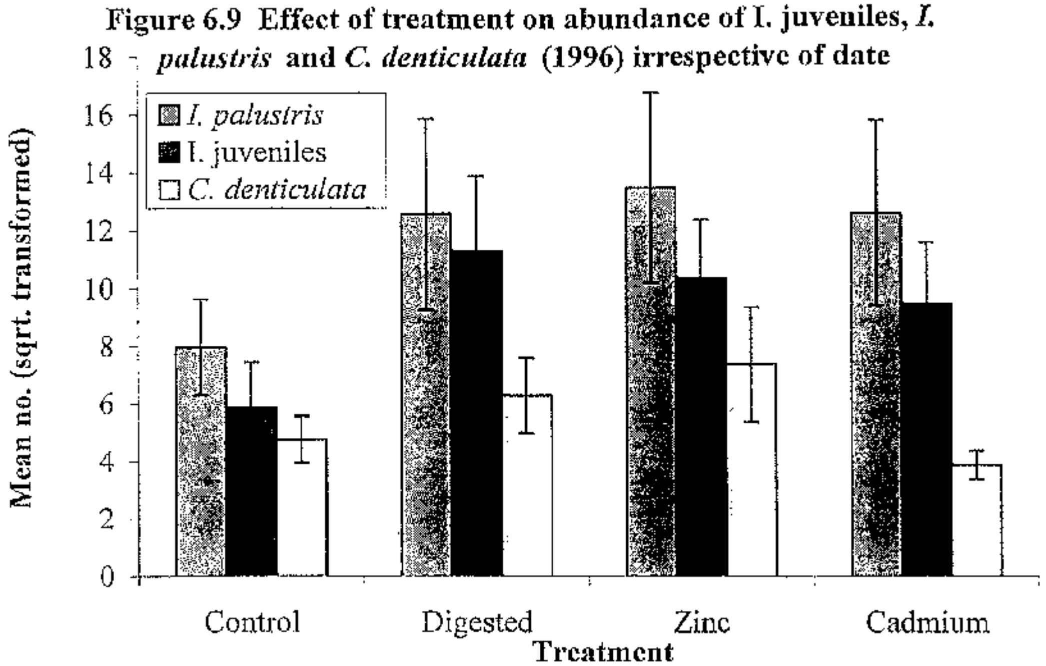


Figure 6.9 The above graphs show the effects of treatment on collembolan species composition caught by pitfall trapping at Auchincruive in 1996. Error bars show standard deviations.

DCA 1995

The 1995 DCA produced eigenvalues of 0.127, 0.057, 0.027 and 0.014 for axes 1-4 respectively. These values indicate that the amount of between-plot variation in collembolan assemblages accounted for by axes 1-4 was 56.4%, 25.3%, 12.0% and 6.2%, respectively. As axes 3 and 4 accounted for so little of this variation, they were not considered further. The samples and species scores were plotted to obtain sample and species ordinations, respectively (see Figures 6.10 and 6.11).

From Figure 6.10 it can be seen that in 1995 the main factor separating samples along axes 1 and 2 was sampling date, with the June plots lying above and to the left of the July plots. The species ordination indicates that the samples taken on 24 July had a higher occurrence of *I. viridis, I. anglicana, S. aureus* and *C. denticulata* (see Figure 6.11) than those taken on 26 June. This is supported by the ANOVAs (see above). The species ordination therefore also indicates that the main driving force influencing the division of samples along axes one was season.

Figure 6.10 Sample DCA ordination for pitfalls 1995 (relative abundance)

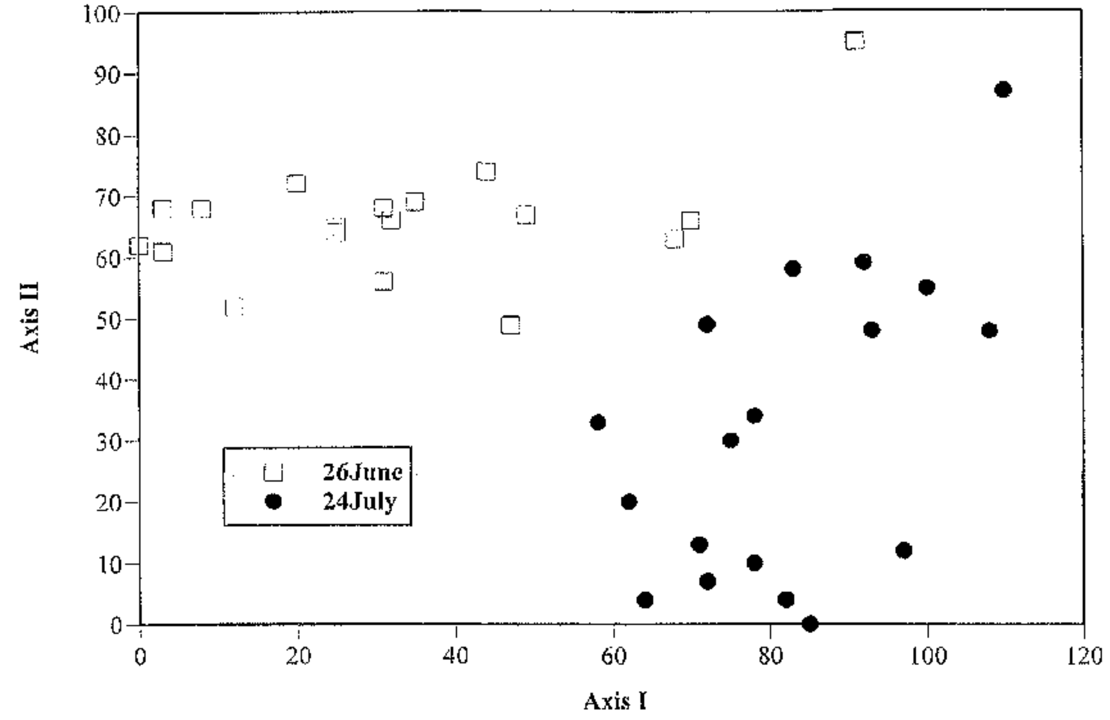
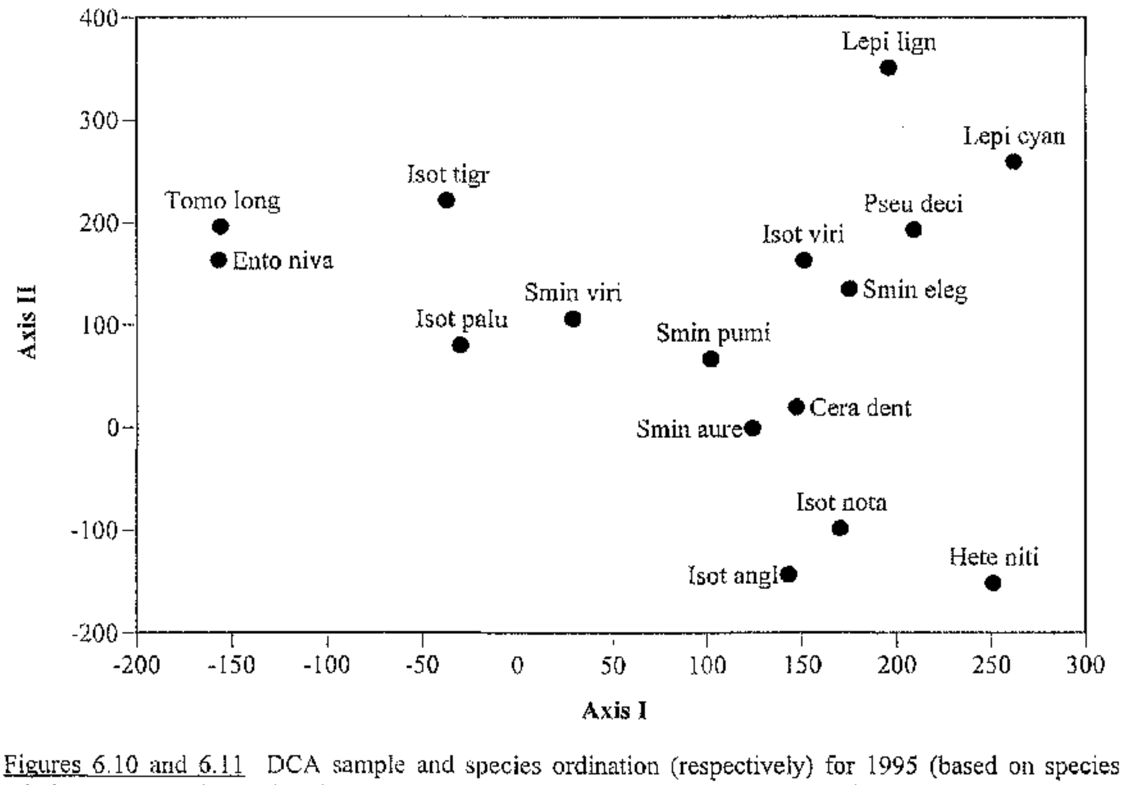


Figure 6.11 DCA species ordination for pitfalls 1995 (relative abundance)

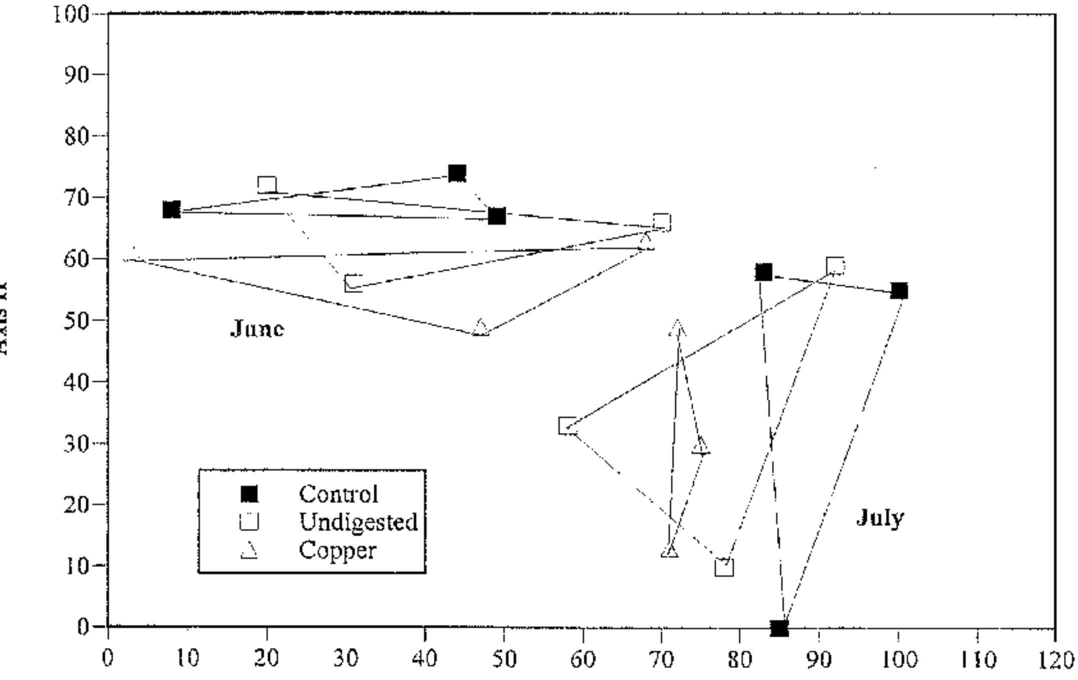


Figures 6.10 and 6.11 DCA sample and species ordination (respectively) for 1995 (based on species relative abundance). Each point represents one plot in sample ordination. Species codes can be found in Appendix IX.

The axes scores derived from the ordination were re-drawn showing undigested and digested treatments separately (see Figures 6.12 & 6.13, respectively). This was done to enable treatment effects to be observed more easily. For the undigested treatments, on both sampling dates, the copper-rich and control plots lie distinct from each other suggesting that the collembolan species composition differed most between these plots (see Figure 6.12). However, on both sampling dates there were also considerable differences between replicas within a treatment, which were as great as the differences between treatments.

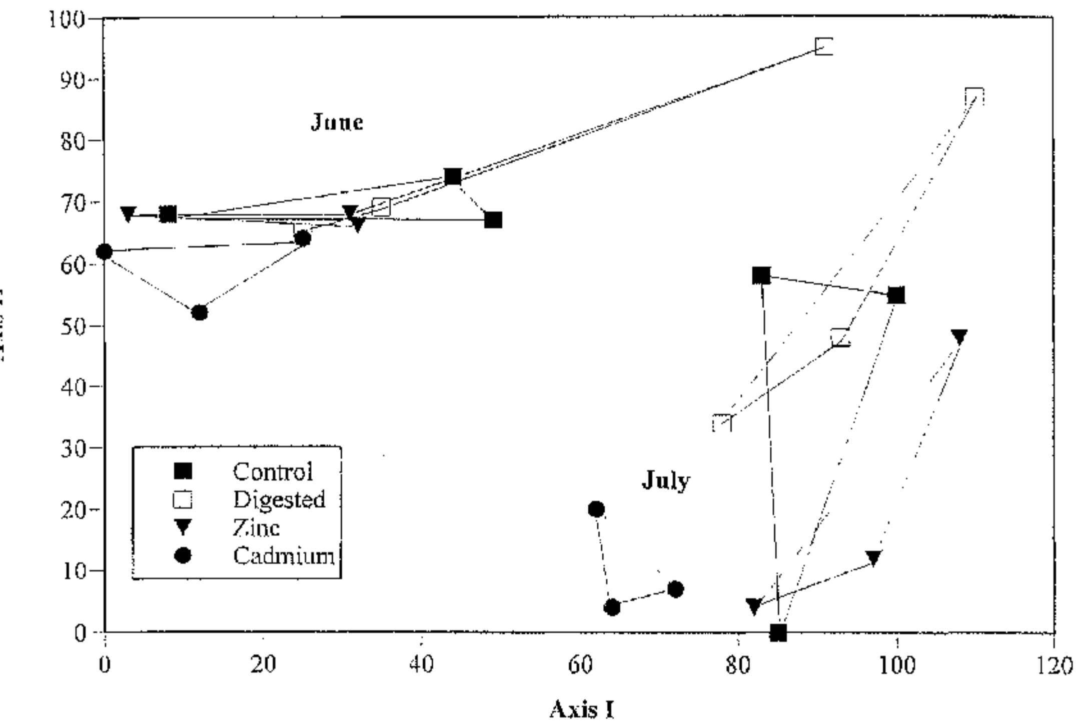
For the digested sludge treatments, the cadmium-rich plots lie distinct from the other treatments on both sampling dates (see Figure 6.13). This indicates that the collembolan composition of cadmium-rich plots differed most from the other treatments. However, as was found for the undigested treatments, considerable differences occurred between replicas of the digested treatments also.

Figure 6.12 DCA ordination for pitfalls 1995: undigested treatments



Figures 6.12 DCA ordinations for the undigested treatments. Each point represents one plot.

Figure 6.13 DCA ordination for pitfalls 1995: digested treatments



Figures 6.13 DCA ordinations for the digested treatments. Each point represents one plot.

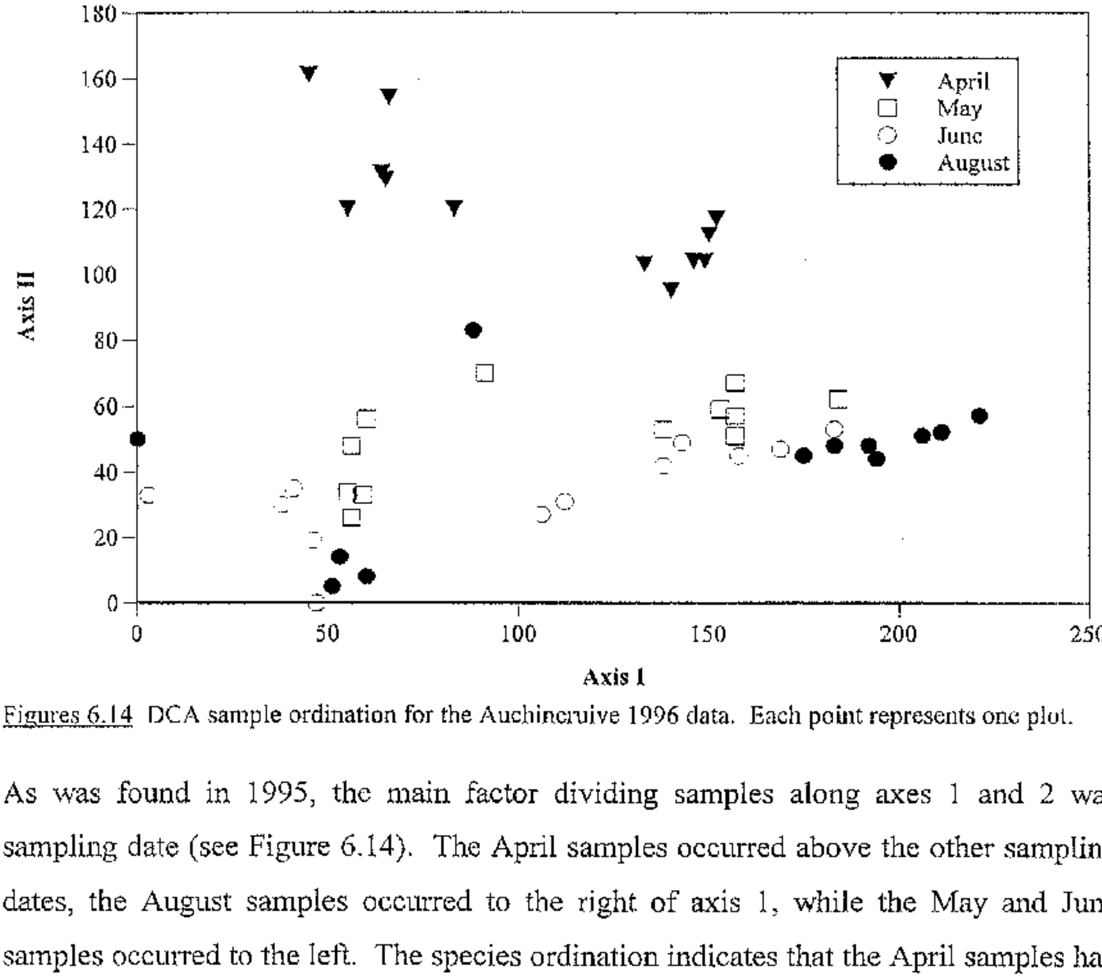
Closer examination of the ordinations found that for almost all treatments (on both sampling dates), block two replicas differed most between the three replicas of a treatment. It would therefore appear that while no individual species was affected by block (see above), the collembolan composition as a whole was. The suction samples and litterbags also indicated that in 1995, block two differed most between replicas of a treatment (see Chapters 4 & 5 respectively). Since this block appears to be different on both sampling dates, irrespective of sampling method, it is suggested that it was a true effect and not merely a consequence of sampling error.

Closer examination of the available soil data for this block indicated that its pH and conductivity was lower than the other blocks prior to sludge application (see Appendix VI). It is possible that the observed differences in block two were a consequence of the soil being more acidic. The species ordination for the suction data indicated that pH may have influenced the separation of plots (see Chapter 5).

DCA 1996

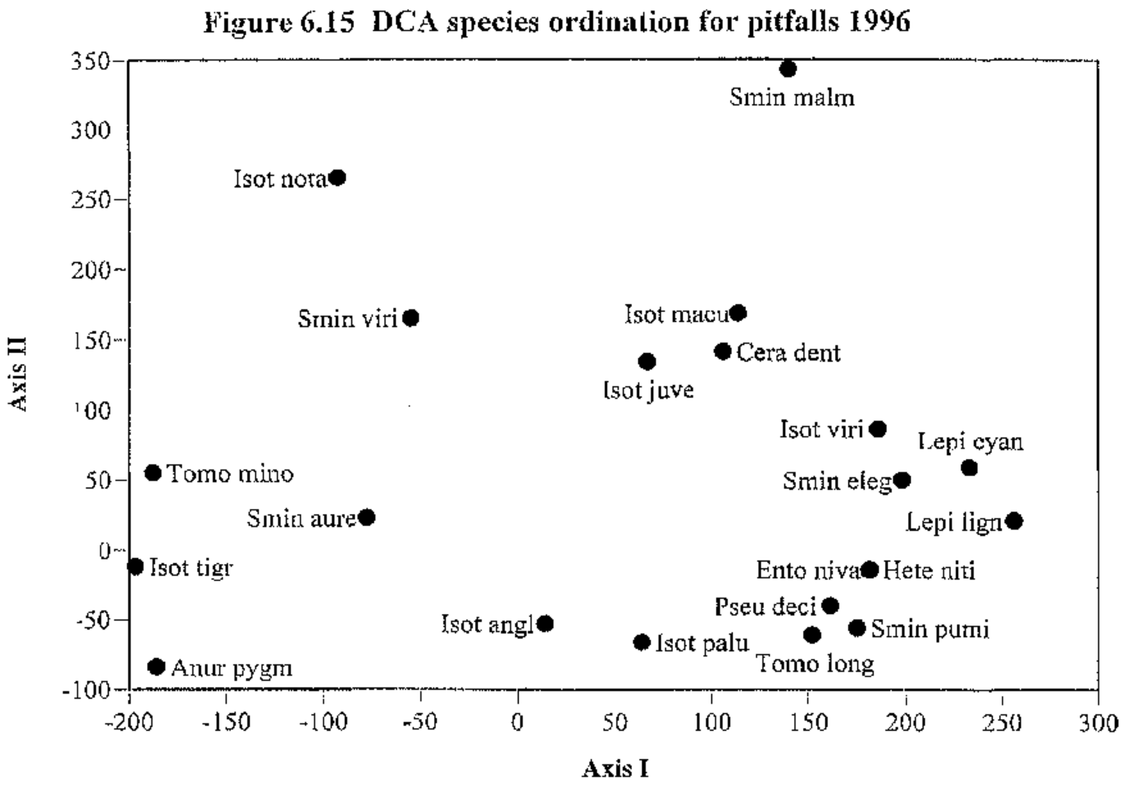
The 1996 DCA produced eigenvalues of 0.422, 0.154, 0.058 and 0.029 for axes 1-4 respectively. The between-plot variation in collembolan community structure accounted for by axes 1-4 was therefore 63.7%, 23.2%, 8.8% and 4.4%, respectively. As in 1995, axes 3 and 4 accounted for little of the observed variation and were not considered further. The species and sample scores for axes 1 and 2 were plotted to obtain species and sample ordinations (see Figures 6.14 & 6.15 respectively).

Figure 6.14 Sample DCA ordination for pitfalls 1996 (relative abundance)



Figures 6.14 DCA sample ordination for the Auchincruive 1996 data. Each point represents one plot.

As was found in 1995, the main factor dividing samples along axes 1 and 2 was sampling date (see Figure 6.14). The April samples occurred above the other sampling dates, the August samples occurred to the right of axis 1, while the May and June samples occurred to the left. The species ordination indicates that the April samples had a higher abundance of *I. maculatus* and *I. notabilis* (see Figure 6.15) than the other sampling dates and this is supported by the ANOVAs (see Table 6.2). The species ordination also indicates that *L. cyaneus, I. viridis* and *H. nitidis* were more abundant in the August samples, while *S. aureus*, *Isotoma/Isotomurus* juveniles and *I. anglicana* were more abundant in the May/June samples (see Figure 6.15). This is again supported by the ANOVAs (see Table 6.2). The species ordination therefore also indicates that date influenced the separation of samples along axes 1 and 2. There was, however, still considerable differences between samples on a particular date.



Figures 6.15 DCA species ordination for the Auchincruive 1996 data (see Appendix IX for species codes).

