Examensarbeten **2008:3**

Institutionen för skogens ekologi och skötsel

Heavy metal accumulation in voles, shrews and snails after fertilisation with pelletized and granulated municipal sewage sludge

Ackumulering av tungmetaller i sork, näbbmus och skogssnigel efter skogsgödsling med pelleterat avloppsslam

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This report presents an MSc thesis at the Department of Forest Ecology and Management, Faculty of Forest Sciences, SLU. The work has been supervised and reviewed by the supervisors, and been approved by the examiner. However, the author is the sole responsible for the content.

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Sammanfattning

Syftet med denna studie är att undersöka om bioindikatorerna skogssork (*Myodes glareolus),* vanlig näbbmus *(Sorex araneus)* och brun skogssnigel *(Arion subfuscus)* ackumulerar tungmetaller efter gödsling av skog med värmebehandlat och pelleterat eller granulerat avloppsslam. Försöksområdet ligger nära Bäcksjön, Umeå, i Västerbottens län. Det dominerande trädslaget är tall (*Pinus sylvestris*) med inslag av gran (*Picea abies*), skogstypen är blåbärsristyp på frisk mark. I maj 2003 anlades ett replikerat randomiserat blockförsök med slampellets och slamgranuler i olika doser, samt kontrollytor. Behandlingsytorna var 46 x 46 meter. Doseringen för pelleterat slam från Umeås reningsverk var 5,3 ton/ha, 6,4 ton/ha och 16,1 ton/ha. Granulerat slam från Himmerfjärdens reningsverk, Stockholm, spreds i doserna 4,5 ton/ha och 13,6 ton/ha. Ett kompletterande försök med behandlingsytor om ett hektar vardera lades ut i juni 2006. Detta försök innefattade försöksleden 12 ton/ha (granulerat slam från Himmerfjärdsverken), samt kontroll, och hade tre upprepningar.

Fångst av bioindikatorer skedde under augusti månad 2006. Njurar från skogssork och vanlig näbbmus, samt hela skogssniglar, analyserades med avseende på tungmetallkoncentrationer.

Resultaten visar få signifikanta skillnader mellan behandlingarna för skogssork och vanlig näbbmus. För brun skogssnigel däremot visar resultaten på förhöjda halter av flertalet ämnen. Anmärkningsvärt för samtliga bioindikatorer är att signifikant skillnader för Cd och Pb visar lägre halter efter gödsling jämfört med kontroll. Som kovariat vid ANOVAn användes kroppsvikten för bioindikatorerna, denna visar på flertalet negativa samband. Speciellt tycks B, Si och Sn minska med ökad kroppsvikt för samtliga bioindikatorer. För vanlig näbbmus tycks även V minska med ökad kroppsvikt.

Abstract

The purpose with the study was to determine whether or not there is any bioaccumulation of heavy metals after fertilising with pelletized or granulated dried sewage sludge. For this purpose, three bioindicators were sampled, i.e. *Myodes glareolus* (bank vole), *Sorex araneus* (common shrew) and *Arion subfuscus* (dusky slug).

The experimental site is situated within the boreal forest zone near Bäcksjön, Västerbotten County. In May 2003 ten plots (46 x 46 meter) were fertilised with different doses of pelletized and granulated sewage sludge and two additional plots were selected as controls. Another trial was established in early June 2006 when three large plots (100 x 100 meter) were selected for fertilising with sludge granules and three were selected as controls.

In this study the analyses of kidneys from bank vole and common shrew show no consistent accumulation of heavy metals after fertilising with sewage sludge. Dusky slug on the other hand had significantly higher concentrations of several elements from fertilised plots. All significant results with Cd and Pb showed a lower concentration from Fertilised plots compared with Control plots. With bodyweight as a covariate several negative correlations were found. In common for all bioindicators is that B, Si and Sn seem to decrease in concentration with higher bodyweight. For common shrew also V seems to decrease with higher bodyweight.

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Introduction

Sludge in forestry

In Sweden, about 1 000 000 ton of fresh weight (f.w.) sewage sludge are produced every year. This equals about 240 000 ton dry weight (d.w.), of which approximately 50 % is organic material. From the year 2005 deposition on landfills is prohibited and hence alternative ways of disposal are needed. Sewage sludge can be used as fertiliser or soil improver, given that it contains about 3 % phosphorus and 3,5 % nitrogen (Naturvårdsverket 2006). The sewage sludge is treated to reduce the number of pathogenic micro organisms and amount of easily decomposed organic material. The treatment creates a more stable sludge with lower risks for spreading of pathogens and a less disturbing odour. The most common technique for stabilisation of sewage sludge is anaerobic decomposition of the organic fractions. Energy is produced from the biogas produced in the process (Naturvårdsverket 2006). The processed sludge is centrifuged and dried before pelletzised or granulated (UMEVA 2006; SYVAB 2006). These pellets and granules can then be spread out in the forest as a fertiliser.

Sewage sludge provides additional organic matter to the forest floor which will increase the water- and nutrient holding capacity. Also, essential base cations (Na, K, Ca and Mg) are added with the application. The sludge also increases the cation and acid/base buffer capacities of the soil and raises the pH, which reduces the loss of base cations (Bramryd 2001). Further, Bramryd (2002) showed that 20 ton/ha, dry weight, of dewatered sewage sludge increased the content of N, P, K, Ca and Na in the soil. In another study by Harrison et al. (1994) on coarse-textured soil, the same pattern was shown except that there was an increase of Mg instead of Na. The increases in nutrients, especially N, gave a significantly higher tree growth rate for Scots pine up to six years after application compared to the control site (Bramryd 2001). Also, Luo & Christie (2001) found a significant increase in annual tree growth after two and three years in plots with sitka spruce *(Picea sitchensis)* when treated with 40 ton of biosolids per ha. (In the previous mentioned study, biosolids is alkaline stabilized sewage sludge solids made from rural and urban sewage sludge with 40-48 % d.w.) The basal area and the volume, of the studied trees, increased significantly after application (Bramryd 2001; Harrison et al. 2002; Kimberley et al. 2004). The height growth, however did not respond in the same extent (Bramryd 2001). Sewage sludge has been found to be a good slow release fertiliser for forestry, but the dose should be designed on a site-by-site basis considering stand age, tree species, distance to wetland, and so forth (Kāposts et al. 2000; Luxmoore et al. 1999; Henry et al. 1994; Dutch & Wolstenholme 1994). Bramryd (2002) stressed that it is a prerequisite that the concentrations of environmental pollutants in the sewage sludge are low enough. Also the location and properties of the fertilised site is important. The suitability of the site for sludge application depends on physical features like topography, transportation and access factors, vegetation, water resources and climate factors (Henry & Cole 1997). Additionally, depth of organic-rich topsoil in a forest would be very important in terms of binding cationic heavy metals applied via sewage sludge or other wastes (Sidle & Kardos 1977).

In Sweden the use of biofuel has doubled since the 1980s and the demand will most likely increase further. The energy production from biofuel is presently 100 TWh, which comprises 20 % of Sweden’s energy consumption. About 50 % of the total biofuel is wood fuel (wood, bark, needles and leaves) (Svenska Naturskyddsföreningen 2006). If sewage sludge can be used as a fertiliser to increase the forest production it would be favourable for the sewage treatments work and for the forest industry.

Even though the sewage sludge is said to be harmless (UMEVA 2006) a certain concern from the society still exists. Studies of vegetation have indicated that some metals increase in concentration after sludge application. Plant uptake of heavy metal is highly dependent on soil pH, organic matter, competing cations and plant species as well as the amount of heavy metals applied (Anthony & Kozlowski 1982; Labrecque et al. 1995). Dutch and Wolstenholme (1994) found increased concentrations of Zn and Cu in the foliage. Kāposts et al. (2000) detected elevated amounts of heavy metals in pine needles, berries and mushrooms. Bramryd (2001) found that in the mor layer the concentrations of Cr, Cu, Pb, Ni and Zn increased after application and still after eleven years significantly increased levels of Cu and Zn were found. Also Luo and Christie (2001) found heavy metal enrichment in the litter layer three years after application of biosolids. Many heavy metals are microelements and in small amounts they represent necessary components for the living organism (Kāposts et al. 2000). But when the concentration increases they can become toxic. The risk of bioaccumulation of heavy metals in the ecosystem is of great concern. Therefore, limit values and target values have been set up for heavy metals and certain organic compounds, respectively, to prevent the use of sewage sludge harmful for the environment (Naturvårdsverket 2006). Since the sludge composition changes between sources, and over time, sludge quality must be continuously monitored. Effect studies need to be expanded. Trophic level of the bioindicator must be considered when studying bioaccumulation because, for instance, Sidle & Sopper (1976) found that Cd could readily be transferred from the soil, to plants, and further on to primary and secondary consumers such as animals or humans.

Aim of the study

The purpose with this study was to determine if any bioaccumulation of heavy metals can be detected in the bioindicators bank vole (*Myodes glareolus* Schreber*)*, common shrew (*Sorex araneus* Linné*)* and dusky slug (*Arion subfuscus* Draparnaud*)*, after forest fertilisation with pelletized and granulated dry sewage sludge.

Bioindicators

In this study bank vole (*Myodes glareolus)*, common shrew (*Sorex araneus)* and dusky slug (*Arion subfuscus*) are used as bioindicators. High levels of heavy metals can cause toxicity in humans and animals, for example Cd has been associated with hypertension, emphysema, chronic bronchitis and in extreme cases death (Sidle & Sopper 1976). Pollutants often concentrate in the liver and kidney of mammals (Sidle & Sopper 1976; Lodenius et al. 2002) and increased quantities of heavy metals in bank vole have been found to extend the liver in size (Milton et al. 2003). Toxic effects in mammals were observed when kidney and liver Cd-levels exceed 274 and 465 µg/g dry weight respectively (Nickelson & West 1996). Johnson & Roberts (1977) found that heavy metals accumulate differently in different types of body tissues, for instance Cd was found to accumulate strongly in liver and kidneys, whereas Pb accumulated in the skeleton. Zn was rather evenly spread in kidney, liver, muscle, hair and bone tissue (Johnson & Roberts 1977).

