Arbejdsrapport fra Miljøstyrelsen

No. 69 1997

Ecotoxicological Assessment of Sewage Sludge in Agricultural Soil

Miljø- og Energiministeriet **Miljøstyrelsen**

Arbejdsrapport fra Miljøstyrelsen
Working Report No. 69 1997

Ecotoxicological Assessment of Sewage Sludge in Agricultural Soil

Paul Henning Krogh, Martin Holmstrup and John Jensen
National Environmental Research Institute.
Department of Terrestrial Ecology

Søren O. Petersen
Danish Institute of Agricultural Sciences.
Department of Soil Science

Ministry of Environment and Energy, Denmark
Danish Environmental Protection Agency

The Danish Environmental Protection Agency will, when opportunity offers, publish reports and contributions relating to environmental research and development projects financed via the Danish EPA.

Please note that publication does not signify that the contents of the reports necessarily reflect the views of the Danish EPA.

The reports are, however, published because the Danish EPA finds that the studies represent a valuable contribution to the debate on environmental policy in Denmark.

Contents

1 Preface

The aim of this project is to contribute to the assessment of ecological effects of sewage sludge on soil organisms and processes in the field. Processes and organisms important to the soil ecosystem have been selected to use them as indicators for possible ecological impacts of sludge. The present ecological risk assessment of sewage sludge in Denmark has not yet been validated in long term field experiments.

The report should help answering questions as concluded in former projects initiated by the Danish EPA (Krogh *et al.* 1996, Kristensen *et al.* 1996). In these reports it was recommended to do field studies to investigate the following themes:

- recovery after short term effects
- long term ecological effects
- the safety factor used for data from laboratory screening

The project was financed by the Danish Environmental Protection Agency, Office for Agriculture and Freshwater. A steering committee included the following persons:

Bettina H. Jensen, Environmental Protection Agency (Chairman)
Lone Schou, Environmental Protection Agency
Alf Aagaard, Environmental Protection Agency
Torben Madsen, Water Quality Institute
John Jensen, National Environmental Research Institute
Paul Henning Krogh, National Environmental Research Institute

Acknowledgements

We thank the following persons:
MSc student Mette Holm for co-operation in the laboratory and field studies.
Elin Jørgensen and Karen Kjær Jacobsen for rearing laboratory animals and doing the preference observations.
Zdenek Gavor is thanked for careful taxonomic investigation and enumeration of soil invertebrates.
John Geert Rytter for doing earthworm sampling and sorting.
Erik Damgård and the staff at Askov Field Research Station for establishment of the field experiments.
Charlotte Bech and Lene Birksø Bødskov for doing the DTP of the report. Kathe Møgelvang and Juana Jacobsen for drawing figures.
Søren Erik Larsen, UNI-C Århus for statistical advice.

2 Dansk resumé

Selv om akutte effekter af slam og kemiske stoffer i slam er observeret i laboratorietests, peger denne undersøgelse på, at sandsynligheden for negative effekter på jordens frugtbarhed som følge af normal anvendelse af spildevandsslam på kort sigt er meget lille i forhold til de ændringer, der opstår ved normal landbrugspraksis, f.eks. som følge af jordbearbejdning, afgrødeskift, pesticidanvendelse m.m. Tilførslen af slam vil uden tvivl ændre sammensætningen af organismer i agroøkosystemet. I de fleste tilfælde med en generel øget aktivitet til følge. Det er ikke muligt med denne undersøgelse at konkludere med sikkerhed, hvorvidt gentagne slambehandlinger på lang sigt kan forårsage uønskede effekter.

Gennem de sidste par årtier er der opstået en voksende bekymring for anvendelse af spildevandsslam og andre affaldsprodukter som gødning på landbrugsarealer. Som et resultat af en løbende debat på området har Miljøstyrelsen i 1995 og 1996 igangsat en række undersøgelser for at belyse problemstillinger ved anvendelsen af slam som gødning i jordbrug. Disse inkluderer udvikling og forbedring af analysemetoder, undersøgelser af stoffernes skæbne i slambehandlingen, udvikling af biologiske screeningstest samt undersøgelser vedrørende planteoptag og eksponering af mennesker via afgrøder. Som en del af dette program har Afdelingen for Terrestrisk Økologi, ved Danmarks Miljøundersøgelser, igangsat langtidsforsøg, hvor økologiske effekter af spildevandsslam undersøges i felten. Nærværende rapport er en fortsættelse af undersøgelser igangsat i 1995 og afrapporteret i 1996 (Krogh *et al.* 1996) og dækker effekter observeret i felten i op til 1 år efter slamudbringning. Undersøgelserne er planlagt at skulle fortsætte endnu i tre år.

Feltstudier

Feltstudier til økologisk effektvurdering af spildevandsslam blev igangsat i oktober 1995 på marker med vinterhvede og i april 1996 på marker med byg og kløvergræsudlæg. Begge forsøgstyper blev udført på to lokaliteter med hhv. sandblandet lerjord (JB5) og sandjord (JB1). Forsøgene blev udført som en sammenligning af effekter af henholdsvis spildevandsslam og kvægmøg. Jordprøver blev indsamlet fra 0-5 cm's dybde i alle tilfælde og i 10-20 cm dybde på udvalgte forsøgsfelter. Slam og kvægmøg blev udbragt i mængderne 3.5, 7 og 21 tons tørstof pr. ha., mens kontrolmarker ikke modtog nogen form for gødning. Vinterhveden fik slam fra Ringkøbing og byggen fik slam fra Herning. Begge slamtyper repræsenterer ikke en "worst case" situation, men må regnes for at være blandt de mere forurenede slamtyper i Danmark (Kristensen *et al.* 1996).

Undersøgelsen omfatter målinger af den mikrobielle aktivitet i jorden og en registrering af ændringerne i sammensætningen af udvalgte grupper af jordbundsdyr. Den mikrobielle aktivitet blev målt ved at bestemme den potentielle ammonium-oxidation i jorden. Nitrificerende bakterier er en af to fysiologiske ens grupper, der er ansvarlig for ammonium- og nitritoxidationen. Da denne måleparameter således dækker en relativt snæver gruppe af bakterier, er metoden mere følsom end f.eks. CO_2 produktionen. Begge gødningsformer viste i alle tilfælde enten ingen eller en stimulerende virkninger på ammonium-oxidationen, men aktiviteten var signifikant større i den horisont, hvor slammet var nedbragt ved pløjningen (10-20 cm), sammenlignet med det øverste jordlag (0-10 cm).

I både vinterhvedemarken og bygmarken var antallet af mikroleddyr øget signifikant henholdsvis et halvt år og ét år efter slamudbringningen. Kvægmøg havde tilsyneladende en større stimulerende virkning end slam. Regnormenes antal og biomasse var forøget ved den laveste slamdosis, hvorimod der, sammenlignet med kontrollen, ingen effekter var at se ved de to højeste doser.

Omend der også blev observeret en øget aktivitet i den øverste del af jorden (0-5 cm), var den relative stimulering af såvel den mikrobielle aktivitet som af en række mikroleddyr, f. eks. springhalen *Folsomia fimetaria*, markant større i 10-20 cm's dybde. I denne dybde var slamklumper endnu synlige et halvt år efter nedpløjningen i bygmarken.

Laboratorietests

I laboratorietests blev effekten af slam fra Herning samt af stofferne LAS og nonylphenol (NP) undersøgt både ved eksponering for stofferne direkte opblandet i jord og ved eksponering for stofferne efter de var opblandet i en svagt kontamineret slamtype fra Skævinge på Sjælland. Springhaler var mindre påvirket af både LAS og NP, når de blev eksponeret for stofferne efter at disse var tilsat slam og opblandet i jorden, end hvis stofferne blev blandet direkte i jorden. Den mængde af de rene stoffer, som gav en 50%'s nedgang i reproduktionen (EC_{50}), gav således kun en 10%'s nedgang i reproduktionen, når de var opblandet i slam ("spiket" i slam). Forskellen skyldes mest sandsynligt en reduceret tilgængelighed af kemikalierne, efter at de blev opblandet i slammet. De effekter, som blev observeret i laboratoriet, starter i alle tilfælde ved et niveau, der er mindst 25-50 gange over de estimerede koncentrationer i jorden, som føl-ge af den mest intensive slamdosering i feltforsøgene, dvs. for LAS cirka 7.5 mg kg^{-1} og for NP cirka 1.0 mg kg^{-1}, begge tørvægt. Den anvendte maksimaldosis i feltforsøgene vil igen som et minimum betyde henholds-vis 2 og 10 gange højere jordkoncentrationer af LAS og NP i forhold til en normal slamudbringning reguleret af slambekendtgørelsen. Ifølge den danske slambekendtgørelse vil den maksimale tilførsel af LAS og NP i år 2000 føre til beregnede jordkoncentrationer, som er henholdsvis ca. 5 og100 gange lavere end de højeste koncentrationer i feltforsøgene i denne rapport.

Reproduktionen og den juvenile vækst af regnorme var stimuleret ved alle koncentrationer af slam fra Herning.

Præferenceforsøg, hvor springhaler blev tilbudt kombinationer af forskellige typer af organisk materiale som fødekilde, har vist:

1. At den mindst foretrukne fødekilde var slam, specielt den mest forurenede slamtype fra Herning, og at springhalerne brugte mindre tid i end uden for fødekilden, når denne var slam i sammenligning med parallelle forsøg med bagegær.

2. Tilstedeværelsen af både slam og kvægmøg forårsagede en meget stærk hæmning af springhalers reproduktion i laboratorieforsøg. Dermod er der i disse forsøg ikke observeret nogen effekt på væksten af

springhaler ved nogen af de anvendte fødekilder. Mekanismen bag hæmningen af reproduktionen er ukendt.

Opsummerende kan det konkluderes, at selv om der i laboratoriet er observeret negative effekter af slam og visse miljøfremmede kemikalier, der forekommer i relativt store mængder i slam, starter disse effekter først ved koncentrationer, der ligger mindst 50 gange over det niveau, der må forventes i jorden efter normal slamudbringning. Feltstudier af mikroorganismer og jordlevende invertebrater har da heller ikke vist nogen former for negative effekter af slamtilførsel, selv om slamdoser 5-10 gange over det tilladelige har været anvendt. Tværtimod viser forsøgene en markant stimulering af en række organismer. Selv om tilførslen af slam således uden tvivl vil ændre sammensætningen af organismer i agroøkosystemet, tyder alt på, at disse ændringer på kort sigt vil være ubetydelige i forhold til de ændringer, der opstår ved normal landbrugspraksis, f.eks. som følge af jordbearbejdning, afgrødeskift, pesticidanvendelse m.m. Denne undersøgelse viser således, på basis af registrering af feltpopulationer af jordbundsorganismer, at sandsynligheden for negative effekter på jordens frugtbarhed som følge af normal anvendelse af spildevandsslam på kort sigt vil være meget lille. Det er ikke muligt at konkludere med sikkerhed, hvorvidt gentagne slambehandlinger på langt sigt kan forårsage uønskede effekter. En fortsættelse af nærværende undersøgelse af økologiske effekter af spildevandsslam kan være med til at kaste lys over langtidseffekterne af gentagne slambehandlinger.

Undersøgelsen problematiserer endvidere anvendelsen af frisk slam i laboratorieundersøgelser, idet denne havde effekter, som ikke kunne genfindes i felten. Det antages, at en del stoffer med giftvirkning over for jordbundsdyr forsvinder efter kort tids videreomsætning m.v. i jorden. Det er uklart i hvor høj grad, at giftighed målt i standardiserede laboratorietests (screeningstests) stammer fra de organiske miljøfremmede stoffer eller fra andre stoffer, som er naturligt forekommende i slam og kvægmøg.

3 Summary

Although acute effects of some xenobiotic chemicals present in relatively large amounts in sewage sludge have been observed in the laboratory, these occur above the concentrations likely to be found in soils treated with Danish sewage sludge. This is confirmed by field experiments, which did not show any negative effects of sewage sludge on soil micro-organisms and fauna the first year after sludge application. Application of sewage sludge will most certainly change the agro-ecosystem. In most cases with an increase in biological activity as a result. However, these changes will not be more significant than changes caused by normal agricultural practice, e.g. use of pesticides and inorganic fertilizers, ploughing etc. Although, the present investigation suggests that the risk of reducing the soil quality by using sewage sludge as fertilizer is very limited on a short term basis, no conclusions concerning long term effects of repeated sludge applications can be made.

In the last couple of decades, a considerable public concern for environmental problems associated with the use of sewage sludge as fertilizer has resulted in a series of restrictions. In 1986 the EU sludge directive was implemented, and since then several regulations of sludge have been implemented in Denmark, the latest directive being from 1996. A series of investigations has been initiated by the Danish EPA. These include development of analytical methods, fate studies of pollutants in sludge, development of biological screening tests and investigations of plant uptake of organic pollutants from soil fertilised with sewage sludge. However, the risk assessment of sewage sludge in Denmark has not yet included long term field experiments. This report presents the results from field and laboratory studies initiated in 1995 by the National Environmental Research Institute, Department of Terrestrial Ecology and the Danish Institute of Plant and Soil Science, Department of Soil Science. The aim is to investigate the long term ecological effects of sewage sludge application. The investigations are planned to continue for another two years, until 1999.

Field tests

Long term field studies were started in 1995 and 1996 to assess the effects of sewage sludge on soil fauna and microbial processes in winter-wheat and barley undersown with clover-grass. Soil samples from a sandy clay and a sandy soil were taken in both the topsoil (0-5 cm) and in a depth of 10-20 cm. Three levels of sludge and cattle manure (3.5, 7, 21 t d.wt. ha^{-1}) were compared with control fields not receiving any fertilizers. Sludge from two different waste water treatment plants was applied to each crop. Although not representing an absolute worst case situation, both sludge types were considered among the more heavily contaminated in Denmark.

Effects on the microbial community were evaluated by measurements of the potential ammonium oxidation. Nitrifying bacteria belong to one of two physiologically uniform groups of autotrophic organisms that are responsible for ammonium oxidation and nitrite oxidation. Therefore these processes are probably more sensitive to adverse environmental conditions than processes which are carried out by a greater variety of microbes. Microarthropods and earthworms were selected as representatives of the soil fauna, because both groups of organisms exist naturally in agricultural soil where they participate in soil ecosystem processes important to the sustainability of soils.

One year after sludge application in the winter wheat field and six months after in the barley fields, no negative effects of sewage sludge or cattle manure were observed. Both fertilizers showed either no effect or a stimulating effect on the ammonium oxidation potential. Cattle manure has a larger positive effect on microarthropods than sludge. For earthworms a significant stimulation in number and biomass was observed six months after application of 3.5 t sludge ha^{-1}, whereas no difference to the control was found at the doses of 7 and 21 t D.W. ha^{-1} . Stimulation of microorganisms and microarthropods by sludge and cattle manure was observed in both soil layers (0-5 and 10-20 cm). However, the stimulation was in general relatively lower in the top soil, where only few sludge lumps were observed half a year after ploughing.