To enable treatment effects to be seen more easily, the axes scores were re-drawn showing each sampling date separately (see Figures 6.16-6.19). For all sampling dates the biggest difference in collembolan community structure occurred between the cadmium-rich and digested plots (see Figures 6.16-6.19). For all dates the separation between these treatments was substantially larger than differences between replicas.

Large differences between replicas, however, occurred for the zinc-rich and control plots. In the case of the control plots, the block two replicas were consistently separated to the left of the other replicas for all sampling dates. Block two was not considerably separated from the block one and three replicas in the other treatments, hence suggesting that the division was the result of this individual plot being different, rather than the entire block. Examination of the available soil data for this plot in 1996 indicated

Figure 6.16 DCA ordination April data (1996)

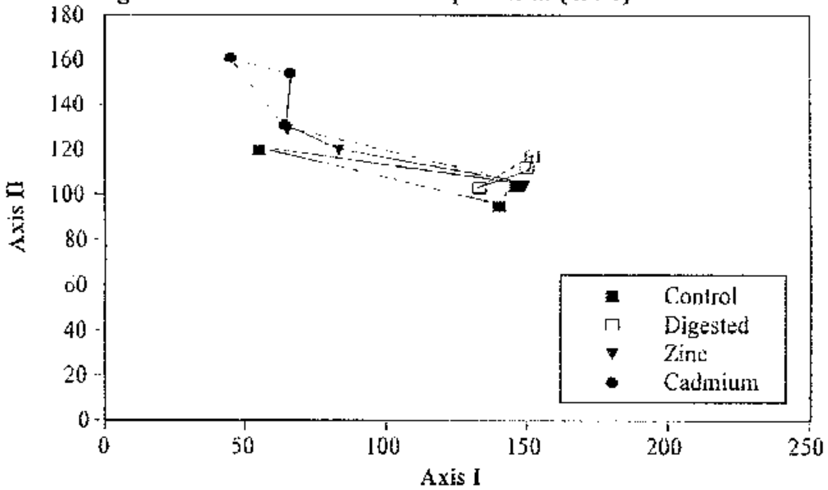


Figure 6.17 DCA ordination May data (1996)

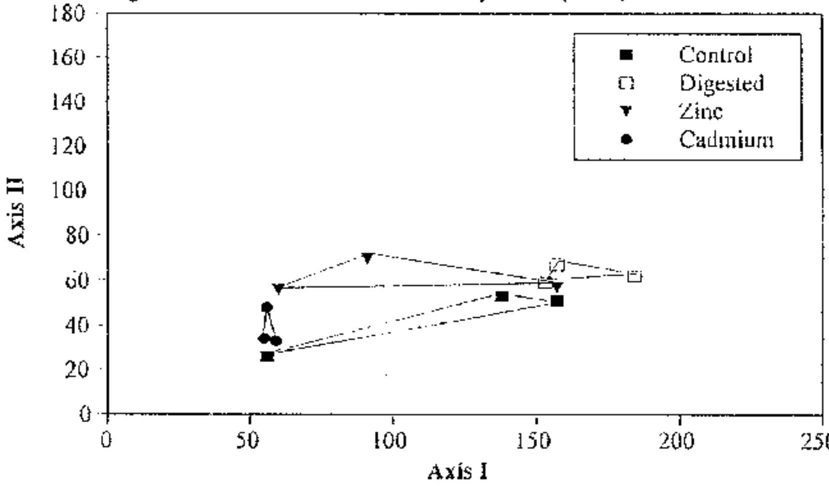


Figure 6.18 DCA ordination June data (1996)

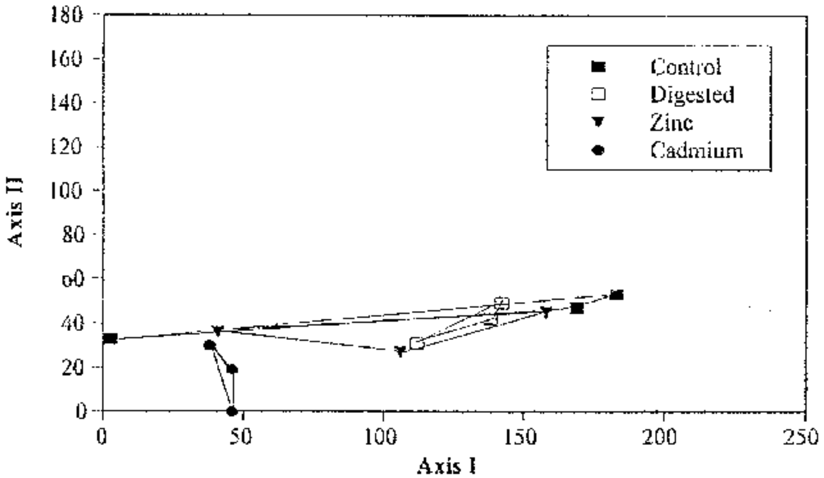
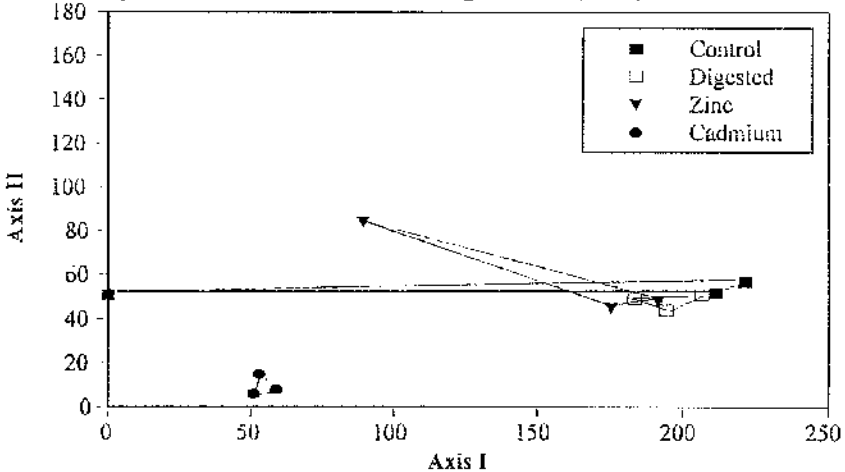


Figure 6.19 DCA ordination August data (1996)



Figures 6.16-6.19 DCA ordinations for 1996 pitfalls at Auchincruive showing April, May, June and August respectively. Each point represents one plot.

that biomass carbon was higher, while pH and grass yield were lower in this replica than the other two control replicas (see Appendices VIII & XI).

For the zinc-rich treatments, block two occurred towards the right of axis one and block one occurred towards the left. In the April and May ordinations, block two differed most between the three replicas of the zinc-rich plots, while in June and August block one differed most. Examination of the soil data did not, however, show any variable that differed consistently between these plots (see Appendix XI).

For both the zinc-rich and control plots, differences between replicas appeared to be consistent between sampling dates. This indicates that the variation was not simply a consequence of sampling error, but a real difference between the plots. It would therefore appear that while treatment was a main factor influencing the collembolan composition in the cadmium-rich and digested plots, other environmental parameters (e.g. pH and grass height) were also important in the zinc-rich and control plots.

Relationship between grass height and species abundance

A product moment correlation was calculated to investigate the relationship between grass height and the abundance of the most frequently occurring species (i.e. those listed in Table 6.3) for the 1996 data set. Results of the correlations can be seen in Table 6.4 below (only species showing a significant correlation are shown). A positive correlation was found between grass height and the abundance of *S. aureus* and *Isotoma/Isotomurus* juveniles, indicating that the abundance of these species increased with grass height. The abundance of *I. viridis* and *S. pumilis* showed a negative correlation with grass height, therefore indicating that the abundance of these species decreased as grass height increased. It is, however, possible that grass height was confounded with sampling date, and care should therefore be taken in interpreting these results.

Table 6.4 Relationship between grass height and species abundance

Species	**Product moment correlation coefficient**	**t-value**	**Probability df=46 n=48**
I. viridis	-0.53	-4.19	< 0.01
I. juvenile	0.32	2.27	< 0.01
S. aureus	0.33	2.39	< 0.01
S. pumilis	-0.39	-2.91	< 0.01

Effect of treatment on size distribution of *I. palustris*

For the August pitfall traps (1996), the size of *I. palustris* was measured, and the distribution plotted as a percentage (see Figure 6.20). This was done to detect any sub-lethal effects of treatment on this metal tolerant species (e.g. changes in growth rate and reproduction). With the exception of the control, all treatments showed two main peaks suggesting that two generations were present in August. In 1996, the number of juvenile *Isotomurus/Isotoma* species peaked in abundance in May (see above). It is therefore suggested that the second peak in August represents this first generation in May, while the first peak represents a second generation that hatched in late July. The second peak is larger than the first peak, suggesting that reproduction was highest in May, and this was in agreement with the results from the ANOVA (see above).

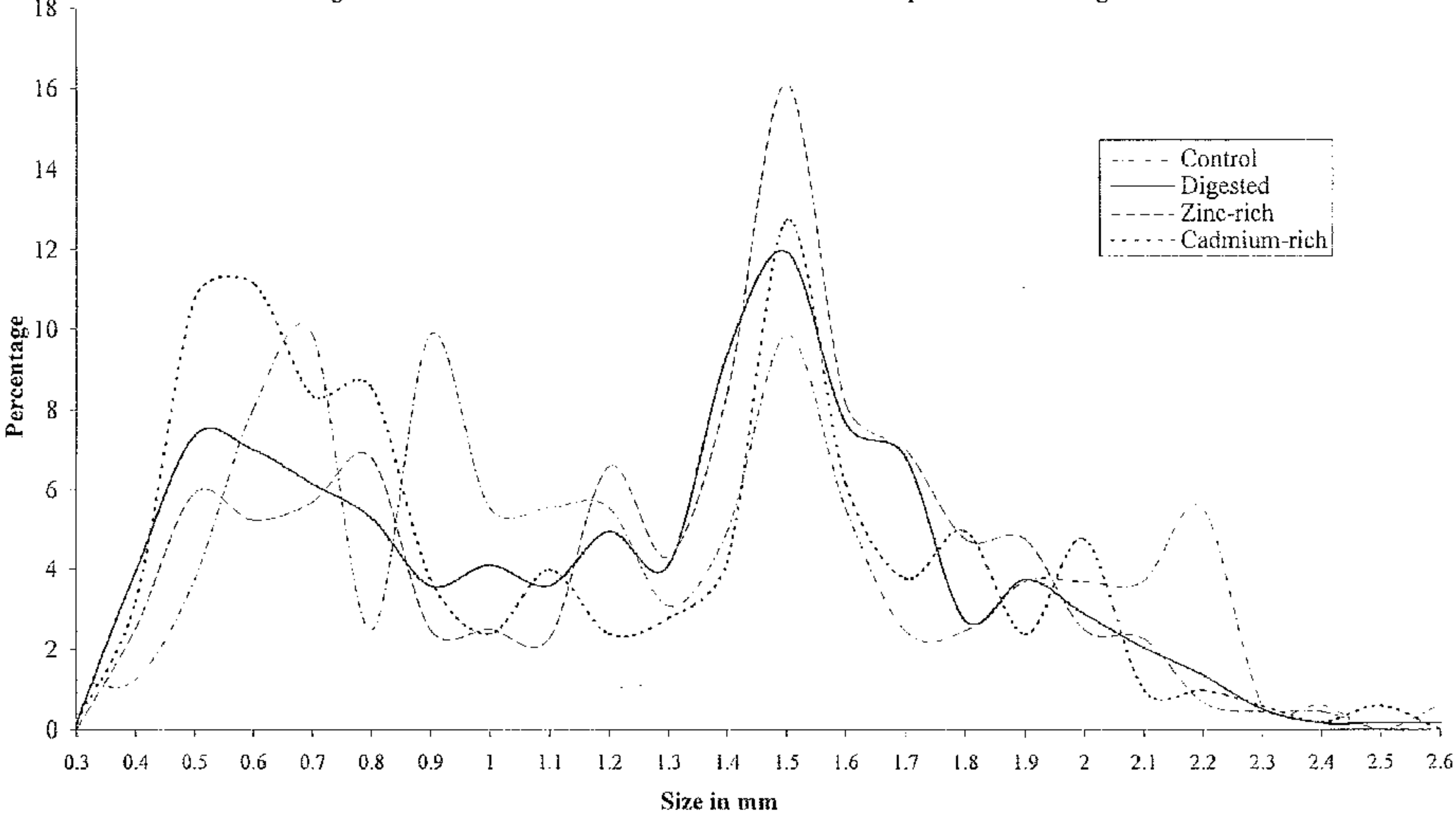
When compared to the zinc-rich and digested plots, a greater proportion of *I. palustris* in the cadmium-rich plots were less than 1mm. This may indicate that reproduction was greater in the cadmium-rich plots. Alternatively it may indicate that survival of juveniles to a size greater than 1mm was lower in the cadmium-rich plots than the digested and zinc-rich plots. It is therefore possible that while the addition of cadmium-rich sludge does not adversely affect reproduction, it may affect growth. The proportion of individuals with a size greater than 2.0 mm is greater in the control plots than on the sludged plots. This suggests that the growth rate (or survival) of *I. palustris* was greater in the control plots than the sludged plots.

6.5 Discussion

Effect of sampling date on species composition

The DCA ordinations indicated that in both 1995 and 1996, date was the main factor influencing collembolan composition with treatment being less important. As was found with the suction data, some species were more abundant in April (e.g. *I. notabilis*), others in May (e.g. *I. anglicana* and *Isotoma/Isotomurus* juveniles) and others in August (e.g. *H. nitidis, I. viridis* and *L. cyaneus*). Results from the suction data indicated seasonal differences in species abundance could only partly be accounted for by humidity preference and this was supported by the pitfall data. For example, both *L.*

Figure 6.20 Effect of treatment on size distribution of *I. palustris* on 1 August



cyaneus and *I. viridis* peaked in August despite *L. cyaneus* preferring dry conditions, while *I. viridis* preferred humid conditions (Joosse 1970). It would therefore appear that other environmental variables were involved in determining seasonal peaks.

Although *I. notabilis* was more abundant in April pitfalls, it occurred in its highest abundance in May suction samples (see Chapter 5). The higher capture rate of this species in the April pitfalls could not therefore be attributed to a higher abundance in April. It is possible that either *I. notabilis* was more active in April than on subsequent sampling dates, or that the 'digging in effect' lead to its higher abundance. The digging in effect is more pronounced for some species (e.g. *T. minor*), than for other species (e.g. *E. nivalis*: Joosse & Kapteijn 1968). It is therefore possible that *I. notabilis* was stimulated, or attracted, more by CO_2 than other species, and was consequently over-represented in the April pitfalls. It would be interesting to quantify any differences in CO_2 attraction/stimulation between this species and the other species present. This exemplifies how, through using both pitfalls and suction samples, a better understanding of collembolan ecology can be achieved.

Berbiers *et al.* (1989) found that while some species were caught more frequently in pitfalls set in long grass (*I. viridis* and *S. pumilis*), other species where caught more frequently in short grass (e.g. *S. aureus* and *S. elegans*). They suggested that differences between species were a consequence of differences in locomotory activity in the varying grass height. Contrary to the findings of Berbiers *et al.* (1989), this study found that *I. viridis* and *S. pumilis* were actually caught more frequently in short grass than long grass. In this study, the higher abundance of *I. viridis* and *S. pumilis* in short grass was thought to be a consequence of an increased sampling efficiency, and the abundance of these species in pitfalls was inversely related to grass height. Greenslade (1964) found that the abundance of carabid beetles caught in pitfall traps was also inversely related to the vegetation cover. The average grass height in Berbiers *et al.* (1989) study was 14.2 cm, and less than 6 cm for long and short grass respectively, whereas, in the present study it was 35 cm and 10 cm for long and short grass respectively. There were therefore considerable differences between the 'long' grass at the Auchincruive site and the 'long' grass at Berbiers's site. It is possible that while the grass at Auchincruive exceeded a threshold height which reduced the sampling efficiency, the grass at Berbiers's site did not.

The abundance of *Isotoma/Isotomurus* juveniles peaked in May in both pitfalls and suction samples. In agreement with other authors, reproduction for *Isotoma* and *Isotomurus* species was therefore greatest in April/May (Hale 1965; Joosse 1969). In August 1996, the size distribution of *I. palustris* individuals was bimodal. This indicates that on this date two generations of *I. palustris* were present, and Joosse (1969) also found that *I. palustris* gave rise to two generations per year in the Netherlands. It would therefore appear that in addition to egg laying in April/May, egg laying also occurred in late July. As *I. palustris* overwinters as half grown juveniles (Joosse 1969), it is suggested that the first generation of juveniles born in April/May were derived from the overwintering population, while the second generation born in late July were derived from the April/May population.

Effect of treatment on species composition

It was found that the collembolan community structure differed most between the cadmium-rich and digested plots and between the copper-rich and control plots. Differences between digested treatments were more pronounced in 1996. However, even in 1996, differences in community structure were apparent between the replicas of a treatment. Differences in replicas were consistent across sampling methods (i.e. suction and pitfalls) and sampling dates, indicating that they were not just a result of sampling error, but a true effect caused by differences in soil parameters (e.g. pH, conductivity and moisture content). Species specific differences in response to changes pH are well documented (Hågvar 1990; Hågvar & Abrahamsen 1980; Hågvar & Kjøndal, 1981), and the suction samples species ordination suggested that pH may have influenced the separation of samples in the ordinations (see Figure 5.15).

Species specific differences in metal susceptibility are also well documented (Bengtsson & Rundgren 1988; Filser *et al.* 1995; Hågvar & Abrahamsen 1990; Posthuma & Van Straalen 1993). In this study, the pitfall traps indicated that *I. viridis* and *L. cyaneus* were adversely affected by cadmium-rich sludge, while *I. palustris* was not. As these results were also found by suction sampling, they must be related to a change in density and not to sub-lethal effects on activity. When sampled by pitfall trapping the abundance of total Collembola and *I. maculatus* were also adversely affected by cadmium-rich sludge. This adverse affect was not, however, significant when these species were sampled by suction, hence suggesting that these results were not related to a change in density. It is therefore possible that the lower capture rate in pitfalls set in cadmium-rich plots was a consequence of a decrease in activity. Hopkin (1989) found that isopods exposed to high concentrations of metals are moribund, and will only move slowly when stimulated. It is therefore possible that metals can have sub-lethal effects on collembolan activity, and this will be investigated more thoroughly in Chapter 7.

In this study, *I. notabilis* and *H. nitidis* were favoured by the addition of digested sewage sludge but not by the addition of metal-rich sludge, suggesting that the presence of metals in sludge adversely affected these species. Furthermore, the abundance of *H. nitidis* was inversely related to the concentration of cadmium in sewage sludge (see Chapter 5). Adverse effects of metals in sewage sludge were also found for *I. notabilis* by Lübben (1989), and this species was rare in heavy metal polluted soil close to Gusum brass mill (Tranvik *et al.* 1993). It would therefore appear that the cadmium-rich sludge adversely affected this species also. Explanations of why some species are more susceptible to metals than others are given in Chapters 4 and 5.

Isotoma/Isotomurus juveniles were found to have a higher abundance on all plots receiving sludge. This suggests that their reproduction was promoted by the addition of sludge and was not affected by cadmium or zinc. However, as the juveniles were not identified to species level, it is possible that metals adversely affected the reproduction of some species, while promoting that of others. The different size classes of *I. palustris* indicated that reproduction in *I. palustris* may have been lower in the digested and zinc-rich plots than the cadmium-rich plots. It is therefore possible that while reproduction in *I. palustris* was adversely affected by digested and zinc-rich sludge, that of other *Isotoma/Isotomurus* species was promoted, and hence no adverse effects on the total abundance of *Isotoma/ Isotomurus* juveniles was detected. It would be interesting to identify juveniles to the species level to determine if reproduction was adversely affected by metal-rich sludge.

A higher proportion of *I. palustris* were larger than 2 mm in the control plots, than on the sludged plots. This may indicate a reduced growth rate (possibly as a consequence of metal pollution), or a reduced survival in sludged plots. Survival may have been reduced due to an increase in predators, and the abundance of carabid beetles has been shown to be higher on sludged plots (Larsen *et al.* 1996). As the total abundance of *I. palustris* was higher on sludged plots than control plots, the influence of density dependant factors (e.g. competition and parasitism) would also be greater on these plots.

Both the suction samples and pitfalls indicated that although some species had a lower abundance in zinc-rich than digested plots, this difference was not significant. It would therefore appear that zinc was less toxic than cadmium. This result may be expected, as zinc is a micro-nutrient which is regulated more efficiently, than nonessential elements such as cadmium (see Chapter 5 for more details). One species, *C. denticulata,* actually occurred in its highest abundance in zinc plots. It is possible that prior to sludge application this species was deficient in zinc, and through the application of zinc-rich sludge this was overcome. It is also possible that *C. denticulata* had a zinc-sensitive gut parasite, and through the addition of zinc this parasite was eliminated favouring *C. denticulata*.

6.6 Main findings

1: There was much agreement with results found by pitfall trapping and suction sampling.

2: Effects of date were more pronounced than those of treatment.

3: *Isotoma notabilis* was more abundant in the April pitfalls which was possibly a consequence of the digging in effect being more pronounced for this species.

4: The abundance of *I. viridis* and *S. pumilis* was inversely related to grass height, and this was thought to be due to a decreased sampling efficiency in long grass.

5: The total collembolan abundance, and the abundance of *L. cyaneus, I. viridis* and *H. nitidis*, were lower in cadmium-rich plots than digested plots.

6: *Isotoma/Isotomurus* juveniles and *I. palustris* appeared to be tolerant of metal-rich sludge, and their abundance was higher on all sludged plots, irrespective of metal contamination.

7: There was evidence that reproduction was higher for *I. palustris* in the cadmium-rich plots than the digested or zinc-rich plots suggesting sub-lethal effects of digested and zinc-rich sludge on reproduction.

8: There was evidence that all sludges adversely affected the growth rate and/or survival of *I. palustris.* This may have been a result of an increase in predators, parasites or competition in the sludged plots.

9: As was found for the suction data, cadmium was more toxic than zinc.

CHAPTER 7. A comparison between litterbags, suction samples and pitfall traps

7.1 Introduction

The previous two chapters concentrated on the response of epigeal and hemiedaphic Collembola to sludge application. In Chapter 5 suction sampling was used to monitor Collembola, while in Chapter 6 pitfall trapping was used. Both methods are considered to be effective for sampling epigeal and hemiedaphic species, but not for euedaphic species (Berbiers *et al.* 1989). In Chapter 4, the effects of sludge application on euedaphic and hemiedaphic Collembola were investigated using litterbags.

Although soil cores are thought to give the most complete method of sampling Collembola (sampling epigeal, hemiedaphic and euedaphic species: Berbiers *et al.* 1989), they may be inefficient at sampling epigeal species (Frampton 1994). Frampton *et al.* (1992) found that for most epigeal species, less than one individual per soil core was recovered. Furthermore, in soils with a high organic content the extraction of Collembola from cores can be very time consuming, as flotation techniques are generally required. A more practical alternative to soil cores may therefore be achieved by combining methods of sampling epigeal/hemiedaphic species with methods of sampling euedaphic species.

Although suction sampling and pitfall traps both sample epigeal/hemiedaphic species, the sampling efficiency of different species may vary between the two methods. Suction sampling gives good quantitative data on collembolan population density (Frampton 1994; Johnson *et al.* 1957) but, as they are predominately taken during the day, nocturnal species can be poorly represented (Joosse 1965). Pitfall trapping, on the other hand, gives poor quantitative data as it is dependant not only on the abundance of each species, but also on their activity. Pitfall traps tend to over-represent the most active species, while under-representing the least active. Catches are therefore influenced by factors which affect the mobility of arthropods (e.g. weather and grass height: Greenslade 1964; Berbiers *et al.* 1989). Pitfall traps are, however, generally left *in situ* for a period of several days, and will efficiently sample both diurnal and nocturnal species. The most effective sampling regime for epigeal and hemiedaphic Collembola is therefore thought to be a combination of both pitfalls and suction samples (Berbiers *et al.* 1989; Frampton 1994).