Bank vole (Picture 1) is a common herbivore in the boreal forest and feed on high quality food such as seeds, fruits and forbs (Löfgren 1995). Herbivores are exposed to heavy metals through food intake and studies have shown that concentration of Zn, Cd and Cu were higher in plants fertilised with sewage sludge compared with plants in control areas (Dressler et al. 1986). In a waste-water irrigated habitat the concentration of heavy metals in meadow vole (*Microtus pennsylvanicus*) indicated no accumulation (Anthony & Kozlowski 1982). But other studies with sewage sludge treatments have shown a significant accumulation of Cd and a slight accumulation of Cu in liver and kidney (Andersson et al. 1982).

Common shrew (Picture 2) is reported to have higher metabolic rate and different diet compared to rodents and this is stated to cause a higher heavy metal accumulation (Hegstrom & West 1989; Świergosz-Kowalewska et al. 2005). It is clear that the accumulation depends on both metal species and trophic level (Hegstrom & West 1989). Cd tends to accumulate more in the liver and kidney of common shrew than bank vole (Świergosz-Kowalewska et al. 2005). Even after 11 and 15 years after application of biosolids (anaerobically digested municipal sewage sludge) the shrews had elevated levels of Cd (Nickelson & West 1996). Since the shrew feed on invertebrates, mainly earthworms, some studies have been made on earthworm's ability to accumulate heavy metals. Kruse & Barrett (1985) reported that earthworms *(Lumbricus rubellus)* tend to bioaccumulate heavy metals such as Cd, Pb and Cu. In that study of municipal sewage sludge fertilisation the Cd level was as much as nine times higher than the values from controls. In a Pb-contaminated environment both earthworms and shrews showed elevated levels of Pb (Reinecke et al. 2000).

Dusky slugs (Picture 3) are detritivorous and herbivorous organisms, and are therefore exposed to both soils and plant contaminants and integrate these two routes of exposure, as concluded from a study of *Helix aspersa* snails (Scheifler et al. 2003). In a comparison between the snail species *Arion subfuscus* and *Deroceras reticulatum* it was evident that both species accumulated metals in a contaminated area (Greville & Morgan 1991). Zinc was shown to be transferred from sewage sludge amended soil to snails, as reported by Scheifler et al. (2003).

Picture 1: *Myodes glareolus*
Photo: http://www.biopix.dk

Picture 2: *Sorex araneus*
Photo: http://www.biopix.dk

Picture 3: *Arion subfuscus*
Photo: http://www.biopix.dk

Materials and methods

Field work

Experimental site

The studied site is situated within the boreal forest zone near Bäcksjön, Västerbotten county (WGS84 Lat/Long: N 63º 56' 40.62" E 20º 24' 30.35") in Sweden. Scots pine *(Pinus sylvestris* Linné*)* is the dominating tree species, but some understory spruce *(Picea abies* (L.) Karst*)* occur especially in the west part of the experimental area. The field layer and ground vegetation is composed of ericaeous dwarf shrubs, feather mosses and some lichens in the drier parts. The soil is a sandy till with a surface layer of coarse sand, gravel and cobbles, due to wave-washing in connection to postglacial land rise.

In May 2003 12 plots, each 2116 m^2, were treated with sewage sludge pellets or granules (Table 1). The fertilisation trial is a randomised block design with two replications (blocks). The plot size is 46 x 46 meters. The pellets were produced at Umeå sewage treatment work and were applied in three different doses (5,3 ton/ha, 6,4 ton/ha and 16,1 ton/ha). The granules originated from Himmerfjärden sewage treatment work, Stockholm, and were applied in two doses (4,5 ton/ha and 13,6 ton/ha). Two block control plots were included in the sampling (Figure 1).

Table 1. Elemental analysis of dry pellets and granules from Umeå and Himmerfjärden respectively. (Data from analyses made 2003 at the Soil Science Laboratory, Dept of Forest Ecology and Management, SLU. (Kenneth Sahlén)).

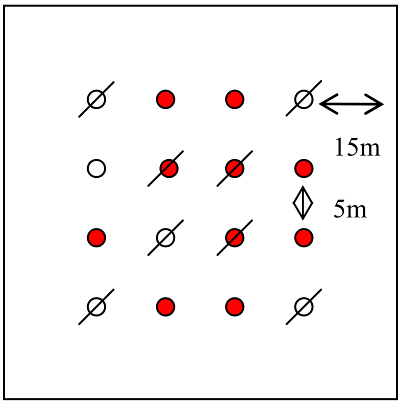
Elements	Pellets (mg/kg) Analyse	Granules (mg/kg) Analyse	Elements	Pellets (mg/kg) Analyse	Granules (mg/kg) Analyse
Al	0,9	1,6	Mn	490	270
As	3,6	4,5	Mo	3,4	6,7
B	17	20	Na	0,03	0,06
Ca	1,8	2,5	Ni	19	24
Cd	0,8	1,3	Pb	15	32
Co	8,6	8,0	P	4,1	4,2
Cr	18	40	S	0,97	1,3
Cu	130	390	Zn	115	870
Fe	13	6,2	NH4	1980	7270
K	0,09	0,20	NO3	0	5
Mg	0,26	0,52	pH	6,32	7,85

In early June 2006 a complementary fertilisation trial, with 1 ha plots, was established in the same experimental area as the old trial. Among six plots three were selected for treatment with 12 ton/ha granulated sewage sludge (92 % d.m.) from Himmerfjärden sewage treatment work and three plots were defined as control plots (Figure 1).

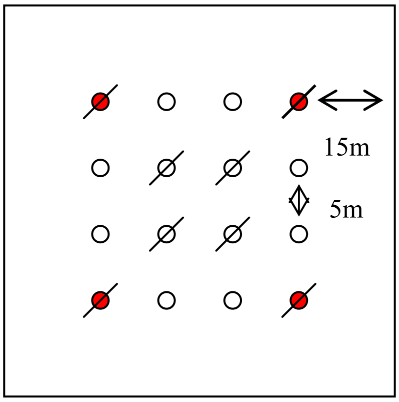
The 2003-fertilised experiment represents a situation where possible treatment effects mainly depend on heavy metal uptake by the vegetation. The sludge material is decomposed, disintegrated, or to some extent buried within the mor layer. In the newly established 2006-fertilised experiment, the sludge granules were exposed and almost unaltered. Therefore, transfer of heavy metals through direct ingestion by animals, if occurring, would be included in this experiment.

Sampling

During try-out capture of voles and shrews on the 3-6 June 2006 live-capture traps were placed out on the 2003-fertilised plots at Bäcksjön. Plastic sticks were placed out at the nodes of a squared grid centred in each plot (Figure 2). The distance from the plot-border to the grid in the plot centre measured about 15 meter. The grid-patterns contained 16 nodes (4 rows and 4 columns), placed 5 meter from each other. At each stick a trap was placed, baited with ¼ of a fresh apple, ~ 4 cm^2 seed-wax bait and a piece of nesting material. The traps were then controlled every morning and every night for three days.



Plot 1-5



Plot 6-12

Figure 2. Grid pattern for 2003-fertilised plots, the lines across the circles shows were the snail-trap cups were placed. The only difference between the two is the colour of the sticks which functioned as a support to identify the individual stick numbers if the tags would disappear.

On the 31 of July the second round of trapping started, but this time with snap traps. In the 2006-fertilised plots a squared grid with 10 meter to the plot border were placed out (Figure 3). The grid contained 64 nodes/sticks, 8 rows and 8 columns, and the distances between the sticks were 10 meter. Plastic cups (210 cl) were placed in the ground in row 3 and 6, for the trapping of snails. The 2003-fertilised areas also got plastic cups beside each corner stick and beside the four sticks in the centre, in total eight in each plot (Figure 2). Three traps at each stick were baited with seed-wax bait and set very carefully before they were placed out under the dwarf shrub. Initially the 2003-fertilised plots were trapped for three nights. The traps were emptied every morning and every evening, and the catch were placed in plastic bags with ID numbers and then placed in a cooling bag. The captured animals were transferred to a -80ºC freezer within two hours, until dissection. The same procedure was subsequently repeated in the 2006-fertilised area.

Regarding the trapping methods used, Hansson and Hoffmeyer (1973) could not find any significant difference in number of trapped bank vole, when comparing snap- and live-traps. The captures of common shrew on the other hand show a significant difference both between traps and season. For common shrew the live-traps were said to be more efficient (Hansson & Hoffmeyer 1973). In this study, however, differences in trapping success between different trapping campaigns were not the issue investigated.

The snail traps, plastic buckets filled with water, glycol, beer and a drop of soap, were placed out August 22 and emptied after three days (August 25). The collected snails were placed in marked plastic bags, placed in a cooling bag and finally transferred to the freezer.