Laboratory experiments

In the laboratory the effects of sewage sludge and the two chemicals linear alkylbenzene sulphonate (LAS) and nonylphenol (NP) were studied, since these compounds are found in high concentrations of most Danish sludge types. For earthworms reproduction and juvenile growth were stimulated, and cocoon hatchability was unaffected at all sludge concentrations. Experiments with Collembola showed that both chemicals were less toxic when applied to the soil via spiked sludge than when the chemicals were mixed directly into the test soil. This is most properly due to sorption of LAS and NP to sludge material leading to reduced bioavailability of the chemicals to Collembola when mixed with sludge. The effect levels observed in the laboratory (EC_{10}, EC_{50}) appeared at concentrations approximately 25-50 times higher than the estimated soil concentrations of 7.5 mg LAS kg^{-1} and 1.0 mg NP kg^{-1} in the field experiment. The levels used in the field experiments are at least 2 and 10 times higher than estimated soil concentrations after the maximum allowable load of sludge in 1997. According to the Danish sludge directive, the maximum allowable load of LAS and NP in year 2000 will lead to soil concentrations approximately 5 and 100 times lower than the estimated concentrations after the highest dose of sludge used in the field studies in this report.

Preference experiments with the Collembola *F. fimetaria* were done with different combination of food sources, including sewage sludge, yeast and cattle manure. The results showed:

1. The least preferred food source was sludge, especially the more contaminated sludge type. The Collembola spent more time outside the food source when offered sludge as compared with parallel experiments with baker's yeast.

2. A very strong inhibition of the reproduction i the laboratory whenever sludge or cattle manure were present. No reduction in growth was observed with any food source. The cause for the observed inhibition of reproduction is presently not known.

In conclusion, acute and chronic effects of LAS and NP present in high concentrations in sewage sludge have been observed in the laboratory, but these starts at concentrations at least 50 times above the concentrations likely to be found in soils treated with sewage sludge. Field experiments showed no negative effects of sewage sludge on soil microorganisms and fauna in the first year after application. On the contrary, application of sludge increased the number and activity of most soil living organisms. Although sludge may alter the structure of the soil ecosystem by favouring some species at the expense of others, these changes seem, when compared to perturbations of agricultural soils caused by for example cultivation and crop rotation, to be of minor practical or environmental concern on a short term basis. The present investigation shows that the risk of reducing the soil quality by using sewage sludge as fertilizer is very limited on a short term basis. However, it is not within the possibilities of this report to make any conclusion concerning the possibility of long term effects of repeated sludge applications. A continuation of the ongoing study of ecological effects of sludge for another two years will help to give more information of the long-term effects of continuous sewage sludge application.

Using fresh sludge in short term laboratory tests for risk assessment of sewage sludge seems problematic because it contains compounds that inhibit the reproduction. But, according to the field experiments this toxicity may only be transient. The toxicity may be caused by either the organic xenobiotic chemicals or by natural constituents occurring in sludge and cattle manure.

4 Introduction

Wastes from human activities have been used as fertilizers in Denmark since historic times, and today approximately 70% of the sewage sludge produced in Denmark is used as agricultural fertilizer. The use of sewage sludge in agriculture is very appropriate, since nutrients in this way can be recycled. However, in the last couple of decades, a considerable public concern for environmental problems first associated with heavy metals and recently with organic chemicals have resulted in a series of restrictions in the agricultural practise of sewage sludge usage.

4.1 National sludge investigations

As a result of an ongoing debate intensified in 1995-1996 concerning the risk of using sewage sludge as fertilizers on arable land, a series of investigations were initiated by the Danish EPA. These include development of analytical methods, fate of pollutants in sludge, development of biological screening tests and investigations of plant uptake of organic pollutants from soil fertilised with sewage sludge. In 1995 the National Environmental Research Institute, Department of Terrestrial Ecology and the Danish Institute of Plant and Soil Science, Department of Soil Science, initiated field and laboratory investigations of the possible ecological impact of the use of sewage sludge (Krogh *et al.* 1996). The present report is a continuation of that investigation.

4.2 Choice of indicators

Nitrification

In Krogh *et al.* (1996) a number of microbial parameters were assessed in a sandy soil after application of sludge. Based on the experience from this and other investigations, the potential ammonium oxidation was considered a suitable parameter for a study of the possible effects of sewage sludge application on microbial communities. The purpose was to evaluate if the amendments had any long-term effects on microbial activities in the soil. Nitrification is recognised as a central process in the cycling of nitrogen in soil systems (Alexander, 1977). The final product of nitrification is nitrate which may be removed from the soil system by plant uptake or microbial immobilisation, or nitrate may be lost through leaching or dissimilatory reduction (denitrification).

Nitrifying bacteria belong to one of two physiologically uniform groups of autotrophic organisms that are responsible for ammonium oxidation and nitrite oxidation. Therefore, these processes are probably more sensitive to adverse environmental conditions than processes carried out by a greater variety of microbes. The nitrifying potential is often used as an indicator of toxic effects on microorganisms of amendments, typically by measuring the potential for ammonium oxidation, the rate limiting step in nitrification (e.g. Torstensson 1993).

Soil fauna

Microarthropods and earthworms have been selected for a study of possible ecological effects after sludge application for several reasons:

- Both groups of organisms exist naturally in agricultural soil where they participate in the soil ecosystem processes important to sustainability of soils. Furthermore, standardised laboratory tests with species of both animal groups exist.
- Terrestrial risk assessment increasingly tends to be based on data derived from laboratory studies of these soil animal groups, and therefore supplementary data on the effects on field populations is needed.
- Laboratory tests have proven feasible, and toxicity is correlated to effects observed in the field.
- The broadness in life forms ensures that effects not observed in one species may be observed in another with a different niche and habitat.

In addition to the field studies that cover the main part of the project, various supplementary laboratory studies have been done to evaluate the toxicity of two sludge types and the compounds LAS and nonyl-phenol. In order to take the laboratory experiments a step further towards realistic conditions, those single compounds have been applied as a slurry, i.e. by spiking a sludge slurry and adding it to the soil.

As a first step towards an understanding of possible adverse effects of sludge, preference experiments have been done to investigate whether sludge is an appropriate food source that enables Collembola to grow and reproduce normally and whether they are able to distinguish between a contaminated sludge and a less contaminated type of sludge.

4.3 Danish sludge regulation

The first regulation of sludge in Denmark addressed, in the same manner as the rest of Europe, the maximum allowable heavy metal concentration. In 1989 the EU sludge directive from 1986 was implemented, but since then several regulations of sludge has been implemented in Denmark, the latest directive being from 1996 (Table 4.1. and 4.2.).

In addition to limit values for heavy metals, the latest Danish sludge directive include cut-off values for four organic chemicals or groups of chemicals (Table 4.1). The cut-off values for cadmium and the organic chemicals will all be lowered by the year 2000.

In addition to limit values and cut-off values for selected heavy metals and organic pollutants, maximum limits for addition of phosphorous to soils via sludge exist. Until July 2000 the limit is 40 kg P ha^{-1} $year^{-1}$ as an average over 3 years, i.e. a total of 120 kg P ha^{-1}, and after that date it will be lowered to 30 kg P ha^{-1} $year^{-1}$. Depending on the P content of the sludge (mean is 30 kg P t^{-1} D.M) this may lead to annual sludge application rates of approximately 1.3 t ha^{-1} y^{-1} or maximum rates at 4 t ha^{-1} (10-95% fractiles is 3-6 t ha^{-1}) in 1997. Assuming no change in the P content in the following years, the total application will as an average be 3 t D.M. ha^{-1} in year 2000. Additionally, a limit for application of sewage sludge expressed as dry matter pr. hectare is laid down in the sludge directive (10 t D.M. $year^{-1}$ as an average during 10 years). However, due to a high amount of phosphorous in almost all Danish sludge, these criteria will only in theory be used to control sewage sludge application, whereas the application of other organic fertilising products, e.g. compost, may be controlled by the dry matter criteria.

Worst case

If considering a normal application of sludge, i.e. a total application of approximately 4 t D.M. $year^{-1}$ today and 3 t D.M. $year^{-1}$ in the year 2000, an average and a worst case situation of the load of pollutants to agricultural land can be made (Table 4.2). Data from routine measurement of heavy metals at the sewage plants have been collected and statistically presented by the Danish EPA, but since routine measurements of the regulated four groups of organics will not start until July 1997, no detailed information about the organic pollutants is available at the moment. However, analyses from 20 selected sewage plants have been reported in Kristensen *et al.* (1996) and the results from these analyses are used as best estimates in Table 4.2.

Table 4.1.
Limit values for heavy metals and cut-off values for organic pollutants in sludge as outlined in the Danish sludge directive from 1996. D.M.:Dry Matter.

Grænseværdier og afskæringsværdier for tungmetaller og organiske stoffer samt indholdet af de samme stoffer i Herning slam.

Chemical	**Limit value 1.7. 1997 mg kg^{-1} D.M.**	**Limit value 1.7. 2000 mg kg^{-1} D.M.**	**Herning sludge[1] mg kg^{-1} D.M.**	**Limit value 1.7. 1997 mg kg^{-1} P**	**Limit value 1.7. 2000 mg kg^{-1} P**	**Herning sludge[1] mg kg^{-1} P**
Heavy metals:						
Cadmium	0.8	0.4	1.11	200	100	33.2
Chromium	100	100	62.4	-[2]	-	
Copper	1000	1000	280	-	-	
Lead	120	120	46	10,000	10,000	1377
Mercury	0.8	0.8	1.09	200	200	32.6
Nickel	30	30	34	2,500	2,500	1,018
Zinc	4000	4000	1660	-	-	
Organic pollutants:						
LAS[3]	2600	1300	1100	-	-	
DEHP[4]	100	50	31	-	-	
Σ PAHs[5]	6	3	3.87	-	-	
Nonylphenol[6]	50	10	140	-	-	

Notes: [1] Heavy metal concentrations are based on measurements of five different batches of sludge from 1995, as presented by Krogh et al. (1996) and the concentrations of organic pollutants can be found in Table 5.4.
[2] No criteria exist, [3] Linear alkylbenzene sulphonates, [4] Di(2-ethylhexyl)phthalate, [5] Sum of 9 PAHs, [6] Including the nonylphenol-ethoxylates with 1 and 2 ethoxy groups

Table 4.2

The load of selected heavy metals and organic pollutants as a result of normal sewage sludge application in 1997, as a worst case situation in 1997 and 2000 according to the latest sludge directive from 1996 and in the highest exposure situation from the present study (Herning sludge, for Ringkøbing sludge, see Krogh et al. 1996).

Normal- (1997) og maximaltilførslen (1997 og 2000) af tungmetaller og organiske stoffer iflg. slambekendtgørelsen, samt tilførslen af samme stoffer ved den højeste dosering i denne undersøgelse.

Compound	**Average load 1997** $g\ ha^{-1}$	**Maximum load 1997** $g\ ha^{-1}$	**Maximum load 2000** $g\ ha^{-1}$	**Load at 21 t D.M. This study**[1] $g\ ha^{-1}$	**% of max. 1997**
Heavy metals:					
Cadmium	6.2	24.0	9.0	23.3	97
Chromium	104	400	400	1310	328
Copper	1040	4000	3000	5880	147
Lead	300	1,200	900	966	81
Mercury	5.6	24.0	18.0	22.9	95
Nickel	90.0	300.0	225.0	714	238
Zinc	3040	16,000	12,000	34,860	218
Organic pollutants:					
LAS	2120	10,400	3900	23,100	222
DEHP	98	400	150	651	162
PAHs	-	24.0	9.0	81.3	339
Nonyl-phenol (+ 1,2 ethoxylates)	31.8	200.0	30.0	2940	1470

Assumptions: In all cases the phosphorus content of the sludge is estimated to 30 mg kg^{-1}. The average and maximum loads in 1997 are estimated by the assumption of application of 4 t ha^{-1} (limited by an allowable application of 120 mg P ha^{-1} once) of a sludge containing average concentration (Heavy metals as reported in 1994 in a memorandum by the Danish EPA; Organics by Kristensen *et al.* 1996; both 50% fractiles) or a maximum concentration (cut-off value) of the compounds in question. The maximum load in year 2000 is estimated by the assumption of application of 3 t ha^{-1} (limited by the allowable application of 90 mg P ha^{-1} at one time).

Notes: [1] The loads in this study represents the heavy metal load calculated from measurements of five different batches of Herning sludge in 1995, see Krogh *et al.* (1996), and for organic pollutants the sludge concentrations can be found in Table 5.4.

5 Materials and methods

5.1 Description of soil, organic fertilizers and crop yield

Soil characteristics have been given in Table 5.1 and nutrient contents of the sludge and the cattle manure have been given in Table 5.2.

The crop yield as determined for one replicate of each treatment was considerably improved by the organic fertilizers (Table 5.3). In the winter wheat field the sludge apparently gave higher yields than the dung. The clover-grass ley was part of the harvested straw and contributed to the yield at the low doses, but was probably suppressed by the wheat at higher dosages.

Sludge types

Sludge from two waste water treatment plants has been used: one from Ringkøbing, Jutland, and one from Herning, Jutland. Sludge nutrient characteristics have been given in Table 5.2. Cattle manure was obtained from a near by cattle farm. Analysis for residues of organic compounds consisting of the major groups of PAH's, persistent pesticides, detergents etc. were provided through a commercial chemical analysis package offered by Miljø-Kemi, Danish Environmental Center Inc. The results of this analysis are given in Table 5.4. Compared with the survey of sludges from a number of waste water treatment plants by Kristensen *et al.* (1996) these sludges are among the mostly polluted in Denmark.

Table 5.1
Soil texture at the two field locations with sandy clay (Askov) and sand (Lundgård). Proportions are given as percentage of weight.

Teksturanalyse af de to forsøgsjorde: Askov (lerjord) og Lundgård (sandjord). Alle tal er opgivet som vægtprocent.

Locality	**Askov**		**Lundgård**	
Depth	**0-23 cm**	**23-50 cm**	**0-23 cm**	**23-50 cm**
Clay (<0.002 mm)	10.6	13.1	4.3	3.3
Silt (0.002-0.02 mm)	11.8	11.9	3.8	3.1
Fine sand (0.02-0.2 mm)	37.0	35.9	26.6	24.7
Coarse sand (0.2-2.0 mm)	37.6	36.6	63.1	67.6
Humus	3.0	2.5	2.2	1.3

Table 5.2.
Nutrient content of cattle manure and sludge from Ringkøbing and Herning waste water treatment plants. For cattle manure and Ringkøbing sludge measurements were done on each batch used for the two localities.

Analyseresultater af kvægmøg og slam fra Ringkøbing og Herning rensningsanlæg. For kvægmøg og Ringkøbing slam findes to værdier, da to forskellige partier er brugt ved udbringning.