By combining information derived from suction sampling and pitfall trapping, it is possible to estimate the activity level of individuals species. A similar approach has been successfully used to investigate the effect of moulting on locomotory activity in the field (With & Joosse 1971). It should therefore also be possible to detect sub-lethal effects of chemicals on collembolan activity by combing pitfall and suction data (Frampton 1994).

In this Chapter, the litterbag, pitfall and suction data sets are compared. Information from pitfalls and suction samples is then combined to investigate sub-lethal effects of cadmium-rich sludge.

7.2 Species caught in litterbags, suction samples and pitfalls (1995)

The species collected by litterbags (22 July), suction samples (25 July) and pitfall traps (24 July) in late July 1995 were compared in order to investigate which species were sampled by each method. The late July dates were selected as this was the only sampling period that included all three methods. This enabled seasonal effects on species composition to be eliminated. As the cadmium-rich plots were not sampled by litterbags, they were omitted from the investigation. A species list and the sampling methods which collected each species is presented in Table 7.1 (species present are marked with an X).

In the late July samples, a total of 25 species were identified (see Table 7.1). Of these, 21 were collected by litterbags (total of 6,362 individuals), 14 by pitfall traps (total of 7,055 individuals) and 15 by suction (total of 9,628 individuals). It would therefore appear that litterbags sampled more species than pitfalls or suction. As less individuals were sampled by litterbags, the greater number of species could not be attributed to more individuals being collected. It would therefore appear that the euedaphic collembolan fauna at this study site was more diverse than the epigeal fauna.

Table 7.1 Comparison between litterbags, suction samples and pitfall traps (July 1995)

Species	Litterbags	Pitfalls	Suction
Anurida pygmaea	X		X
Friesea claviseta	X		
Ceratophysella denticulata	X	X	X
Folsomia candida	X		X
Folsomia fimetarioides	X		
Folsomia fimetaria	X		
Isotoma anglicana	X	X	X
Isotomurus palustris	X	X	X
Isotomodes productus	X		
Isotoma tigrina	X	X	
Isotoma notabilis	X	X	X
Isotoma viridis		X	X
Heteromurus nitidis	X	X	X
Lepidocyrtus cyaneus	X	X	X
Lepidocyrtus lignorum		X	X
Pseudosinella decipiens	X	X	X
Tomocerus longicornis			X
Sminthurinus aureus	X	X	X
Sminthurinus elegans	X	X	X
Sminthurides pumilis	X	X	
Sminthurus viridis		X	X
Neelus minimus	X		
Mesaphorura spp.	X		
Protaphorura cancellata	X		
Onychiurus circulans	X		

Sminthurinus elegans, *S. aureus*, *L. cyaneus*, *I. palustris*, *P. decipiens*, *I. notabilis*, *I. anglicana*, *C. denticulata* and *H. nitidis* were collected by all three sampling methods, and as expected all these species are hemiedaphic. Likewise, the eight species which were only collected by litterbags were all euedaphic (namely *N. minimus*, *Mesaphorura* spp. *P. cancellata*, *O. circulans*, *F. claviseta*, *F. fimetarioides*, *F. fimetaria*, *I. productus*). The euedaphic species *A. pygmea* and *F. candida* were collected by suction sampling and litterbags. The litterbags, however, appeared to be more efficient at sampling these species and sampled 21 specimens of *F. candida* and 49 specimens of *A. pygmea*, whereas, only one specimen of each of these species was caught by suction. As expected, euedaphic species were sampled more efficiently by litterbags than pitfall traps or suction.

Four species were only collected by suction and pitfalls (namely *S. viridis*, *T. longicornis*, *I. viridis* and *L. lignorum*). Of these species *T. longicornis* and *S. viridis* are strictly epigeal (Frampton 1994; Ellis 1974), and were consequently never collected by litterbags (see Appendix IV). *Isotoma viridis* and *L. lignorum* are generally considered to be hemiedaphic (but *I. viridis* is known to climb vegetation: Ellis 1974; Curry 1994), and although they did not occur in the late July litterbags, they occurred in litterbags collected on other dates in 1995 (see Appendix IV). It would therefore appear that the species sampled by the three methods under investigation was primarily dependant on ecomorphology.

A more detailed investigation was then conducted on the species caught by suction and pitfall traps (see Table 7.2). A comparison was made between the samples taken in 1996, when all suction samples were taken during the week that pitfall traps were *in situ*. From Table 7.2, it can be seen that the majority of species were sampled by both suction and pitfall trapping. In 1996, a total of 22 species were collected by suction sampling and 20 were collected by pitfall trapping. The suction samplers also collected more individual Collembola than pitfall traps (122,323 were collected by suction and 44,745 by pitfalls). As the number of species collected by any sampling method invariably increases with sample size (Magurran 1988), the larger number of species in suction samples was expected simply as a consequence of more individuals being collected.

In 1996, the species *F. fimetarioides*, *F. candida*, *Dicyrtoma ornata* (Nicoleti) and *Dicyrtoma saundersi* (Lubbock) were only caught by suction sampling, while the species *T. minor* and *A. pygmea* were only caught by pitfall trapping. Of these species, *F. fimetarioides*, *F. candida* and *T. minor* were represented by only one individual, and it is therefore likely that they were sampled as a consequence of chance.

The species *D. ornata* and *D. saundersi*, on the other hand, occurred in several suction samples but were absent from pitfall traps. It would therefore appear that suction was more efficient at sampling these species. Species of the genera *Dicyrtoma* are typically herbage dwellers (Curry 1994). As pitfall traps only sample ground active invertebrates, they are less efficient, when compared to suction, at sampling epigeal insects (Duffield & Aebischer 1994). It is therefore likely that *D. ornata* and *D. saundersi* were sampled more efficiently by suction as a consequence of their predominately epigeal existence.

Table 7.2 Species caught by suction samples and pitfalls 1996

Species	Pitfalls	Suction
Anurida pygmaea	X	
Ceratophysella denticulata	X	X
Folsomia candida		X
Folsomia fimetarioides		X
Isotoma anglicana	X	X
Isotomurus palustris	X	X
Isotomurus maculatus	X	X
Isotoma tigrina	X	X
Isotoma notabilis	X	X
Isotoma viridis	X	X
Entomobrya nivalis	X	X
Heteromurus nitidis	X	X
Lepidocyrtus cyaneus	X	X
Lepidocyrtus lignorum	X	X
Pseudosinella decipiens	X	X
Sminthurinus aureus	X	X
Sminthurinus elegans	X	X
Sminthurides pumilis	X	X
Sminthurides malmgreni	X	X
Sminthurus viridis	X	X
Dicyrtoma saundersi		X
Dicyrtoma ornata		X
Tomocerus longicornis	X	X
Tomocerus minor	X	

The species *A. pygmea*, which occurred in several pitfalls, was absent from suction samples. Pitfall traps tend to over-represent the most active species. *Anurida pygmea* is euedaphic, and therefore has morphological adaptations to a subterranean existence including reduced furca and legs. It is therefore unlikely that *A. pygmea* is particularly mobile, and consequently it was unlikely to be present in the pitfalls as a result of it's high activity level. It is possible that *A. pygmea* is only present on the surface at night and, as the suction samples were taken during the day, it was not collected by suction.

7.3 Comparison between relative abundance of species caught in suction samples and pitfall traps

Wilcoxon's matched pairs tests were conducted (on the relative abundance for dominant species) to investigate if species were caught proportionally more often by suction or pitfalls. These tests were performed on the most frequently occurring species (i.e. those occurring in over half of either suction or pitfall samples) caught by suction and pitfall trapping on the sampling period commencing 1 August 1996. It was decided to standardise across grass height, as this was thought to have a greater influence on sampling efficiency than treatment (see Chapters 5 and 6). 1 August was chosen as the grass was cut on 26 July and species specific differences in sampling efficiency as a consequence of grass height were minimised. This, however, did not correct for some plots having a denser vegetation than others (i.e. cadmium-rich plots tended to be less dense) which may also have influenced the sampling efficiency. It was necessary to use the relative abundance rather than the absolute abundance because the suction samples collected more individuals than the pitfalls.

The relative abundance for each species caught by suction sampling and pitfall trapping was calculated for a specific plot on 1 August. Pitfall traps and suction samples for each plot were then paired, and a Wilcoxon matched pairs test conducted. Differences between suction and pitfall abundances which equalled zero were ignored and the number of observations adjusted appropriately. The results of these tests can be seen in Table 7.3 together with the appropriate number of observations and median percentage per plot.

Tomocerus longicornis, *I. palustris*, *I. anglicana*, *S. viridis*, *I. viridis* and *I. notabilis* had a higher relative abundance in suction samples than pitfalls (see Table 7.3). Some of these species (e.g. *Sminthurus viridis* and *I. viridis*) are known to climb grass (Curry 1994; Frampton 1994). As pitfall traps rely on the species being ground active, epigeal species such as *I. viridis* and *S. viridis* are likely to be sampled more efficiently by suction than pitfalls. *Isotoma notabilis* is, however, strictly hemiedaphic (Curry 1994) and the higher relative abundance of this species in suction samples could not be the consequence of an epigeal existence. It is possible that this species is diurnal and, as the suction samples were taken during the day, diurnal species were over-represented.

Table 7.3 Comparison of pitfall traps and suction samples with respect to species relative abundance

Species	Median percentage in suction	Median percentage in pitfalls	T-value	Significance level
I. palustris	19.76	12.71	7 (n =12)	= 0.01
I. maculatus	1.25	1.96	26 (n =12)	N.S.
I. viridis	5.78	2.76	7 (n =12)	= 0.01
I. anglicana	4.32	1.42	1 (n =12)	= 0.001
I. notabilis	3.33	0.50	0 (n =12)	< 0.001
T. longicornis	0.76	0.08	1 (n =12)	= 0.001
H. nitidis	1.09	1.02	34 (n =12)	N.S.
L. cyaneus	55.14	61.01	49 (n =12)	N.S.
S. viridis	2.87	0.60	2 (n =12)	< 0.01
S. aureus	0.11	0.98	10 (n =12)	<0.05
S. elegans	0.08	0.58	0 (n =9)	< 0.01
S. pumilis	0.00	0.74	0 (n =11)	= 0.001
C. denticulata	0.00	4.15	0 (n =11)	= 0.001

The relative abundance of *S. aureus*, *S. elegans*, *S. pumilis* and *C. denticulata* was higher in pitfalls than suction samples (see Table 7.3). This may be the result of these species being strictly hemiedaphic, and therefore more active on the soil surface than epigeal species. These species would also be caught more frequently in pitfall traps than suction samples if they were nocturnal. *Orchesella cincta* and *T. minor* are both thought to be predominately nocturnal (Joosse 1965), and this study suggests that this is the case for *C. denticulata* (which was rarely caught by suction, but frequently by pitfalls).

7.4 Effect of cadmium on activity

The number of individuals of a particular species collected by suction is directly related to the density of that species, while the number of individuals caught by pitfall trapping is related to both its density and activity (Berbiers *et al.* 1989). It is therefore possible to combine information derived from pitfall trapping and suction sampling to obtain an indication of the activity level of a species. By dividing the number of individuals caught by pitfall trapping by an estimate of the population density (calculated from the suction sampling data), an indication of activity level can thus be obtained (With & Joosse 1971). The resultant activity level will be most accurate when environmental variables which affect sampling efficiency are kept constant. Using this information, it is possible to detect sub-lethal effects of chemicals on activity under near normal conditions in the field (Frampton 1994). The presence of high body burdens of zinc and cadmium (1.5% and 0.5% respectively) in isopods renders them moribund and they will only move weakly when stimulated (Hopkin 1989). It may therefore be expected that heavy metals also reduce the activity of Collembola.

Using the above approach, sub-lethal effects of the cadmium-rich sludge were investigated for the total Collembola, a cadmium sensitive (*L. cyaneus*) and a cadmium tolerant species (*I. palustris*). The abundance of these species caught by pitfall traps on 1 August in digested and cadmium-rich plots was divided by the abundance caught by suction sampling in the same plot on the same date (the median indices are presented in Table 7.4). As grass height can affect the activity of certain species of Collembola (Berbiers *et al.* 1989), 1 August was chosen to standardise grass height across treatments. Differences in activity level, or sampling efficiency, as a consequence of grass height were therefore eliminated.

Table 7.4 Effect of cadmium on activity level

Species	Median index for digested plots	Median index for cadmium-rich plots
Total	0.76	0.60
I. palustris	0.40	0.37
L. cyaneus	0.63	0.09

From Table 7.4 it can be seen that activity level of total Collembola and *I. palustris* did not appear to be affected by cadmium-rich sludge. For *L. cyaneus*, on the other hand, the median value for the pitfall/suction index was substantially higher in the digested plots than the cadmium-rich plots. This indicates that cadmium-rich sludge adversely affected the activity level of *L. cyaneus*. However, as too few replicas were available to merit statistical analysis, a definite conclusion cannot be drawn.

The detoxification of heavy metals is thought to be energetically costly, and a decrease in glycogen reserves after metal exposure has been found for several invertebrate species (Richards & Ireland 1978; Reddy & Bhagyalakshmi 1994; Bodar *et al.* 1990). The presence of metals may therefore be expected to reduce activity by decreasing the energy available. Metals may also directly interfere with the biochemical pathways involved in metabolism. Sørensen *et al.* (1997) observed reduced activity in a metal polluted population of the isopod *Oniscus asellus* (Linné). Laboratory studies have also found reduced activity in the Collembola *Folsomia fimetaria* at high levels of the pesticide dimethoate (Fábián & Petersen 1994). Computer-automated video tracking may therefore provide a quick and effective method of monitoring pollution (Sørensen *et al.* 1997), and this study suggests that the species *L. cyaneus* has the potential to act as a test organism.

7.5 Discussion and recommendations for future study

The litterbags collected almost all species found in pitfalls and suction samples, in addition to several species which were not sampled by either of these methods (e.g. *Mesaphorura* spp. and *N. minimus*). It was, however, found that the number of hemiedaphic individuals collected by litterbags was generally lower than the number collected by suction or pitfall trapping (e.g. in late July 1995, 354 individuals of *L. cyaneus* were collected by suction, while only 61 were collected with litterbags). Furthermore, strictly epigeal species (e.g. *S. viridis*) were never found in litterbags. It is therefore concluded that to obtain the most complete picture of a collembolan community, it is necessary to combine methods which sample euedaphic/hemiedaphic species with methods which sample hemiedaphic/epigeal species. The most effective sampling regime for Collembola would therefore be accomplished by combining soil cores or litterbags with pitfall traps or suction samples.

When concentrating on the hemiedaphic and epigeal species, the two most effective sampling methods appear to be pitfall trapping and suction sampling (Frampton 1994). Frampton (1994) found more statistically significant effects of fungicides when Collembola were sampled by suction than when they were sampled by pitfalls, hence suggesting that suction was the better sampling method. Plant climbing species such as *S. viridis* are, however, not only sampled more efficiently by suction, but are also likely to be more susceptible to pesticide sprays (Frampton 1994). This would explain why Frampton (1994) found more significant effects of fungicides with suction.

In this study, the suction DCA for 1995 generally showed a better treatment split than the pitfall DCA for the same year (see Chapters 5 and 6 respectively). This suggests that suction was better at discriminating between communities. In 1996, however, the pitfall and suction DCA showed similar treatment effects, suggesting that with more intensive sampling both methods become equally good at detecting changes in community structure. For both the pitfall and suction data, three significant treatment effects were found in 1995 and 9 were found in 1996. With respect to sludge application, neither sampling method indicated more treatment effects. It is, however, important to remember that in 1996 almost three times more Collembola were collected by suction. This implies that more work was involved in sorting the suction data to achieve the same end point (i.e. nine significant differences), hence suggesting that in this study the most efficient method may have been pitfall trapping.

Although both sampling methods detected similar treatment effects for several species (see Chapter 5 and 6), effects for some species were only detected by suction (e.g. *S. viridis* and *I. anglicana*) or by pitfalls (e.g. *Isotoma/Isotomurus* juveniles and *I. notabilis*). The most effective sampling regime for epigeal and hemiedaphic Collembola would therefore appear to be a combination of pitfall traps and suction samples (Berbiers *et al.* 1989; Frampton 1994). As resources are often limiting, this option may not always be practical and in such cases only one method can be selected. When selecting a sampling method, the efficiency of each method in collecting specific groups of species should be considered and the influence of treatment on these groups taken into account. For example if a treatment predominantly affects epigeal species (e.g. some pesticides), suction sampling would be the preferred method because it is more efficient at sampling such species.

This investigation also suggests that metals may have sub-lethal effects on activity, particularly in metal sensitive species. By combining information derived from suction samples and pitfalls the detection of such effects are possible, hence indicating a further advantage in using both methods. It is also possible to collect individuals that have been exposed to a certain chemical in the field, and use computer-automated video tracking in the laboratory to determine sub-lethal effects (Sørensen *et al.* 1997). It is suggested that such behavioural analysis is less time and resource consuming than conventional toxicity tests with end points such as growth, survival and reproductive output (Sørensen *et al.* 1997).

CHAPTER 8. Effects of pharmaceutical waste on euedaphic and hemiedaphic Collembola

8.1 Pharmaceutical wastes

The pharmaceutical industry produces organic acids, steroids and antibiotics using a variety of fermentation processes that involve micro-organisms (Volz & Heichel 1979). The fermentation products are generally produced in large vats containing a carbon source (such as molasses to act as food for the micro-organisms), lime (to maintain the pH) and in some cases zinc (to control growth rates). When fermentation is complete, the fermentation product is extracted by distillation, filtration and/or precipitation. The large quantities of waste that remains contain cellular material from the fermentation micro-organisms, unused substrate and filter aids (e.g. perlite), and is therefore considered to be non-toxic (Larsen *et al.* 1991). Disposal, however, poses a problem since the high levels of organically degradable material can cause eutrophication if the waste enters aquatic environments (Larsen *et al.* 1991).

Pharmaceutical wastes generally have large BOD (biochemical oxygen demand) and COD (chemical oxygen demand) levels, that exceed the maximum for disposal of effluents in water. Activated sludge treatment, although expensive, will significantly decrease the BOD and the treated sludge can sometimes be discharged into a stream (Lederman *et al.* 1975). Alternative methods of disposing of pharmaceutical waste include incineration, landfill (both problematic due to environmental impact: Wright 1978), livestock feeding supplement and fertiliser. When added as a dietary supplement for livestock, the growth rate has been found to increase due to an unidentified growth factor (Lederman *et al* 1975). This practice is now, however, no longer advised (Aitken 1994).

Pharmaceutical sludge can contain high concentrations of nitrogen, phosphorus and potassium (plant macro-nutrients), and lower concentrations of micro-nutrients such as zinc, magnesium and calcium (Larsen *et al.* 1991). Corn (De Roo 1975) and soybeans (Volz & Heichel 1979) have been found to achieve significantly higher yields when fertilised with pharmaceutical waste compared to inorganic fertiliser. Some pharmaceutical sludges can also improve the structure of sandy soils as a consequence of their high organic matter content (Larsen *et al.* 1991). In addition, the heavy metal content has been found to be lower in pharmaceutical sludge than in sewage sludge (Larsen *et al.* 1991). Pharmaceutical sludge would therefore appear to comply with the Waste Management Licensing Regulations 1994, which state that sludges from biological treatment plants may be used as agricultural fertiliser if they benefit agriculture and promote ecological improvement without causing pollution.

Pharmaceutical sludge can, however, contain a number of compounds that have the potential to be sources of pollution, e.g. zinc, high salt concentrations and/or antibiotic residues (De Roo 1975). Tomatoes were found to be particularly sensitive to high salt concentrations and showed symptoms indicating nitrogen deficiency when fertilised by pharmaceutical sludge (De Roo 1975). The high salt concentration in the sludge was thought to suppress nitrification in the substrate, uptake of nitrogen by the plants, and nitrogen metabolism in the plant (De Roo 1975).

Only a few studies have looked at the effects of land application of pharmaceutical waste (Aitken 1994) and, with the exception of nematodes (De Roo 1975) and earthworms (Aitken 1993), there is little information concerning the effects on invertebrates. Such studies are necessary if their usage as fertilisers is to be promoted. At SAC Auchincruive, two small plot trials were instigated in 1993 and 1994 to investigate the fertilising potential of pharmaceutical wastes. Advantage was taken of the presence of these trials to gather some information on the effects of these wastes on Collembola.

8.2 Pharmaceutical Trial 1 - Introduction

The SmithKline and Beecham pharmaceutical plant situated near Irvine, produces large quantities of aerobically treated sludge (ATS) as a by-product of antibiotic production (Aitken 1994). ATS has significant quantities of nitrogen, phosphorus and potassium and the dry solids content for these elements is similar to other organic wastes (e.g. sewage sludge: Aitken 1994). Aitken (1994) found that plots receiving ATS waste had grass yields 66% higher than unfertilised plots, indicating ATS has fertilising potential. However, this increase was significantly less than the increase of 245% which was achieved in inorganically fertilised plots, suggesting macro-nutrients are less available in ATS (Aitken 1994). Current legislation allows ATS to be used as an agricultural fertiliser providing land application does not result in pollution (Aitken 1994).

ATS has low levels of heavy metals and organic contaminants, and has a neutral pH (Aitken 1994). These factors indicate that ATS does not therefore pose a threat to the environment. Furthermore, because the waste has been aerobically treated, the BOD level has been significantly reduced from 5,850 mg l^{-1} to 220 mg l^{-1} (Aitken 1994). There is therefore less threat, in comparison to undigested waste, of pollution to nearby water courses. A possible source of pollution, however, is the residual antibiotics (namely penicillin) which are present in this waste (Aitken 1994). Bewick (1978) found that high levels of antibiotic waste (4 tonnes ha^{-1}) decreased the soil microbial activity. Through adversely affecting the soil microbes, the decomposition rate and hence the recycling of plant nutrients, are likely to be retarded (Bewick 1978). Furthermore, the antibiotic streptomycin, was found to decrease soil nitrate-N levels suggesting nitrifying bacteria are particularly susceptible to streptomycin (Ingham & Coleman 1984).

Aitken (1994) found that the microbial biomass of ATS treated plots was reduced by about 20% when compared to inorganically fertilised plots. The microbial biomass levels in ATS plots were, however, within levels commonly found for grassland. It is unknown if biomass differences were the result of antibiotic residue in the waste, or a decrease in material available for microbial breakdown (Aitken 1994). It is likely that any adverse affects on microbial biomass would also adversely affect the Collembola that feed on the soil micro-flora (Seastedt 1984).

8.3 Pharmaceutical Trial 1 - Methods

ATS waste

Sludge analysis was conducted at SAC Auchincruive (Aitken 1994). Analysis of the ATS waste confirmed that heavy metals (Cu, Zn, Cd, Cr, Ni and Pb) were at low concentrations (see Appendix XV). The ATS waste had a neutral pH and a BOD value of 220 mg l^{-1}. The levels of nitrogen, phosphorus and potassium in the waste were 0.8 g kg^{-1}, 0.3 g kg^{-1} and 0.5 g kg^{-1} fresh weight sludge, respectively. Low levels of residual antibiotic were present in this waste (Aitken 1994).

Inorganic fertiliser (NPK)

Three different inorganic fertilisers provided similar levels of plant macro-nutrients (specifically nitrogen, phosphorus and potassium) to the ATS waste. The fertilisers chosen were ammonium nitrate, triple superphosphate and muriate of potash.

Trial site

The trial site was situated at Holms Field, SAC Auchincruive. The soil was a sandy loam of satisfactory drainage and the test crop was Timothy grass (*Phleum bertolonii*). The plots (1.5 m x 3 m) ran continuously in two blocks of three (see Figure 8.1).