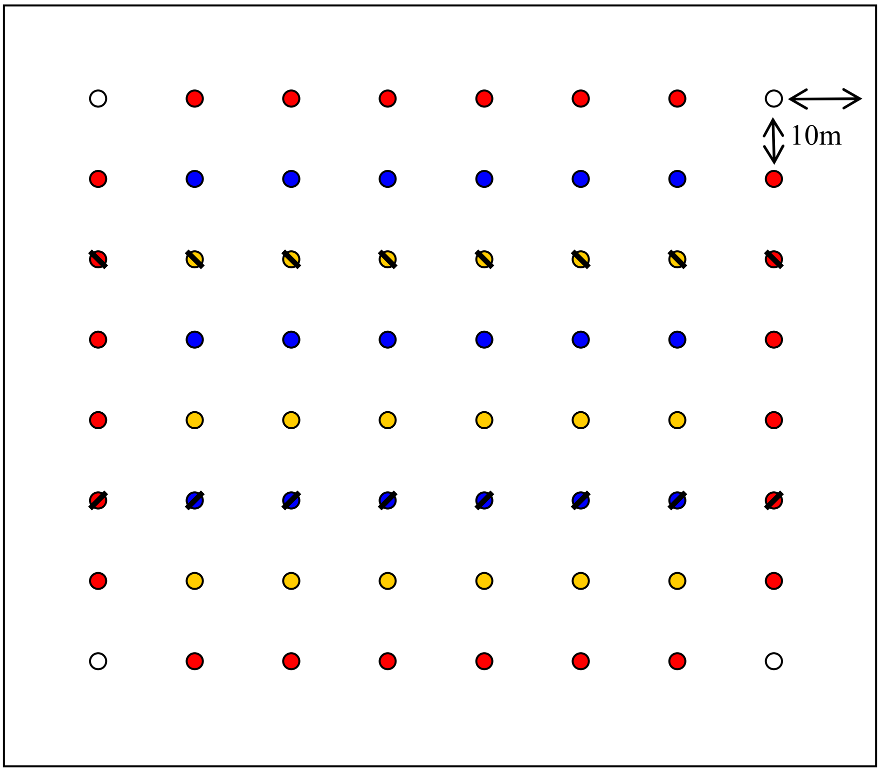
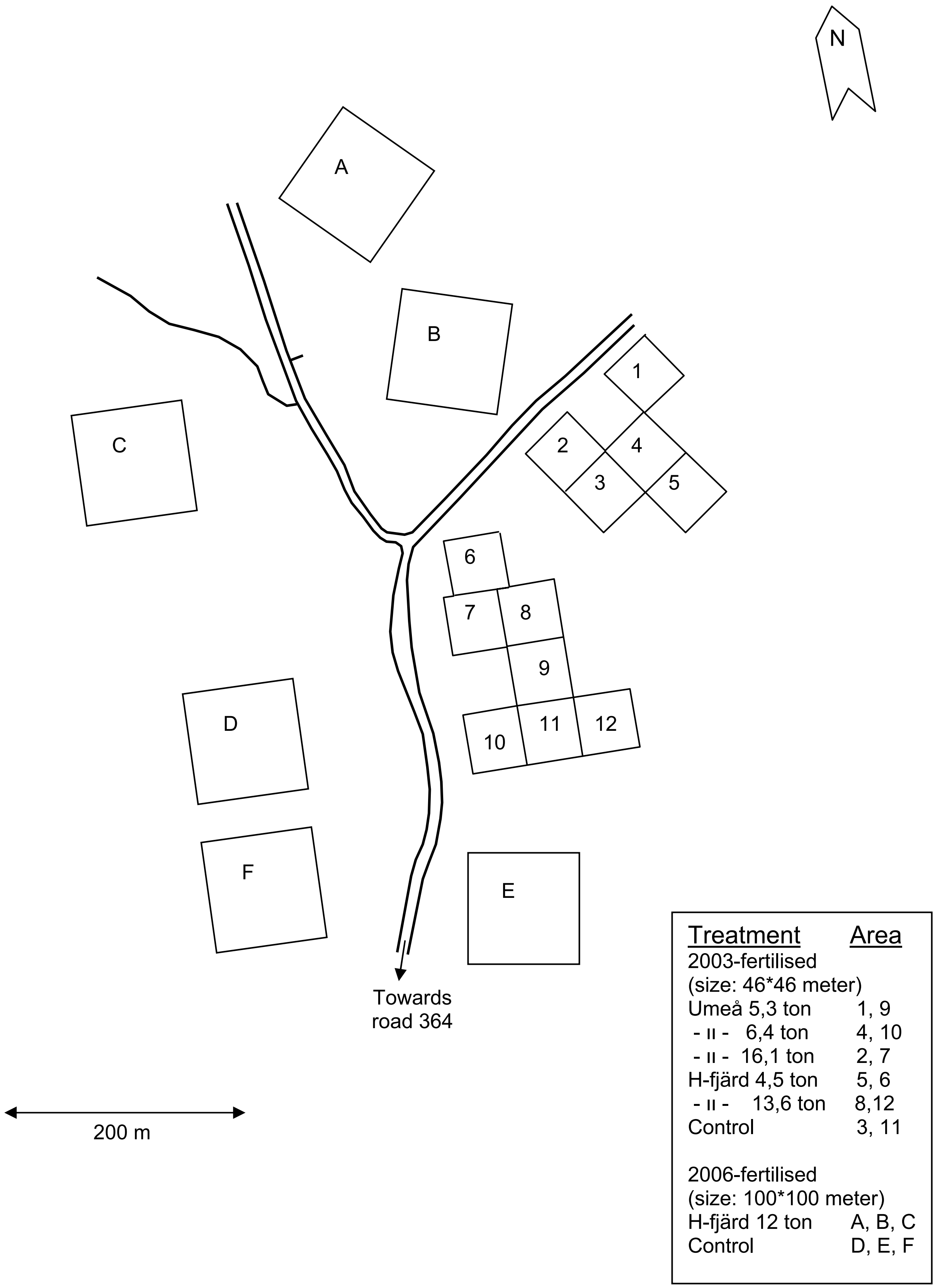


Figure 3. Grid pattern for 2006-fertilised plots, the lines across the circles shows were the snail-trap cups were placed. The different colours of the sticks functioned as a support to identify the individual stick numbers if the tags would disappear.

Figure 1.

Bäcksjön- experimental site map



Sample treatment

Dusky slugs were cut out from the plastic bags with a piece of plastic left underneath them and then placed in aluminium forms. They were dried in + 70º C for 48 hours. After drying they were weighed, grained in a ball mill and placed in Eppendorf tubes. All dusky slugs from one plot were combined into one composite sample. For the 2003-fertilised plots one or two subsamples were analysed (two subsamples if sufficient amount of material) and for the 2006-fertilised plots four subsamples were analysed from each plot.

Bank vole and common shrew were dissected, the kidneys were placed in separate Eppendorf tubes and dried in + 70º C for about 72 hours. All mammals were analysed as individual samples but for the plots were more than five individuals were caught only five individuals were randomly selected for analysis. Every available kidney-pair were analysed from the 2003-fertilised plots and for the 2006-fertilised plots five samples were analysed at the most.

Analytical method

The samples were dissolved in 2 ml HNO_3, heated to + 180º C for 15 minutes and + 180º C for 9,5 minutes. After that the samples were analysed with ICP/MS-DRC – Elan 6100, PerkinElmer. The recommended amount of dried analysing material was 500mg, but since the dry weight of kidneys and snails was low the amount of digesting solution was proportionally adjusted.

The analysed substances were aluminium (Al), iron (Fe), boron (B), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), silicon (Si), tin (Sn), vanadium (V) and zinc (Zn). In the following text the entire group of analysed microelements will somewhat incorrectly be included in the term “heavy metals”, although Al and B are no heavy metals.

Certified calibration substances used were BCR 185 Bovine liver and NIST 1515 Apple. The measurement uncertainty for the analysis is the combined sum of instrument-related error components that contributes to the uncertainty in the analyse value. The estimation of the uncertainty follows the international recommendations of GUM (Guide to the expression of uncertainty in measurements). It is calculated as the standard deviation of repeated determinations, multiplied by two. The detection limit is here defined as the measurement uncertainty multiplied by two. The approximate detection limits, for low levels of each element, were as follows (mg kg^{-1}, d.w.): Al=1.0; B=2.2; Cd=0.30; Co=0.04; Cr=1.1; Cu=1.3; Fe=44; Mn=0.0.75; Mo=0.38; Ni=1.0; Pb=0.14; Se=1.4; Si=20; Sn=2.5; V=0.20; Zn=11.5. For bank vole and common shrew and the elements Ni, Sn and V, a large part of the analysed objects were lower than the defined detection limits, but higher than the measurement uncertainty (i.e. greater than zero). Also dusky slug had elements below detection limits but above zero, i.e. Se and Sn. This means that the reported concentration levels for these elements are more uncertain (cp. detection levels and results in Appendix Tables 5 – 10).

Statistical analysis

Treatment effects were evaluated, for each indicator species/each trial, in ANOVA models (General Linear Model). The main model used had the element as a response variable and sludge treatment/dose as the main factor. In another round of ANOVA (General Linear Model) tests the type of sludge (pellets or granules) was used as the main factor. Both trials were evaluated with bodyweight of indicator organism as covariate. Also, bodyweight was used as a coarse assessment of specimens' age. Significance tests of differences between factor levels were done with Tukey's multiple comparison tests. As a complement to the ANOVA linear regression analyses with element versus fertilising dose in N (kg/ha) were done for the 2003-fertilised plots including the extern control. Basic data treatment was done in Microsoft Excel and the statistical analyses in Minitab 14.

When statistically analysing the 2003-fertilised plots the number of observations per species at each treatment level were somewhat unbalanced and significant treatment effects were not apparent (Appendix Table 1 and 3). Treatments were then re-grouped into "High" and "Low" doses to find out if there was a difference between these groups. The new treatment groups included a greater number of observations (bioindicators), which might give more reliable results. The Low dose include 5,3 ton/ha, 6,4 ton/ha and 4,5 ton/ha and the High dose include 13,6 ton/ha and 16,1 ton/ha. A further fusion of treatments, i.e. all observations from sludge Fertilised plots versus all observations from Control plots, was also tried. For the 2006-fertilised plots the treatments were grouped into Control (K 2) and Fertilised, the number of observations per species is presented in Appendix Table 2 and 4.

Both control plots (K 1) in the 2003-fertilised trial are bordering heavily fertilised plots in one direction, giving some uncertainty regarding their status as controls for vole and shrews (Figure 1). Therefore, the controls (K 2) for the 2006-fertilised trial were included in the statistical evaluation of the 2003-trial as external controls to check if K1 controls were valid. These external controls were located ca 100-300 meter S-W of the 2003-fertilised trial area.

Results

2003- Fertilised plots

M. glareolus (bank vole)

In the comparison between Fertilised and Control for bank vole, significant difference ($p<0,05$) between the treatments were revealed for three elements, i.e. B, Se and Zn (Table 2). The results for a fixed set of toxic heavy metals, i.e. Cu, Zn, Cd, Pb, Cr, Ni, B, Mo, Se and Sn are illustrated in figure 4-8. The ANOVA model comparing High dose, Low dose and separate Controls (K 1 and K 2) was significant for four elements, but only for three elements were any factor levels significantly different according to Tukey´s multiple comparison test (Table 3). Adding bodyweight as a covariate factor to this model, the five elements B, Cu, Si, Sn and Zn all showed significantly lower concentrations with increasing bodyweight. Another ANOVA model was tested to find out if there were any differences between pellets and granules, but no significant differences was found. Linear regressions analyses with the different elements versus the fertiliser dose of N (kg/ha), were also made. Se, B and V concentrations increased with N dose, among which Se had the most significant regression ($n=28$; $r2= 0,301$; $p= 0,003$) (Figure 9). Thus, Se consequently increases with higher amount of sewage sludge applied.