	Cattle manure		**Ringkøbing sludge**		**Herning sludge**	
	Clay	**Sand**	**Clay**	**Sand**	**Clay**	**Sand**
Dry matter % of total	20.69	20.54	23.15	19.46	19.5	18.9
Ash ($g\ kg^{-1}$)	361.2	354.7	497.4	464.3	347.5	352.0
Total nitrogen ($g\ kg^{-1}$)	33.8	34.6	41.0	41.1	50.3	51.9
Nitrate ($g\ kg^{-1}$)	0.0	0.0	0.0	0.0	4.05	5.03
Ammonia ($g\ kg^{-1}$)	11.1	11.7	3.8	0.9	3.24	5.82
Phosphorous ($g\ kg^{-1}$)	9.2	9.3	41.9	43.7	39.02	39.18
Kalium ($g\ kg^{-1}$)	23.7	23.9	1.7	1.8	2.52	2.70
Carbon ($g\ kg^{-1}$)	320	320	258	264	339.3	338.6
pH_{H2O}	7.03	7.05	7.81	5.93	6.59	7.32

Table 5.3
Crop yield for the two types of sludge and dung at the sandy and loamy field locations

Udbytter for strå og kerner ved de to slamtyper og kvægmøg på sandjord og lerjord.

	t D.M. ha^{-1}	**Herning** Loam	**Herning** Sand	**Ringkøbing** Loam	**Ringkøbing** Sand
Straw hkg ha^{-1}					
Control	0.0	37.4	56.9	20.9	12.4
Sludge	3.5	40.0	57.3	32.6	15.4
	7.0	36.2	42.2	36.8	18.0
	21.0	45.2	60.8	53.0	23.0
Cattle manure	3.5	40.1	45.9	25.3	11.6
	7.0	35.6	53.0	35.0	16.5
	21.0	44.4	45.1	53.3	23.9
Grain hkg ha^{-1}					
Control	0.0	26.2	4.42	30.43	17.3
Sludge	3.5	34.7	22.2	45.35	25.6
	7.0	40.9	26.5	55.76	35.9
	21.0	57.6	48.7	77.38	61.6
Cattle manure	3.5	36.9	17.2	37.34	22.2
	7.0	39.7	26.9	45.60	31.9
	21.0	56.6	51.2	71.07	54.6

Tabel 5.4
Content of xenobiotic organic compounds in the sludge used in the field experiments.

Indhold af miljøfremmede organiske stoffer i Ringkøbing-slam d. 3. okt. 1995 og Herning-slam d. 29. april 1996. Alle mængder er i µg kg⁻¹ tørstof, hvis ikke andet er anført.

Compound	Ringkøbing- sludge oktober 1995 µg kg⁻¹ D.M.	Herning-sludge, april 1996 µg kg⁻¹ D.M.	Detection limit µg kg⁻¹ D.M.
PAH:			
naphtalene	260	180	20
methylnaphtalene	800	400	20
dimethylnaphthalenes	2800	960	20
trimethylnaphthalenes	3100	900	20
acenaphtylene	71	48	20
acenaphtene	57	-	20
fluorene	290	78	20
phenanthrene	500	310	20
anthracene	*	52	20
fluoranthene	340	170	20
pyrene	340	270	20
benz(a)anthracene	120	56	20
chrysen/triphenylene	170	120	20
benzfluoranthenes (b+j+k)	380	99	20
benz(a)pyrene	170	73	20
benz(ghi)perylene	22	51	20
indeno(1,2,3-cd)pyrene	91	29	20
dibenz(a,h)anthracene	20	-	20
Sum of PAH's	9500	3870	
PCB-congeners:			
No. 28	-	<10***	5
No. 52	28	8.9	5
No. 101	26	13	5
No. 118	17	11	5
No. 138	42	6.2	5
No. 153	49	5.5	5
No. 180	26	-	5
Sum of 7 congeners	170	45	
Chlorinated pesticides			
alfa-HCH	-	-	30
beta-HCH	-	-	30
gamma-HCH (lindane)	-	-	30
delta-HCH	-	-	30
o,p'-DDE	-	-	30
p,p'-DDE	-	-	30
o,p'-DDD	-	-	30
p,p'-DDD	-	-	30
o,p'-DDT	-	-	30

Compound	Ringkøbing- sludge oktober 1995 $\mu g\ kg^{-1}$ D.M.	Herning-sludge, april 1996 $\mu g\ kg^{-1}$ D.M.	Detection limit $\mu g\ kg^{-1}$ D.M.
p,p'-DDT	-	-	30
Chlorobenzenes:			
1,4-dichlorbenzene	91	16	5
1,2,4-trichlorbenzene	12	34	5
1,2,3,4-tetrachlorbenzene	-	-	5
pentachlorbenzene	-	-	5
hexachlorbenzene	-	7.9	5
Chlorphenols			
2,4-dichlorphenol	40	130	10
2,4,6-trichlorphenol	-	250	10
2,3,4,6-tetrachlorphenol	-	-	5
pentachlorphenol	-	12	5
4-chlor-3-methylphenol	-	-	25
Phthalater:			
di-n-butylphthalate (DBP)	340	350	300
butylbenzylphthalate	130	170	50
di(2-ethylhexyl)phthalate(DEHP)	1	31000	100
di(2-ethylhexyl)adipate	-	2700	100
Di-n-octylphthalate	400	480	50
P-triesters:			
tri-n-butylphosphate	-	670	50
triphenylphosphate	160	87	50
tricresylphosphate	-	-	50
Nonylphenol, mg kg^{-1} (+1 and 2-ethoxylates)	35	140	0,5
LAS, mg kg^{-1}	1700	1100	100
Dry matter %	17.8	32.0	

* Is included in phenanthrene
** Interference, no result
*** Increased detection limit
\- Below detection limit

5.2 Field investigation

5.2.1 Experimental design

The experiment is conducted as a randomized blocks design with seven treatments and six replicates. The seven treatments consist of 3.5, 7 and 21 t D.M. sludge ha^{-1} and 3.5, 7 and 21 t D.M. cattle manure ha^{-1} and one control treatment receiving no fertilizer at all. The experiment with Ringkøbing sludge was initiated in October 1995 and the sludge was spread in $7.5 \times 12\ m^2$ plots manually by fork sowing winterwheat. The sludge from Herning was applied to $5 \times 14\ m^2$ plots in April 1996 before sowing spring barley with clover-grass ley. Before sowing, the sludge was ploughed down to a depth of 15 cm. See Table 5.5 for overview of the time schedule. The experiments have been done on a sandy loam and a sandy soil (Table 5.1). The distance between plots for the Herning sludge experiment was 1 m between the plots lying side by side along their longest side.

5.2.2 Sampling and treatment of samples

Microbial activity

Soil for potential ammonium oxidation was sampled from the two experimental sites at Askov and Lundgaard in November 1996. In each plot a 30-mm diam. soil corer with an open slit was used to take six 0-20 cm subsamples which were pooled. The co-ordinates for each subsample were selected using a random number table. At the Askov site the subsamples from the unamended control plots and from the plots with 21 t D.M. sludge were divided in two and the 0-10 cm and 10-20 cm depth intervals pooled separately. The soil samples were stored in loosely closed plastic bags at 2°C and analysed within two weeks after sampling. The soil samples were sieved (<4 mm) the day before the analysis was carried out.

Microarthropods

Samples for microarthropod population analysis were taken to a depth of 5 cm with a soil corer with an inner diameter of 5.8 cm. The Ringkøbing sludge experiment was sampled 10 November, 1995, 2 May, 1996 and 9 September, 1996. The Herning sludge experiment was sampled 22 June and 2 September, 1996.

On 22 October, 1996, additional sampling was done in plots with the highest sludge dose and in the control. These soil cores taken in the 10 to 20 cm soil horizon were cut into two samples 5 cm each. The samples should give information of possible extreme conditions in this soil horizon as it was realised that the sludge when it was ploughed in was strictly confined to a narrow horizontal zone within this part of the soil profile.

A total of 990 samples for microarthropod density estimation were sampled and analysed for the purpose of the present project. Microarthropods were extracted in a high gradient temperature and moisture extractor of the MacFadyen type.

Earthworms

A survey of the earthworm population at Askov was conducted on 21 October 1996, around the peak season for earthworm activity. From each plot a single soil sample, 25 cm · 25 cm · 25 cm, was taken. Individual soil samples were wet sieved through a series of four box sieves of decreasing mesh size (viz. 10, 4, 2 and 1 mm), with a careful handsorting of all material, ensuring an efficient extraction of cocoons, juveniles and adults. Species were identified according to Sims and Gerard (1985). The worms were kept for 2 days in Petri dishes with moist filter paper at 5°C so gut material was voided. After this, number and individual fresh weight of worms were determined to provide an estimate of number and biomass per square meter.

5.2.3 Potential ammonium oxidation

For the measurements of potential ammonium oxidation activity, 5 g fresh wt. soil was suspended in 100 ml 0.5 mM $(NH_4)_2SO_4$ / 1.0 mM K_2HPO_4 (pH 7.2) containing 10 mM $NaClO_3$ to inhibit nitrite oxidation (Belser & Mays, 1980). The soil slurries were incubated at 20°C in 250-ml Erlenmeyer bottles on a rotary shaker in the dark. After ca. 15 min and then 5 hours later a 5-ml sample was removed, centrifuged at 2°C, filtered (Advantec GF75), and analyzed for nitrite after reaction with sulphanil amide (Schmidt & Belser, 1982). Soil moisture was determined by drying a separate soil sample for 24 hours at 105°C.

5.3 Laboratory studies

5.3.1 Preference/avoidance

The acute behavioural response to pairs of fresh organic fertilizers was investigated for the following materials: Herning sludge, Skævinge sludge, cattle manure, Bakers' yeast and 'nill' (no material). The materials were offered in all possible pairwise combinations to the springtail Collembola *F. fimetaria*. Table 5.6 shows the content of selected organic pollutants for the two types of sludge.

About five mg of each type of material was placed on plaster of Paris with 10% charcoal by weight in Petri dishes. A 'wall' was placed in the plaster to divide the petri dish into two parts. The wall was just as long to allow for an opening of 1 cm in one side of the dish (Figure 5.1).
The Collembola were observed three times during the day for three days. Ten adult females of age 28-31 days, starved for five days before the experiment, were added to each dish.

Table 5.5
Schedule for agricultural measures and the sampling program for earthworms, microarthropods and nitrifiers.

Praktisk landbrugsmæssig forløb og prøvetagningsprogram for regnorme, mikroleddyr og nitrifikanter.

	Winter wheat Ringkøbing sludge	**Barley clovergrass Herning sludge**
Application of sludge and cattle manure; plowing	Oct. 4.-11. 1995	April 30.-May 7. 1996
Sowing	Oct. 11.-12. 1995	May 1.-7. 1996
First sampling	Nov. 10. 1995	June 22. 1996
Second sampling	May 2. 1996	Sept. 2. 1996
Third sampling	Sept. 9. 1996	
Earthworm sampling		Oct. 21 1996
Nitrification		Nov. 1996
Harvest	Aug. 23.-27. 1996	Aug. 23.-27. 1996

Tabel 5.6
Content of selected xenobiotic organic compounds in the two sludge types used in the laboratory experiments.

Indhold af udvalgte miljøfremmede organiske stoffer i Skævinge-slam og Herning-slam anvendt til laboratoriforsøg.

Compound	Skævinge-sludge	Herning-sludge	Detection limit
PAH: μg kg^{-1} D.M.			
acenaphtene	-	50	20
fluorene	140	170	20
phenanthrene	100	740	20
fluoranthene	210	450	20
pyrene	400	650	20
benzfluoranthenes (b+j+k)	300	370	20
benz(a)pyrene	200	220	20
indeno(1,2,3-cd)pyrene	90	120	20
benz(ghi)perylene	150	220	20
sum of PAH's	**1,590**	**2,990**	
Di(2-ethylhexyl)phthalate, DEHP **mg kg^{-1} D.M.**	56	42	100
Nonylphenol, (+ 1 and 2-ethoxylates) **mg kg^{-1}**	15	190	0.5
LAS, **mg kg^{-1}**	130	2,600	50
Dry matter, %	22.7	24.9	

- : Below detection limit

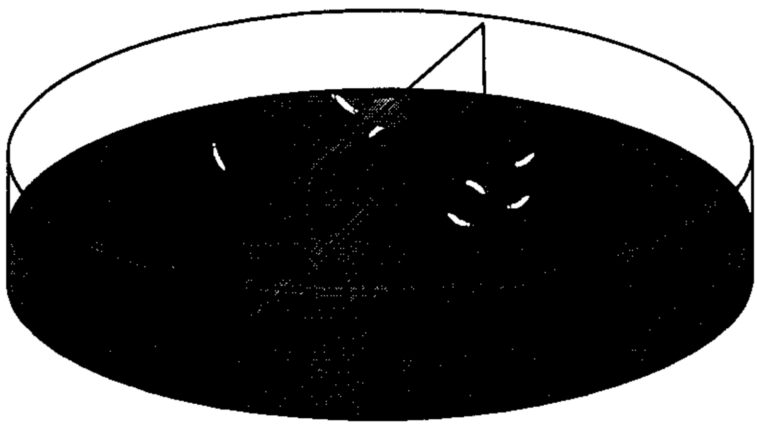


Figure 5.1
Petri dish used to study food preference/avoidance. A transverse wall divides the dish into two sections and inhibits random passage.

Petriskål anvendt til at undersøge springhalers preference for slam, kvægmøg og gær. En tværgående væg hæmmer tilfældig passage mellem de to sektioner.

5.3.2 **Collembolan reproduction**

In laboratory tests the springtail *Folsomia fimetaria* L. was used as described by Krogh (1995). Ten males and ten females were added to each replicate. The standard test procedure has been described in Kula *et al.* (1995). The animals were 23-26 days old at the start of the experiments lasting for three weeks. The animals were fed dried Baker's yeast at the start and after two weeks. Replicates were incubated at 20°C. At the termination of the test the samples were extracted in a high gradient extractor beginning with 25°C and increasing by 5°C every 12 hours to 40°C. Counting and size enumeration were done with a digital image processing equipment. If nothing else has been stated, the sludge or compound have been homogeneously mixed into the soil.

The soil was dried at 80°C for 24 hours and then stored at 5°C until use. One experimental unit contains 26.5 g soil + 3.5 g water + sludge or chemical. The sludge had been stored at -18°C until use.

Experiments with nonylphenol and linear alkyl sulfonate (LAS) (MARLON® A 350, 50% active ingredient with mean carbon chain length of 11.53 and mean molecular mass of 344 g mol^{-1}) were performed as standardised laboratory experiments.

5.3.3 **Earthworms**

Reproduction

Effects on earthworm reproduction were assessed by a modified version of the method described by van Gestel *et al.* (1989). The worms used in this study were adult *Aporrectodea calignosa* Savigny collected from an agricultural soil near Aarhus, Denmark. Fresh sludge from Herning was mixed with dried soil (24 hrs at 105°C) from Lundgård to give six concentration levels of sludge (viz. 0, 0.7, 1.7, 3.3, 6.7 and 10.0 g dry sludge·kg^{-1} dry soil). The mixture was moistened with demineralized water to give a final water content of 16% based on fresh weight. Amounts of ca. 1 kg of the mixture were placed in 1-litre plastic pots with lids, small holes allowing ventilation. On top of the soil, to serve as food for the worms, were placed amounts of ca. 25 g of a mixture of ground cattle manure and soil, previously dried at 105°C and remoistened to 50% of fresh weight. To each of four replicates 4 adult worms were added and the pots were incubated at 15°C in the dark for 21 d. After this period the contents of the pots were wet sieved through a 2 mm mesh and the number of worms and cocoons recorded. Hatchability of cocoons was assessed by incubating them at 20°C in Petri dishes with moist filter paper.