Experimental design

Three treatments were studied and each treatment was replicated giving a total of six plots (see Figure 8.1 and Table 8.1). ATS was applied using calibrated watering cans, while NPK was applied by hand. Application occurred on 28 April 1994.

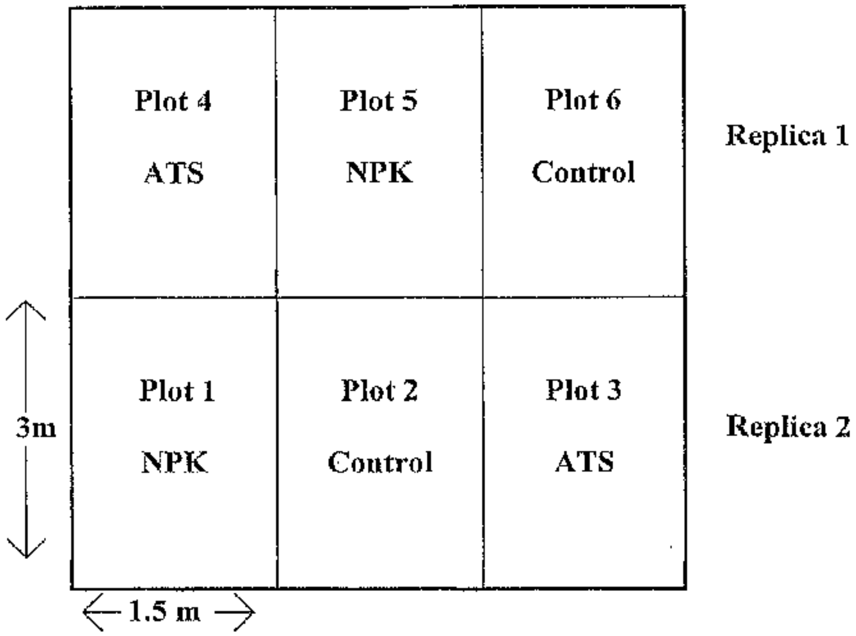
Table 8.1 Treatments studied

Plot Number	Treatment
2 & 6	Control (no fertiliser)
1 & 5	Ammonium nitrate (160 kg ha^{-1} N), triple superphosphate (130 kg ha^{-1} P_2O_5) and muriate of potash (40 kg ha^{-1} K_2O).
3 & 4	ATS (100 m^3 ha^{-1})

Sampling technique

Seven litterbags (see page 44) of 15 cm x 15 cm squares (mesh size 1 mm), were buried at a depth of 2-5 cm. Each bag contained 2.50 g of dried sycamore leaves and were re-hydrated before burial. Litterbags were buried on 27 April 1994 and, after 15 weeks, the bags were removed and the Collembola extracted by modified Tullgren funnels (see page 45) over a period of six days. They were extracted into alcohol, prepared and identified primarily following Fjellberg (1980) and Gisin (1960). See Chapter 3 for further information on identification techniques.

Figure 8.1 Layout of trial site



Statistical analysis

All samples within a plot were pooled (total of seven samples) to obtain an overall indication of collembolan community structure for each plot. Chi-squared tests were performed on the pooled data to test for treatment effects on: total collembolan abundance; the frequency of dominant species (i.e. those occurring in over 50% of samples); the relative frequencies of dominant species; euedaphic and hemiedaphic frequencies; and sexual and asexual frequencies. A DCA (Hill 1979) was also performed on the pooled data (using the relative abundance and without down-weighting) to provide a visual impression of the relationship between plots. Collembolan diversity was compared between the plots using the following measures of diversity: **S** (number of species), **d** (Berger-Parker), N_2 (Hill's) and α (Fisher's log series index). See pages 30-32 for information on diversity indices.

8.4 Pharmaceutical Trial 1 - Results

Abundance

A total of 1,905 individuals were collected belonging to 19 species (see Appendix XVI for species list). Between-plot difference in the total number of Collembola and the number of frequently occurring species were tested for using chi-squared tests for homogenous frequencies (Fowler & Cohen 1992). Results of these tests, the number of total Collembola, and the number of frequently occurring species can be seen in Table 8.2.

The frequency of total Collembola and the frequencies of dominant species were not evenly distributed between plots (see Table 8.2). The total collembolan abundance was lower than expected in both ATS replicas, and higher than expected in both control replicas (see Table 8.2). In addition, *I. notabilis, F. candida, A. pygmea*, *C. denticulata* and *Mesaphorura* spp. (a combination of *M. macrochaeta* and *M. hylophila*) were less abundant in both ATS replicas (see Table 8.2). This suggests that ATS adversely affected the collembolan abundance and, with the exception of *L. cyaneus*, the abundance of the frequently occurring species (see Table 8.2).

Lepidocyrtus cyaneus and *A. pygmea* were favoured in plots fertilised by NPK, but not those fertilised by ATS (see Table 8.2). However, the NPK plots were similar to the control plots with respect to these two species. *Ceratophysella denticulata* was more abundant on NPK plots than control plots (see Table 8.2). The disproportionately large number of *C. denticulata* on replica two, however, gave a higher expected value than would otherwise have been attained and, as a consequence, the frequency in NPK replica one was only marginally greater than the expected (see Table 8.2). A disproportionately high number of *Mesaphorura* spp. in replica two of the control also caused higher expected values than would otherwise have been achieved (see Table 8.2).

It must also be noted that replicas of a treatment were different. In particular, for the NPK plots replica one had more total Collembola and *I. notabilis* than expected, whereas, replica two had less. Furthermore, replica two of the control was found to have more *Mesaphorura* spp. than expected while replica one had less. The DCA ordination indicates that differences between replicas were as large, if not larger than, differences between treatments and this is clearly seen in the DCA ordination (see Figure 8.2). Ideally, if more replicas had been available or if samples had been taken on several sampling dates, this variation may have been reduced.

Table 8.2 Comparing abundance between treatments

	Control Rep 1	ATS Rep 1	NPK Rep 1	Control Rep 2	ATS Rep 2	NPK Rep 2	Expected	X^2-value	Significance (5 d.f.)
Total Collembola	389	51	531	507	146	281	371.5	593.3	< 0.001
Mesaphorura spp.	12	2	48	154	3	22	40.2	422.8	< 0.001
Isotoma notabilis	337	28	268	267	106	81	181.2	432.4	< 0.001
Folsomia candida	7	3	23	10	2	5	8.3	35.9	< 0.001
Lepidocyrtus cyaneus	3	2	10	6	5	12	6.3	12.2	< 0.05
Ceratophysella denticulata	1	8	28	9	8	106	26.7	298.6	< 0.001
Anurida pygmea	14	6	28	31	5	37	20.2	46.2	< 0.001

Table 8.2 This table shows the abundance of total Collembola and the abundance of each of the frequently occurring species across the three treatments and two replicas. It also shows the expected value, the X^2 value and the probability value obtained when the abundance was compared between treatments and replicas.

Figure 8.2 DCA Ordination for pharmaceutical trial 1

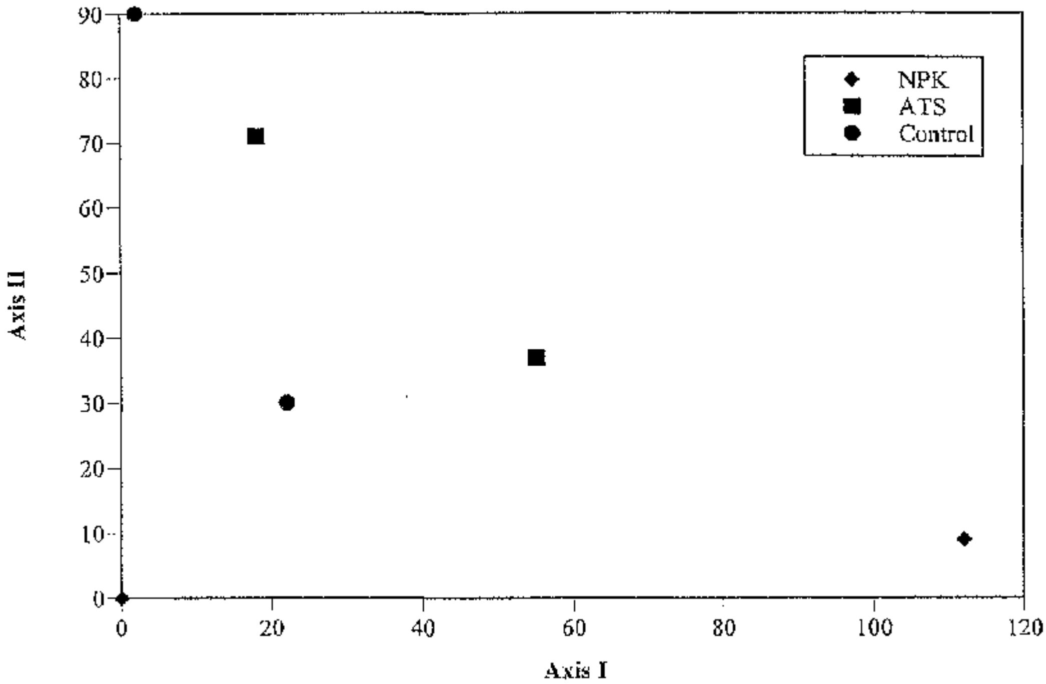


Figure 8.2: DCA ordination performed on species relative abundance. Each point represents one plot.

Relative frequencies

The relative frequencies of the dominant species were compared using a 6 x 6 contingency table (25 degrees of freedom). The result of the contingency table, and the observed and expected abundance of each species, are presented in Table 8.3. The relative frequencies of dominant species were found to be dependant on plot ($P < 0.001$). The disproportionately higher abundance of *Mesaphorura* spp. in replica two of the control, and of *C. denticulata* in replica two of the NPK plots, were the main contributors to the X^2 value (see Table 8.3). This again illustrates that there were differences between replicas. It was, however, interesting to note that the proportion of *C. denticulata* was less in the control plots than in the ATS and NPK plots. This suggests that this species was favoured by fertilisation.

Table 8.3 Comparing relative frequencies of species between treatments

Species	Control (R1)	ATS (R1)	NPK (R1)	Control (R2)	ATS (R2)	NPK (R2)	X^2_{25} value	Significance (25 d.f.)
Mesaphorura spp.	12 *53.1*	2 *7.0*	48 *57.5*	154 *67.7*	3 *18.3*	22 *37.4*		
Isotoma notabilis	337 *239.6*	28 *31.4*	268 *259.4*	267 *305.5*	106 *82.6*	81 *168.4*		
Folsomia candida	7 *11.0*	3 *1.4*	23 *11.9*	10 *14.1*	2 *3.8*	5 *7.8*		
Lepidocyrtus cyaneus	3 *8.4*	2 *1.0*	10 *9.1*	6 *10.7*	5 *2.9*	12 *5.9*		
Ceratophysella denticulata	1 *35.3*	8 *4.6*	28 *38.2*	9 *45.0*	8 *12.2*	106 *24.8*		
Anurida pygmea	14 *26.7*	6 *3.49*	28 *28.88*	31 *34.0*	5 *9.2*	37 *18.8*	656.1	< 0.001

Table 8.3 This table shows the abundance of the frequently occurring species across the three treatments and replicas. It also shows the expected value (in italics) and probability value obtained when the abundance was compared between treatments and replicas.

Diversity

The following measures of diversity were calculated for each of the six plots: **S** to measure richness, **d** to measure evenness, and α and $\mathbf{N_2}$ to measure diversity (see pages 30-32 for information on diversity indices).

Table 8.4 Comparing the diversity between plots

Diversity Index	**Control R1**	**ATS R1**	**NPK R1**	**Control R2**	**ATS R2**	**NPK R2**
S (no. species)	11	8	14	14	12	10
N_2 (Hill's)	1.33	2.88	3.28	2.66	1.86	3.96
d (Berger-Parker)	0.866	0.549	0.505	0.527	0.726	0.377
α (Fisher's)	2.11	2.66	2.64	2.66	3.1	2.02

From Table 8.4 it can be seen that the number of species (**S**) recorded was independent of treatment. Hill's $\mathbf{N_2}$ found the NPK plots to be most diverse and Berger-Parker's **d** found these plots to be most even (indicated by the low value of **d**). It would therefore appear that NPK increased diversity.

Sexual and asexual

The relative frequencies of sexual and asexual individuals were compared between plots using a 2 x 6 contingency table (5 degrees freedom). The results are presented in Table 8.5 below. A significant difference was found in the frequencies of asexual and sexual individuals between treatments ($P < 0.001$). The control plots had a higher number of asexually reproducing individuals than expected, while the NPK plots had a higher number of sexually reproducing individuals (see Table 8.5). This is in agreement with Siepel's (1995) predictions that fertilisation increases the number of sexual species. However, fertilisation by ATS waste did not have the same effect, and instead the frequencies of sexual and asexual individuals were as expected.

Table 8.5 Comparing the relative frequencies of sexual and asexual individuals

Reproduction	**Control R1**	**ATS R1**	**NPK R1**	**Control R2**	**ATS R2**	**NPK R2**	
Sexual	33.0 *106.4*	18.0 *14.0*	191.0 *145.2*	76.0 *138.7*	35.0 *39.9*	168.0 *76.9*	
Asexual	356.0 *282.6*	33.0 *37.1*	340.0 *385.8*	431.0 *368.3*	111.0 *106.1*	113.0 *204.2*	X^2_5= 279.8

Table 8.5 This table shows the observed and expected (shown below in italics) frequencies of asexually and sexually reproducing Collembola found in each plot. It also shows the X^2 value (5 d.f.) obtained when the relative frequencies of sexual and asexual individuals were compared.

Euedaphic and hemiedaphic

The relative frequencies of euedaphic and hemiedaphic species were compared between treatments again using a 2 x 6 contingency table (5 degrees of freedom). The results are presented in Table 8.6.

Table 8.6 Comparing the frequencies of euedaphic and hemiedaphic individuals

Ecomorphology	**Control R1**	**ATS R1**	**NPK R1**	**Control R2**	**ATS R2**	**NPK R2**	
Hemiedaphic	354.0 *279.6*	39.0 *36.7*	321.0 *381.6*	309.0 *364.4*	134.0 *104.9*	212.0 *201.9*	
Euedaphic	35.0 *109.5*	12.0 *14.4*	210.0 *149.4*	198.0 *142. 7*	12.0 *41.1*	69.0 *79.1*	X^2_5= 165.5

Table 8.6 This table shows the observed and expected (shown below in italics) frequencies of euedaphic and hemiedaphic Collembola found in each plot. It also shows the X^2 value (5 d.f.) obtained when the relative frequencies of sexual and asexual individuals were compared.

The relative frequencies of euedaphic and hemiedaphic individuals were dependent on plot ($P < 0.001$). There were more hemiedaphic and less euedaphic species than expected in both replicas of the ATS treatments. However, in replica one the difference was only marginal and did not contribute significantly to the chi-squared value (see Table 8.6). Differences between the control and NPK treatments were not consistent between replicas (although they contributed most to the chi-squared value), and therefore no conclusions could be drawn concerning the effects of these treatments on ecomorphology.

8.5 Pharmaceutical Trial 1 - Discussion

It has generally been found that inorganic fertilisers increase the collembolan abundance (Marshall 1977, Siepel & Van de Bund 1988). No increase was found in this study, and the collembolan abundance was similar in the inorganically fertilised plots (NPK) and the control plots. This may have been the result of an increase in salt concentration or impurities in the fertiliser (e.g. copper or chlorine: Marshall 1977). Although the density of Collembola was similar in the NPK and control plots, the species composition was different. *Ceratophysella denticulata*, *A. pygmea*, and *L. cyaneus* were favoured by inorganic fertiliser. Gunhold (1957) also found that *L. cyaneus* favoured fertilised plots and it has been called a manure-loving species (Marshall 1977).

Ceratophysella denticulata was more abundant under conventional management (with increased fertiliser) than under integrated farming (with reduced fertiliser: Brussaard *et al.* 1990).

Siepel (1995) predicted that because fertilisation decreases the predictability of an environment, asexual species, being less adapted to cope with fluctuating environments, will be more susceptible to fertilisation than sexual species. The results for this trial agree with Siepel's prediction, in that the control had a higher number of asexually reproducing individuals, while the NPK treatment had a higher number of sexually reproducing individuals.

Aerobically treated sludge (ATS) plots had a significantly lower density of total Collembola, and the majority of collembolan species, than the inorganically fertilised and control plots. The lower grass yield in the ATS plots when compared to the NPK plots (Aitken 1994), would probably be accompanied by a decrease in the litter layer and an increase in environmental fluctuations in the soil. This in turn would be likely to affect Collembola. While the abundance of Collembola was lower in the ATS plots than the control plots, the grass yield in ATS plots was actually greater than control plots (Aitken 1994). This suggests that effects of ATS on Collembola could not be entirely attributed to changes in the vegetation height.

The soil microbial biomass was found to be lower in the ATS plots than the NPK plots suggesting that ATS adversely affected the microbial community (Aitken 1994). This may have been a direct effect of antibiotic residues in the waste, or an indirect effect of a lower root yield in the ATS plots resulting in less available material for microbial breakdown (Aitken 1994). Bewick (1978) also found a decrease in soil microbial activity in plots receiving antibiotic waste. It is likely that through adversely affecting the microbial community of the soil, the Collembola, which live in close association with the soil micro-flora and fauna, would also be adversely affected. These results indicate that the application of ATS waste to agricultural land may, at least in the short-term, be deleterious to soil collembolan and microbial communities. Through adversely affecting these communities, it is likely that a retardation in the decomposition rate would also occur counteracting the fertilising potential of ATS.

8.6 Pharmaceutical Trial 2 - Introduction

A large quantity of liquid sodium ammonium sulphate (SAS) solution is also produced by the SmithKline and Beecham pharmaceutical plant, Irvine. SAS has the potential to 'fertilise or beneficially condition the land' as it contains high concentrations of nitrogen, sodium and sulphur, all of which are important plant nutrients. SAS application has already been shown to increase the herbage yield by up to 46% (Aitken & Kirkland 1993). However, before SAS can be used as an agricultural fertiliser, it must be demonstrated that there are no adverse environmental effects resulting from its application. If no pollution occurs, current legislation permits such wastes to be applied as fertilisers without a licence (Aitken & Kirkland 1993).

Potential problems for utilising SAS waste as a fertiliser may arise from it's low concentration of the salt sodium thiogllycollate (Aitken & Kirkland 1993). This salt is an 'oxygen scavenger' and partly accounts for the high BOD of the sludge. The sludge could therefore be environmentally harmful if it entered an aquatic environment (e.g. through 'run-off': Aitken & Kirkland 1993). High levels of SAS waste (250 kg ha^{-1}) were found to reduce the pH value of the soil at a depth of 0-10 cm, from pH 5.63 to 5.32 (Aitken & Kirkland 1993). In addition, high application rates elevated the salinity at a depth of 0-10 cm, from 0.34 ms cm^{-1} to 0.88 ms cm^{-1} (Aitken & Kirkland 1993).

Adverse effects of SAS on earthworm abundance were apparent even at the lowest application rate, and their abundance did not attain pre-treatment levels after a period of nine months (Aitken & Kirkland 1993). Clover was also found to be adversely affected by SAS waste, with scorching of leaves apparent 11 days after application (Aitken 1993). The adverse affects on both earthworms and clover were thought to be caused by the high salt concentration (Aitken & Kirkland 1993). Changes in salt and pH levels as a consequence of applying SAS waste, were generally restricted to the top 10 cm of the soil (Aitken & Kirkland 1993). Since this is where the majority of euedaphic and hemiedaphic collembolan activity occurs, it was also possible that these chemical changes could adversely affect Collembola. This experiment aimed to investigate the effect of land application of SAS waste on: the total collembolan abundance; individual species abundance; relative frequencies of dominant species; diversity of Collembola; proportion of sexual and asexual individuals; and the proportion of euedaphic and hemiedaphic individuals.

8.7 Pharmaceutical Trial 2 - Methods

SAS waste

Analysis of SAS was conducted at SAC Auchincruive (Aitken 1993). Analysis confirmed that heavy metals (Cd, Ni, Cr, Pb, Cu and Zn) were at low levels (see Appendix XV). Furthermore, no bacteria (salmonella, coliforms or closridia) were present. The dry matter content was low (380 g l^{-1}) and the BOD was high (2,340 mg l^{-1}). The waste was acidic (pH 4.3) with a high salt content (1.27 g cm^3). The sludge contained significant amounts of nitrogen and sodium (51.5 g l^{-1} nitrogen and 73.8 g l^{-1} sodium).

Ammonium nitrate

The inorganic fertiliser ammonium nitrate (AN) was used to evaluate the fertilising potential of SAS waste.

Trial site

This investigation was part of a larger experiment conducted at Holms Field, Auchincruive, during 1993. A total of 46 plots, consisting of recently reseeded late perennial ryegrass (*Lolium pernne*) and a moderately high content of clover were established. A dressing of phosphorus and potassium was applied to all plots (50 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O). The soil type was a sandy loam of satisfactory drainage. The plots, each 1.5 m x 3 m, ran contiguously in 2 blocks of 23 (see Figure 8.3). Within this larger experiment four application rates of AN and SAS were examined, in addition to a no treatment control. The fertilisers were either applied early (2 April 1993) or late (28 April 1993).

Experimental design

The highest application levels of AN and SAS were chosen for this study (plots are marked with an asterix on Figure 8.3). The early fertilised plots were studied and hence AN and SAS were applied on 2 April 1993, the former by hand and the latter using calibrated watering cans. Each treatment was replicated, and occurred randomly within block one. The treatments studied can be seen in Table 8.7.

Table 8.7 Treatments studied

Plot Number	Treatment
9 & 14	Control
5 & 21	Early maximum AN (250 Kg/ha N)
10 & 17	Early maximum SAS (250 Kg/ha N)

Sampling technique

Seven litterbags (see page 44), of 15 cm x 15 cm squares (mesh size 1 mm), were buried at a depth of 2-5 cm in the selected plots. Each bag contained 2.50 g of dried sycamore leaves and were re-hydrated before burial. Litterbags were buried almost a year after fertiliser application, on 22 March 1994. After 12 weeks the bags were removed and the Collembola extracted by modified Tullgren funnels (see page 45) over a period of six days. They were extracted into alcohol, prepared and identified primarily following Fjellberg (1980) and Gisin (1960: see Chapter 3 for further details on identification techniques).

Statistical analysis

All samples within a plot were pooled (total of seven samples) to obtain an overall indication of collembolan community structure for each plot. Chi-squared tests were conducted on the pooled data to test for treatment effects on: total collembolan abundance; abundance of individual species; relative frequencies of dominant species; proportion of sexual and asexual individuals; and proportion of euedaphic and hemiedaphic individuals. A DCA was performed on the species relative abundance (without downweighting: Hill 1979). Four measures of diversity were also examined specifically; **S** (number of species), **d** (Berger-Parker), $\mathbf{N_2}$ (Hill's) and α (Fisher's log series index). See pages 30-32 for information on diversity indices.

Figure 8.3 Pharmaceutical trial 2 site plan

					*				*	*				*			*				*		
Plot Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
AN: kg/haN	0	0		0	250	100	150	0	0	0	200	0	0	0	200	0	0	150	0	100	250	0	0
SAS:kg/haN	200	100		250	0	0	0	200	0	250	0	100	150	0	0	200	250	0	100	0	0	150	150

Plot Number	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
AN: kg/haN	0	0		0	150	200	150	250	250	100	200	0	100	200	100	150	250	100	0	250	200	150	0
SAS:kg/haN	100	200		150	150	200	0	250	0	100	0	0	0	200	0	150	250	100	0	0	0	0	250

Fence Line

Treatments (April)

AN - Ammonium nitrate (0-250 kg/ha N)
SAS - Sodium/Ammonium Sulphate (0-250 kg/ha N)

Test Crop

Grass Silage

8.8 Pharmaceutical Trial 2 - Results

Abundance

Sixteen species of Collembola (a total of 1,943 individuals) were collected by the litterbags (see Appendix XVII for species list). Differences in the number of total Collembola, and the number of the most frequently occurring species, were compared between the six plots using chi-squared tests for homogenous frequencies (Fowler & Cohen 1992). Results of the chi-squared tests, and abundance of total Collembola and dominant species, can be seen in Table 8.8.