Table 2. Elements with significantly different mean values in the comparison between Fertilised and Control (K 1 + K 2).

Element (response variable)	Significant difference
B	Fertilised > Control
SE	Fertilised > Control
ZN	Fertilised < Control

Table 3. Elements with significantly different mean values in the comparison between High dose, Low dose and Controls (K 1 and K 2 separately).

Element (response variable)	Significant difference
B	no difference
SE	High and Low dose > K 2
SN	High dose < Low dose > K 2
V	High dose > K 1

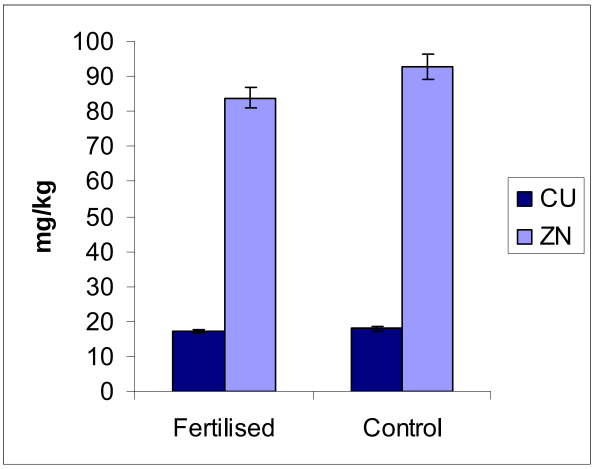


Figure 4. *M. glareolus* mean value and SE for Cu and Zn. The Control for Zn is significantly higher than Fertilised. (Fertilised n=15, Control n=13).

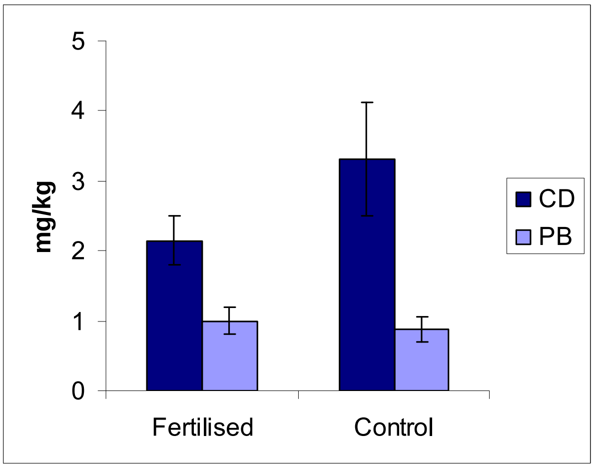


Figure 5. *M. glareolus* mean value and SE for Cd and Pb. In Fertilised plots n=15 and in Control plots n=13.

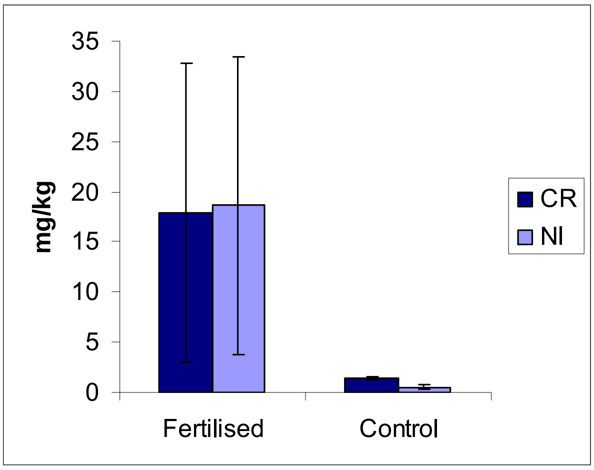


Figure 6. *M. glareolus* mean value and SE for Cr and Ni. In Fertilised plots one vole (the same as for Mo had extremely high concentration both Cr and Ni relative to the other voles. In Fertilised plots n=15 and in Control plots n=13.

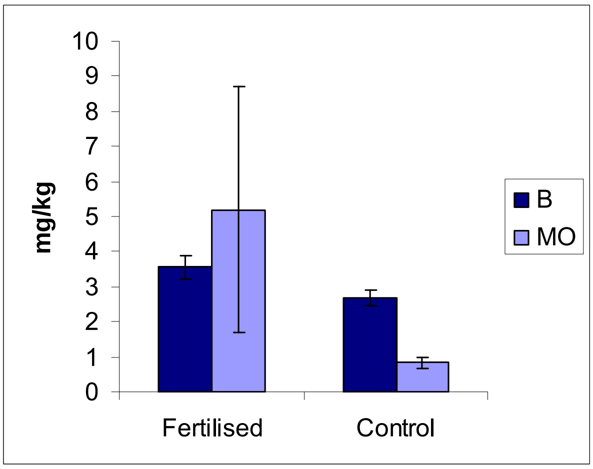


Figure 7. *M. glareolus* mean value and SE for B and Mo. In Fertilised plots one vole (the same as for Cr and Ni) had extremely high concentration of Mo relative to the other voles. In Fertilised plots n=15 and in Control plots n=13.

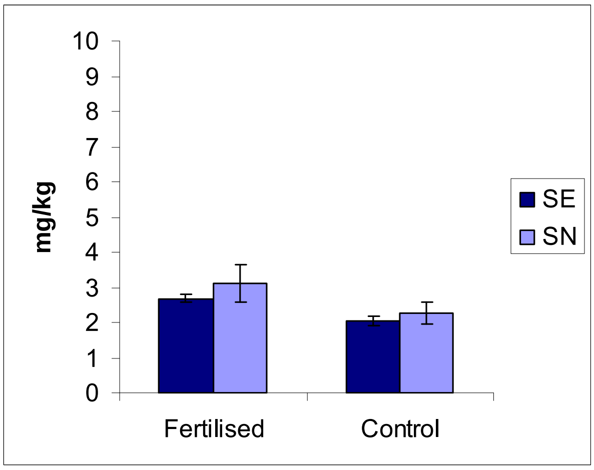


Figure 8. *M. glareolus* mean value and SE for Se and Sn. In Fertilised plots n=15 and in Control plots n=13.

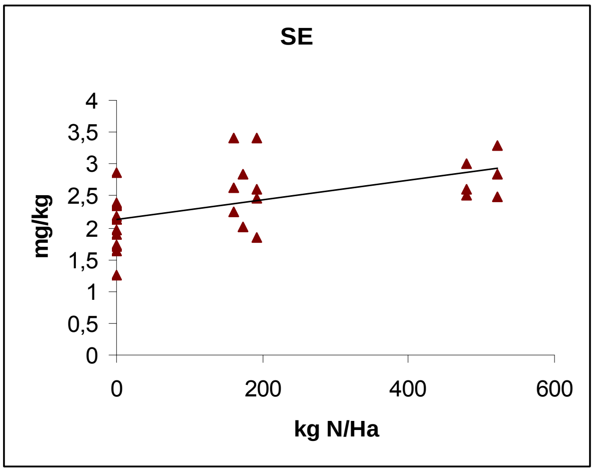


Figure 9. *M. glareolus* Regression analyse for Se.

S. araneus (common shrew)

Significant differences were found between treatment levels Fertilised and Control, both showing elevated levels from Control plots (Table 4). Mean values for the elements with significant difference are illustrated in figure 10, 11 and 13. For the other heavy metals no significant treatment difference were found (Figure 10-14). In the ANOVA with High dose, Low dose and Controls (K 1 and K 2), there were three elements with significant differences between levels (Table 5). With bodyweight as a covariate Al, B, Si, Sn and V gave significant p-values. They all showed that a higher bodyweight gives a lower concentration of the element in the kidneys. In the comparison between pellets and granules there were no significant differences. A linear regression analysis was made between elements and amount of N applied per ha. Fe and Pb showed negative correlations with increasing N dose of which Pb was the most significant (n= 30 ; r2= 0,247 ; p= 0,005) (Figure 15), thus the amount of Fe and Pb decreases with higher dos of N and consequently a higher dose of sewage sludge.

Table 4. Elements with significantly different mean values in the comparison between Fertilised and Control (K 1 + K 2).

Element (response variable)	Significant difference
CU	Fertilised < Control
PB	Fertilised < Control

Table 5. Elements with significantly different mean values in the comparison between High dose, Low dose and Control (K 1 and K 2 separately).

Element (response variable)	Significant difference
B	Low dose > K2
MN	High, Low and K 2 < K 1
PB	High and Low dose < K 1

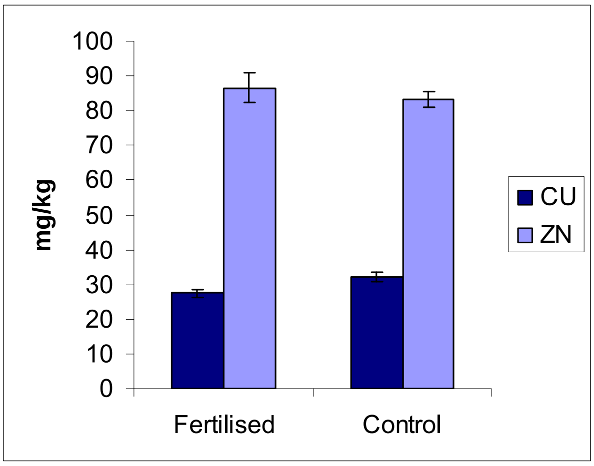


Figure 10. *S. araneus* mean value and SE for Cu and Zn. The Control for Cu is significantly higher than Fertilised. Fertilised n=21 and Control n=9.