Growth

Effects of sludge on growth of juvenile *A. calignosa* were studied during a 105 d period. The juvenile worms (8 to 10 individuals at each concentration) were kept singly in 150 ml plastic beakers with about 100 g soil (fresh weight) with concentrations of sludge as in the reproduction experiment. Instead of applying food to the surface, the soil was enriched with 1% dried cattle manure before mixing with sludge. The fresh weight (including gut contents) of each worm was determined at the beginning of the experiment and at 21 days intervals.

5.4 Statistical analysis

The nitrification results from each site were analyzed for treatment effects by one-way ANOVA followed by Tukey's HSD multiple comparison test if significant variation was observed. A table-wide error rate of 0.05 was used (Rice, 1989). The 0-10 and 10-20 cm depth intervals were compared by a paired-sample *t*-test (Zar, 1984).

The field experiments with soil fauna were analyzed by PROC GLM paying due attention to the possibility of interactions between the blocks and the treatments. If these interactions were estimated to be less than 5% it was used as an error term for the calculation of the F value for the main effects instead of the default error mean square (PROC GLM, SAS Institute, 1990). Testing for differences between pairs of the six organic matter treatments with the control was done using Dunnett's t-test. Overall differences between the sludge and the cattle manure treatments was tested by contrasting the three levels of sludge with the three levels of cattle manure.

For the preference experiment a Bradley-Terry model was used within PROC CATMOD (Agresti 1995).

EC_X (X: % decrease relative to the control) values were estimated either by fitting a sigmoid curve to the dose-response data using the SAS Procedure NLIN or, if this was not possible the ICp approach was used (Norberg-King 1993).

6 Results

6.1 Field investigations

6.1.1 Ammonium oxidation potential

Average rates of ammonium oxidation potential after application of Herning sludge are shown in Fig. 6.1. The ammonium oxidation potential in the sandy loam at Askov was 2-3 times higher than the potential in the coarse sandy soil at Lundgaard (see Fig. 6.1; Control), where the level of activity corresponded to that obtained in a previous study at the same site (Krogh *et al.*, 1996). It is well established that finer textured soils contain a higher proportion of protected pore space where microbes are able to escape predation or harsh environmental conditions, and that such soils therefore contain a larger microbial biomass (Ladd *et al.*, 1993).

Site specific response

The effects of organic amendments differed between the two sites. At Lundgaard the ammonium oxidation potential was significantly elevated at the highest sludge application rate, while no response to cattle manure at any of the three application rates was detected (Fig. 6.1). This difference could be due to the higher C/N ratio of the cattle manure (9.4) compared with the sewage sludge (6.3) which may have given a lower net release of ammonium. At Askov no long-term response of neither sewage sludge nor solid cattle manure was observed. The lack of response at the Askov site may be due to the higher 'standing crop' of nitrifying bacteria, possibly combined with a delayed transport of ammonium to the sites of nitrification.

Vertical distribution

The potential ammonium oxidation activity in 0-10 cm and 10-20 cm depth was determined separately in the control plots and in the plots receiving 21 t D.M. sludge at the Askov site (Tab. 6.1). In the unamended control there was no significant difference between the two depth intervals ($0.2<P<0.5$; n=6). In the sludge treatment, however, the activity at 10-20 cm depth was significantly higher than in the top layer ($0.01<P<0.02$; n=6). This is probably due to the fact that sludge and manure were incorporated by ploughing. Hence, mixing the 0-20 cm soil layer prior to analysis caused a dilution of treatment effects from the organic amendments.

Table 6.1
Potential ammonium oxidation activity in 0-10 and in 10-20 cm depth in the loamy soil. The rates were compared within treatment by a paired-sample t-test.

Potentiel ammnonium oxidations aktivitet i 0-10 og 10-20 cm's dybde i lerjord Raterne er sammenlignet indenfor behandlingerne med en parret t-test.

Block	**Treatment**			
	Control		**21 t D.M. sewage sludge**	
	0-10 cm	**10-20 cm**	**0-10 cm**	**10-20 cm**
		(nmol nitrite g^{-1} dry wt. soil h^{-1})		
1	17.9	19.4	24.9	26.9
2	18.9	17.7	20.4	26.1
3	15.9	15.5	15.0	17.7
4	13.1	14.3	15.5	21.1
5	20.6	18.5	20.0	22.0
6	19.6	16.6	16.5	24.6
t-value	-0.74		3.48	
P	$0.2<P<0.5$		$0.01<P<0.02$	

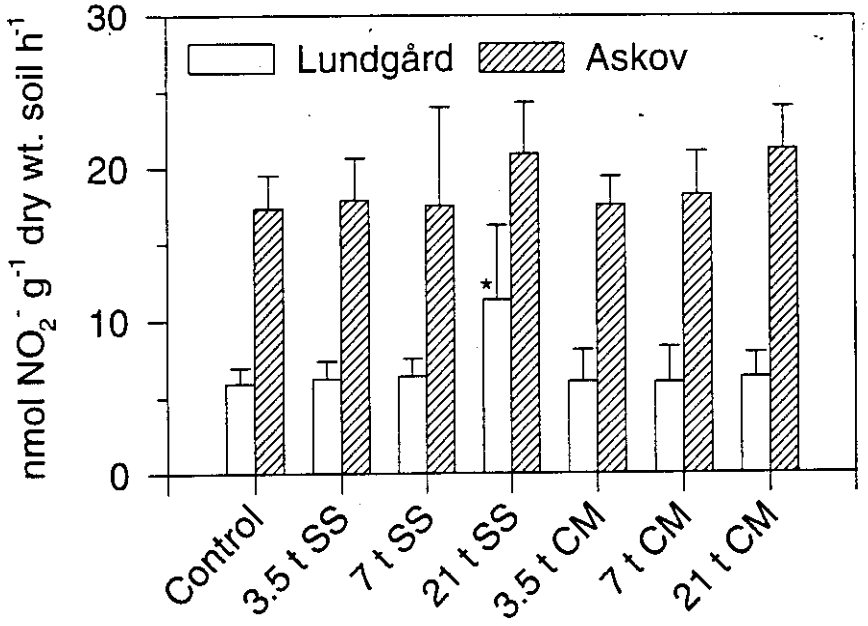


Figure 6.1
*Ammonium oxidation potential in 0-20 cm depth at Askov and Lundgaard (n=6) after application of sewage sludge (SS) from Herning or cattle manure (CM). The vertical lines represent standard deviations, a significant difference (P<0.05) within each site is indicated by an *.*

*Ammonium oxidations potentialet i 0-20 cm´s dybde i lerjord og sandjord (n=6) efter tilførsel af Herning slam (SS) eller kvægmøg (CM). Lodrette linjer på søjler er standard afvigelsen. Signifikante forskelle (P<5%) på hver jordtype er angivet med *.*

6.1.2 **Microarthropods**

The changes in the microarthropod populations in the winter wheat up to one year after application of Ringkøbing sludge or cattle manure are presented in Figure 6.2 and 6.3. The changes in microarthropod populations during the first six months in the barley/clover-grass fields treated with Herning sludge or cattle manure are shown in Figure 6.4 and 6.5.

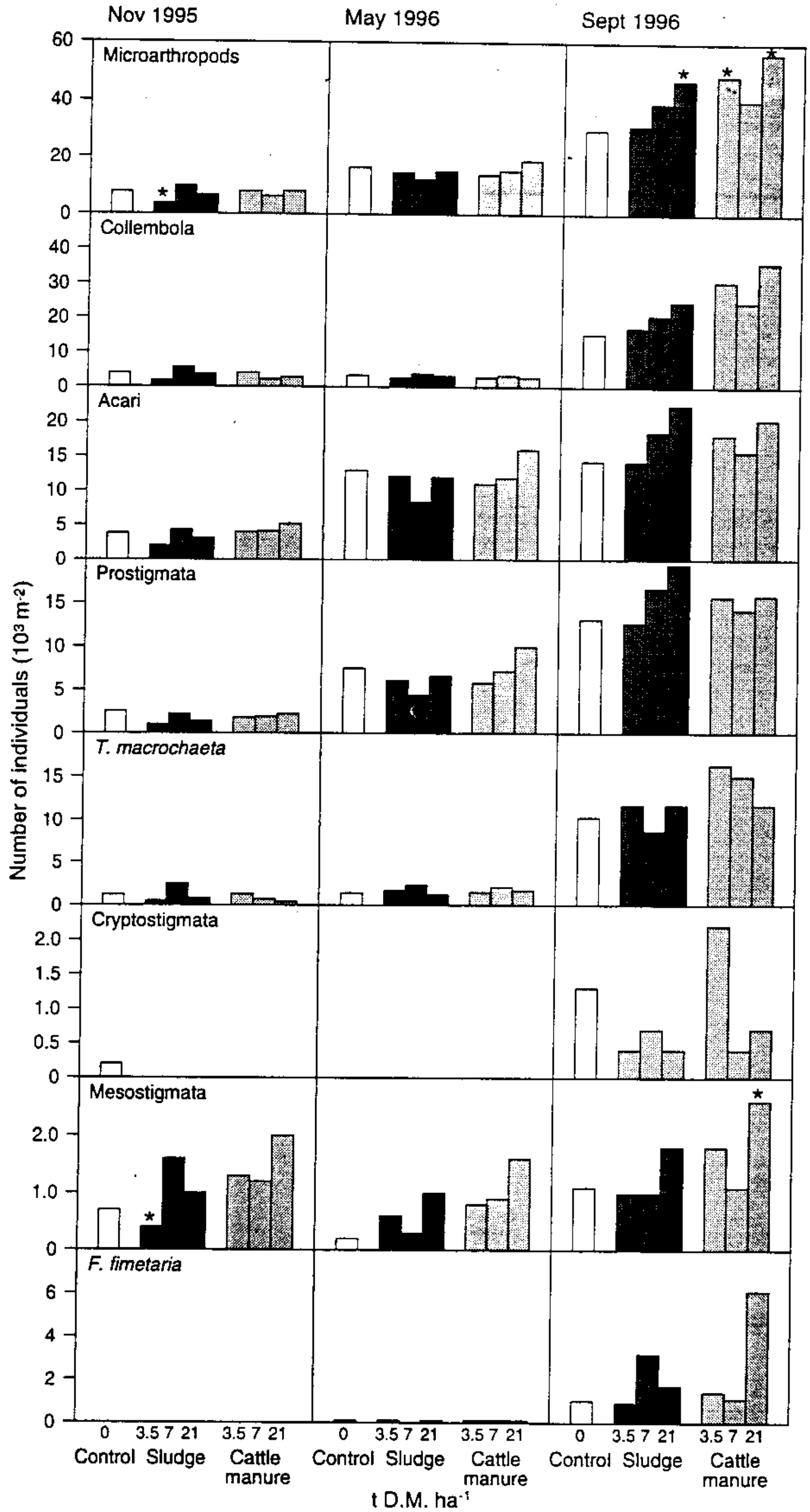


Figure 6.2

*Microarthropod population abundances in the loamy soil location during three sampling occasions in the winter wheat field. * indicates significant difference from control (Dunnett's test, P<5%).*

Mikroarthropod populationsudvikling i lerjorden ved tre prøvetagninger i vinterhvedemarken.

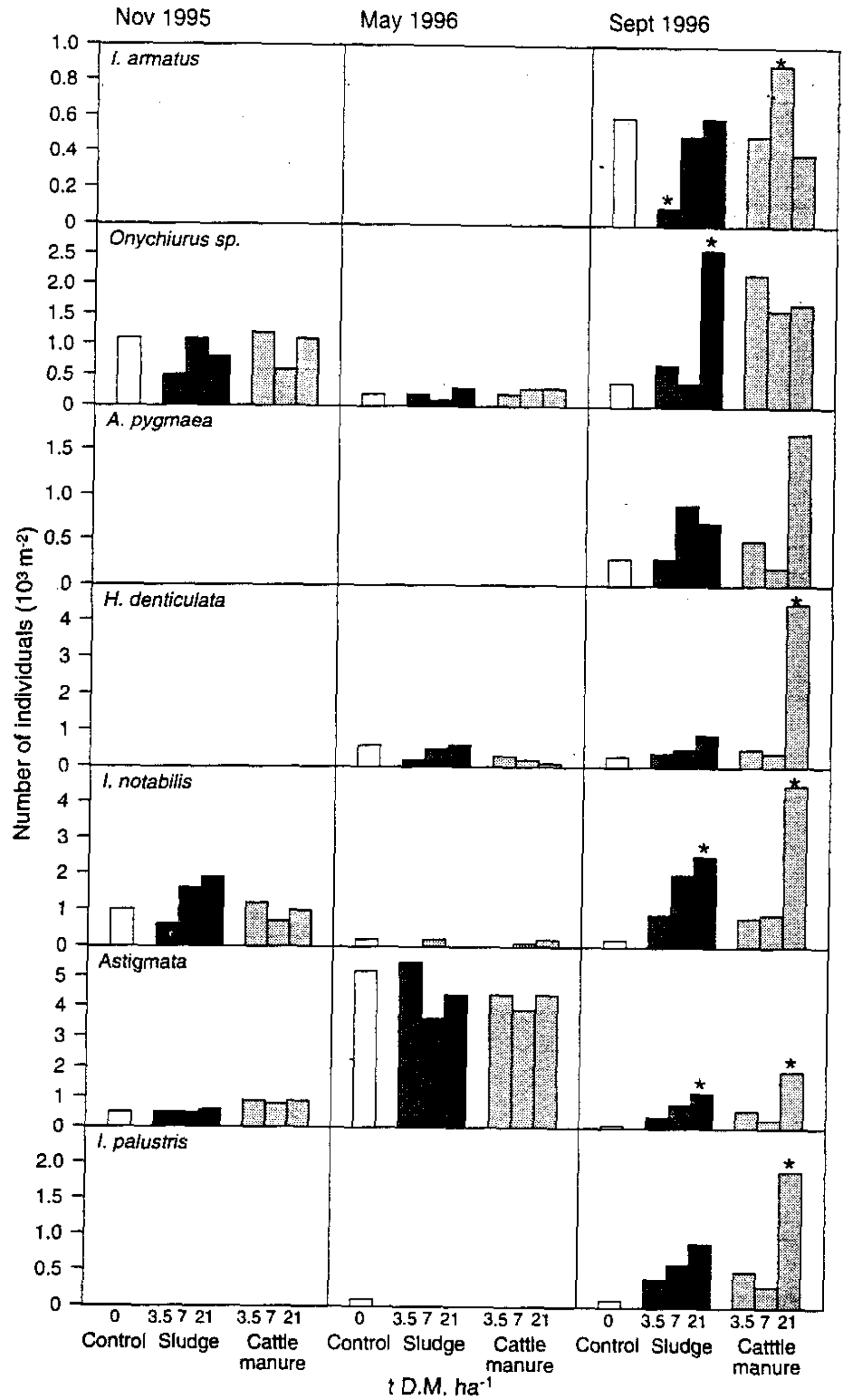


Figure 6.2 (continued)