The abundance of total Collembola and, with the exception of *I. viridis* and *P. decipiens*, the abundance of the dominant species were not evenly distributed between plots (see Table 8.8). There was significantly more total Collembola in the SAS plots than expected and less in the control plots (see Table 8.8). The results for AN plots are inconclusive, with only replica one having more Collembola than expected. It can, however, be see that both the AN plots had a higher abundance of total Collembola than the control plots (see Table 8.8). *Folsomia candida* and *Mesaphorura* spp. (composed of *M. macrochaeta* and *M. hylophila*) occurred more frequently in SAS plots than expected and less frequently in control plots (see Table 8.8). It is interesting to note that no species occurred more frequently in the control, suggesting that in the long-term no species was adversely affected by either of the fertilisers (see Table 8.8).

Differences in the frequencies of *F. fimetarioides, I. palustris* and *I. tigrina* could not be attributed to treatment. *Isotoma tigrina* showed marked differences between AN replicas, with replica one having less than expected and replica 2 having more (see Table 8.8). For *I. palustris*, all the treatments had lower abundances in replica 2, hence indicating there was a significant replica effect for *I. palustris* (see Table 8.8). Although the numbers of *C. denticulata* tended to be lower in the control replicas than the SAS and AN replicas, the chi-squared test did not show this difference (see Table 8.8). The disproportionately small number of *C. denticulata* in replica 2 of the control gave a lower expected value than would otherwise have been attained. As a consequence of this, the numbers on control replica one were not lower than the expected. The DCA

Table 8.8 Comparing abundance between treatments

Species	Control Rep 1	SAS Rep 1	AN Rep 1	Control Rep 2	SAS Rep 2	AN Rep 2	Expected	X^2-value	Significance (5 d.f.)
Total Collembola	240	516	385	131	380	291	323.83	275.19	< 0.001
Mesaphorura spp.	6.00	35.00	28.00	13.00	62.00	27.00	28.50	67.14	< 0.001
Isotoma tigrina	10.00	11.00	4.00	8.00	16.00	27.00	12.67	25.53	< 0.001
Isotomurus palustris	22.00	14.00	19.00	5.00	7.00	7.00	12.33	20.38	< 0.005
Isotoma viridis	9.00	17.00	9.00	6.00	8.00	9.00	9.67	7.38	N.S.
Folsomia candida	20.00	152.00	14.00	12.00	56.00	14.00	44.67	340.42	< 0.001
Folsomia fimetarioides	7.00	13.00	30.00	5.00	1.00	13.00	11.50	45.17	< 0.001
Pseudosinella decipiens	6.00	13.00	3.00	5.00	10.00	7.00	7.33	8.91	N.S.
Ceratophysella denticulata	158.00	249.00	242.00	76.00	198.00	182.00	109.30	184.17	< 0.001

Table 8.8 This table shows the abundance of total Collembola and the abundance of each of the frequently occurring species across the three treatments and two replicas. It also shows the expected value, the X^2 value and the probability value obtained when the abundance was compared between treatments and replicas.

ordination (see Figure 8.4) indicates that differences in collembolan composition between replicas were as great, if not greater than, the differences between treatments.

Relative frequencies

The relative frequencies of the dominant species were compared using a 8 x 6 contingency table (35 degrees of freedom). The result of the contingency table, and the observed and expected abundance of each species, are presented in Table 8.9. The relative frequencies of dominant species were found to be dependant on plot ($P < 0.001$). The main contributors to the X^2 value were found to be the higher proportion of *Mesaphorura* spp. in SAS replica 2, and of *F. candida* in SAS replica one (see Table 8.9). It is possible that this was the result of *F. candida* having a competitive advantage in SAS replica one, whereas, the *Mesaphorura* spp. had the advantage in replica two. This again illustrates that there were differences between replicas. *Folsomia candida* was, however, found to have lower relative frequencies in the AN replicas than expected, indicating that it was adversely affected by AN (see Table 8.9).

Figure 8.4 DCA ordination for pharmaceutical trial 2

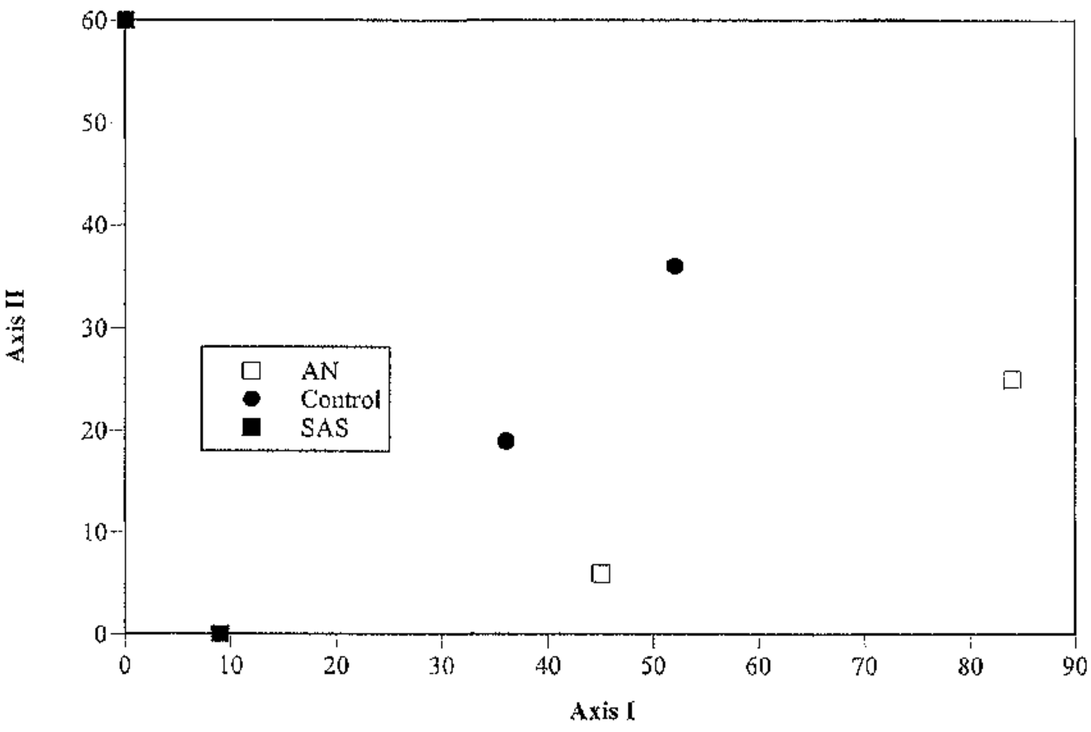


Table 8.9 Comparing relative frequencies of species between treatments

Species	Control (R1)	SAS (R1)	AN (R1)	Control (R2)	SAS (R2)	AN (R2)
Mesaphorura spp.	6 *21.8*	35 *46.2*	28 *32.0*	13 *11.9*	62 *32.8*	27 *26.2*
Isotoma tigrina	10 *9.7*	11 *20.5*	4 *14.2*	8 *5.3*	16 *14.6*	27 *11.7*
Isotomurus palustris	22 *9.4*	14 *20.0*	19 *13.9*	5 *5.2*	7 *14.2*	7 *11.4*
Isotoma viridis	9 *7.4*	17 *15.7*	9 *10.9*	6 *4.0*	8 *11.1*	9 *8.9*
Folsomia candida	20 *34.2*	152 *72.4*	14 *50.2*	12 *18.7*	56 *51.4*	14 *41.1*
Folsomia fimetarioides	7 *8.8*	13 *18.7*	30 *12.9*	5 *4.8*	1 *13.3*	13 *10.6*
Pseudosinella decipiens	6 *5.6*	13 *11.9*	3 *8.2*	5 *3.1*	10 *8.5*	7 *6.8*
Ceratophysella denticulata	158 *141.0*	249 *298.6*	242 *206.8*	76 *77.0*	198 *212.1*	182 *169.5*

X^2_{35} value	Significance (35 d.f.)
302.4	< 0.001

Table 8.9 This table shows the abundance of the dominant species across the three treatments and two replicas. It also shows the expected value (in italics), and probability value obtained when the abundance was compared between treatments and replicas.

Diversity

Although no individual species was adversely affected by SAS, it is possible the diversity of the community was. Diversity values were therefore calculated for the six plots (see Table 8.10).

Table 8.10 Comparing the diversity between treatments

Diversity Index	**Control R1**	**AN R1**	**SAS R1**	**Control R2**	**AN R2**	**SAS R2**
S (no. species)	10	13	12	9	11	10
N_2 (Hill's)	2.2	2.4	3.05	2.74	2.41	3.07
α (Fisher's)	2.11	2.6	2.2	2.19	2.26	1.88
d (Berger-Parker)	0.658	0.629	0.483	0.580	0.625	0.521

The plots receiving AN fertiliser were more diverse when measure by α (Fisher's). However, when diversity was measured by $\mathbf{N_2}$ (Hill's), the SAS plots had the higher diversity. This is likely to be a consequence of α being unaffected by the dominance of single species, whereas, $\mathbf{N_2}$ is sensitive to single species dominance (Magurran 1988). As may be expected from comparing $\mathbf{N_2}$ and α, the SAS plots had a lower **d** (Berger-Parker) value than the AN (or control) plots. This indicates that the evenness of the SAS plots was greater which would contribute to the higher $\mathbf{N_2}$ value. It is interesting to note that no diversity index ranked the control plots as the most diverse, and hence it would appear that fertilisation increased the diversity of the collembolan community within this trial.

Sexual and asexual

The numbers of Collembola with asexual and sexual reproduction were compared between treatments using a 6 x 2 contingency table (5 degrees of freedom). The results are presented in Table 8.11.

Table 8.11 Comparing the frequencies of sexual and asexual individuals

Reproduction	**Control R1**	**SAS R1**	**AN R1**	**Control R2**	**SAS R2**	**AN R2**	
Sexual	206 *176.4*	313 *379.2*	310 *283.0*	101 *96.3*	261 *279.3*	237 *213.9*	
Asexual	34 *63.6*	203 *136.8*	75 *102.1*	30 *34.7*	119 *100.7*	54 *77.1*	X^2_5= 87.0

Table 8.11 This table shows the observed and expected (shown below in italics) frequencies of asexually and sexually reproducing Collembola found in each plot. It also shows the X^2 value (5 d.f.) obtained when the relative frequencies of sexual and asexual individuals were compared.

From Table 8.11 it can be seen that there was a significant difference in the frequencies of sexual and asexual individuals between treatments ($P < 0.01$). In particular, the control plots and AN plots had a higher frequency of sexual species and a lower frequency of asexual species than expected, whereas, the SAS plots had a higher frequency of asexual species and a lower frequency of sexual species. This does not support Siepel's (1995) hypothesis that fertilisation favours sexual individuals over the asexual individuals.

Euedaphic and hemiedaphic

The numbers of euedaphic and hemiedaphic individuals were compared between the treatments in order to test for a difference in ecomorphology. Again a 6 x 2 contingency table (5 d.f.) was used to test for differences. The results are presented in Table 8.12.

Table 8.12 Comparing the frequencies of euedaphic and hemiedaphic individuals

Ecomorphology	**Control R1**	**SAS R1**	**AN R1**	**Control R2**	**SAS R2**	**AN R2**	
Hemiedaphic	207 *170.0*	309 *365.4*	286 *272.7*	100 *92.8*	241 *269.1*	233 *206.1*	
Euedaphic	33 *70.0*	207 *150.6*	99 *112.4*	31 *38.2*	139 *110.9*	58 *84.9*	$X^2_5 =$ 83.8

Table 8.12 This table shows the observed and expected (shown below in italics) frequencies of euedaphic and hemiedaphic Collembola found in each plot. It also shows the X^2 value (5 d.f.) obtained when the relative frequencies of sexual and asexual individuals were compared.

A significant difference in the frequency of euedaphic and hemiedaphic individuals between plots was found ($P < 0.01$). Euedaphic individuals were over-represented in the SAS plots and under-represented in the AN and the control plots.

8.9 Pharmaceutical Trial 2 - Discussion

Both fertilisers were found to increase the abundance and diversity of Collembola over the unfertilised control a year after treatment. Several studies have found organic waste and inorganic waste to increase the abundance of Collembola (Lübben 1989; Siepel & Van de Bund 1988: for organic and inorganic, respectively). This is thought to be principally the result of enhanced macro and micro-floral communities, which increases the food and cover for Collembola.

As found in other studies, fertilisation altered the species composition (Marshall 1977). *Folsomia candida* and *Mesaphorura* spp. were favoured by SAS, while the former species was adversely affected by AN. *Folsomia candida* and *Mesaphorura* spp. were also favoured by heavy metal contaminated sludge (Lübben 1989) and *F. candida* occurs more frequent in intensely managed plots (higher fertiliser, pesticide and ploughing) than less intensely managed plots (Brussaard *et al.* 1990). It would appear that *F. candida* and *Mesaphorura* spp. benefit most from fertilisation under conditions that other species find adverse (e.g. the presence of heavy metals and pesticides). However, in Chapter 4 *Mesaphorura* spp. were found to be adversely affected by the addition of metal-rich sludge.

The earthworm abundance was adversely affected by SAS for at least nine months following application, and this was thought to be the consequence of the high salt concentration of SAS (Aitken 1993). Collembola have been shown to be adversely affected by salinity in the laboratory (Hutson 1978). However, if the high salinity of the SAS waste adversely affected Collembola, they appeared to be short-lived and the abundance and diversity were enhanced by SAS in the long-term.

Siepel & Van de Bund (1988) predicted that fertilisation would favour hemiedaphic individuals over euedaphic. Contrary to this prediction, euedaphic individuals were actually favoured by SAS. Furthermore, the SAS waste had a lower occurrence of sexual individuals and a higher occurrence of asexual individuals than the AN or the control. This is again contrary to Siepel's (1995) predictions that fertilisation increases sexual species. It is likely that the increase in asexual species and in euedaphic species in the SAS plots resulted from the increase in *F. candida* and *Mesaphorura* spp. in this treatment, as these species are both euedaphic and asexual.

It would therefore appear that the application of SAS did not adversely affect the collembolan abundance or diversity in the long-term. The species composition was, however, changed with *F. candida* and *Mesaphorura* spp. being promoted by SAS. This change in community structure affected the soil ecosystem which in turn could affect decomposition and mineralisation.

8.10 General discussion

For both pharmaceutical trials treatment and plot effects were apparent. Aerobically treated sludge (ATS) significantly reduced the abundance of Collembola, and the detrimental effects were non-specific, with almost all investigated species being adversely affected. Both the inorganic fertilisers (AN and NPK) had a higher diversity when measured by Hill's N_2, suggesting that inorganic fertilisers promote diversity both in the long-term and the short-term. Furthermore, sodium ammonium sulphate (SAS) was found to increase the diversity and the total abundance of Collembola in the long-term, and *Folsomia candida* and *Mesaphorura* spp. particularly benefited from SAS application. Although the collembolan community was promoted by SAS in the long-term, adverse effects were noted following application for clover and earthworms, and may also have occurred for Collembola (Aitken & Kirkland 1993).

These results must, however, be analysed with caution as differences between replicas were sometimes as great, if not greater than, differences between treatments. Exceptionally high numbers of certain species (e.g. *C. denticulata*) were still found in some plots, and this caused a higher expected value than would of otherwise been reached. Ideally, if more replicas had been available, the problem of aggregation could have been minimised and stronger statistical tests applied.

Both pharmaceutical trials were part of a larger scale study, and it was therefore necessary to sample Collembola using minimal disturbance techniques. Because litterbags cause minimum disruption, these were chosen instead of soil cores. It is important to note that litterbags do not give a true indication of the soil fauna because Collembola in the litterbags are not solely determined by the external soil fauna, but also by colonisation and successional effects (see page 44). Furthermore, as litterbags provide a food source and exclude larger predators, the number of Collembola in litterbags are frequently higher than the abundance in the external soil (Crossley & Hoglund 1962).

Although SAS promoted collembolan communities in the long-term, earthworms and clover (and possibly Collembola) were adversely affected directly following application

(Aitken 1993). Furthermore, the application of ATS was found to adversely affect Collembola for at least a period of three months following application. It is likely that wastes from different processes will affect the soil community differently, and therefore the use of pharmaceutical waste should be more carefully regulated than current legislation demands.

CHAPTER 9. General Conclusions

9.1 The influences of metal-rich sludges on Collembola

At the Auchincruive site a total of 24 species were caught by pitfall trapping and suction sampling in 1995 and 1996. Of these, 16 species occurred frequently enough for statistical tests to be applied. Adverse effects of zinc and copper-rich sludge were not found for any of these species. It is however, important to note that only initial work was conducted on the copper-rich plots, and it is possible that a more detailed investigation would have shown effects. *Lepidocyrtus cyaneus, I. viridis, I. maculatus* and *H. nitidis*, on the other hand, were found to be sensitive to the application of cadmium-rich sludge. Four out of a possible 16 (or 25%) epigeal/hemiedaphic species (sampled by suction and pitfalls) were adversely affected by the application of cadmium-rich sludge.

A total of 26 species were sampled by litterbags at Auchincruive in 1995 and of these, 10 species occurred frequently enough for statistical tests to be applied. *Neelus minimus* and *Mesaphorura* spp. were found to be adversely affected by the application of zinc-rich sludge, and *Mesaphorura* spp. were also adversely affected by copper-rich sludge. It would therefore appear that at Auchincruive euedaphic Collembola were more susceptible to the zinc and copper-rich sludges than hemiedaphic/epigeal Collembola. As the cadmium-rich plots were not sampled by litterbags, no conclusions can be drawn concerning the effects of cadmium-rich sludge on euedaphic Collembola. It is possible to combine information derived from the three sampling methods to determine what percentage of Collembola were adversely affected by the copper-rich and zinc-rich sludges. Through doing this, it can be concluded that 2 out of 20 (or 10%) species were adversely affected by zinc-rich sludge, while 1 out of 14 (or 7%) were adversely affected by copper-rich sludge.

To assess the ecological risks of applying metal-rich sludge to agricultural land, it is necessary to relate the above percentages to a more meaningful term. Van Straalen (1993) suggests that since it is rarely possible to protect 100% of species, one should aim to protect 95%. All three metal-rich sludges were found to adversely affect over 5%

of the species studied, hence suggesting they were not safe for land application (at least with respect to Collembola).

The 95% protection level does, however, have its problems, principally the implication that 5% of species are 'expendable', irrespective of what these species are (Hopkin 1993). It is possible that 'expendable' species are vital for ecosystem functioning, or that they are Red Data Book species (Hopkin 1993). The 95% protection level value, although being a useful concept, should therefore not be treated as a 'holy grail' (Hopkin 1993).

The CEC Directive 86/278/EEC concerns the control of heavy metal accumulation in the soil, and provides recommended maximum values for soil, sludge and rates of addition (CEC 1986). This Directive stipulates that all Member states must observe the maximum soil metal concentrations, and that soil levels must be monitored. In addition to setting maximums for soil metal levels, the Directive also stipulates that Member states must control the rate of addition of heavy metals to the soil (Smith 1996). This may be achieved by either controlling the concentration of metals present in the sludge, or by limiting the amount of heavy metals added annually (CEC 1986). This latter approach has been adopted by the UK.

The maximum sludge concentrations, soil levels and application rates set by the CEC (1986) are shown in Table 9.1, alongside the levels present at Auchincruive. From this table it can be seen that the soil concentrations of all three metals were below the maximum permissible levels set under the CEC Directive (1986). This study suggests that Collembola exhibited adverse affects to copper, zinc and particularly cadmium, at soil concentrations deemed safe by current UK legislation. Furthermore, because the UK did not adopt the safe metal loadings for sludge (Smith 1996), sludges with metal concentrations in excess of those used at SAC may be applied to agricultural land in the UK.

Instead of setting a maximum metal concentration in sludge, the UK adopted the approach of limiting the annual application rate of metals. At Auchincruive, the application rates of cadmium, zinc and copper were far in excess (five times in the case of copper to 25 times in the case of cadmium) of application rates permissible by UK legislation (see Table 9.1). At safe metal application rates it would take over 50 years for the soil metal concentrations to build up to the maximum permissible levels (Aitken personnel communication). It was therefore necessary to use high application rates to enable the maximum soil concentrations to be achieved within a realistic number of years. Furthermore, by raising the soil levels quickly genetic adaptation, which may obscure real effects, was less likely to occur.

Table 9.1 Maximum metal concentrations set by CEC (1986) and concentrations at SAC

Metal	Soil concentration	Sludge concentration	Annual application rate
Cadmium	SAC 2.2 mg kg^{-1} Maximum 3 mg kg^{-1}	SAC 48.9 mg kg^{-1} Maximum 40 mg kg^{-1}	SAC 4.3 kg ha^{-1} yr^{-1} Maximum 0.15 kg ha^{-1} yr^{-1}
Zinc	SAC 270 mg kg^{-1} Maximum 300 mg kg^{-1}	SAC 7,036 mg kg^{-1} Maximum 4,000 mg kg^{-1}	SAC 288.5 kg ha^{-1} yr^{-1} Maximum 30 kg ha^{-1} yr^{-1}
Copper	SAC 69 mg kg^{-1} Maximum 140 mg kg^{-1}	SAC 3,394 mg kg^{-1} Maximum 1,750 mg kg^{-1}	SAC 67.9 kg ha^{-1} yr^{-1} Maximum 12 kg ha^{-1} yr^{-1}

Table 9.1 This table shows the maximum soil concentrations set by the CEC Directive 86/278/EEC (1986). It shows the mean cadmium and zinc concentrations in 1996 and the mean copper concentration in 1995 for the SAC Auchincruive trial site. It also shows the mean sludge concentrations and application rates at SAC.

It would therefore appear that Collembola were adversely affected by soil concentrations of copper, zinc and cadmium below the maximum permissible levels set by Directive 86/278/EEC (CEC 1986). Furthermore, as the UK does not place any restrictions on maximum metal concentrations in sludge (the approach adopted by most European countries: Smith 1996), adverse effects were found at sludge concentrations permissible for land application in the UK. Caution must, however, be taken as the annual application rates were up to 25 times higher than the maximum acceptable levels.

As sensitivity depends on the metal in question, it is important to monitor which metals are present in sludge. Cadmium-rich sludge was found to be particularly toxic to Collembola and it is therefore suggested that levels of cadmium should be carefully controlled. The levels of cadmium in sewage effluent from industrial sources have declined markedly in the last decade, and consequently most sludges currently spread on agricultural land contain background levels of cadmium (Smith 1996). It is also possible to include metal removal stages (e.g. acid extraction techniques) in sludge treatment to ensure that sludges spread on agricultural land are safe.

9.2 Recommendations for future studies

As found by several authors, Collembola were heterogeneously distributed in the study areas (Hughes 1962; Krogh 1991; Heungens & Van Daele 1984; Winter *et al.* 1990; Frampton 1988). Frampton (1994) concluded that to obtain an accurate estimation of Collembola populations, an impracticably high number of suction or pitfall samples (over 200) would have to be taken, except at times of peak abundance. In this study, the five samples (litterbag, suction or pitfall) taken on a plot on a specific sampling date were combined to help overcome problems of spatial heterogeneity and to give an indication of overall community structure per plot (see Chapter 3). The abundances were then square root transformed to normalise the data and the probability was also set at 0.01 to avoid Type one errors (i.e. wrongly rejecting the null hypothesis: Day & Quinn 1989). By taking these precautions it could be safely concluded that results were real and not just a consequence of sampling error.