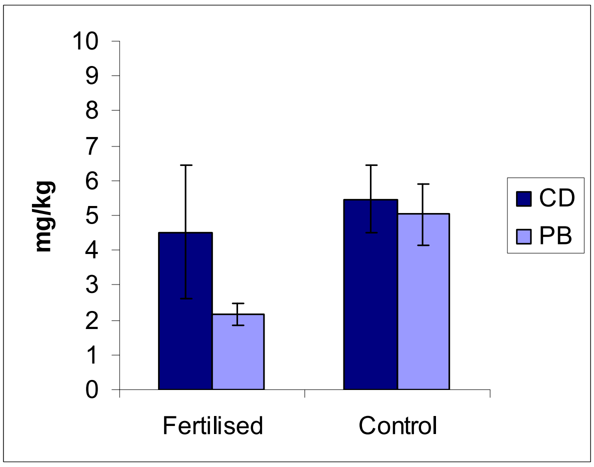


Figure 11. *S. araneus* mean value and SE for Cd and Pb. The Control for Pb is significantly higher than Fertilised. Fertilised n=21 and Control n=9.

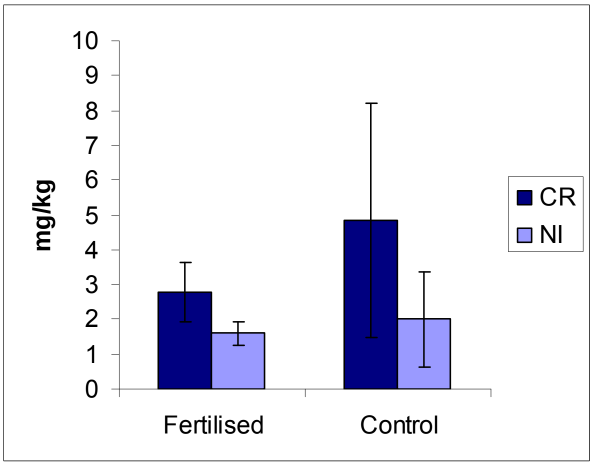


Figure 12. *S. araneus* mean value and SE for Cr and Ni. In Control plots one common shrew had much higher concentration of Cr compared to the other from Control plots. In Fertilised plots n=21 and in Control plots n=9.

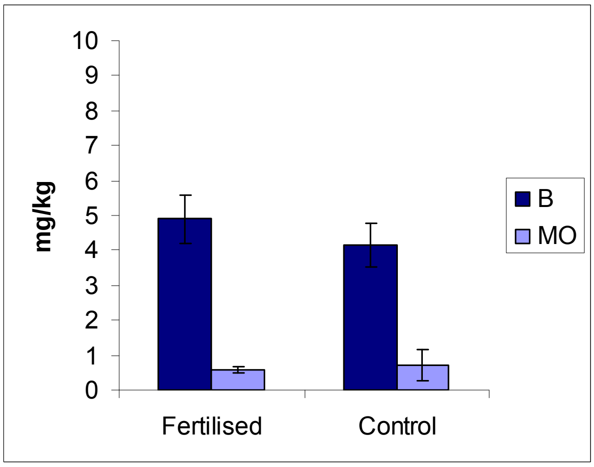


Figure 13. *S. araneus* mean value and SE for B and Mo. In Fertilised plots n=21 and in Control plots n=9.

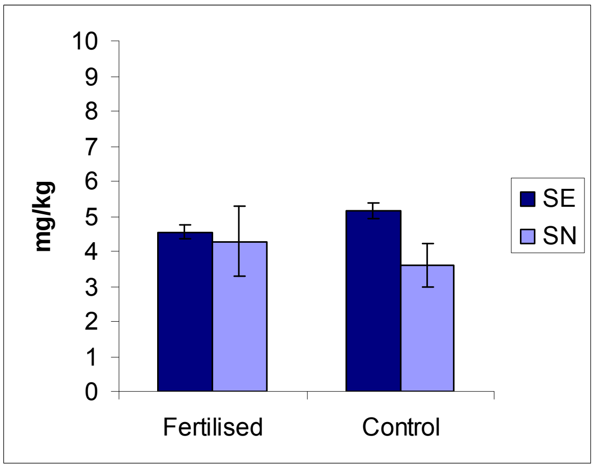


Figure 14. *S. araneus* mean value and SE for Se and Sn. In Fertilised plots n=21 and in Control plots n=9.

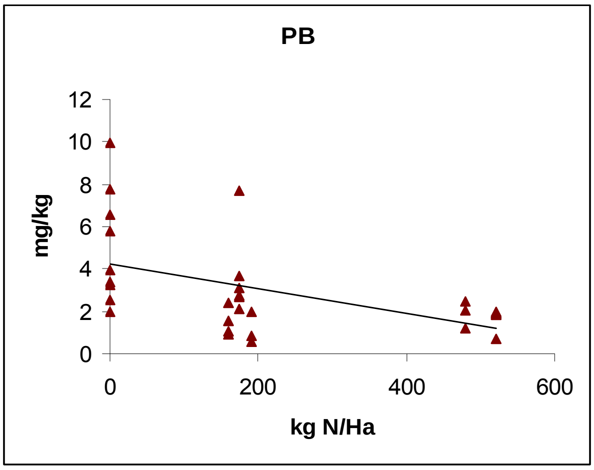


Figure 15. *S. araneus* Regression analyse for Pb.

A. subfuscus (dusky slug)

The slugs generally accumulated some elements much stronger than the mammals. Concentrations of Al, Cr and Mn were two to three orders of magnitude higher in the slugs. However, this strong enrichment occurred in both control and sludge treated plots.

The analyses of variance for dusky slug resulted in several significant ($p<0,05$) differences (Table 6; Figure 16-20). The ANOVA for High dose, Low dose and Control showed a similar result as for Fertilised versus Control (Table 7). Bodyweight as covariate gave a significant negative correlation for B, Co, Cr, Fe, Ni, Pb, Si, Sn and V, thus, the concentration decreases with increasing bodyweight. For Cd the significant correlation showed an increasing amount of Cd with higher bodyweight. In the comparison between pellets and granules, granule treatment resulted in significantly higher kidney concentration of Cu and Mn. In the linear regression analyse Cr, Fe, Mo, Ni, Si, Sn and V increased with increased amount of N and consequently higher amount of sewage sludge applied. The most significant positive correlations were shown by Ni ($n= 32$; $r2= 0,213$; $p= 0,008$), followed by Si ($n= 32$; $r2= 0,173$; $p= 0,018$). Cd on the other hand showed a significant decreasing trend with a higher amount applied ($n= 32$; $r2= 0,393$; $p= 0,000$) (Figure 21).

Table 6. Elements with significantly different mean values in the comparison between Fertilised and Control (K 1 + K 2).

Element (response variable)	Significant difference
AL	Fertilised > Control
CD	Fertilised < Control
CO	Fertilised > Control
CR	Fertilised > Control
FE	Fertilised > Control
MO	Fertilised > Control
NI	Fertilised > Control
SI	Fertilised > Control
V	Fertilised > Control
ZN	Fertilised > Control

Table 7. Elements with significantly different mean values in the comparison between High dose, Low dose and Control (K 1 and K 2 separately).

Element (response variable)	Significant difference
AL	Low dose > K 2
CD	Low and High dose < K 2
CO	Low dose > K 2
CR	Low dose > K 2
CU	Low dose > High dose
FE	Low dose > K 2
MN	High dose < K 1
MO	Low dose > K 2
NI	Low and High dose > K 2
V	Low dose > K 2

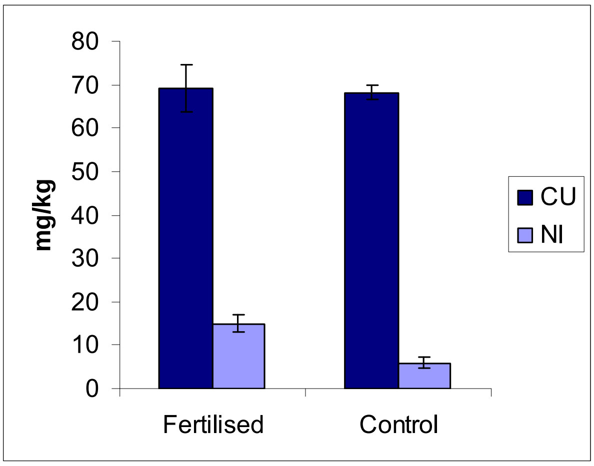


Figure 16. *A. subfuscus* mean value and SE for Cu and Ni. Ni has significantly higher value in Fertilised plots. For Fertilised and Control n=16.

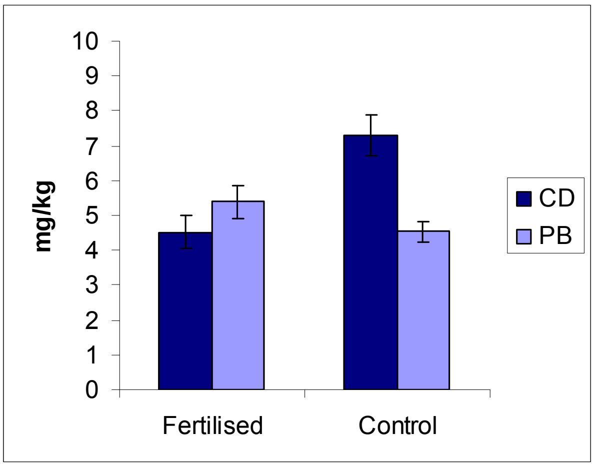


Figure 17. *A. subfuscus* mean value and SE for Cd and Pb. Cd have significantly higher value in Control plots. In Fertilised plots n=16 and in Control plots n=16.

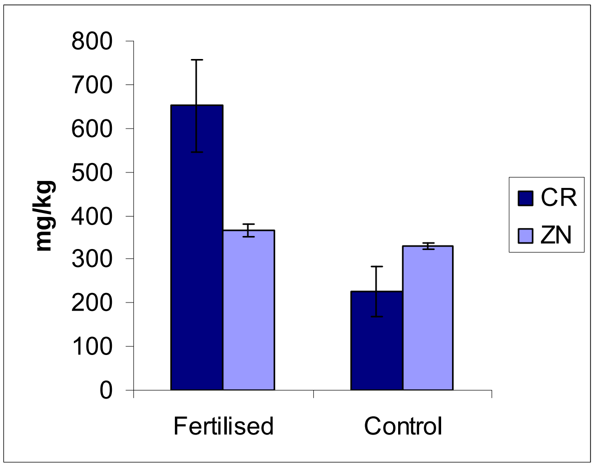


Figure 18. *A. subfuscus* mean value and SE for Cr and Zn. Both Cr and Zn have significantly higher values in Fertilised plots. For Fertilised and Control n=16.