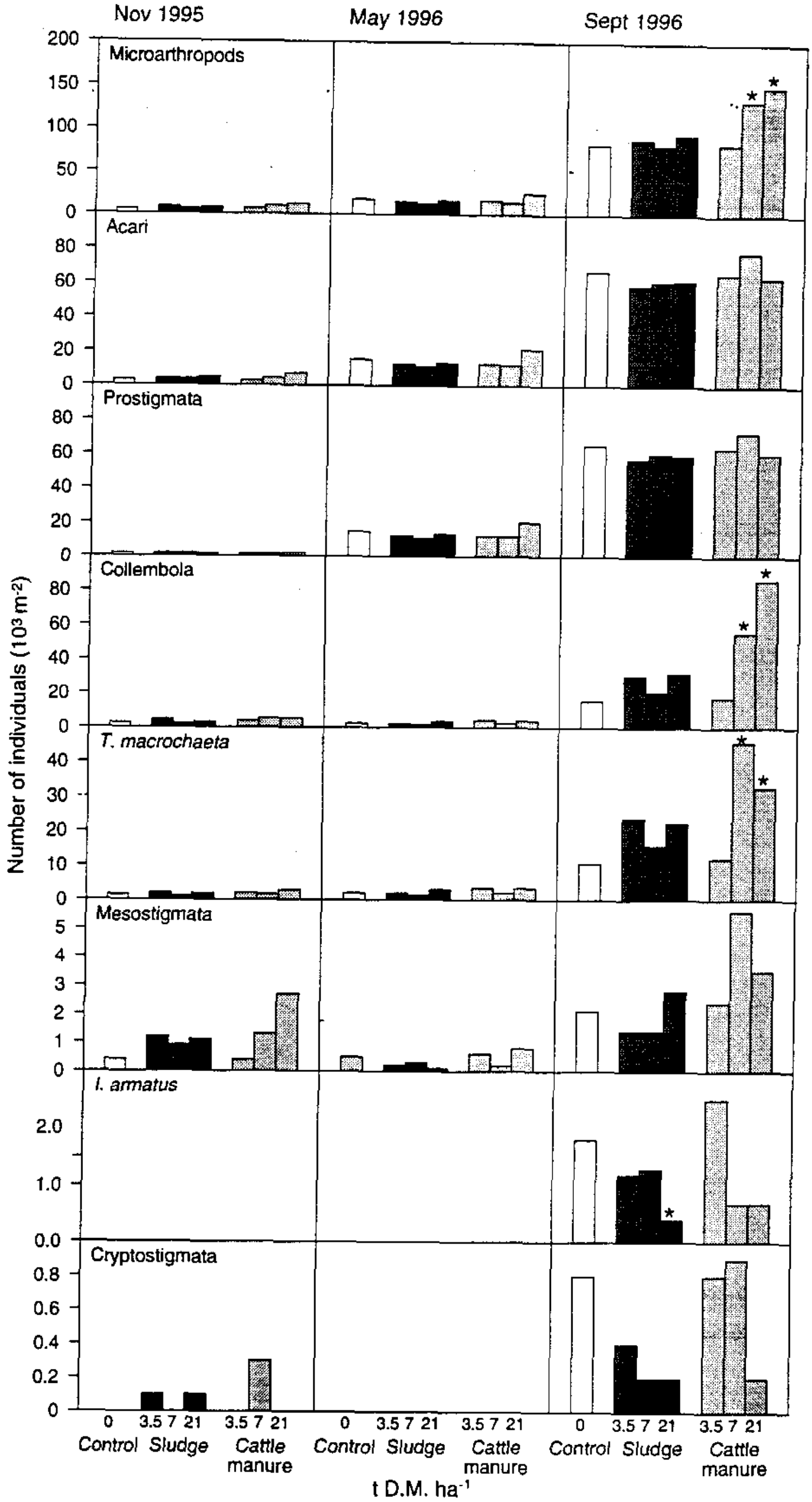


Figure 6.3

*Microarthropod population abundances in the sandy soil location during three sampling occasions in the winter wheat field. * indicates significant difference from control (Dunnett's test, P<5%).*

Mikroarthropod populationsstørrelser i sandjorden med vinterhvede ved tre prøvetagningstidspunkter.

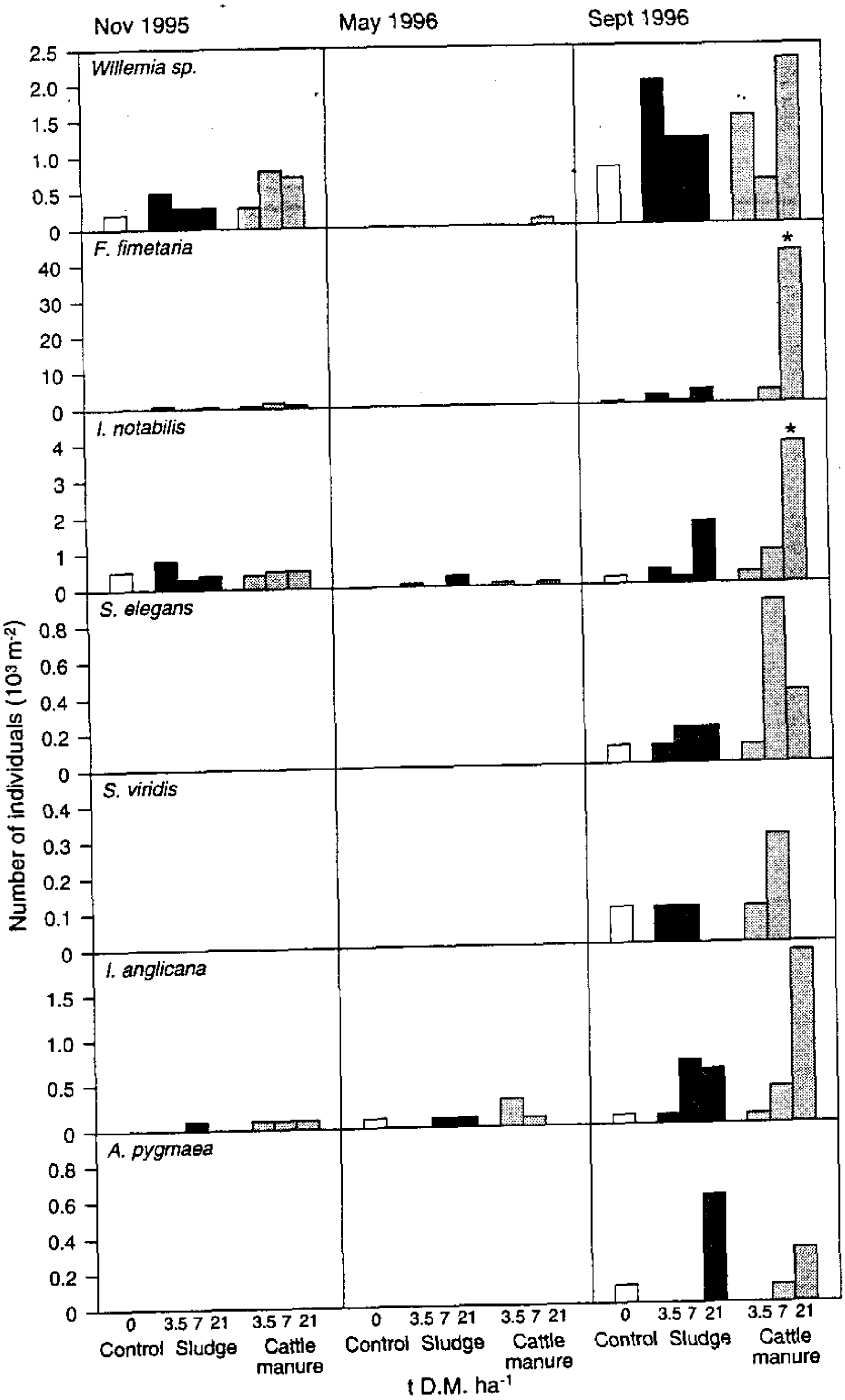


Figure 6.3 (continued)

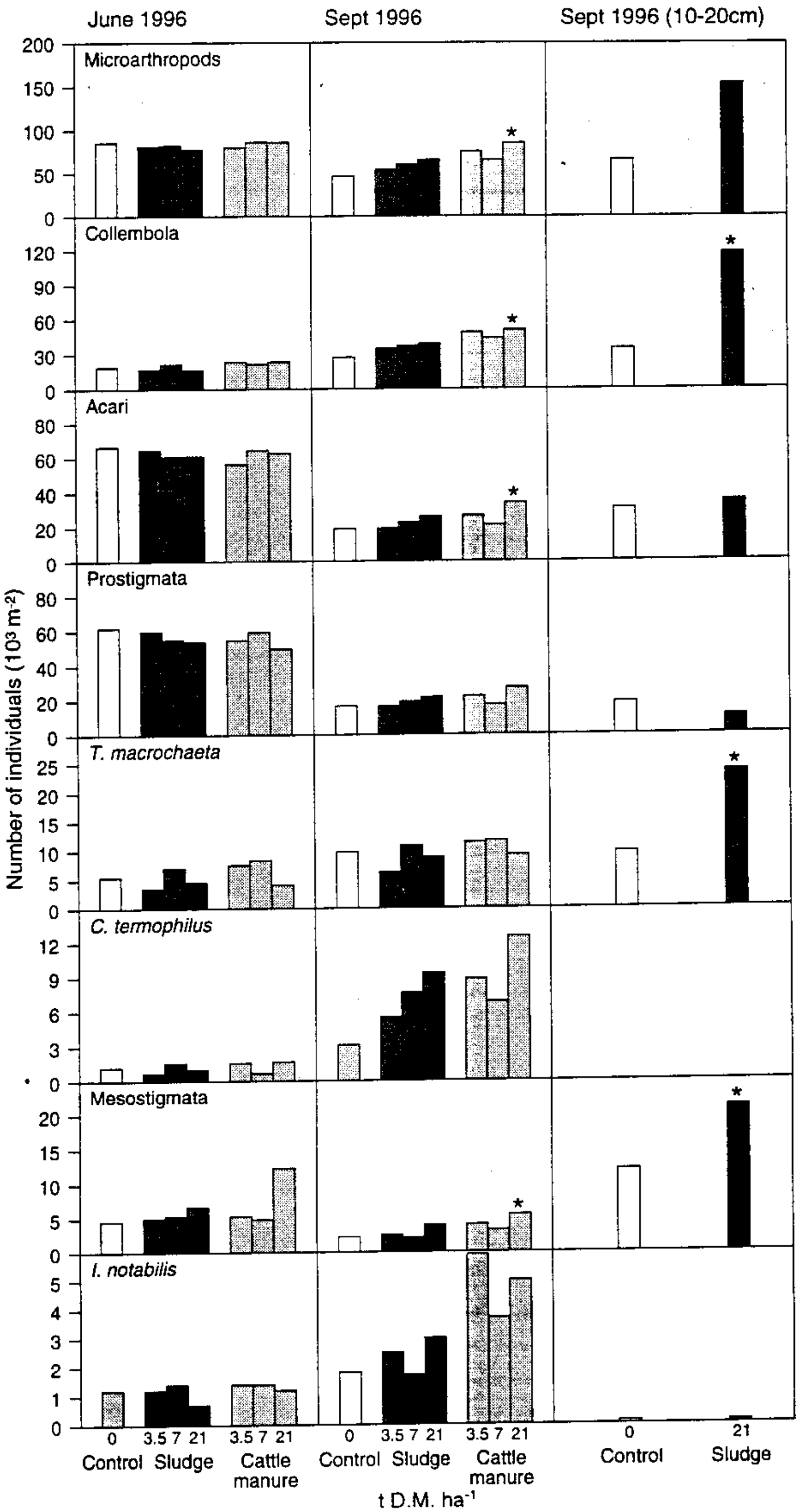


Figure 6.4
*Microarthropod population abundances in the loamy soil location during two sampling occasions in the barley/clover-grass field. The last samples were taken at a soil depth of 10-20 cm. * indicates significant difference from control (Dunnett's test, P<5%).*

Mikroarthropod populationsstørrelser i lerjorden med byg/kløvergræs. De sidste prøver stammer fra 10-20 cm's dybde.

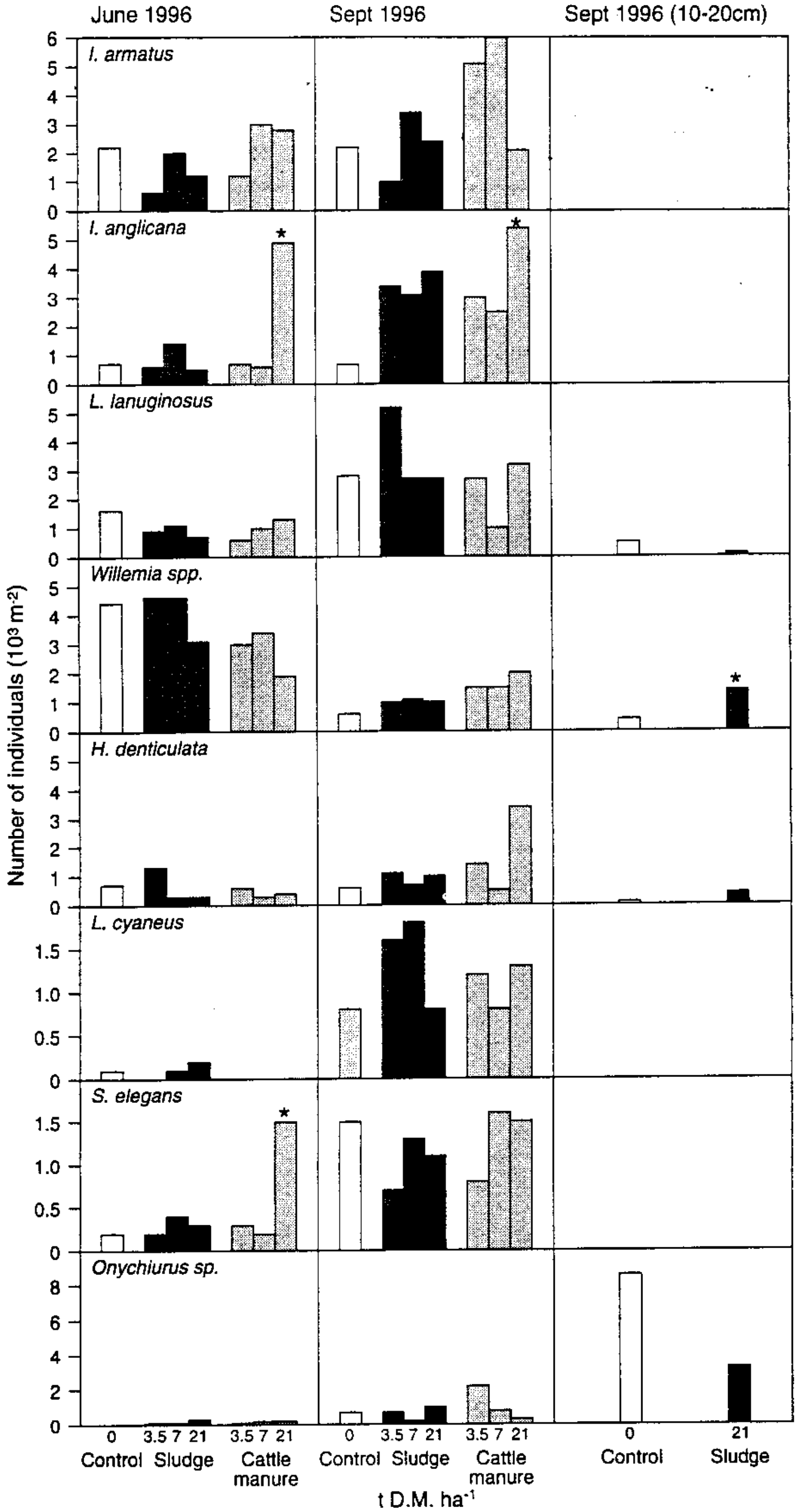


Figure 6.4 (continued)