In this study, pitfall trapping and suction sampling was conducted from April to August. As species specific differences in seasonality are well documented (Joosse 1969; Niijima 1975, Brussaard *et al.* 1990), it is possible that winter species were omitted by this sampling regime. Sampling by litterbags was, however, conducted during the winter of 1995, and no hemiedaphic species was sampled exclusively by winter litterbags. It is therefore probable that, at least for hemiedaphic Collembola, species present in winter were also present in summer. It would appear that sampling from April to August included most (if not all) species, and minimised problems associated with sampling in wet weather (e.g. pitfall traps filling with rain). An increased sampling efficiency in shorter grass was found for several species sampled by pitfalls or suction. It is therefore suggested that the best time to sample hemiedaphic and epigeal species is when the grass is short.

Identification of Collembola is labour intensive and requires taxonomic expertise. It would therefore be convenient to be able to classify Collembola at a level other than species. As a consequence of species specific differences in metal sensitivity within genera (e.g. *F. quadrioculata* has been described as a metal sensitive species: Filser *et al.* 1995, while *F. candida* is described as tolerant: Lübben 1989), effects of metals may not be detected at the genus level and identification just to this level should be avoided. The ecological classification of Collembola into epigeal, euedaphic and hemiedaphic individuals is reasonably straight forward requiring little expertise (see Chapter 2), and could therefore provide an alternative to species identification. Differences in susceptibility of euedaphic and hemiedaphic Collembola have been found for pesticides, with hemiedaphic species being more susceptible as a result of the pesticide concentrating in the litter layer (Krogh 1991). In this study no treatment effect was found when the total Collembola were grouped in euedaphic and hemiedaphic individuals, hence suggesting that neither ecomorphology was more susceptible (see Chapter 4). The application of SAS (but not ATS) pharmaceutical waste was found to promote euedaphic species at the cost of hemiedaphic species (see Chapter 8). It would therefore appear that the classification of Collembola into hemiedaphic and euedaphic individuals is of little use when looking at the effects of metal-rich sludge, but may be of use when looking at other agricultural practices.

While the copper and zinc-rich sludges did not affect epigeal and hemiedaphic species, adverse effects were found for euedaphic species, hence suggesting that these species were more susceptible to metal-rich sludge. However, the manipulation and identification of epigeal species is generally easier than euedaphic species as they tend to be larger. Furthermore, adverse effects of cadmium-rich sludge were found for several hemiedaphic and epigeal species. By combining methods which sample euedaphic species (e.g. litterbags and soil cores) with methods which sample epigeal species (e.g. suction samples and pitfalls), the most effective sampling regime for studying metal-rich sludge is achieved.

The species *F. candida* is the subject of numerous toxicity tests (Gestel & Hensbergen 1997; Crommentuijn *et al.* 1993). *Folsomia candida* is an ideal organism for conducting laboratory experiments as cultures are easily maintained and they have a short reproductive cycle at 20°C (Hopkin 1997). The International Standardisation Organisation (ISO) has now produced guidelines for a standard toxicity test using *F.*

candida as a test organism (International Standardisation Organisation 1994). It is therefore likely that *F. candida* will become a routine organism for ecotoxicology testing. Lübben (1989) found that *F. candida* was actually favoured by the addition of metal-rich sludge. It would therefore appear that metal levels deemed safe for this species are likely to adversely affect more sensitive species. Care must therefore be taken in extrapolating results from laboratory experiments on *F. candida* to other species of Collembola in the field. In this study the species *H. nitidis*, which is also relatively easy to culture, was found to be negatively correlated with cadmium concentration (see Chapter 5). It is therefore possible that this species is more sensitive to metals than the relatively insensitive *F. candida* and may therefore be a useful organism for ecotoxicology testing.

Under conditions currently deemed safe by CEC legislation it would take many years for the soil metal levels to reach the levels present in this experiment. As metals are persistent and accumulate with prolonged sludge application, it is likely that adverse affects on invertebrates would not become apparent until several years of repeated application. It is therefore suggested that monitoring programs should be conducted on a long-term basis, and should investigate sub-lethal effects on activity and reproduction to enable earlier detection of ecosystem disturbance.

The phyto-availability of heavy metals in sludge is dependant on several factors including soil structure, pH and cation exchange capacity (Smith 1996). Further work is therefore required to investigate how these factors influence the availability of metals to invertebrates. Soil pH is thought to have the greatest influence on metal availability, and ecosystem recovery may be promoted by elevating the pH (through the addition of lime) to render heavy metals less available (Benninger-Traux & Taylor 1993). Since Collembola are influenced by both metals and pH, it would be interesting to determine how liming contaminated land influences their community.

Microarthropods which possess the enzyme chitinase, are able to ingest fungal cell walls where heavy metals are frequently concentrated (Siepel 1994; Siepel & Ruiter-Dijkman 1993). It is therefore likely that such feeding guilds will be particularly susceptible to heavy metals (Siepel 1994). By testing for the presence of chitinase, it may be possible to determine if *I. viridis* and *L. cyaneus* were sensitive to cadmium as a consequence of their feeding guild. It would also be interesting to use acid digestion techniques to determine if sensitive species had higher body burdens of metals than tolerant species (Van Straalen & Van Wensem 1986). This would help determine if metals were directly or indirectly toxic.

Previous studies have found Collembola to be effective indicators of pesticide use (Frampton 1994), and the findings of this research suggests that they can also be used as effective monitors of heavy metal pollution. The identification of Collembola is assumed to be a difficult task, and this may be considered a drawback in using them as indicators. However, with some expertise the majority of species can be identified with relative ease, and although a current British key is lacking, good continental keys are available (Gisin 1960; Fjellberg 1980). Collembola also have the advantage over most arthropods that several species are present in a recognisable form throughout the year. Furthermore, as a consequence of their small size, they are particularly useful for small plot trials where the use of larger invertebrates would be inappropriate. They are also equally useful for field scale studies. As a consequence of their more direct association with contaminants, Collembola may give an earlier indication of pollution. Finally, Collembola are an interesting and diverse group and, because they are relatively understudied, there is still plenty to discover about their behaviour and ecology.

REFERENCES

Aitchison, C.W. 1983. Low temperature and preferred feeding by winter active Collembola (Insecta, Apterygota). *Pedobiologia*, **25**: 27-36.

Aitken, N.M. 1993. *The Effect of Applying Sodium Ammonium Sulphate Pharmaceutical Waste to Agricultural Land.* Report to SmithKline Beecham.

Aitken, N.M. & Kirkland, I.J. 1993. *The Effect of Applying Sodium Ammonium Sulphate Pharmaceutical Waste to Agricultural Land - Phase 2.* Report to SmithKline Beecham.

Aitken, N.M. 1994. The effect of applying aerobic treatment sludge waste to agricultural land. *SAC Report*: C94/107/230.

Aitken, N.M., Bromilow, T.H.W. & Merrilees, D.W. 1995. The uptake of metals in plants from sewage sludge and the distribution of metals in the soil. In *Soil Management and Sustainable Agriculture* (eds. Cook, H.F. & Lee, H.C.) pp. 534-537. Wye College Press, London.

Andrzejewska, L. 1979. Herbivorous fauna and its role in the economy of grassland ecosystems. I. Herbivores in natural and managed meadows. *Polish Ecological Studies*, **5**: 5-44.

Anderson, J.M. 1975. Succession, diversity, and trophic relationships of some soil animals in decomposing leaf litter. *Journal of Animal Ecology*, **44**: 475-495.

Anderson, J.M & Bignell, O.E. 1980. Bacteria in the food, gut contents and faeces of the litter-feeding millipede *Glomeris marginata* (Villers). *Soil Biology and Biochemistry*, **12**: 251-254.

Badejo, M.A. & Straalen, N.M. Van. 1992. Effects of atrazine on growth and reproduction of *Orchesella cincta* (Collembola). *Pedobiologia*, **36**: 221-230.

Badejo, M.A. & Olaifa, J.I. 1996. Effects of precultivation and weed control practices on the activity of the epigeic collembolan fauna of cowpea. In *Ninth International Colloquium on Apterygota: Abstracts* (eds. Purvis, G., Bolger, T. & Kaye, B.) p27. University College Dublin: Dublin.

Bardgett, R.D., Frankland, J.C. & Whittaker, J.B. 1993. The effects of agricultural management on the soil biota of some upland grasslands. *Agriculture, Ecosystems and Environment*, **45**: 25-45.

Beckett, P.H.T. & Davis, R.D. 1979. The disposal of sewage sludge onto farmland: the scope of the problem of toxic elements. *Water Pollution Control*, **78**: 419-445.

Begon, M. & Mortimer, M. 1981. *Population Ecology: A Unified Study of Animals and Plants.* Blackwell Scientific Publications: Oxford.

Bengtsson, G & Rundgren, S. 1988. The Gusum case: a brass mill and the distribution of soil Collembola. *Canadian Journal of Zoology*, **66**: 1518-1526.

Bengtsson, G., Gunnarsson, T. & Rundgren, S. 1983. Growth changes caused by metal uptake in a population of *Onychiurus armatus* (Collembola) feeding on metal polluted fungi. *Oikos*, **40**: 216-225.

Bengtsson, G., Ohlsson, L. & Rundgren, S. 1985a. Influence of fungi on growth and survival of *Onychiurus armatus* (Collembola) in a metal polluted soil. *Oecologia*, **68**: 63-68.

Bengtsson, G., Gunnarsson, T. & Rudgren, S. 1985b. Influence of metals on reproduction, mortality and population growth in *Onychiurus armatus* (Collembola). *Journal of Applied Ecology*, **22**: 967-978.

Benninger-Truax, M. & Taylor, D.H. 1993. Municipal sludge metal contamination of old-field ecosystems: Do liming and tilling affect remediation? *Environmental Toxicology and Chemistry,* **12**: 1931-1943.

Berbiers, P., Maelfait, J.P. & Mertens, J. 1989. Evaluation of some sampling methods used to study Collembola (Insecta, Apterygota) in a pasture. *Revue Écologie et de Biologie du Sol,* **26**: 305-320.

Berger, W.H. & Parker, F.L. 1970. Diversity of planktonic Foraminifera in deep sea sediments. *Science,* **168**: 1345-1347.

Bewick, M.W.M. 1978. Effects of tylosin and tylosin fermentation waste on microbial activity of soil. *Soil Biology and Biochemistry,* **10**: 403-407.

Bird, G.A. & Chatarpaul, L. 1986. Effect of whole-tree and conventional forest harvest on soil microarthropods. *Canadian Journal of Zoology,* **64**: 1986-1993.

Blair, J.M. & Crossley, D.A. Jr. 1988. Litter decomposition, nitrogen dynamics and litter microarthropods in a Southern Appalachian hardwood forest 8 years following clearcutting. *Journal of Applied Ecology,* **25**: 683-698.

Bodar, C.W.M., Donselaar, E.G. & Herwig, H.J. Van. 1990. Cytopathological investigations of digestive tract and storage cells in *Daphnia magna* exposed to cadmium and tributyltin. *Aquatic toxicology,* **17**: 325-338.

Bolger, T. 1985. The rate of disappearance of *Salix* litter on cutaway bog and the microarthropods associated with it. *Pedobiologia,* **28**: 145-153.

Boyd, H. P. 1995. Arthropods taken in pitfall traps in the pine barrens of New Jersey. *Entomological News,* **106**: 45-56.

Bruce, L.J., McCracken, D.I., Foster, G.N. & Aitken, M.N. 1997. The effects of cadmium and zinc-rich sewage sludge on epigeic Collembola populations, *Pedobiologia,* **41**: 167-172.

Brussaard, L.; Bouwman, L.A.; Geurs, M.; Hassink, J. & Zwart, K.B. 1990. Biomass, composition and temporal dynamics of soil organisms of a silt loam soil under conventional and integrated management. *Netherlands Journal of Agricultural Sciences*, **38**: 283-302.

Burnett, G.F. 1968. The effects of irrigation, cultivation and some insecticides on the soil Arthropods of an East African dry grassland. *Journal of Applied Ecology*, **5**: 141-156.

Carapelli, A., Fanciulli, P.P., Frati, F. & Dallal, R. 1996. Estimating levels of gene flow between natural populations in the genus *Isotomurus* (Collembola, Isotomidae). In *Ninth International Colloquium on Apterygota: Abstracts* (eds. Purvis, G., Bolger, T. & Kaye, B.) p6. University College Dublin: Dublin.

Carlton-Smith, C.H. 1987. *Effects of Metals in Sludge Treated-Soils on Crops.* Technical Report TR 251. WRc Medmanham: Marlow.

CEC (Commission of European Communities). 1986. Council directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture (86/278/EEC). *Official Journal of the European Communities* No. L 181: 6-12.

CEC (Commission of European Communities). 1991. Council directive of 21 May 1991 concerning urban waste water treatment (91/271/EEC). *Official Journal of the European Communities* No. L 135: 40-52.

Chaney, W.R., Kelly, J.M. & Strickland, R.L. 1978. Influence of cadmium and zinc on carbon dioxide evolution from litter and soil from a black oak forest. *Journal of Environmental Quality*, 7: 115-119.

Chang, A.C., Page, A.L., Warneke, J.E., Resketo, M.R. & Jones, T.E. 1983. Accumulation of cadmium and zinc in barley grown on sludge-treated soils: A long-term field study. *Journal of Environmental Quality*, **12**: 391-397.

Christiansen, K. 1964. Bionomics of Collembola. *Annual Review of Entomology*, **9**: 147-178.

Coghlan, A. 1997. Lamb's liver with cadmium garnish... *New Scientist*: 22 March p4.

Comber, S.D.W. & Gunn, A.M. 1994 *Diffuse Sources of Heavy Metals to Sewers.* Report No. FR 0470. Foundation for Water Research: Marlow.

Critchley, R.F. & Agg, A.R. 1986. *Sources and Pathways of Trace Metals in the United Kingdom.* WRc Report No. ER 822-M. WRc Medmenham: Marlow.

Crossley, D.A. Jr. & Hoglund, M.P. 1962. A litter-bag method for the study of microarthropods inhabiting leaf litter. *Ecology*, **43**: 571-573.

Crommentuijn, T., Brils, J. & Straalen, N.M. Van. 1993. Influence of cadmium on life history characteristics of *Folsomia candida* (Willem) in an artificial soil substrate. *Ecotoxicology and Environmental safety*, **26**: 216-227.

Cuendet, G. & Ducommun, A. 1990. Peuplements lombriciens et activité de surface en relation avec les boues d'épuration et autres fumures. *Revue Suisse de Zoologie*, **97**: 851-869.

Culliney, T.W. & Pimentel, D. 1986. Effects of chemically contaminated sewage sludge on an aphid population. *Ecology*, **67**: 1665-1669.

Curl, E.A. & Harper, J.D. 1990. Fauna - microflora interactions. In *The Rhizosphere* (Ed. Lynch, J.M.) pp. 369-388. John Wiley & Sons: Chichester.

Curry, J.P. 1970. The effects of different methods of new sward establishment and the effects of the herbicides paraquat and dalapon on the soil fauna. *Pedobiologia,* **10**: 329-361.

Curry, J.P. 1994. *Grassland Invertebrates: Ecology, Influence on Soil fertility and Effects on Plant Growth.* Chapman & Hall: London.

Curry, J.P. & Cunningham, C. 1978. A comparison of the epigeal arthropod fauna of old pasture and new leys of various floral types. *The Scientific Proceedings of the Royal Dublin Society, Series A*, **6**: 305-316.

Curry, J.P. & Tuohy, C.F. 1978 Studies on the epigeal microarthropod fauna of grassland swards managed for silage production. *Journal of Applied Ecology*,**15**: 727-741.

Davidson, R.L., Hilditch, J.A., Wiseman, J.R. & Wolfe, V.G. 1979. Growth, fecundity, and mortality responses of Scarabaeidae (Coleoptera) contributing to population increases in improved pastures. In *Proceedings of the 2nd Australasian Conference on Grassland Invertebrate Ecology,* (eds Crosby, T.K. & Pottinger, R.P.) pp. 66-70. Government Printer: Wellington.

Day, R.W. & Quinn, G.P. 1989 Comparisons of treatments after an analysis of variance in ecology. *Ecological Monographs*, **59**: 433-463.

Dietrick, E.J., Schlinger, E.I. & Bosch, R. Van Den. 1959. A new method for sampling arthropods using a suction collecting machine and modified Berlese funnel separator. *Journal of Economic Entomology*, **52**: 1085-1091.

De Roo, H.C. 1975. Agricultural and horticultural utilisation of fermentation residues. *Connecticut Experimental Station Bulletin* **750**.

Dhillon, B.S. & Gibson, N.H.E. 1962. A study of the Acarina and Collembola of agricultural soils. I. Numbers and distribution in undisturbed grassland. *Pedobiologia*, **1**: 189-209.

Digby, P.G.N. & Kempton, R.A. 1987. *Multivariate Analysis of Ecological Communities.* Chapman & Hall: London.

Duffield, S.J. & Aebischer, N.J. 1994. The effect of spatial scale of treatment with dimethoate on invertebrate population recovery in winter wheat. *Journal of Applied Ecology*, **31**: 263-281.

Duffy, E. 1974. Comparative sampling methods for grassland spiders. *Bulletin of the British Arachnological Society*, **3**: 34-37.

Edwards, C.A. & Lofty, J.R. 1975. The influence of cultivation on soil animal populations. In *Progress in Soil Zoology* (ed. Vanek, J.) pp. 399-407. Academia: Prague.

Ellis, W.N. 1974. Ecology of epigeic Collembola in the Netherlands. *Pedobiologia*, **14**: 232-237.

Engelmann, M.D. 1961. The role of soil arthropods in the energetics of an old field community. *Ecological Monographs*, **31**: 221-238.

Fábián, M. & Petersen, H. 1994. Short-term effects of the insecticide dimethoate on activity and spatial distribution of a soil-inhabiting collembolan *Folsomia fimetaria* Linné (Collembola : Isotomidae). *Pedobiologia*, **38**: 289-302.

Filser, J. Fromm, H., Nagel, R.F. & Winter, K. 1995. Effects of previous intensive agricultural management on microorganisms and the biodiversity of soil fauna. *Plant and Soil*, **170**: 123-129.

Fisher, R.A., Corbet, A.S. & Williams, C.B. 1943. The relation between the number of species and the number of individuals in a random sample of an animal population. *Journal of Animal Ecology*, **12**: 42-58.

Fjellberg, A. 1980 *Identification Keys to Norwegian Collembola.* Norsk Entomologisk Forenging: Ås-NLH.

Foster, G.N., McCracken, D.I., Luff, M.L., Rushton, S.P. & Eyre, M.D. 1990. Pesticide studies on non-target invertebrates in a wider environmental context. *Proceedings Crop Protection in Northern Britain 1990*, 153-158.

Fowler, J. & Cohen, L. 1992. *Practical statistics for field biology.* John Wiley & Sons: Chichester.

Fox, C.J.F. 1964. The effects of five herbicides on the numbers of certain invertebrate animals in grassland soil. *Canadian Journal of Plant Science*, **44**: 405-409.

Frampton, G.K. 1988. The effects of some commonly-used foliar fungicides on Collembola in winter barley: laboratory and field studies. *Annals of Applied Biology*, **113**: 1-14.

Frampton, G.K. 1994. Sampling to detect effects of pesticides on epigeal Collembola (springtails). *Aspects of Applied Biology*, **37**: 121-130.

Frampton, G.K., Langton, S.D., Greig-Smith, P.W. & Hardy, A.R. 1992. Changes in the soil fauna at Boxworth. In Pesticides, Cereal Farming and the Environment: The Boxworth Project (eds. Greig-Smith, P.W., Frampton, G.K. & Hardy, T.) pp. 132-143. HMSO: London.

Gast, C.H., Janssen, E., Bierling, J. & Haanstra, L. 1988. Heavy metals in mushrooms and their relationship with soil characteristics. *Chemosphere*, **17**: 789-799.

Gauch, H.G. 1982. *Multivariate Analysis in Community Ecology.* Cambridge University Press: Cambridge.

Gestel, C.A.M. Van, & Hensbergen, P.J. 1997. Interaction of Cd and Zn toxicity for *Folsomia candida* Willem (Collembola: Isotomidae) in relation to bioavailability in soil. *Environmental Toxicology and Chemistry*, **16**: 1177-1186.

Gill, R.W. 1969. Soil microarthropod abundance following old-field litter manipulation. *Ecology,* **50**: 805-816.

Gillot, C. 1995 *Entomology* (2nd Edition). Plenium Press: New York.

Gisin, H. 1960. *Collembolenfauna Europas.* Museum D'Histoire Naturelle: Genève.

Glockemann, B. & Larink, O. 1989. Einfluß von klärschlammdüngung und schwermetallbe lastung auf milben, speziell Gamasiden, in einem ackerboden. *Pedobiolgia*, **33**: 237-246.

Goodman, D. 1975. The theory of diversity-stability relationships in ecology. *Quarterly Review of Biology*, **50**: 237-266.

Gough, H.J. 1977. A key for the identification of the families of Collembola recorded from the British Isles. *Entomologist's Monthly Magazine*, **113**: 193-197.

Goulden, C.E. 1969. Developmental phases of the biocoenosis. *Proceedings of the Natural Academy of Science*, **62**: 1066-1073.

Greenslade, P. 1974. Ecological and zoogeographical notes on Collembola of Kangaroo Island, South Australia. *Pedobiologia*, **14**: 256-265.

Greenslade, P.J.M. 1964. Pitfall trapping as a method for studying populations of Carabidae (Coleoptera). *Journal of Animal Ecology*, **33:** 301-310

Gunhold, P. 1957. Untersuchungen über den einfluss der Gülledüngung auf die biologischen, chemischen and physikalischen eigenschaften des bodens. I. Der einfluss der gülle auf das bodenleben. *Zeitschrift fuer Aker-und Pflanzenbau*, **102**: 461-480.

Gusenleitner, J. 1959. Versuche über den einfluss von düngemitteln auf die bodentierwelt bei grünland. *Festschrift Sechsigjähr Best Landwirtschaftsgesetz Chemische Bundesversuchsinstitut fuer Anst Linzer*, **60**: 155-170.

Haarløv, N. 1947. A new modification of the Tullgren apparatus. *Journal of Animal Ecology*, **16**: 115-121.

Haarløv, N. 1960. Microarthropods from Danish soils: ecology, phenology. *Oikos Supplement*, **3**: 176.

Hågvar, S. 1990. Reactions to soil acidification in microarthropods: Is competition a key factor? *Biology and Fertility of Soils*, **9**: 178 - 181.

Hågvar, S. & Abrahamsen, G. 1980. Colonisation by Enchytraeidae, Collembola and Acari in sterile soil samples with adjusted pH levels. *Oikos*, **34**: 245-258.

Hågvar, S. & Abrahamsen, G. 1990. Microarthropods and Enchytraeidae (Oligochaeta) in naturally lead-contaminated soil: A gradient study. *Environmental Entomology*, **19**: 1263-1277.

Hågvar, S. & Kjøndal, B.R. 1981. Effects of artificial acid rain on the microarthropod fauna in decomposing birch leaves. *Pedobiologia*, **22**: 409-422.

Hale, W.G. 1965. Observations on the breeding biology of Collembola (II). *Pedobiologia*, **5**: 161-177.

Hale, W.G. 1967. Collembola. In *Soil Biology* (eds. Burges, A. & Raw, F.) pp. 397-411. Academic Press, London.