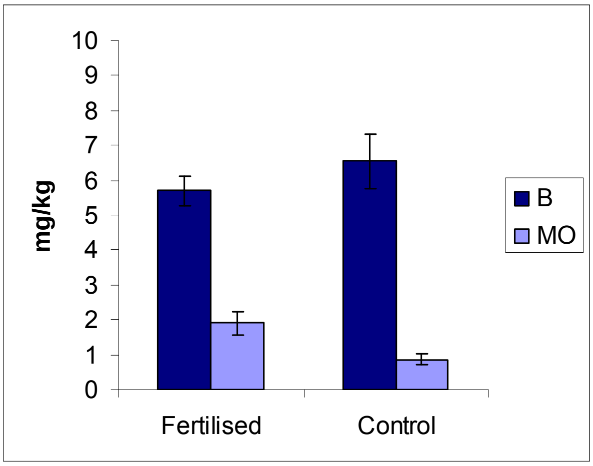


Figure 19. *A. subfuscus* mean value and SE for B and Mo. Mo have significantly higher value in Fertilised plots. For both Fertilised and Control n=16.

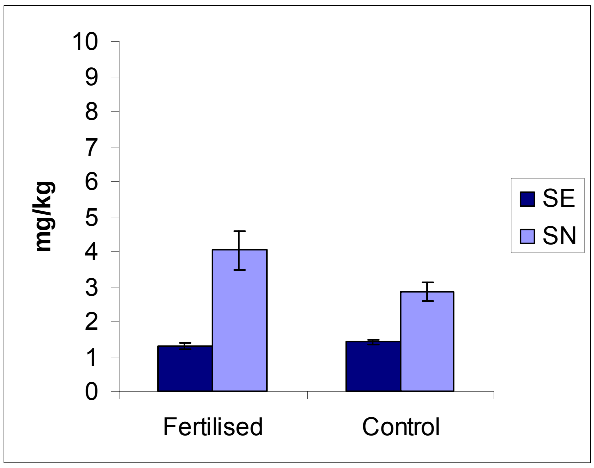


Figure 20. *A. subfuscus* mean value and SE for Se and Sn. For both Fertilised and Control n= 16.

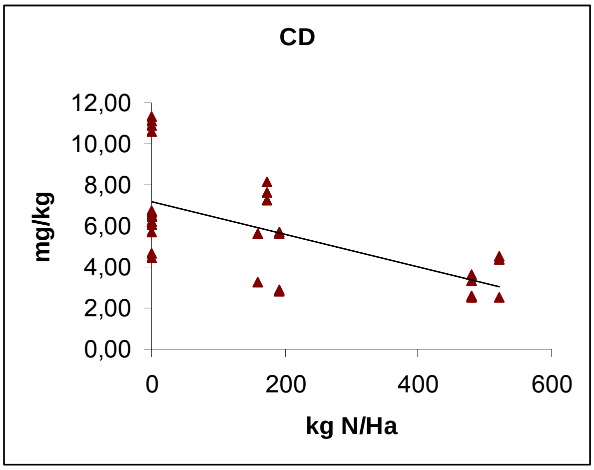


Figure 21. *A. subfuscus* Regression analyse for Cd.

2006- Fertilised plots

M. glareolus (bank vole)

There was only one significant difference between treatments for bank vole (Table 8). Illustrations of mean values for some of the analysed elements are shown in figure 22-26. Mean values for all elements are found in the Appendix Table 8. Bodyweight was a significant covariant factor for some elements. The concentration of B, Si and Sn decreased with higher bodyweight while the concentration of Cd and Fe increased.

Table 8. Elements with significantly different mean values in the comparison between Fertilised and Control (K 2).

Element (response variable)	Significant difference
CD	Fertilised < Control

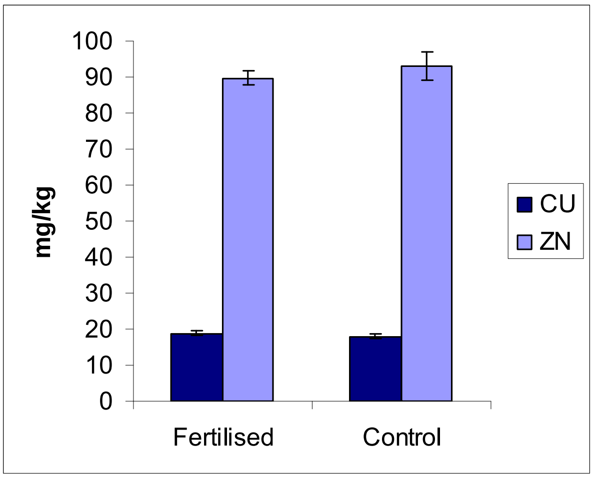


Figure 22. *M. glareolus* mean value and SE for Cu and Zn. Fertilised n= 15 and Control n= 12.

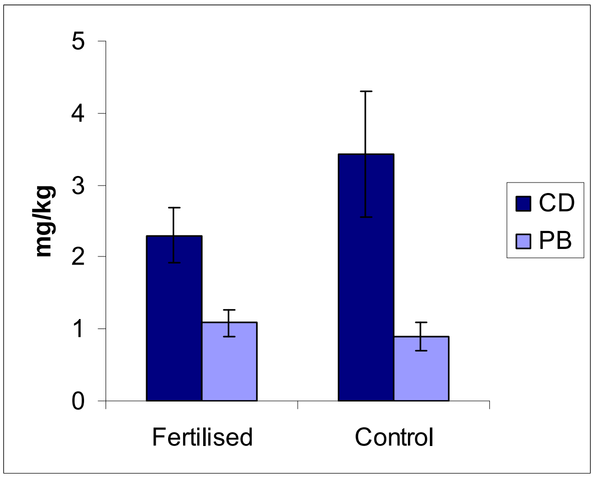


Figure 23. *M. glareolus* mean value and SE for Cd and Pb. Cd has significantly higher value for Control. Fertilised n= 15 and Control n=12.

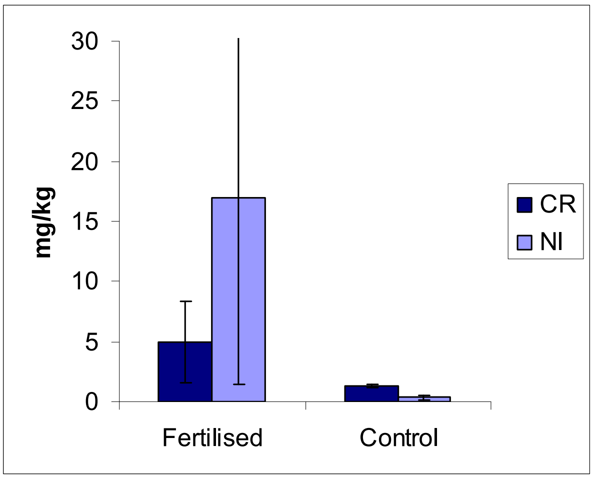


Figure 24. *M. glareolus* mean value and SE for Cr and Ni. In Fertilised plots one vole (the same as for Mo) had extremely high values of Ni relative to the other voles from fertilised plots. Fertilised n=15 and Control n=12.

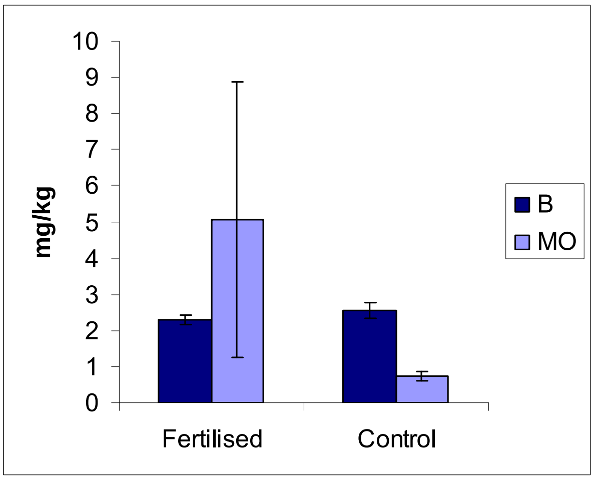


Figure 25. *M. glareolus* mean value and SE for B and Mo. In Fertilised plots one vole (the same as for Ni) had higher values of Mo relative to the other voles from Fertilised plots. Fertilised n= 15 and Control n=12.

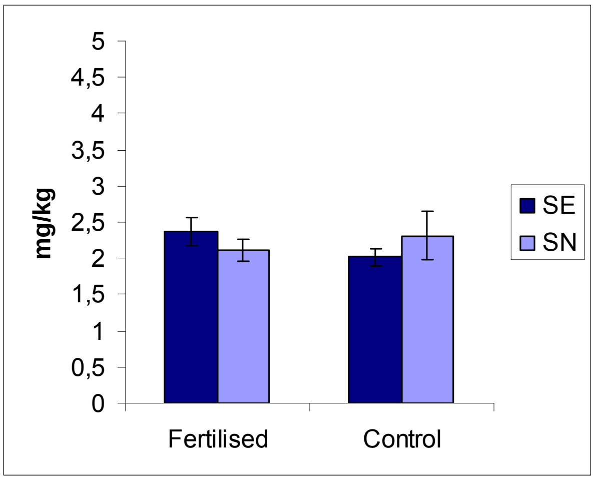


Figure 26. *M. glareolus* mean value and SE for Se and Sn. Fertilised n= 15 and Control n=12.

S. araneus (common shrew)

According to ANOVA, sludge treatment did not result in significant differences between means for any element (Figure 27-31). Bodyweight, however, was a significant covariate for several elements. Cr and Mo showed that a higher bodyweight give a higher concentration of the elements. For B, Se, Si, Sn and V a higher bodyweight decreases the concentration of the elements.