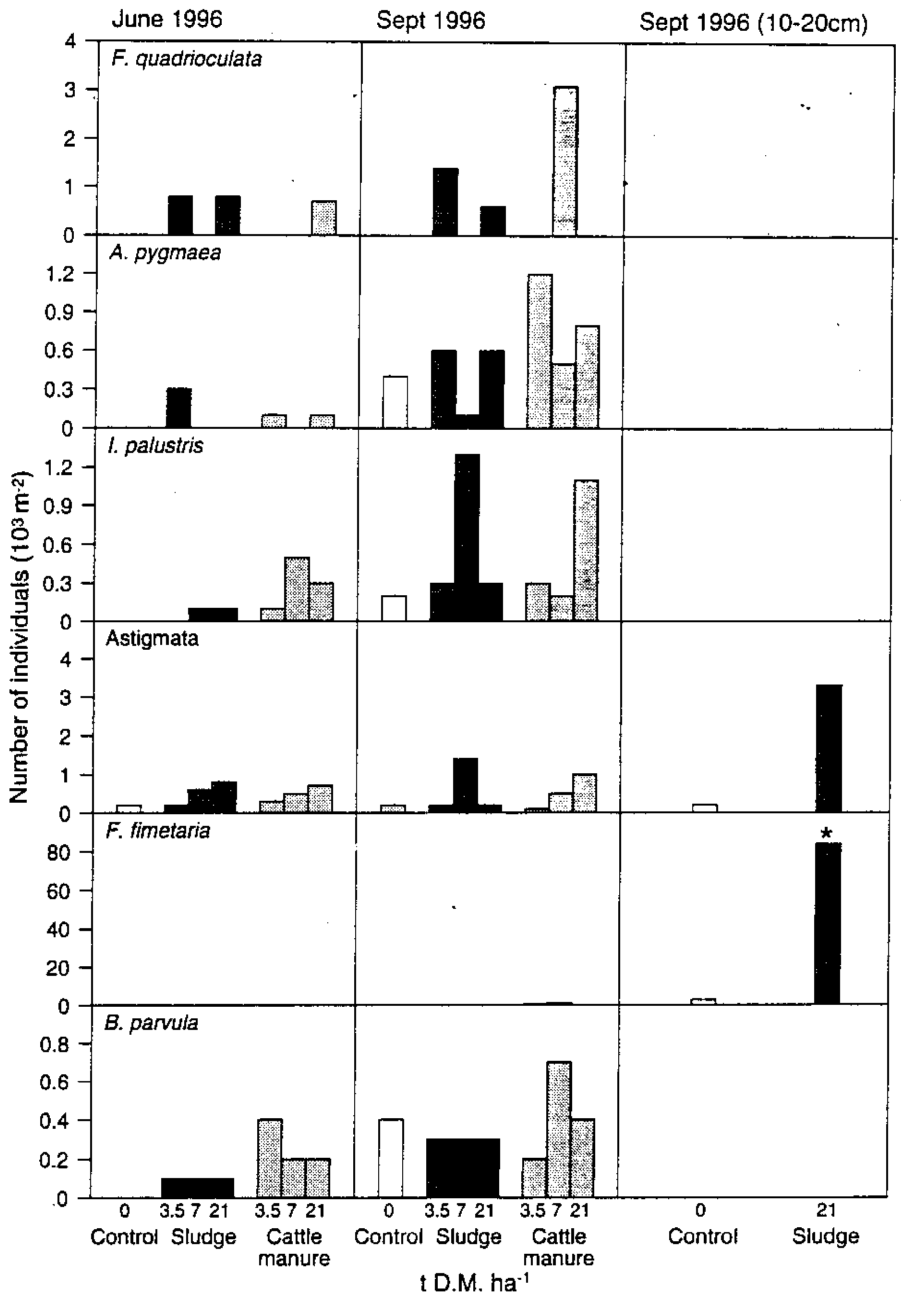


Figure 6.4 (continued)

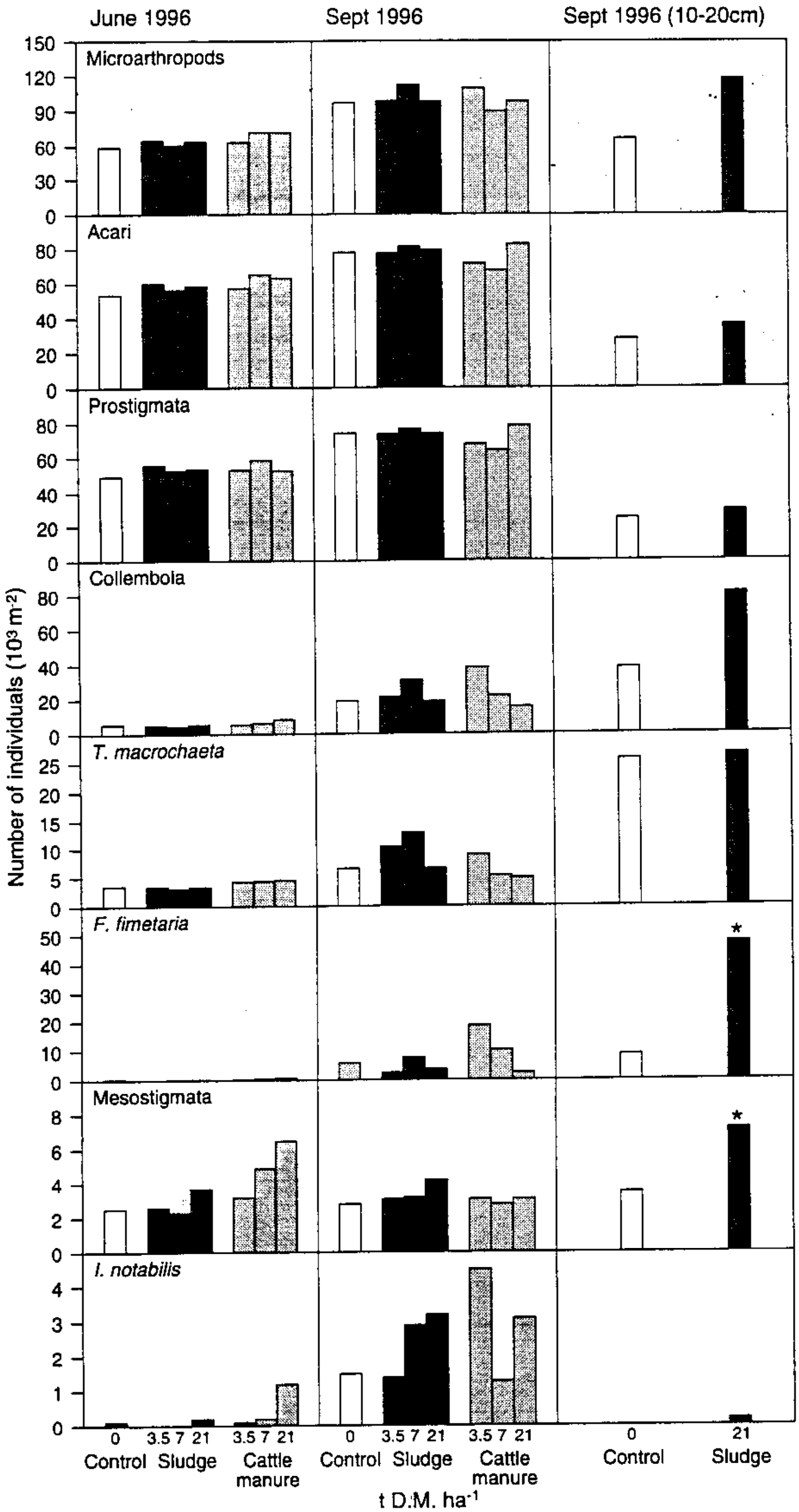


Figure 6.5

*Microarthropod population abundances in the sandy soil location during two sampling occasions in the barley/clover-grass field. The last samples were taken at a soil depth of 10-20 cm. * indicates significant difference from control (Dunnett's test, P<5%).*

Mikroarthropod populationsstørrelser i sandjorden med byg/kløvergræs. De sidste prøver blev taget i 10-20 cm's dybde.

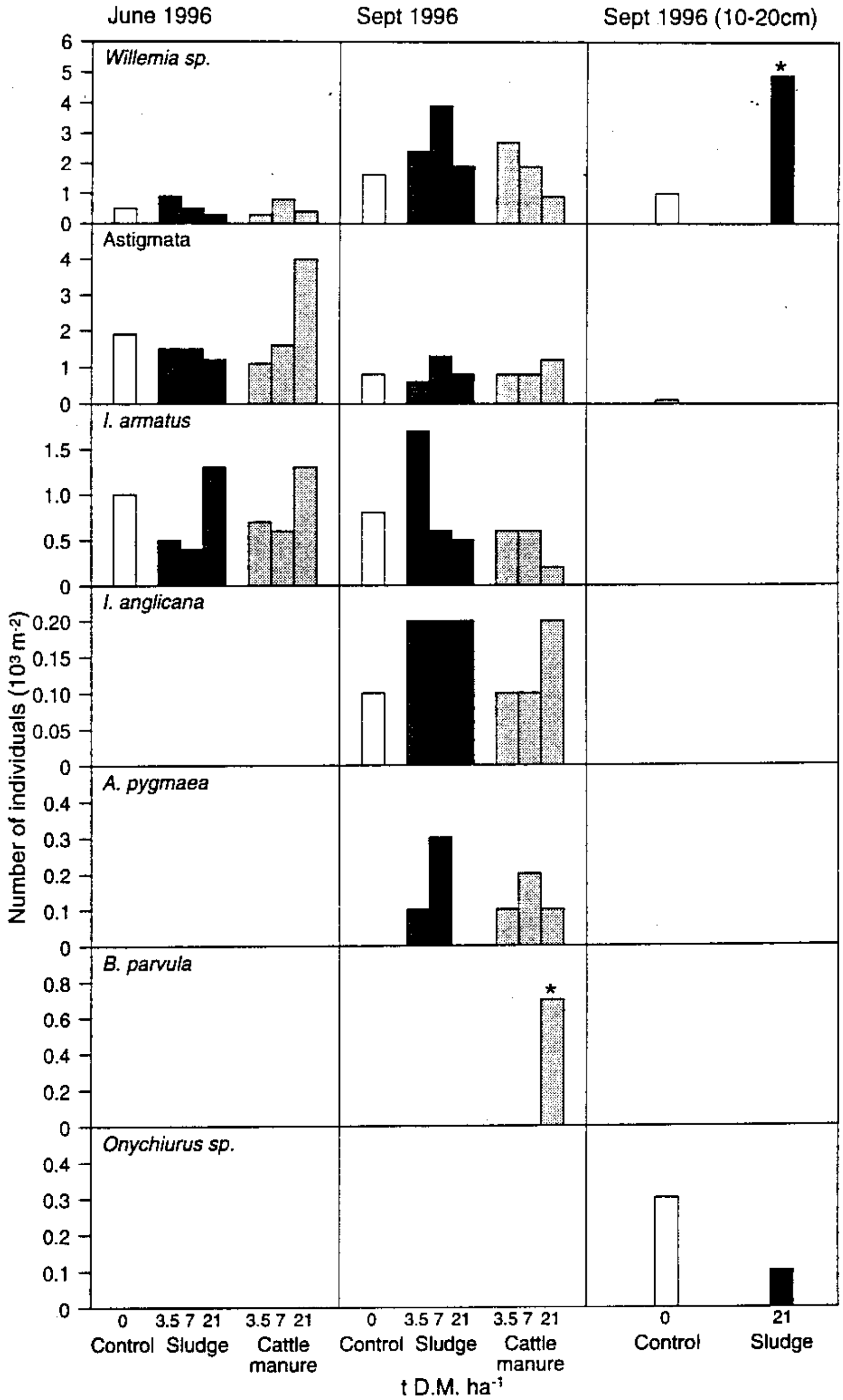


Figure 6.5 (continued)

Winter wheat

The microarthropod populations in the 0-5 cm soil depth in the winter wheat increased about four-fold in the loamy soil and about 15 fold in the sandy soil from plouging in the sludge and cow manure in the autumn of 1995 to the autumn of 1996 in the stubble. At the last sampling both treatments had led to a significant increase in microarthropod population abundance as well as species diversity in the loamy soil but only the cattle manure led to significant increases in abundances of some groups of species and of the overall diversity in the sandy soil (Fig. 6.2 and 6.3).

The overall difference between the two types of organic matter was significant for microarthropods and Collembola, suggesting a general difference in the mechanisms underlying the observed stimulations. This difference apparently includes the larger increase in populations due to the dung compared with the sludge.

The highest concentrations of sludge and cattle manure in the sandy soil led to a doubling and a five-fold increase of Collembola, respectively (Fig. 6.3). This was mainly due to the two dominating Collembola, *T. macrochaeta* and *F. fimetaria*.

Barley clover-grass

In the barley field microarthropod populations were sampled after five months which hardly is enough time to establish large populations after ploughing in the spring. In fact, the control populations were smaller after the five months at the loamy site, presumably due to the dry summer. In the sandy soil they had increased two-fold. Both kinds of treatments led to stimulations, but only the dung significantly increased the populations at the highest dose (Fig. 6.4 and 6.5). No significant effects could be detected on diversity.

10-20 cm.

The additional sampling set covering a depth from 10-20 cm contained about 8 species while the samples from 0-5 cm contained about 14 species. Neither species richness, Shannon-Wiener diversity index nor the equability was significantly changed by any of the treatments in either of the depths. Microarthropods below 5 cm contributed at least 60% to the total population abundance in the loamy 0-20 cm profile and at the highest dosage of sludge they contributed >70%.

The Collembola *T. macrochaeta* and *F. fimetaria* were still dominating in this soil horizon and together with Mesostigmata, *Willemia* spp. and Astigmata they were very stimulated by the presence of sludge. In particular *F. fimetaria* were 30 times more abundant in the sludge treated zone compared with the control in the loamy soil (Fig. 4C).

6.1.3 Earthworms

Abundancy

The species composition and the size of the earthworm population found at the barley clovergrass field at Askov was typical for agricultural soils of that soil type and crop rotation (Edwards and Bohlen 1996). The total number of earthworms ranged from 184 to 264 m^{-2}. Sludge treatment effected a significant increase of the population at 3.5 $t \cdot ha^{-1}$ whereas plots dressed with higher doses had the same number of earthworms as control plots (Figure 6.6). The number of cocoons was not significantly different between treatments even though the average number of cocoons in plots applied 3.5 $t \cdot ha^{-1}$ was somewhat higher than in other plots (Figure 6.6). The earthworm biomass ranged from 92 to 171 g $fw \cdot m^{-2}$. Also here, plots dressed with 3.5 $t \cdot ha^{-1}$ had significantly higher biomass than plots with other treatments (Figure 6.6).