Hanlon, R.D.G. & Anderson, J.M. 1979. The effects of Collembola grazing on microbial activity in decomposing leaf litter. *Oecologia*, **38**: 93-99.

Harper, J.L. 1969. The role of predation in vegetational diversity. *Brookhaven Symposia in Biology*, **22**: 48-62.

Harwood, R.W.J. 1994. *The Management of Arable Field Margins to Enhance Natural Populations of Beneficial Arthropods*. Unpublished PhD thesis. University of Southampton.

Hassall, M., Visser, S. & Parkinson, D. 1986. Vertical migration of *Onychiurus subtenuis* (Collembola) in relation to rainfall and microbial activity. *Pedobiologia*, **29**: 175-182.

Helmke, P.A., Robarge, W.P., Korotev, R.L. & Schomberg, P.J. 1979. Effects of soil-applied sewage sludge on concentrations of elements in earthworms. *Journal of Environmental Quality*, **8**: 322-327.

Heungens, A. & Daele, E.Van. 1984. The influence of some acids, bases and salts on the mite and Collembola population of a pine litter substrate. *Pedobiologia*, **27**: 299-311.

Hill, M.O. 1973. Diversity and eveness: a unifying notation and its consequences. *Ecology*, **54**: 427-431.

Hill, M.O. 1979 *DECORANA - a FORTRAN Program for Detrended Correspondence Analysis and Reciprocal Averaging*. Cornell University, Ithaca: New York.

Hill, M.O. & Gauch, H.G.Jr. 1980. Detrended correspondence analysis: an improved ordination technique. *Vegetatio*, **42**: 47-58.

Hintzpeter, U. & Bauer, T. 1986. The antennal trap of the ground beetle *Loricera pilicornis*, a specialisation for feeding on Collembola. *Journal of Zoology*, **208**: 615-630.

Höller-Land, G. 1959. Über die besiedlung des bodens mit Collembolen bei düngung mit verscheiden behandeltem klärschlamm. *Zeitschrift fuer Angewandte Entomologie*, **44**: 425-444.

Hopkin, S.P. 1989. *Ecophysiology of Metals in Terrestrial Invertebrates*. Elsevier Applied Science: Barking.

Hopkin, S.P. 1993. Ecological implications of '95% protection levels' for metals in soils. *Oikos*, **66**: 137-141.

Hopkin, S.P. 1997. *Biology of the Springtails (Insecta: Collembola)*. Oxford University Press: Oxford.

Hughes, R.D. 1962. The study of aggregated populations. In *Progress in Soil Zoology* (ed Murphy, P.W.) pp. 51-55 Pitman Pess: Bath.

Humbert, W. 1978. Cytochemistry and X-ray microprobe analysis of the midgut of *Tomocerus minor* Lubbock (Insecta, Collembola) with special reference to the physiological significance of the mineral concentrations. *Cell and Tissue Research*, **187**: 397-416.

Hutson, B.R. 1978. Influence of pH, temperature and salinity on the fecundity and longevity of four species of Collembola. *Pedobiologia*, **18**: 163-179.

Ingham, E.R. & Coleman, D.C. 1984. Effects of streptomycin, cycloheximide, fungizone, captan, carbofuran, cygon, and PCNB on soil microbe populations and nutrient cycling. *Micorbial Ecology*, **10**: 345-358.

Ireland, M.P. & Richards, K.S. 1977. The occurrence and localisation of heavy metals in the earthworms *Lumbricus rubellus* and *Dendrobaena rubida* in a heavy metal site. *Histochemistry,* **51**: 153-166.

International Standardisation Organisation. 1994. *Draft Soil Quality - Effects of Soil Pollutants on Collembola (Folsomia candida): Method for the Determination of Effects on Reproduction.* ISO/CD 11267. London.

Janssen, M.P.M. & Hogervorst, R.F. 1993. Metal accumulation in soil arthropods in relation to micro-nutrients. *Environmental Pollution,* **79**: 181-189.

Johnson, C.G., Southwood, T.R.E. & Entwistle, H.M. 1957. A new method of extracting arthropods and molluscs from grassland and herbage with a suction apparatus. *Bulletin of Entomological Research,* **48**: 211-218.

Joosse, E.N.G. 1965. Pitfall-trapping as a method for studying surface dwelling Collembola. *Zeitschrift für Morphologie und Okologie der Tiere,* **57**: 320-328.

Joosse, E.N.G. 1969. Population structure of some surface dwelling Collembola in a coniferous forest soil. *Netherlands Journal of Zoology,* **19**: 621-634.

Joosse, E.N.G. 1970. The formation and biological significance of aggregations in the distribution of Collembola. *Netherlands Journal of Zoology,* **20**: 299-314.

Joosse, E.N.G. & Buker, J.B. 1979. Uptake and excretion of lead by litter-dwelling Collembola. *Environmental Pollution,* **18**: 235-240.

Joosse, E.N.G. & Kapteijn, J.M. 1968. Activity-stimulating phenomena caused by field-disturbances in the use of pitfall traps. *Oecologia,* **1**: 385-392.

Joosse, E.N.G. & Verhoef, S.C. 1983. Lead tolerance in Collembola. *Pedobiologia,* **25**: 11-18.

Kappelle, M., Kennis, P.A.F. & Vries, R.A.J. 1995. Changes in diversity along a successional gradient in a Costa Rican upper montane *Quercus* forest. *Biodiversity and Conservation,* **4**: 10-34.

Kiekens, L. 1984. Behaviour of heavy metals in soils. In *Utilisation of Sewage Sludge on Land: Rates of Application and Long-term Effects of Metals* (eds. Berglund, S., Davis, R.D. & L'Hermite P.) pp. 126-134D. Reidel Publishing Company: Dordrecht.

Klironomos, J.N., Widden, P. & Deslandes, I. 1992. Feeding preferences of the Collembolan *Folsomia candida* in relation to microfungal successions on decaying litter. *Soil Biology and Biochemistry,* **24**: 685-692.

Komulainen, M. & Mikola, J. 1995. Soil processes as influenced by heavy metals and the composition of soil fauna. *Journal of Applied Ecology,* **32**: 234-241.

Krogh, P.H. 1991. Perturbation of the soil microarthropod community with the pesticides benomyl and isofenphos. I. Population changes. *Pedobiologia,* **35**: 71-88.

Kruse, E.A. & Barrett, G.W. 1985. Effects of municipal sludge and fertilizer on heavy metal accumulation in earthworms. *Environmental Pollution (Series A),* **38**: 235-244.

Larink, O., Lübben, B., Glockemann, B. Prescher, S. 1990. Klärschlamm, schwermetalle und bodentiere. *NNA-Berichte*, **3**: 77-80.

Larsen, A.B. Funch, F.H. & Hamilton, H.A. 1991. The use of fermentation sludge as a fertilizer in agriculture. *Water Science and Technology,* **24**: 33-42.

Larsen, K.J., Purrington, F.F., Brewer, S.R. & Taylor, D.H. 1996. Influence of sewage sludge and fertilizer on the ground beetle (Coleoptera: Carabidae) fauna of an old-field community. *Environmental Entomology*, **25**: 452-459.

Lederman, P.B., Skovronek, H.S. & Rosiers, P.E. Des. 1975. Pollution abatement in the pharmaceutical industry. *Chemical Engineering Progress*, 71: 93-97.

Loring, S.J., Snider, R.J. & Robertson, L.S. 1981. The effects of three tillage practices on Collembola and Acarina populations. *Pedobiolgia,* **22**: 172-184.

Lübben, B. 1989. Influence of sewage sludge and heavy metals on the abundance of Collembola on two agricultural soils. In *Third International Seminar on Apterygota* (ed. Dallai, R.) pp. 419-428. University of Siena: Siena

Luff, M.L. 1975. Some features influencing the efficiency of pitfall traps. *Oecologia,* **19**: 345-357.

MacFadyen, A. 1961. Improved funnel-type extractors for soil arthropods. *Journal of Animal Ecology,* **30**: 171-182.

MacFadyen, A. 1978. The role of the fauna in decomposition processes in grassland. *The Scientific Proceedings of the Royal Dublin Society, Series A*, **6**: 197-206.

MacLeod, A., Wratten, S.D. & Harwood, R.W.J. 1994. The efficiency of a new lightweight suction sampler for sampling aphids and their predators in arable land. *Annals of Applied Biology*, **124**: 11-17.

Magurran, A.E. 1988. *Ecological diversity and its Measurements.* Princeton University Press: Princeton.

Mallow, D., Snider, R.J. & Robertson, L.S. 1985. Effects of different management practices on Collembola and Acarina in corn production systems: II. The effects of molboard plowing and atrazine. *Pedobiologia,* **28**: 115-131.

Marshall, S.A., Anderson, R.S., Roughley, R.E., Behan-Pelletier, V. & Danks, H.V. 1994. Terrestrial arthropod biodiversity: planning a study and recommended sampling techniques. Supplement to the *Bulletin of the Entomological Society of Canada*, **26**.

Marshall, V.G. 1972. Comparison of two methods of estimating efficiency of funnel extractors for soil microarthropods. *Soil Biology and Biochemistry*, **4**: 417-426.

Marshall, V.G. 1974. Seasonal and vertical distribution of soil fauna in a thinned and urea-fertilised Douglas-fir forest. *Canadian Journal of Soil Science*, **54**: 491-500.

Marshall, V.G. 1977. *Effects of Manures and Fertilizers on Soil Fauna: A Review.* Commonwealth Agricultural Bureaux: Slough.

May, R.M. 1975. Patterns of species abundance and diversity. In *Ecology and Evolution of Communities* (eds. Cody, M.L. & Diamond, J.M.) pp. 81-120. Harvard University Press: Cambridge.

McCracken, D.I. & Foster, G.N. 1993. Surface-active invertebrate communities and the availability of potential food for the Chough, *Pyrrhocorax pyrrhocorax* L., on pastures on north-west Islay. *Pedobiologia,* **37**: 141-158.

McGrath, S.P. 1987. Long-term studies of metal tranfers following application of sewage sludge. In *Pollutant Transport and Fate in Ecosystems* (eds. Coughtrey, P.J., Martin, M.H. & Unsworth, M.H.) pp. 301-317. Blackwell Scientific Publications; Oxford.

Mebes, K.H. & Filser, J. 1997. A method for estimating the significance of surface dispersal for population fluctuations of Collembola in arable land. *Pedobiologia,* **41**: 115-122.

Mills, J.T. & Sinha, R.N. 1971. Interactions between a springtails, *Hypogastrura tullbergi*, and soil-borne fungi. *Journal of Economic Entomology*, **64**: 398-401.

Milne, S. 1962. Phenology of a natural population of soil Collembola. *Pedobiologia*, **2**: 41-52.

Mola, L., Sabatini, M.A., Fratello, B. & Bertolani, R. 1987. Effects of atrazine on two species of Collembola (Onychiuridae) in laboratory tests. *Pedobiologia*, **30**: 145-149.

Moreby, S.J. Aebischer, N.J., Southway, S.E. & Sotherton, N.W. 1994. A comparison of the flora and arthropod fauna of organically and conventionally grown winter wheat in southern England. *Annals of Applied Biology*, **125**: 13-27.

Muskett, C.J. & Jones, M.P. 1980. The dispersal of lead, cadmium and nickel from motor vehicles and effects on roadside invertebrate macrofauna. *Environmental Pollution (series A)*, **23**: 231-242.

Niijima, K. 1975. Seasonal changes in collembolan populations in a warm temperate forest of Japan: II. Population dynamics of the dominant species. *Pedobiologia*, **15**: 40-52.

O'Connor, F.B. 1957. An ecological study of the Enchytraeid worm population of a coniferous forest soil. *Oikos*, **8**: 162-199.

Odum, E.P. 1969. The strategy of ecosystem development. *Science*, **164**: 262-270.

Odum, E.P. 1985. Trends expected in stressed ecosystems. *Bioscience*, **35**: 419-422.

Petruzzelli, G., Guidi, G. & Lubrano, L. 1978. Cadmium occurrence in soil organic matter and its availability to wheat seedlings. *Water, Air and Soil Pollution*, **9**: 263-269.

Petruzzelli, G., Ottaviani, M., Lubrano, L., & Veschetti, E. 1994. Characterization of heavy metal mobile species in sewage sludge for agricultural utilization. *Agrochimica*, **38**: 277-284.

Pielou, E.C. 1975. *Ecological Diversity*. Wiley: New York.

Pielou, E.C. 1977. *Mathematical Ecology*. John Wiley & Sons: New York.

Pimentel, D. & Warneke, A. 1989. Ecological effects of manure, sewage sludge and other organic wastes on arthropod populations. *Agricultural Zoology Reviews*, **3**: 1-30.

Pokarzhevskii, A.D. & Straalen, N.M. Van. 1996. A multi-element view on heavy metal biomagnification. *Applied Soil Ecology*, **3**: 95-98.

Posthuma, L. & Straalen, N.M. Van. 1993. Heavy-metal adaptation in terrestrial invertebrates: a review of occurrence, genetics, physiology and ecological consequences. *Comparative Biochemistry and Physiology*, **106**: 11-38.

Posthuma, L., Verweij, R.A., Widianarko, B. & Zonneveld, C. 1993a. Life-history patterns in metal-adapted Collembola. *Oikos*, **67**: 235-249.

Purvis, G. & Curry, J.P. 1978. The effects of grazing and silage production systems on the epigeal fauna of grassland. *The Scientific Proceedings of the Royal Dublin Society, Series A*, **6**: 317-325.

Rabitsch, W.B. 1995. Metal accumulation in arthropods near a lead/zinc smelter in Arnoldstein, Austria. I. *Environmental Pollution*, **90**: 221-237.

Reddy, P.S. & Bhagyalakshmi, A. 1994. Changes in oxidative metabolism in selected tissue of the crab (*Scylla serrata*) in response to cadmium toxicity. *Ecotoxicology and Environmental Safety*, **29**: 255-264.

Richards, K.S., & Ireland, M.P. 1978. Glycogen-lead relationship in the earthworm *Dendrobaena rubida* from a heavy metal site. *Histochemsitry*, **56**: 55-64.

Rund, T. 1995. Heavy metals - finding the status quo. *Arable farming*, **9**: 30-34.

Rühling, A. & Tyler, G. 1973. Heavy metal pollution and decomposition of spruce needle litter. *Oikos*, **24**: 402-416.

Ruppel, H. 1953. Physiologische untersuchungen über die bedeutung der ventraltubus und die atmung der Collembolen. *Zoologische Jahrbuecher Abteilung fuer Allgemeine Zoologie und Physiologie der Tier*, **64**: 430-469.

SAC. 1997. Use of sewage sludge on agricultural land. *SAC Technical Note* T450.

Schaffer, W.M. 1974. Optimal reproductive effort in fluctuating environments. *American Naturalist*, **108**: 783-790.

Seastedt, T.R. 1984. The role of microarthropods in decomposition and mineralization processes. *Annual Review of Entomology*, **29**: 25-46.

Sheals, J.G. 1957. The Collembola and Acarina of uncultivated soil. *Journal of Animal Ecology*, **26**: 125-134.

Siepel, H. 1990. Decomposition of leaves of *Avenella flexuosa* and microarthropod succession in grazed and ungrazed grasslands. I. Succession of microarthropods. *Pedobiologia*, **34**: 19-30.

Siepel, H. 1994. Heavy-metal pollution changes the composition of soil micro-arthropod life-history tactics and feeding guilds. *Pedobiologia*, **34**: 19-30.

Siepel, H. 1995. Applications of microarthropod life-history tactics in nature management and ecotoxicology. *Biology and Fertility of Soils*, **19**: 75-83.

Siepel, H. & Maaskamp, F. 1994 Mites of different feeding guilds affect decomposition of organic matter. *Soil Biology and Biochemistry*, **26**: 1389-1394.

Siepel, H. & Bund, C. Van de. 1988. The influence of management practices on the microarthropod community of grassland. *Pedobiologia*, **31**: 339-354.

Siepel, H. & Ruiter-Dijkman, E.M. de. 1993. Feeding guilds of oribatid mites based on their carbohydrase activities. *Soil Biology and Biochemistry*, **25**: 1491-1497.

Siepel, H., Meijer, J., Mabelis, A.A.. & Boer, M.H. Den. 1989. A tool to assess the influence of management practices on grassland surface macrofaunas. *Journal of Applied Entomology*, **108**: 271-290.

Sievers, H & Ulber, B. 1990. The effects of organic manure applications on Collembola and other small arthropods as seedling pests in sugar beet. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, **97**: 588-599.

Simpson, K. 1986. *Fertilisers and manures*. Longman Group Ltd: New York.

Smith, S.R. 1996. *Agricultural Recycling of Sewage Sludge and the Environment*. CAB International: Oxon.

Sokal, R.R. & Rohlf, F.J. 1995. Biometry: *The Principles and Practice of Statistics in Biological Research 3rd Edition*. W.H. Freeman and Company: New York.

Sørensen, F.F., Weeks, J.M. & Baatrup, E. 1997. Altered locomotory behaviour in woodlice (*Oniscus asellus* (L.)) collected at a polluted site. *Environmental Toxicology and Chemistry*, **16**: 685-690.

Sotherton, N.W., Moreby, S.J. & Langley, M.G. 1987. The effects of the foliar fungicide pyrazophos on beneficia arthropods in barley fields. *Annals of Applied Biology*, **111**: 75-87.

Southwood, T.R.E. 1971. *Ecological Methods: With Particular Reference to the Study of Insect Populations*. Chapman & Hall: London.

Stach, J. 1954. The apterygoten fauna of Poland in relation to the world fauna of this group of insects: Family Onychiuridae. *Polska Akademia Nauk Instytut Zoologiczny, Krakow*, **1951**: 1-97.

Stach, J. 1956. The apterygoten fauna of Poland in relation to the world fauna of this group of insects: Family Sminthuridae. *Polska Akademia Nauk Instytut Zoologiczny, Krakow*, **1956**: 1-287.

Straalen, N.M. Van 1985. Size-specific mortality patterns in two species of forest floor Collembola. *Oecologia*, **67**: 220-223.

Straalen, N.M. Van 1989. Production and biomass turnover in two populations of forest floor Collembola. *Netherlands Journal of Zoology*, **39**: 156-168.

Straalen, N.M. Van 1993. Soil and sediment quality criteria derived from invertebrate toxicity data. In *Ecotoxicology of Metals in Invertebrates* (eds. Dallinger, R. & Rainbow, P.S.) pp. 427-441. Lewis: Chelsea.

Straalen, N.M. Van, & Wensem, J., Van. 1986. Heavy metal content of forest litter arthropods as related to body-size and trophic level. *Environmental Pollution (Series A)*, **42**: 209-221.

Straalen, N.M. Van, Burghouts, T.B.A., Doornhof, M.J., Groot, G.M., Janssen, M.P.M., Joose, E.N.G., Meerendonk, J.H. Van, Theeuwen, J.P.J.J., Verhoef, H.A., & Zoomer, H.R. 1987. Efficiency of lead and cadmium excretion in populations of *Orchesella cincta* (Collembola) from various contaminated forest soils. *Journal of Applied Ecology*, **24**: 953-968.

Strojan, C.L. 1978a. The impact of zinc smelter emissions on forest litter arthropods. *Oikos*, **31** : 41-46.

Strojan, C.L. 1978b. Forest leaf litter decomposition in the vicinity of a zinc smelter. *Oecologia*, **32**: 203-212.

Subagja, J. & Snider, R.J. 1981. The side effects of the herbicides atrazine and paraquat upon *Folsomia candida* and *Tullbergia granulata* (Insecta, Collembola). *Pedobiologia*, **22**: 141-152.

Suffling, R. & Smith, D.W. 1974. Litter decomposition studies using mesh bags: spillage inaccuracies and the effects of repeated artificial drying. *Canadian Journal of Botany*, **52**: 2157-2163.

Sunderland, K.D. 1975. The diet of some predatory arthropods in cereal crops. *Journal of Applied Ecology*, **12**: 507-515.

Taylor, L.R. 1978. Bates, Williams, Hutchinson, - a variety of diversities. In *Diversity of Insect Faunas - 9th Symposium of the Royal Entomological Society* (eds. Mound, L.A. & Warloff, N.) pp. 1-18. Blackwell: Oxford.

Tranvik, L. & Eijsackers, H. 1989. On the advantage of *Folsomia fimetarioides* over *Isotomiella minor* (Collembola) in a metal polluted soil. *Oecologia*, **80**: 195-200.

Tranvik, L., Bengtsson, G. & Rundgren, S. 1993. Relative abundance and resistance traits of two Collembola species under metal stress. *Journal of Applied Ecology*, **30**: 43-52.

Tullgren, A. 1918. Ein sehr einfacher Ausleseapparat für terricole Tierfaunen. *Zeitschrifts fuer Angewandte Entomologie*, **4**: 149-150.

Tyler, G., Bengtsson, G., Folkeson, L., Gunnarsson, T., Rundgren, S., Rühling, A. & Söderström, B. 1984. *Metallförorening I Skogsmark-Biologiska Effekter.* Lund, Statens Naturvardsverk PM 1910.

Volz, M.G. & Heichel, G.H. 1979. Nitrogen transformations and microbial population dynamics in soil amended with fermentation residue. *Journal of Environmental Quality*, **8**: 434-439.

Wallwork, J.A. 1970. *Ecology of Soil Animals.* McGraw-Hill; London.

Walsingham, J.M. 1978. Effect of sheep grazing on the invertebrate population of agricultural grassland. *The Scientific Proceedings of the Royal Dublin Society, Series A*, **6**: 297-304.

Wiles, J.A. & Frampton, G.K. 1996. A field bioassay approach to assess the toxicity of insecticide residues on soil to Collembola. *Pesticide Science*, **47**: 273-285.

Winklehner, R., Winkler, H. & Kampichler, C. 1997. Estimating local species richness of epigeic Collembola in temperate dry grassland. *Pedobiologia*, **41**: 154-158.

Winter, J.P., Voroney, R.P. & Ainsworth, D.A. 1990. Soil microarthropods in long-term no-tillage and conventional tillage corn production. *Canadian Journal of Soil Science*, **70**: 641-653.

With, N.D.de. & Joose, E.N.G. 1971. The ecological effects of moulting in Collembola. *Revue Écologie et de Biologie du Sol*, **8**: 111-117

Witkamp, M. & Olson, J.S. 1963. Breakdown of confined and nonconfined oak litter. *Oikos*, **14**: 139-147.

Wright, W.R. 1978. Laboratory and field mineralization of nitrogen from fermentation residues. *Journal of Environmental Quality*, 7: 343-346.

Zar, J.H. 1984. *Biostatistical Analysis 2^{nd} Edition.* Prentice-Hall Inc.: New Jersey.

APPENDIX I. Equations for Diversity Indices

a) $\mathbf{N_2}$ - reciprocal of Simpson's index (Hill 1973)

$$N_2 = \left(\sum_i p^2_i\right)^{-1} \qquad (i = 1,2,3,\ldots,S),\ 0 \leq N_2 \leq S$$

Where S = Number of species and p_i = Percentage of the i-th species

b) α - Fisher's log series index

$$\alpha = \frac{[N(1-x)]}{x}$$

Where N = Sum of Collembola and x is estimated from the iterative solution of

$$\frac{S}{N} = [(1-x)/x][-\ln(1-x)]$$

c) d - Berger-Parker's dominance index

$$\mathbf{d} = \frac{N_{max}}{N} \quad 0 \leq \mathbf{d} \leq 1$$

APPENDIX II. Sludge analysis from the Auchincruive contaminated sewage sludge experiment (1994)

Table II.1 Content of plant macro-nutrients, and dry matter content of the sludges 1994

Sewage works	Treatment process	Dry matter (%)	Organic C (%)	N (%ds)	NH4-N (%ds)	P (%ds)	K $mgkg^{-1}$
Banbury	Mesophilic anaerobic digestion (belt pressed)	17.9	36.7	5.20	0.39	1.79	2793
Carterton	Primary settlement (belt pressed)	25.2	47.3	4.76	1.19	1.87	1190
Coleshill	Mesophilic anaerobic digestion (belt pressed)	30.3	32.6	3.63	0.26	2.71	2970
Selkirk	Primary settlement (lagooned)	17.2	41.5	4.59	0.93	1.10	3488
Perry Oaks	Mesophilic anaerobic digestion (belt pressed)	49.9	18.1	1.86	0.02	2.42	4208

Table II.1 This table shows the mean dry matter content and the mean levels of plant macro-nutrients for the 4 sludges in 1994. The abbreviation ds stands for dry solids.