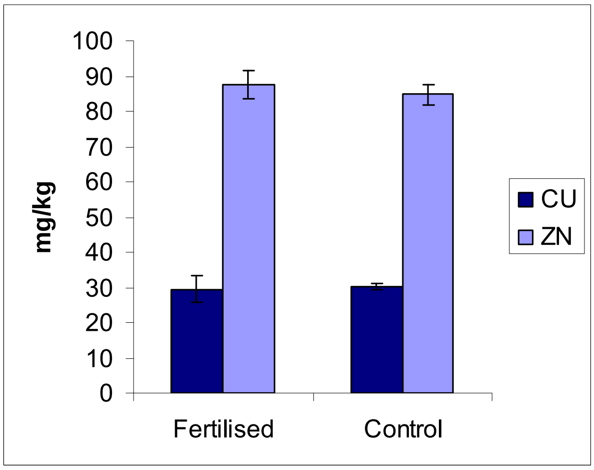


Figure 27. *S. araneus* mean value and SE for Cu and Zn. Fertilised n= 13 and Control n=5.

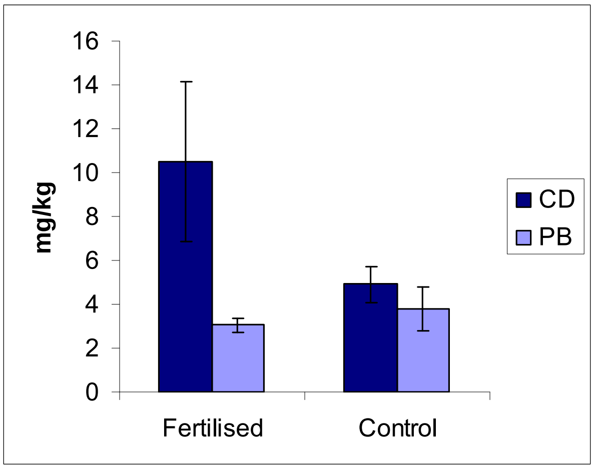


Figure 28. *S. araneus* mean value and SE for Cd and Pb. Fertilised n= 13 and Control n= 5.

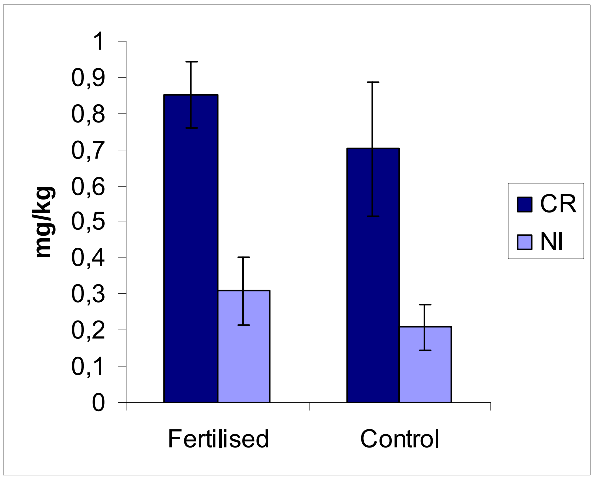


Figure 29. *S. araneus* mean value and SE for Cr and Ni. Fertilised n= 13 and Control n= 5.

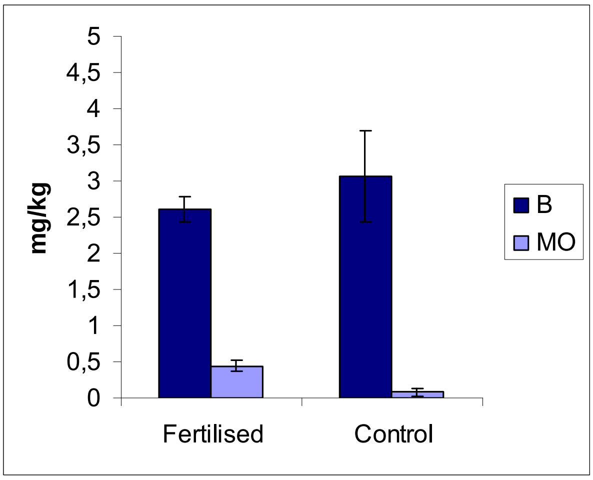


Figure 30. *S. araneus* mean value and SE for B and Mo. Fertilised n= 13 and Control n=5.

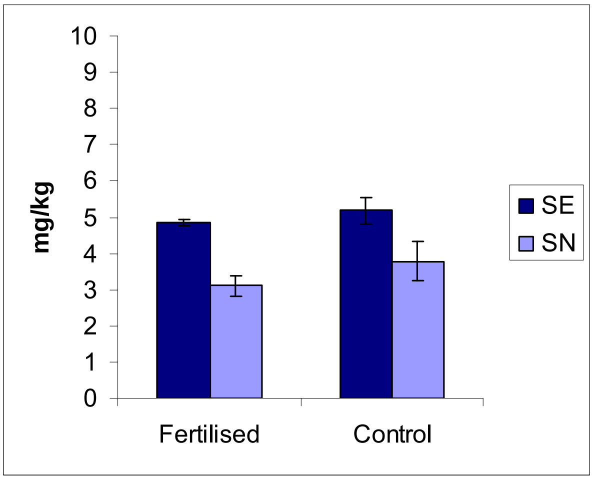


Figure 31. *S. araneus* mean value and SE for Se and Sn. Fertilised n= 13 and Control n= 5.

A. subfuscus (dusky slug)

Sludge treatment was a significant factor for several heavy metals in dusky slug (Table 9). In figure 32-36 some elements mean values is illustrated and a complete table with mean values are found in the Appendix Table 10. With bodyweight as a covariate Si and Sn gave a significant negative correlation, consequently, decreases with higher bodyweight.

Table 9. Elements with significantly different mean values in the comparison between Fertilised and Control (K 2).

Element (response variable)	Significant difference
AL	Fertilised > Control
CD	Fertilised < Control
CR	Fertilised > Control
FE	Fertilised > Control
MN	Fertilised > Control
PB	Fertilised < Control
SI	Fertilised > Control
SN	Fertilised > Control
V	Fertilised > Control
ZN	Fertilised > Control

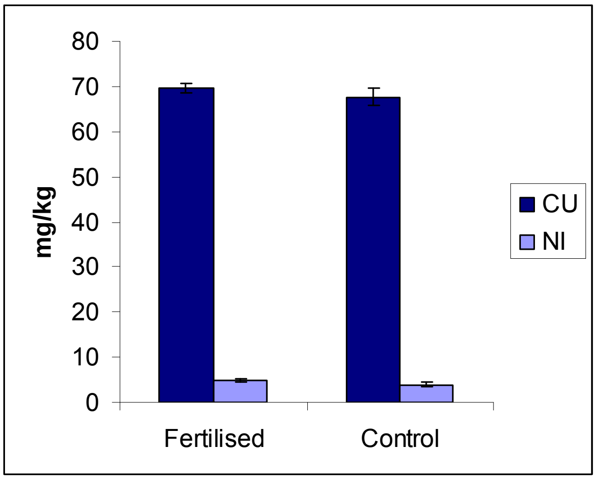


Figure 32. *A. subfuscus* mean value and SE for Cu and Ni. Fertilised and Control n=12.

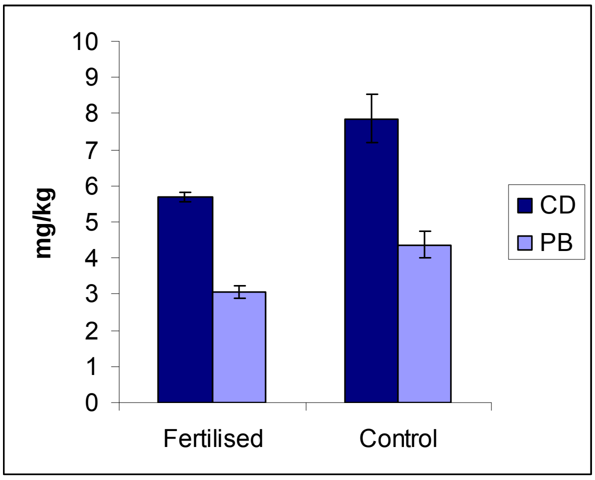


Figure 33. *A. subfuscus* mean value and SE for Cd and Pb (n= 12). .Both Cd and Pb got significantly higher values for control compared with Fertilised.

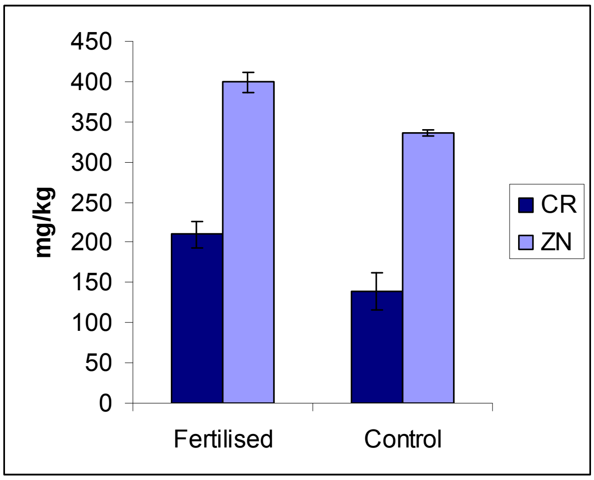


Figure 34. *A. subfuscus* mean value and SE for Cr and Zn. Fertilised is significantly higher than Control for both Cr and Zn. Fertilised and Control n= 12.

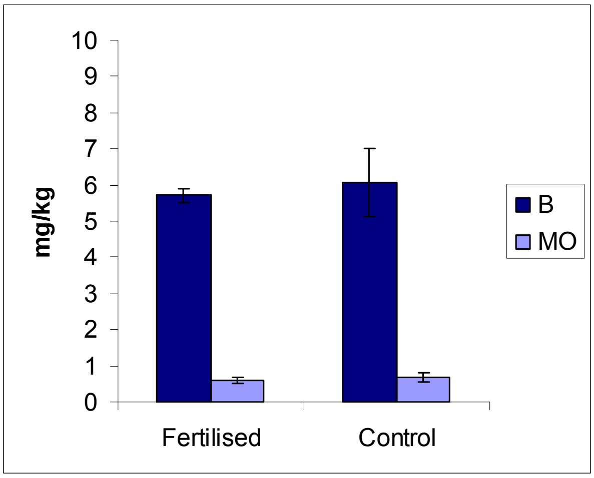


Figure 35. *A. subfuscus* mean value and SE for B and Mo. Fertilised and Control n= 12.