Dominating species

The population was dominated by *Aporrectodea calignosa* and *A.longa* which made up about 75 % of the whole population based on numbers as well as on biomass (Table 6.2 and 6.3). *A. rosea* and *Lumbricus terrestris* were also common and in a few samples contained *Allolobophora chlorotica*. Cocoons of all species except *A. chlorotica* were found but in numbers too low to allow a sound estimation of reproduction and hatchability rates. The stimulation by sludge seen in plots applied 3.5 t ha^{-1} was due to an increase in numbers and biomass of *A. calignosa* and *A. longa* (Figure 6.2). Higher doses of sludge apparently did not cause increased stimulation. As opposed to this, increasing doses of sludge seemed to stimulate *L. terrestris* although this tendency was not statistically significant. *A. rosea* also seemed to be stimulated by lower doses of sludge but negatively affected by the highest dose (Figure 6.7).

Size and age composition

A comparison was made of adult fresh weight and adult-to-juvenile ratio between treatments for *A. calignosa* (this was the only species with sufficient data). Adults from plots dressed with sludge seemed to be larger than adults from control plots and plots applied 21 t·ha^{-1}, but this was not statistically significant (Student's T-test). Based on the available data no remarkable changes appeared in adult-to-juvenile ratio for *A. calignosa* due to sludge.

Table 6.2
Species composition of earthworms (% of total number) in relation to sludge application rate (t dw ha^{-1}).

Artssammensætningen af regnorme (% af total antal) for hver behandling

Species	0 t·ha^{-1}	3.5 t·ha^{-1}	7 t·ha^{-1}	21 t·ha^{-1}
L. terrestris	6.9	6.1	10.8	11.6
A. longa	25.0	25.2	21.5	26.1
A. caliginosa	56.9	51.5	35.4	53.6
A. rosea	9.8	17.2	27.7	8.7
A.chlorotica	1.4	0	4.6	0
Total no. of observations	72	99	65	69

Table 6.3
Species composition of earthworms (% of total biomass) in relation to sludge application rate (t dw ha^{-1})

Artssammensætningen (% af total biomasse) for hver behandling

Species	0 t·ha^{-1}	3.5 t·ha^{-1}	7 t·ha^{-1}	21 t·ha^{-1}
L. terrestris	7.6	16.4	10.8	18.3
A. longa	41.0	41.1	45.8	36.0
A. caliginosa	47.7	39.1	32.2	43.5
A. rosea	3.3	3.4	8.7	2.2
A.chlorotica	0.7	0	2.4	0

Table 6.4
Mean adult fresh weight (± S. E.) and adult-to-juvenile ratio of A. caliginosa in relation to application dose of sludge. The number of observations is indicated in parenthesis.

Middel friskvægt for voksne grå orm og forholdet mellem voksne og unge ved de fire behandlinger. Antal observationer i parentes.

Dose (t·ha^{-1})	Adult fw (mg)	Adult:juvenile-ratio
0 (control)	980 ± 87 (n=9)	0.41 (n=38)
3.5	1022 ± 57 (n=16)	0.46 (n=51)
7	1171 ± 143 (n=4)	0.64 (n=23)
21	1062 ± 76 (n=10)	0.48 (n=37)

Figure 6.6
Effects of sludge on total number, cocoons and biomass of earthworms in the loamy soil. Different letters above bars indicates significant differences between treatments (LSD, P<5%).

Effekt af slam på total antal, kokonner og biomasse for regnorme.

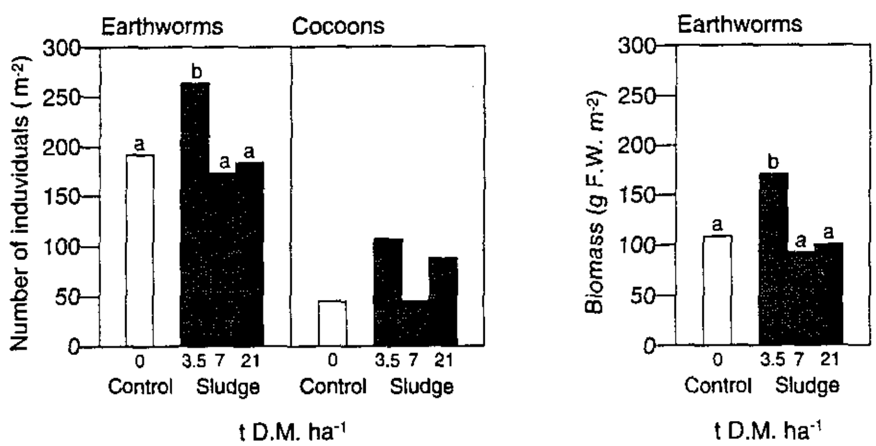
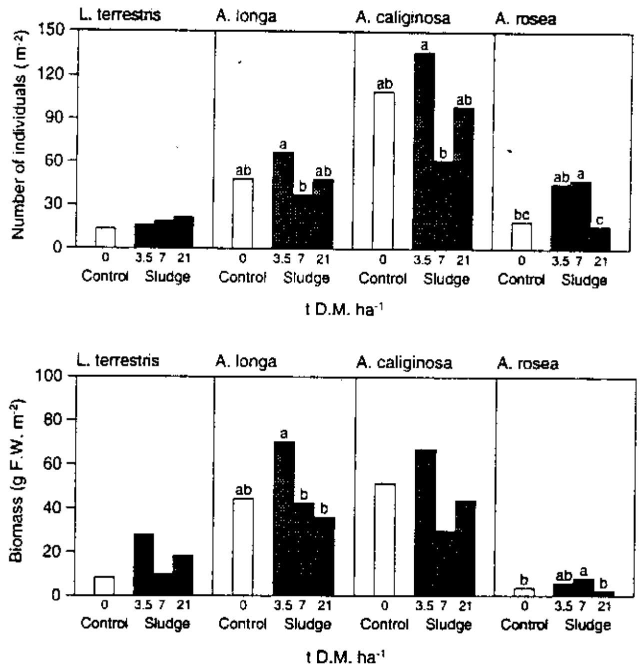


Figure 6.7
Number and biomass of earthworms in the loamy soil treated with sludge at three levels. Bars with different letters are significantly different (LSD, P<5%).

Antal og biomasse for regnorme i lerjorden behandlet med slam.



6.2 Laboratory investigations

6.2.1 Collembolan reproduction in soil

Effects of the two sludge types could only be detected at the sublethal level, even an LC_{10} was not observed.

LAS and NP

The effect of mixing LAS and NP into sludge (Skævinge) decreased the toxicity (EC_{10}) by 300% and 50%, respectively (Table 6.3). The amount of sludge used for the spiking, corresponding to 6 t D.M. ha^{-1} was shown not to have any effects. However, due to the need to make a slurry of the sludge to be able to mix the LAS or NP homogeneously into the sludge the effect of exposure to a sludge slurry was tested and it turned out that the slurry was approximately twice as toxic as sludge itself. Although only half of the slurry causing 10% effect was used in the spiking experiments, a minor stress effect of the slurry itself cannot be completely excluded.

The effect of LAS was close to the results reported by Holmstrup & Krogh (1996), where a German sandy soil (LUFA 2.2) was used. Also the results with NP agreed with the data presented by Krogh *et al.* (1996).

Table 6.5
Results from the laboratory tests with F. fimetaria *in the sandy soil (Lundgård). Sludge in t* D.M.ha^{-1}*;LAS and NP mg* kg^{-1}*soil. Numbers in brackets 95% confidence intervals.*

Resultater fra F. fimetaria *reproduktionstests med slam, LAS og nonylphenol (NP) i sandjord (Lundgård).*

Treatment	EC_{10}	LC_{10}	EC_{50}	LC_{50}
Sludge (Herning)	8.6 [0.1;32]	>50	26.1 [14;34]	>50
Sludge (Skævinge)	25.2 21;31]	>50	36,4 [30;41]	>50
Sludge slurry (Skævinge)	≈ 14	>50	≈ 17	>50
LAS	208 [143;273]		444 [379;509]	>2000 (♀)
Sludge spiked with LAS (6 t D.M ha^{-1})	757 [500;1.014]	N.D.	1.102 [934;1269]	>2000
NP	27.2 [22;28]	42.7 [8.7;77]	44.2 [41;48]	85.9 [59;113]
Sludge spiked with NP (6 t D.M. ha^{-1})	48.7 [47;70]		58.4 [47;70]	>100

6.2.2 Preference experiment

The mean number of Collembola situated on either of the two types of organic material or situated in either side of the Petri dish were calculated from the 9 observations for each petri dish and presented in Table 6.4.

When *F. fimetaria* is in the sludge/sludge situations, it spends less time at the organic matter than outside the organic matter.

The Bradley-Terry test resulted in the following ordering of the types of organic matter:

Cattle manure (71.5%) > Yeast (64%) > Skævinge sludge > (57,3%) > Herning sludge (32,5%) > Nill (25%).

This demonstrates that the sludge is the least preferred material.

Table 6.6
Numbers of F. fimetaria *observed in petridishes with pairwise combinations of different kinds of fresh organic materials. Numbers of* F. fimetaria *on material F1 or F2 and numbers in the same side of the dish R1 or R2 as the material but not in contact with the material. Herning, Skævinge: two sludge types (see Table 5.4). Nil: no organic material.*

Antal af springhalen F. fimetaria *i petriskåle med parvise kombinationer af forskellige typer organisk materiale. F1, F2: antal dyr på fødeemne 1 og 2; R1, R2: Antal dyr i sektion 1 eller 2 men ikke i kontakt med føden.*

Numbers of F. fimetaria Comparison F1/F2	**F1**	**F2**	**R1**	**R2**	**F1+R1**	**F2+R2**
Yeast/Herning	7.3	0.1	1.4	1.2	7.4	1.3
Yeast/Nil	6.9		1.8	1.3	8.7	1.3
Cattle manure/Nil	6.7		0.4	2.8	7.1	2.8
Cattle manure /Herning	6.2	0.6	2.1	1.2	8.3	1.8
Skævinge/Yeast	4.6	1.1	2.4	1.8	7.0	2.9
Cattle manure /Skævinge	4.3	1.9	2.4	1.0	6.7	2.9
Cattle manure /Yeast	4.1	2.4	2.0	1.4	6.1	3.8
Cattle manure /Cattle manure	4.1	3.6	1.4	0.9	5.5	4.5
Skævinge/Nil	4.1		2.6	3.3	6.7	3.3
Herning/Nil	3.0		2.2	4.8	5.2	4.8
Yeast/Yeast	2.8	3.6	1.7	1.8	4.5	5.4
Skævinge/Skævinge	1.6	2.3	2.3	3.8	3.9	6.1
Herning/Herning	1.4	1.6	2.5	4.5	3.9	6.1
Herning/Skævinge	0.8	1.7	4.4	3.1	4.2	5.8
Nil/Nil			5.5	4.5	5.5	4.5

Table 6.7
Final size and reproduction of F. fimetaria *when offered types of organic fertilizers either alone or in combinations in petri dishes.*

Slutstørrelse og formering, når sprighalen F. fimetaria *tilbydes forskellige typer organisk gødning enkeltvis eller i kombinationer.*

	Females		Males		Juveniles	
	Number	**Length mm**	**Number**	**Length mm**	**Number**	**Length mm**
None	10.3	0.9^{c}	10.5^{a}	0.8^{c}	15.8^{a}	0.2
Yeast	9.5	1.2^{b}	9.3^{ab}	1.0^{ab}	63.8^{b}	0.3
Dung	8.3	1.4^{a}	3.8^{ab}	1.1^{a}	2.0^{a}	0.5
Skævinge	10.0	1.3^{ab}	6.3^{ab}	1.0^{ab}	2.5^{a}	0.4
Herning	10.0	1.2^{b}	8.3^{ab}	0.9^{bc}	0.0^{a}	.
Yeast/Dung	4.8	1.3^{ab}	2.5^{b}	1.0^{ab}	0.0^{a}	.
Yeast/Herning	9.8	1.3^{ab}	6.3^{ab}	1.0^{ab}	0.0^{a}	.
Yeast/Skævinge	9.5	1.3^{ab}	7.3^{ab}	1.0^{ab}	4.0^{a}	0.5

6.2.3 Reproduction in petri dishes

The yeast resulted in the highest reproduction and although the Collembola were starved for 5 days before the experiment, they reproduced to a small extent compared to the 200-400 produced in our standard tests (Table 6.5). In all the treatments where sludge and dung were present the reproduction was zero or negligible. Only one clutch was produced during the incubation period of three weeks.

The growth was normal and the sludge and the manure was in some cases even a better food source than yeast when measured by this variable.

6.2.4 Earthworm tests

In the laboratory, the reproduction of *A. calignosa* was stimulated by sludge added to the test soil (Fig 6.8). More than twofold increase of the reproductive rate was observed at 6.7 dry sludge kg^{-1} dry soil but at the highest concentration, 10 $g \cdot kg^{-1}$, this positive effect seemed to level off. However, there were no statistically significant differences between control and any of the sludge concentrations used. Cocoon hatchability was unaffected by sludge concentration, always being higher than 90%. Also, growth of juveniles was stimulated by sludge in the concentrations tested. After 42 and 63 d, worms cultured in 6.7 and 10 g kg^{-1} had significantly larger fresh weight than the control worms (Figure 6.9). After 84 d, the worms cultured at 6.7 g kg^{-1} had a significantly larger fresh weight than control worms, whereas worms at higher concentrations were not significantly larger than control worms any more.

Figure 6.8
Effect of Herning sludge on the mean (±SEM) reproductive rate of A. calignosa.

Effekt af Herning slam på reproduktionsraten for grå orm.

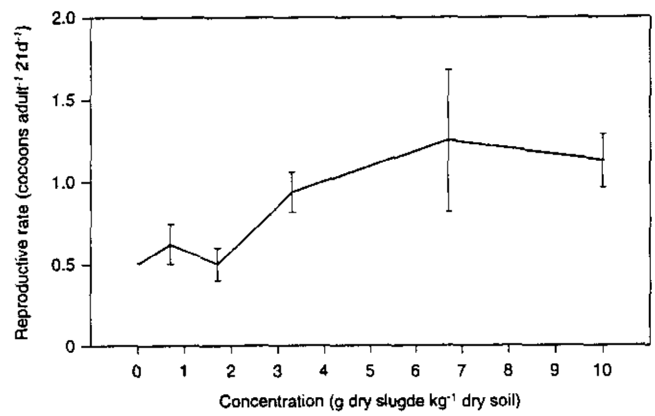
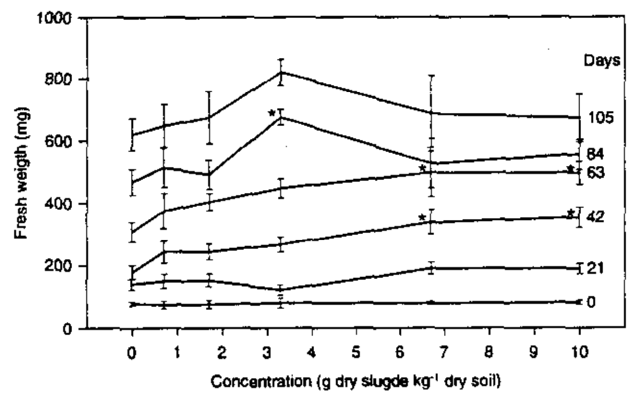


Figure 6.9
Growth of A. Calignosa *during 105 days at increasing doses of sludge. * indicates significant difference from control (ANOVA/LSD).*

*Væksten af grå orm gennem 105 dage ved forskellige mængder slam tilsat jord. * indikerer en signifikant forskel fra kontrol. (ANOVA/LSD).*



7 Discussion

The concerns arising from the use of sewage sludge as fertilizer in agriculture have mainly been addressing two aspects:

1. The risk of uptake and bioconcentration of xenobiotics (e. g. heavy metals and PAHs) in crops, thereby exposing humans, or the transport and accumulation in food chains via uptake in soil invertebrates, e. g. insects and earthworms.