Table II.2 Content of metals in sludges 1994

Sewage works	Zn $mgkg^{-1}$	Cu $mgkg^{-1}$	Cd $mgkg^{-1}$	Ni $mgkg^{-1}$	Pb $mgkg^{-1}$	Cr $mgkg^{-1}$	Al $mgkg^{-1}$	Fe $mgkg^{-1}$	Hg $mgkg^{-1}$	S $mgkg^{-1}$	Mg $mgkg^{-1}$
Banbury	849	693	5.98	41.3	196	69.8	17,877	469	7.71	13,408	1,117
Carterton	532	456	3.77	17.1	55	23.8	14,286	4,000	4.21	8,095	397
Coleshill	5,238	1,376	13.83	584.2	1,000	1,184.8	38,284	22,667	5.13	12,442	990
Selkirk	529	4,331	0.58	918.6	750	348.8	10,465	11,378	1.17	6,570	2,326
Perry Oaks	1,238	671	45.89	224.4	549	633.3	21,443	18,667	6.24	9,018	1,603

Table II.2 This table shows the mean levels of plant micro-nutrients and metals for the 4 sewage sludges in 1994.

APPENDIX III. Soil analysis from the Auchincruive contaminated sewage sludge experimental site (1995)

Table III.I Soil Analysis 1995

Treatment	Block	Biomass C ($ugCg^{-1}$ ODsoil)	*Rhizobia* (10e4 g^{-1}fresh wt)	Total N (%)	Soil respiration ($mgCO_2$-C kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Cd (mg kg^{-1})	pH
Control	1	317.9	93000	0.23	0.49	79.30	22.40	<.33	6.29
Control	2	295.7	75000	0.28	0.5	87.60	22.10	<.33	5.84
Control	3	479	93000	0.29	0.48	84.90	22.00	0.33	6.18
Digested	1	335.6	21000	0.28	0.47	95.60	32.00	0.33	6.18
Digested	2	284.9	215000	0.3	0.42	88.20	29.30	0.33	5.97
Digested	3	515.4	11000	0.33	0.45	89.20	29.50	0.33	6.07
Undigested	1	589.5	149000	0.27	0.51	87.20	28.50	0.33	6.20
Undigested	2	619.9	23000	0.26	0.47	89.60	27.30	<.33	6.40
Undigested	3	640.3	38000	0.32	0.49	86.90	25.50	0.33	6.15

APPENDIX IV. Species list for litterbags 1995 (Auchincruive contaminated sewage sludge experiment)

Anurida pygmaea (Börner)
Ceratophysella denticulata (Bagnall)
Ceratophysella succinea Gisin
Friesea claviseta Axelson
Folsomia candida (Willem)
Folsomia fimetaria (Linné)
Folsomia fimetarioides (Axelson)
Isotomodes productus (Axelson)
Isotoma anglicana Lubbock
Isotomurus palustris (Müller)
Isotoma tigrina (Nicolet)
Isotoma notabilis Schäffer
Isotoma viridis Bourlet
Heteromurus nitidis (Templeton)
Lepidocyrtus cyaneus Tullberg
Lepidocyrtus lignorum (Fabricicus)
Pseudosinella decipiens Denis
Sminthurinus aureus (Lubbock)
Sminthurinus elegans (Fitch)
Sminthurides pumilis (Krausbauer)
Mesaphorura hylophila (Rusek)
Mesaphorura macrochaeta (Rusek)
Stenaphorura denisi (Bagnall)
Onychiurus circulans Gisin
Protaphorura cancellatus Gisin
Neelus minimus (Willem)

APPENDIX V. Diversity Values for Litterbags (Contaminated Sewage Sludge Experiment Auchincruive: 1995)

Table V.1 Comparing diversity across plots and sampling dates (litterbags 1995)

Diversity Index	Date	CB1	CB2	CB3	DB1	DB2	DB3	UB1	UB2	UB3	ZnB1	ZnB2	ZnB3	CuB1	CuB2	CuB3
S	14/2/95	6	3	5	5	7	6	3	4	9	6	3	4	4	7	8
(no. of	25/4/95	9	5	10	5	7	9	4	6	10	6	7	6	3	9	8
species)	3/7/95	8	8	11	9	11	11	9	10	14	7	9	8	6	9	10
	22/7/95	11	10	10	10	12	11	9	12	12	8	8	10	12	12	12
N_2	14/2/95	2.57	1.24	1.01	2.00	1.04	1.41	1.01	1.13	2.99	1.23	1.06	1.58	3.56	1.28	1.66
(Hill's)	25/4/95	3.82	2.55	1.23	1.56	1.27	1.17	1.03	2.26	4.70	1.24	1.19	1.48	1.37	1.18	1.22
	3/7/95	3.32	4.59	5.98	2.94	4.41	4.04	2.59	5.99	4.56	4.01	5.08	3.99	3.35	3.71	2.15
	22/7/95	3.58	3.38	2.18	2.24	2.46	2.44	1.52	2.03	3.75	2.06	1.49	2.17	5.24	2.09	1.91
α	14/2/95	1.51	1.00	0.68	0.95	1.04	0.97	0.44	0.64	2.43	1.33	0.49	0.68	3.18	1.50	1.77
(Fisher's)	25/4/95	2.67	1.69	2.25	1.14	1.46	1.83	0.58	1.41	3.00	1.35	1.34	1.19	1.22	1.68	1.90
	3/7/95	1.75	6.97	4.58	2.39	3.47	3.26	2.43	2.82	3.03	2.16	3.92	2.41	2.30	3.24	2.61
	22/7/95	2.37	1.93	1.88	1.99	2.48	1.97	1.42	2.20	2.04	1.51	1.51	1.79	3.05	2.29	2.17
d	14/2/95	0.58	0.90	0.99	0.66	0.98	0.83	0.99	0.94	0.48	0.90	0.97	0.78	0.38	0.88	0.77
(Berger-	25/4/95	0.44	0.58	0.90	0.79	0.89	0.92	0.98	0.63	0.39	0.90	0.92	0.82	0.85	0.92	0.91
Parker)	3/7/95	0.46	0.40	0.33	0.55	0.41	0.45	0.58	0.24	0.35	0.32	0.34	0.44	0.48	0.45	0.67
	22/7/95	0.36	0.48	0.66	0.64	0.61	0.61	0.81	0.69	0.42	0.67	0.82	0.66	0.28	0.68	0.70

Table V.1 This table shows the values for four measures of diversity for the 15 plots (five treatments and three replicas) and for the four sampling dates. The following abbreviations are used: C = control, D = digested sludge, U = undigested sludge, Zn = zinc-rich sludge, Cu = copper-rich sludge and B = block.

APPENDIX VI. Block Analysis from the Auchincruive Contaminated Sewage Sludge Experiment (1994)

Table VI.1 Block analysis prior to sludge application 1994

Block	pH	Conductivity (uS)	CEC (meq kg^{-1})	Organic C (%)	N (g kg^{-1})	NO3-N (ppm)	Total N (%)	P (mg kg^{-1})	K (mg kg^{-1})	Mn (mg kg^{-1})	Mg (mg kg^{-1})	%Sand	%Silt	%Clay
1	6.2	1800	127.8	2.42	2.2	7	0.21	37	44	0.12	288	52.9	28.8	18.3
2	5.9	1500	129.4	2.52	2	<1	0.21	24	48	0.12	248	50.9	29.2	19.9
3	6	1700	140.2	2.74	2.1	<1	0.24	22	44	0.13	256	48.3	29.8	21.9

Table VI.2 Block analysis prior to sludge application 1994 (continued)

Block	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Cd (mg kg^{-1})	Ni (mg kg^{-1})	Pb (mg kg^{-1})	Cr (mg kg^{-1})	Hg (mg kg^{-1})	Biomass C (ugC g^{-1}ODsoil)	*Rhizobia* No.	Soil respiration (mgCO_2-C kg^{-1})
1	3.38	80.55	22.50	0.33	30.0	43.5	47.3	0.08	399.40	65330.4	0.50
2	3.55	81.83	21.95	0.33	30.5	45.1	46.0	0.05	462.91	124730.4	0.47
3	3.68	84.95	23.23	0.33	28.0	46.9	44.6	0.07	424.30	106087.0	0.46

APPENDIX VII. Soil Analysis from the Auchincruive Contaminated Sewage Sludge Experiment (1994)

Table VII.1 Analysis of plots prior to sludge application

Treatment	Block	Biomass C ($ugC\ g^{-1}$ ODsoil)	Rhizobia No.	Soil respiration ($mgCO_2$-C kg^{-1})
Control	1	431.66	93,000	0.49
Control	2	425.11	75,000	0.50
Control	3	475.36	93,000	0.48
Digested	1	435.18	21,000	0.47
Digested	2	462.40	215,000	0.42
Digested	3	430.67	11,000	0.45
Undigested	1	466.13	149,000	0.51
Undigested	2	459.89	23,000	0.47
Undigested	3	432.78	38,000	0.49
Zinc	1	357.82	93,000	0.47
Zinc	2	513.92	4,400	0.45
Zinc	3	384.86	43000	0.53
Copper	1	375.08	23,000	0.48
Copper	2	517.40	20,000	0.46
Copper	3	375.27	75,000	0.42
Cadmium	1	404.78	75,000	0.50
Cadmium	2	449.31	23,000	0.50
Cadmium	3	438.47	5,600	0.46

APPENDIX VIII. Grass Yield & Grass Height from the Auchincruive Contaminated Sewage Sludge Experiment

Table VIII.1 Grass yields for 1995 and 1996

Treatment	Block	1995 Cut 1 (fresh wt. kg)	1995 Cut 2 (fresh wt. kg)	1996 Cut 1 (fresh wt. kg)
Control	1	6.9	21.0	16.1
Control	2	5.5	17.0	13.0
Control	3	3.4	20.6	14.0
Digested	1	17.3	20.1	40.8
Digested	2	12.5	21.6	32.5
Digested	3	13.5	22.2	31.8
Undigested	1	16.7	22.2	35.1
Undigested	2	15.0	17.8	34.4
Undigested	3	9.1	24.3	42.8
Zinc	1	21.0	19.1	33.6
Zinc	2	15.5	28.4	30.1
Zinc	3	12.0	22.6	28.6
Copper	1	17.2	15.9	29.2
Copper	2	18.4	20.2	38.4
Copper	3	12.8	27.4	37.0
Cd (very low)	1	22.3	35.7	32.4
Cd (very low)	2	20.0	24.8	36.0
Cd (very low)	3	20.0	28.8	33.9
Cd (medium)	1	21.5	39.4	35.6
Cd (medium)	2	16.7	32.7	39.1
Cd (medium)	3	17.4	36.5	34.2
Cd (high)	1	17.7	32.8	38.5
Cd (high)	2	11.9	31.1	37.0
Cd (high)	3	12.1	29.0	34.3
Cd (very high)	1	10.5	22.3	28.2
Cd (very high)	2	9.2	21.0	27.3
Cd (very high)	3	11.2	27.3	30.5

Table VIII.2 Grass Heights (in mm) 1996

Treatment	Block	30 April	31 May	22 June	4 August
Control	1	133.2	116.6	173.6	90.6
Control	2	121.0	105.8	153.0	84.6
Control	3	135.8	104.1	173.5	99.0
Digested	1	307.7	106.0	442.6	82.5
Digested	2	309.1	110.1	404.2	62.8
Digested	3	327.3	87.5	441.8	77.6
Zinc	1	298.3	100.5	443.7	3.3
Zinc	2	299.0	104.8	405.9	81.4
Zinc	3	324.2	98.7	460.9	76.6
Cadmium	1	271.5	87.3	405.0	78.0
Cadmium	2	277.3	104.3	384.3	97.6
Cadmium	3	264.0	84.1	385.4	79.3

APPENDIX IX. Codes for DCA species ordination

Anur pygm	*Anurida pygmaea*
Cera dent	*Ceratophysella denticulata*
Cera succ	*Ceratophysella succinea*
Frie clav	*Friesea claviseta*
Fols cand	*Folsomia candida*
Fols fime	*Folsomia fimetaria*
Fols firo	*Folsomia fimetarioides*
Isot prod	*Isotomodes productus*
Isot juve	*Isotomurus/Isotoma juvenile*
Isot angl	*Isotoma anglicana*
Isot macu	*Isotomurus maculatus*
Isot palu	*Isotomurus palustris*
Isot tigr	*Isotoma tigrina*
Isot nota	*Isotoma notabilis*
Isot viri	*Isotoma viridis*
Ento niva	*Entomobrya nivalis*
Hete niti	*Heteromurus nitidis*
Lepi cyan	*Lepidocyrtus cyaneus*
Lepi lign	*Lepidocyrtus lignorum*
Pseu deci	*Pseudosinella decipiens*
Smin aure	*Sminthurinus aureus*
Smin eleg	*Sminthurinus elegans*
Smin malm	*Sminthurides malmgreni*
Smin pumi	*Sminthurides pumilis*
Smin viri	*Sminthurus viridis*
Tomo long	*Tomocerus longicornis*
Tomo mino	*Tomocerus minor*
Mesa hylo	*Mesaphorura hylophila*
Mesa macr	*Mesaphorura macrochaeta*
Sten deni	*Stenaphorura denisi*
Onyc circ	*Onychiurus circulans*
Prot canc	*Protaphorura cancellata*
Neel mini	*Neelus minimus*

APPENDIX X. Sludge analysis from the Auchincruive contaminated sewage sludge experiment (1995)

Table X.1 Content of plant macro-nutrients, and dry matter content of the sludges 1995

Sewage works	Treatment process	Dry matter (%)	Organic C (%)	N (%ds)	NH4-N (%ds)	P (%ds)	K $mgkg^{-1}$
Banbury	Mesophilic anaerobic digestion (belt pressed)	24.8	33.4	5.22	0.14	2.34	2,800
Carterton	Primary settlement (belt pressed)	81.9	37.0	4.00	0.34	1.92	2,000
Coleshill	Mesophilic anaerobic digestion (belt pressed)	22.5	32.4	4.19	0.60	3.39	3,500
Selkirk	Primary settlement (lagooned)	24.5	32.0	3.76	0.64	0.77	5,000
Perry Oaks	Mesophilic anaerobic digestion (belt pressed)	76.5	11.6	1.25	0.00	1.59	4,500

Table X.1 This table shows the mean dry matter content and the mean levels of plant macro-nutrients for the 4 sludges in 1995. The abbreviation ds stands for dry solids.

Table X.2 Content of metals in sludges 1995

Sewage works	Zn $mgkg^{-1}$	Cu $mgkg^{-1}$	Cd $mgkg^{-1}$	Ni $mgkg^{-1}$	Pb $mgkg^{-1}$	Cr $mgkg^{-1}$	Al $mgkg^{-1}$	Fe $mgkg^{-1}$	Hg $mgkg^{-1}$	S $mgkg^{-1}$	Mg $mgkg^{-1}$
Banbury	725	750	1.94	48.8	126	45.0	18,060	11,380	2.18	12,400	1,400
Carterton	584	584	1.93	32.7	75	59.6	12,990	6,870	2.97	7,840	1,000
Coleshill	8,000	1,500	20.60	675.0	1,150	1,750.0	19,580	52,750	2.62	13,700	2,500
Selkirk	700	3,550	0.88	45.0	850	550.0	13,290	14,380	2.33	6,770	2,500
Perry Oaks	1,250	563	51.90	151.0	438	650.0	20,990	23,750	2.66	6,350	1,100

Table X.2 This table shows the mean levels of plant micro-nutrients and metals for the 4 sewage sludges in 1995.

APPENDIX XI. Soil analysis from the Auchincruive contaminated sewage sludge experiment (1996)

Table XI.1 Soil analysis 1996

Treatment	Block	Biomass C ($ugC\ g^{-1}$ OD soil)	Soil respiration ($mgCO_2$-$C^{-1}kg$)	Zn ($mg^{-1}kg$)	Cu ($mg^{-1}\ kg$)	Cd ($mg^{-1}kg$)	pH	Total N (%)
Control	1	353.75	0.45	85.20	21.00	<0.33	6.20	0.2
Control	2	379.25	0.45	82.60	20.30	<0.33	5.74	0.2
Control	3	331.17	0.44	88.60	20.00	0.33	6.13	0.21
Digested	1	393.09	0.63	97.90	31.60	0.33	6.07	0.24
Digested	2	395.93	0.6	89.20	30.60	0.33	6.00	0.21
Digested	3	418.62	0.61	97.20	32.00	0.33	5.85	0.28
Undigested	1	493.93	0.64	164.00	41.30	0.33	6.10	0.26
Undigested	2	487.61	0.71	95.2	31.6	0.33	6.31	0.23
Undigested	3	488.26	0.56	95.20	29.60	0.33	6.10	0.26
Cadmium	1	399.52	0.55	122.00	42.30	1.33	6.61	0.24
Cadmium	2	506.76	0.71	136.00	45.30	2.00	6.36	0.24
Cadmium	3	434.31	0.53	139.00	46.60	1.67	6.3	0.28

APPENDIX XII. Species list for suction samples taken at the Auchincruive contaminated sewage sludge experiment

1995

Anurida pygmaea (Börner)
Ceratophysella denticulata (Bagnall)
Folsomia candida (Willem)
Isotoma anglicana Lubbock
Isotomurus palustris (Müller)
Isotoma notabilis Schäffer
Isotoma viridis Bourlet
Heteromurus nitidis (Templeton)
Lepidocyrtus cyaneus Tullberg
Lepidocyrtus lignorum (Fabricicus)
Pseudosinella decipiens Denis
Sminthurinus aureus (Lubbock)
Sminthurinus elegans (Fitch)
Sminthurus viridis (Linné)
Tomocerus longicornis (Müller)

1996

Ceratophysella denticulata (Bagnall)
Folsomia candida (Willem)
Folsomia fimetarioides (Axelson)
Isotoma anglicana Lubbock
Isotomurus palustris (Müller)
Isotomurus maculatus Schaeffer
Isotoma notabilis Schäffer
Isotoma viridis Bourlet
Isotoma tigrina (Nicolet)
Entomobrya nivalis (Linné)
Heteromurus nitidis (Templeton)
Lepidocyrtus cyaneus Tullberg
Lepidocyrtus lignorum (Fabricicus)
Pseudosinella decipiens Denis
Sminthurinus aureus (Lubbock)
Sminthurinus elegans (Fitch)
Sminthurides pumilis (Krausbauer)
Sminthurides malmgreni (Tullberg)
Sminthurus viridis (Linné)
Dicyrtoma saundersi (Lubbock)
Dicyrtoma ornata (Nicoleti)
Tomocerus longicornis (Müller)

APPENDIX XIII. Species list for suction samples taken at the Hartwood contaminated sewage sludge experiment (1996)

Ceratophysella denticulata (Bagnall)
Isotoma anglicana Lubbock
Isotomurus palustris (Müller)
Isotomurus maculatus Schaeffer
Isotoma notabilis Schäffer
Isotoma viridis Bourlet
Isotoma tigrina (Nicolet)
Heteromurus nitidis (Templeton)
Lepidocyrtus cyaneus Tullberg
Lepidocyrtus lignorum (Fabricicus)
Pseudosinella decipiens Denis
Sminthurinus aureus (Lubbock)
Sminthurinus elegans (Fitch)
Sminthurides pumilis (Krausbauer)
Sminthurides malmgreni (Tullberg)
Sminthurus viridis (Linné)
Tomocerus minor (Lubbock)

APPENDIX XIV. Species list for pitfall traps taken at the Auchincruive contaminated sewage sludge experiment

1995

Ceratophysella denticulata (Bagnall)
Isotoma anglicana Lubbock
Isotomurus palustris (Müller)
Isotoma tigrina (Nicolet)
Isotoma notabilis Schäffer
Isotoma viridis Bourlet
Entomobrya nivalis (Linné)
Heteromurus nitidis (Templeton)
Lepidocyrtus cyaneus Tullberg
Lepidocyrtus lignorum (Fabricicus)
Pseudosinella decipiens Denis
Sminthurinus aureus (Lubbock)
Sminthurinus elegans (Fitch)
Sminthurides pumilis (Krausbauer)
Sminthurus viridis (Linné)
Tomocerus longicornis (Müller)

1996

Anurida pygmea (Börner)
Ceratophysella denticulata (Bagnall)
Isotoma anglicana Lubbock
Isotomurus palustris (Müller)
Isotomurus maculatus Schaeffer
Isotoma notabilis Schäffer
Isotoma viridis Bourlet
Isotoma tigrina (Nicolet)
Entomobrya nivalis (Linné)
Heteromurus nitidis (Templeton)
Lepidocyrtus cyaneus Tullberg
Lepidocyrtus lignorum (Fabricicus)
Pseudosinella decipiens Denis
Sminthurinus aureus (Lubbock)
Sminthurinus elegans (Fitch)
Sminthurides pumilis (Krausbauer)
Sminthurides malmgreni (Tullberg)
Sminthurus viridis (Linné)
Tomocerus longicornis (Müller)
Tomocerus minor (Lubbock)

APPENDIX XV. Analyses of pharmaceutical wastes applied at Holms Field Auchincruive

Table XV.1 Metal content of ATS waste 1994

Metal	Concentration (mg kg^{-1})
Cadmium	< 0.01
Nickel	0.04
Chromium	0.15
Lead	0.14
Copper	0.25
Zinc	0.63

Table XV.II Metal content of SAS waste 1993

Metal	Concentration (g l^{-1})
Cadmium	0.05
Nickel	0.35
Chromium	0.80
Lead	0.05
Copper	0.06
Zinc	18

APPENDIX XVI. Species present in Litterbags (1995) in ATS experiment Holms Field Auchincruive

Anurida pygmaea (Börner)
Ceratophysella denticulata (Bagnall)
Folsomia candida (Willem)
Folsomia quadrioculata (Tullberg)
Folsomia fimetarioides (Axelson)
Isotomodes productus (Axelson)
Isotoma anglicana Lubbock
Isotomurus palustris (Müller)
Isotoma notabilis Schäffer
Isotoma viridis Bourlet
Heteromurus nitidis (Templeton)
Lepidocyrtus cyaneus Tullberg
Lepidocyrtus lignorum (Fabricicus)
Pseudosinella decipiens Denis
Sminthurinus aureus (Lubbock)
Sminthurides pumilis (Krausbauer)
Mesaphorura macrochaeta (Rusek)
Stenaphorura denisi (Bagnall)
Onychiurus circulans Gisin

APPENDIX XVII. Species present in Litterbags (1995) in SAS experiment Holms Field Auchincruive

Anurida pygmaea (Börner)
Ceratophysella denticulata (Bagnall)
Friesea claviseta Axelson
Folsomia candida (Willem)
Folsomia fimetarioides (Axelson)
Isotomodes productus (Axelson)
Isotoma anglicana Lubbock
Isotomurus palustris (Müller)
Isotoma tigrina (Nicolet)
Isotoma notabilis Schäffer
Isotoma viridis Bourlet
Lepidocyrtus cyaneus Tullberg
Lepidocyrtus lignorum (Fabricicus)
Pseudosinella decipiens Denis
Mesaphorura macrochaeta (Rusek)
Onychiurus circulans Gisin