2. The risk of sewage sludge being toxic to soil organisms, thereby affecting the functioning of the soil ecosystem, in particular the decomposition of dead organic matter and the release of nutrients for plant growth.

The present investigation addresses only the latter of these two aspects. Nearly all earlier investigations on the effects of sewage sludge have focused either on heavy metals or have not included any identification or quantification of the organic chemical compounds in the sludge. In this respect the present study is unique because it makes it possible to relate observed effects to the actual concentrations of a number of xenobiotic compounds introduced to the soil system via sludge. Such documentation is important when trying to assess the risk of using sewage sludge as fetilizers on arable land.

Microorganisms

Data on effects of organic wastes on soil microbes are scarce but a few investigations of sludge have been done in agricultural soils. Fließbach *et al.* (1994) reported that sludge deposited for ten years at rates of 5 or 15 t D.M. ha^{-1} yr^{-1} increased microbial biomass and decreased the bacterial activity relative to the fungal activity. Contrariwise, Brendecke *et al.* (1993) found that applications of 2 or 6 t ha^{-1} yr^{-1} for four years did not result in any detectable long-term changes in microbial populations and activity. Sewage sludge is a complex substrate, since it may both stimulate and inhibit microbial activities in the soil upon application. This is also true for the nitrification process, since some ammonium is present in the sludge and more is produced by N mineralization in the soil which will stimulate nitrifying bacteria. On the other hand, pollutants like heavy metals or xenobiotic organic compounds may inhibit nitrifiers. In the present study solid cattle manure was included in separate treatments to provide a nutrient source that was comparable to the sludge, but with reduced load of pollutants. Any adverse effects from toxic substances were expected to give negative response to sewage sludge amendment compared to manure amendment because of the higher load of pollutants in the sludge. However, field measurements of ammonium oxidation potential resulted in either no response or a positive response of sludge compared to manure. It should be recognised that a difference in net mineralization of nitrogen between sludge and manure may have confounded this picture. So accumulated effects after repeated sludge applications cannot be excluded on a long-term basis, although no toxic effects on ammonia oxidising bacteria were found six months after sludge application in our study.

Soil fauna

Studies of how the soil fauna is influenced by application of sewage sludge and cattle manure have predominantly shown increases in the populations of different taxonomic groups. Sludge has been shown to stimulate mites (Glockemann and Larink 1989), Collembola (Lübben 1989; Pimentel and Warneke 1989), nematodes (Weiss and Larink 1991, Stevenson *et al.* 1984) and Gamasida (Lübben and Glockemann 1993). The stimulation of these groups is explained by an increase in microbial biomass, which is in accordance with the general knowledge of the trophic link between microarthropods and microorganisms. Furthermore, it is supported by observations of increases in fungal and bacterial feeding nematodes and presumably microbivore Prostigmata (Scutacaridae) (Glockemann and Larink 1989, Weiss and Larink 1991). The present field investigation is completely in line with these former investigations, since almost all species showed positive response to the sludge and manure applications. In a few occasions *I. armatus* significantly reduced their numbers after sludge application (Figure 6.2-6.5). In any case, manure did not reduce the number of microarthropods significantly.

Vertical differences

After ploughing, a clear band of sludge or manure could be observed in the depth of 10-20 cm. Due to this uneven vertical distribution, the observed stimulation of microorganisms and microarthropods were generally more prevailing in this horizon. Observations half a year after ploughing showed e.g. large numbers of *F. fimetaria* together with enchytraeids and nematodes in the sludge lumps. Lübben and Glockemann (1993) reported similar increases in *F.* fimetaria with extremely high stimulation by sludge. In their case the sludge was milled into the top soil. Similar huge increases in *F. fimetaria* have been observed on manure clods (Van de Bund 1965) and is probably also found on the remains of the manure lumps in our study. Although, based on less data in 10-20 cm, stimulation was observed in both horizons. Therefore, it is likely that the conclusion made on the observations in the first 5 cm can be extrapolated to the deeper layer. However, possible vertical differences is an aspect that may needs further investigation in a later project, e.g. is it not possible to exclude a build up of toxicant in the unmixed layer just below the ploughing layer.

Reproduction of Collembolan in the laboratory

Laboratory tests using the Collembolan *F. fimetaria* have shown negative effects of fresh sludge within an acute time perspective (this report and Krogh *et al.* 1996). However these adverse effects occurred at levels of sludge which are above the normal used dosage of 3-4 t ha^{-1}. When *F. fimetaria* was forced to live in close contact with a lump of sludge or cattle manure in Petri dishes, an almost complete inhibition of the reproduction was observed. This may have been caused by ammonia or other volatile compounds disturbing the sensory system, thereby changing the sexual behaviour, or by direct effect on the reproduction by trace pollutants present in the sludge. In contrast to reproduction, growth in *F. fimetaria* was not affected, and although the Collembola, when compared to baker´s yeast, reduced the time spend in contact with sludge as the food source, they were apparently feeding on the lumps of sludge and cattle manure. Therefore, the inhibition of reproduction is not necessarily only a result of a lower nutritive value of sludge and cattle manure, but may just as well be caused by toxic compounds or a combination of these and other aspects. The exact mechanism should be investigated in detailed experiments carefully designed to answer the question. For earthworms similar inhibition and even mortality have been reported for fresh sludge (e.g. Edwards and Bohlen 1996), suggesting that perhaps very basic chemical characteristics of fresh sludge and manure inhibit some larger invertebrates (meso- and macrofauna).

When the reproduction of Collembola is tested in laboratory studies, the general trend is an increasing inhibition of reproduction with increasing dose of sludge, whereas no effects were seen for survival and growth of adults (Krogh *et al.* 1996). Thus, there is some discrepancy between the conclusions derived from the laboratory studies and the observations made in the field.This may be explained by the homogeneous mixing of sludge in the laboratory, which may results in a rapid exposure to the total load of toxic chemicals compared to a slower release from the lumps present in the field after ploughing. Furthermore, the distribution of sludge lumbs in a relatively fixed layer in the field gives the springtails the choice of only seeking the sludge when feeding and by this behaviour reduce the time spend in contact with the sludge as observed in the preference experiments.This will by all means reduce the exposure to the toxic chemicals found in sludge.

Earthworms

Studies of earthworms have shown that species common in agricultural soil (*Lumbricus terrestris, Allolobophora chlorotica*) are stimulated by sludge application, probably because the high content of fats, sugars and protein in sludge is a good food source for earthworms (Hamilton *et al.* 1988, Tomlin *et al.* 1993, Cuendet and Ducommun 1990, Kobel-Lamparski and Lamparski 1986). Most of the sludge produced in sewage plants is anaerobic, and when fresh, it is toxic to earthworms. However, when the sludge is spread on land or mixed into soil the toxicity rapidly disappears (Edwards and Bohlen 1996). Results from the present investigation shows the same overall picture: both in field experiments and in laboratory studies of reproduction and growth of *A. calignosa* only positive effects from sludge were seen. Doses up to 21 t ha^{-1} applied in the field had caused an increase of the number and biomass of earthworms after six months. Growth and reproduction were increased at sludge concentrations corresponding to the doses used in the field. The effects of cattle manure on the earthworm populations were not tested in this study.

Conclusions

In the present study two types of sludge was used, both having a relatively high content of heavy metals, nonylphenol, LAS and phthalates (Table 4.1 and 5.4). At doses up to 21 t ha^{-1}, which are 5 to 10 times higher than the average application rate used in Denmark, there were apparently no negative effects on soil fauna or microbial ammonium oxidation rate. Based on these results and results of other published investigations, it is therefore not likely that sludge application to Danish soils will decrease the decomposition rate in the soil. On the contrary, application of sludge increased the standing crop of soil fauna which, from the farmers point of view, is beneficial. Nevertheless, sludge application will most certainly alter the structure of the soil ecosystem, by being beneficial to particular species or group of organisms. For example, the stimulation of earthworms will influence the soil structure by bioturbation (soil tunnel-ling, creation of a better aggregate stability etc.), which may alter the composition of species in other groups. However, compared to the perturbations otherwise present in agricultural soils due to e.g. cultivation and crop rotation, the change caused by sludge application seems on short term basis to be of minor practical or environmental concern. A continuation of the ongoing study of ecological effects of sludge for another two/three years will help to give more information of the long-term effects of continuous sewage sludge application.

8 References

Agresti, A. (1990). Categorical data analysis. Wiley.

Alexander, M. (1977). *Introduction to Soil Microbiology*, 2nd ed., J. Wiley & Sons, New York.

Belser, L.W. and Mays, E.L. (1980). Specific inhibition of nitrite oxidation by chlorate and its use in assessing nitrification in soils and sediments. *Appl. Environ. Microbial.* **39**, 505-510.

Brendecke, J.W., Axelson, R.D., and Pepper, I.L. (1993). Soil microbial activity as an indicator of soil fertility: Long-term effects of municipal sewage sludge on an arid soil. *Soil Biol. Bioc.* **25**, 751-758.

Cuendet, G. and Ducommun, A. (1990). Peuplements lombriciens et activité de surface en relation avec les boues d'épuration et autres fumeres. *Revue suisse Zool.* **97**, 851-869.

Edwards, C.A. and Bohlen, P.J. (1996). Biology and Ecology of Earthworms. Chapman & Hall, NY. Pp. 426.

Fließbach, A., Martens, R., and Reber H.H. (1994). Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. *Soil Biol. Bioc.* **26**, 1201-1205.

Glockemann, B., and Larink, O. (1989). Einfluß von Klärschlammdüngung und schwermetallbelastung auf Milben, speziell Gamasiden, in einem Ackerboden. *Pedobiologia* **33**, 237-246.

Hamilton, W.E., Dindal, D.L., Parkinson, C.M. and Mitchell, M.J. (1988). Interaction of earthworm species in sewage sludge amended soil microcosms: *Lumbricus terrestris* and *Eisenia fetida*. *Journal of Applied Ecology* **25**, 847-852.

Holmstrup, M. & Krogh, P.H. (1996). Effects of an Anionic Surfactant, Linear Alkylbenzene Sulfonate, on Survival, Reproduction and Growth of the Soil-living Collembolan *Folsomia fimetaria*. *Environmental Toxicology and Chemistry*, **15**, 1745-1748.

Kobel-Lamparski, A. and Lamparski, F. (1986). Die Auswirkung von Klärschlamm auf Oberbodenstruktur und Regenwürmerfauna eines Buchenwaldes. *Verhandlungen der Gesellschaft für Ökologie* (Hohenheim 1984). Band XIV 1986.

Kristensen, P., Tørslev, J., Samsøe-Petersen, L. and Rasmussen, J.O. (1996). Use of organic waste products for application on farmland. [Anvendelse af affaldsprodukter til jordbrugsformål]. With an English summary and recommendations. Report from the Danish EPA]. Miljøprojekt 328. Miljø- og Energiministeriet, Miljøstyrelsen.

Krogh, P.H. (1995). Does a heterogeneous distribution of food or pesticide affect the outcome of toxicity tests with Collembola? *Ecotoxicology & Environmental Safety* **30**, 158-163.

Krogh, P.H. & Bruus Pedersen, M. (1997). Ecological effects assessment of industrial sludge for microarthropods and decomposition in a spruce plantation. *Ecotoxicology & Environmental Safety. In press.*

Krogh, P.H., Holmstrup, M., Jensen, J. & Petersen, S.O. (1996). Økotoksikologisk vurdering af spildevandsslam i landbrugsjord. [Ecological assessment of sewage sludge on farm land - Report from the Danish EPA. In Danish with English summary]. Arbejdsrapport Nr. 43, 53 pp. Miljø- og Energiministeriet, Miljøstyrelsen

Kula, H., Heimbach, U. & Løkke, H. (Eds). (1995). Progress Report 1994 of: SECOFASE. Third Technical Report. National Environmental Research Institute, Denmark. 166pp.

Ladd, J.N., Foster, R.C. and Skjemstad, J.O. (1993). Soil structure: carbon and nitrogen metabolism. Geoderma 56: 401-434.

Lübben, B. (1989). Influence of sewage sludge and heavy metals on the abundance of Collembola on two agricultural soils. In: Dallai, R. (Ed.). University of Siena. 3rd International seminar on Apterygota. Pp. 419-428.

Lübben, B. and Glockemann, B. (1993). Untersuchungen zum Einfluß von Klärschlamm und Schwermetallen auf Collembolen und Gamasiden im Ackerboden.In: Ehrnsberger, R. (Ed.). Bodenfauna und Naturschutz. Bedutung und Auswirkungen von Antropogenen Maßnahmen. Informationen zu Naturschutz und Landschaftspflege **6**, 261-278.

Norberg-King, T.J. (1993). A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach. (Version 2.0). July 1993. National Effluent Toxicity Assessment Center. Technical Report 03-93 Environmental Protection Agency. Environmental Research Laboratory-Duluth, Duluth, Minnesota 55804.

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

Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution* 43, 223-225.

SAS Institute Inc. (1990). SAS/STAT® User's Guide, Version 6 Fourth Edition, Volume 2, Cary NC: SAS Institute Inc., 846 pp.

Schmidt, E.L. & Belser, L.W. (1982). Nitrifying bacteria. In: A.L. Page *et al.* (ed.) *Methods of Soil Analysis Part 2, Chemical and Microbiological Properties*, Am. Soc. Agron., Madison, WI, pp. 1027-1042.

Tomlin, A.D., Protz, R., Martin, R.R., McCabe, D.C. and Lagace, R.J. (1993). Relationship amongst organic matter content, heavy metal concentrations, earthworm activity, and soil microfabric on a sewage sludge disposal site. *Geoderma* **57**, 89-103.

Torstensson, L. (1993). MATS Guideline - Test 04. Ammonium oxidation, a rapid method to estimate potential nitrification in soil. In: Torstensson, L. (Ed.). Guidelines. Soil biological variables in environmental hazard assessment. Swedish Enviroinmental Protection Agency. Report 4262.

Van De Bund, C.F. (1965). Changes in the soil fauna caused by application of insecticides. Bolletino di Zoologia Agraria e di Bachicoltura, **7**, 185-212.

Weiss, B., and Larink, O. (1991). Influence of sewage sludge and heavy metals on nematodes in an arable soil. *Biology and Fertility of Soils* **12**, 5-9.

Zar, J.H. (1984). *Biostatistical Analysis*, 2nd Ed., Prentice-Hall Int. Ltd., London, UK.

ISSN 0908-9195 ISBN 87-7810-865-9
Price (incl 25% VAT): DKK 75,-
Distributed by: "Miljøbutikken" - Books and Information
Phone: +45 33 37 92 92 Fax: +45 33 92 76 90

Miljø- og Energiministeriet **Miljøstyrelsen**
Strandgade 29 · 1401 København K · Telefon 32 66 01 00