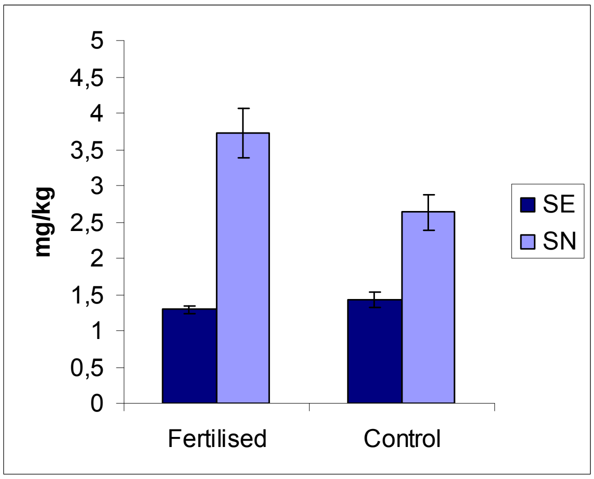


Figure 36. *A. subfuscus* mean value and SE for Se and Sn. Fertilised and Control n= 12.

Discussion

Most trace elements are highly toxic to plants and animals. However, Co, Cr, Cu, Fe, Mn, Mo, Se and Zn, are also essential micronutrients to mammals at somewhat lower concentrations (McBride 1994). The interval between "deficiency" and "toxicity" may be very narrow for some of these elements. Andersson et al. (1982) have indicated that Cd and Cu accumulate in kidneys of meadow vole (*Microtus pennsylvanicus*) when spreading sewage sludge. Also, studies have been done considering accumulation on Pb in shrew (*Myosorex varius*) and an accumulation were found to take place (Reinecke et al. 2000). However, in the present study the few significant differences between treatments for Cd, Pb and Cu rather indicated that bioindicator concentrations were highest in the Control plots. Thus, this study does not show any accumulation of Cd, Pb and Cu after fertilising with sewage sludge.

The result for bank vole had few significant treatment effects. In the 2003-fertilised plots the only elevated levels with significance indicated by both types of ANOVA test, was that of Se. In the Fertilised/Control comparisons B were higher from Fertilised plot and there were significantly higher concentrations of Sn and V in fertilised plots when testing with two fertilisation levels (High/Low/Control). However, since these differences did not appear in the High/Low/Control and Fertilised/Control groupings respectively, and since many of these data were below the estimated detection level results are less reliable. Also, in the regression analyses the increases of B and V with N dose were very weakly significant (and low r^2), i.e. results were not very confirmative. Se, on the other hand, showed a clear and significant positive correlation to N dose in the regression. Thus, for bank vole the only element that shows a clear accumulation tendency is Se. It should be added that Se also is a micronutrient and therefore an accumulation in smaller quantities is not alarming. In another study from a waste-water–irrigated area Anthony & Kozlowski (1982) did not find any elevated levels of heavy metals in meadow vole *(Microtus pennsylvanicus)*. Also, in a study with ash application no elevated levels of Cd were found in the treated plots (Lodenius et al. 2002). In the present study, Zn concentration in bank vole was significantly lower in Fertilised compared to Control in the 2003-fertilised plots. This pattern was also found in bank vole collected from an abandoned lead mine (Milton et al. 2003).

For common shrew in the 2003-fertilised plots the only similarity between results from the two-level ANOVAs (Fertilised/Control) and the three-level ANOVAs (High dose/ Low dose/Controls), was a significantly higher value for Pb in Control plots. The only element with higher value from Fertilised plots than Controls was B in High/Low/Control ANOVAs. Instead, Mn and Cu showed a lower value from the Fertilised plots in High/Low/Control and Fertilised/Control ANOVAs respective. The regression analyses for the common shrew data showed significant negative relationships between sludge N additions and the elements Fe and Pb. The 2006-fertilised plots had no significant treatment effect at all. All together, no clear heavy metal accumulation occurs in the common shrew either. This controverts results from a study with Trowbridge´s shrew *(Sorex trowbridgii)* where elevated levels of Cd, Pb, Zn and Cu were found after fertilising with sewage sludge (Hegstrom & West 1989). To be added, the concentration of Cd, Pb, Zn and Cu in the sludge from the present study is remarkably lower than the average concentration of elements in the sludge used by Hegstrom & West (1989).

The study of dusky slug resulted in more significant results than the other bioindicators. For the 2003-fertilised plots, the two-level ANOVAs (Fertilised/Control) and the three-level ANOVAs (High dose/ Low dose/Controls), results were similar. The elements Al, Cd, Co, Cr, Fe, Mo, Ni and V showed significant treatment effects in both tests. All those, except Cd, had a higher value in Fertilised plots compared to Control plots. These elements should be the most probable to accumulate in slugs. The repeated indication of the Low dose having higher values than High dose, for the 2003-fertilised plots, could not be explained. The linear regressions analyses showed similar significances as the ANOVA. Cd decreased with higher amount of fertiliser N, whereas the other significant elements increased with higher N dose (Table 10). Co, Fe and Mo are micronutrients and not as toxic as Cr and V, and therefore higher levels of these might not be as alarming. Hence, most alarming in the 2003-fertilised plots were the increased levels of Cr, Ni and V in the dusky slugs.

Dusky slug were directly exposed to the sewage sludge in the 2006-fertilised plots, while the slugs in the 2003-fertilised plots were exposed through contaminated vegetation tissues and mor layer. Even though the type of exposures differed some patterns were similar between 2003- and 2006-fertilised plots. Significantly different means between treatments were found for Al, Cd, Cr, Fe and V in both fertilisation trials (Table 10). Looking at the toxic heavy metals, Cr and V, they both accumulated in dusky slugs from Fertilised plots. Apparently, elevated levels of these elements were maintained in the system for some years. On the contrary, additions of sewage sludge consistently led to lower levels of Cd compared to Control plots. Other studies (Scheifler et al. 2003) have shown a slight increase of Cd in slugs after fertilising with sewage sludge. Fe is an important micronutrient and therefore the increased tissue concentration might not be harmful at all. Scheifler et al. (2003) studied *Helix aspersa* and found a clear transfer of Zn from forest soil fertilised with sewage sludge. In the current study Zn were increasing in some trials (Table 10) but this increase is not clear enough to be stated as an accumulation. In a study of *A. subfuscus* in an environment with elevated levels of metals, Greville & Morgan (1991) claimed that slugs adjust to the elevated level of metals (Zn and Pb). Out of the other elements that showed elevated levels in the 2006-fertilised plots, but not in the 2003-fertilised plots, only Sn is very toxic. Though, the result from Sn is somewhat uncertain since many of its data were close or below the estimated detection level. Consequently, this study indicates that sludge fertilisation may cause some additional accumulation of trace elements in dusky slug.

Table 10. Significant results from the trials for *A. subfuscus* where the symbol "+" before the element represents a significantly higher concentration of the element from fertilised plots and a "–" represent a significantly lower concentration of the element from the fertilised plots compared to the control plots. For the regression analysis the symbol represents the slope of the regression line.

Fertilised versus Control -03	High/Low versus Control -03	Fertilised versus Control -06	Regression analysis -03
+ AL	+ AL	+ AL	
- CD	- CD	- CD	CD -
+ CO	+ CO		
+ CR	+ CR	+ CR	CR +
+ FE	+ FE	+ FE	FE +
	+ MN	+ MN	
+ MO	+ MO		MO +
		- PB	
+ NI	+ NI		NI +
+ SI		+ SI	SI +
		+ SN	SN +
+ V	+ V	+ V	V +
+ ZN		+ ZN	

Concentrations of several elements in dusky slug are considerably higher compared to the concentrations in the other bioindicators (Appendix Table 5-10). Also, Scheifler et al. (2003) concluded that slugs are good indicators (considering changes in metal concentration in soil).

For bank vole, common shrew and dusky slug in both the 2003- and the 2006-fertilised trial, the levels of several elements were significantly negatively correlated to bodyweight, i.e. the majority of the significant correlations were negative. Also some elements were in common for all groups. Thus, B, Si and Sn concentrations decrease with increasing bodyweight for all bioindicators, and the same trend is clear for V in common shrews. The only positive correlation in the 2003-fertilised trials was an increase of Cd with higher bodyweight for dusky slug. In the 2006-fertilised trials, however, Cd and Fe increased with higher bodyweight for bank vole and Cr and Mo increased with higher bodyweight for common shrew. For bank vole an increasing amount of Cd and Pb with bodyweight were found in a study by Milton et al. (2003).

A source of error for the 2003-fertilised plots could be that treatment plots are only 2116m^2 and are located close to each other. For the bordering plots the closest traps are only about 30 meters apart. The average range size of bank vole females is 4240m^2 and the size does not change significantly from breeding to post- breeding period (Löfgren 1995). This might mean that bank vole have its territory across several plots and different treatments, which will introduce errors in the determined concentration levels and a loss of precision in the overall treatment comparisons. Looking at the experimental site map (Figure 1) the most affected plots would be plot nine and plot six since they are close to High dose plots. However, a comparison of mean values for plot six and nine with their respective replicate plots, do not reveal any systematic differences. Also, if the small plot areas seriously affected the concentration levels in the K 1 Control, values should be significantly different from K 2 Control values, but they were not.

Since the number of males and females are rather similar within each mammal bioindocator and fertilisation trial, possible systematic differences between sexes should have caused only minor errors. Also, Lodenius et al. (2002) found no significant difference between male and female or between age classes when studying Cd concentration.

Conclusion

For bank vole and common shrew there were no consistent treatment effects on heavy metal concentrations in body tissues. In the case of dusky slug, sludge treatment result in higher levels of several elements. Thus, it appears that dusky slug could be an interesting (numerous and easy to catch) bioindicator for heavy metals.

Acknowledgement

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Appendix

Table 1. 2003-fertilised plots, total collection of mammals.

Treatment (ton/Ha)	Sludge origin	Species	Plot	Male	Female	Number of mammals
4,5	Himmerfjärden	*M. glareolus*	5	0	0	0
4,5	Himmerfjärden	*S. araneus*	5	2	3	5
4,5	Himmerfjärden	*M. glareolus*	6	2	0	2
4,5	Himmerfjärden	*S. araneus*	6	0	1	1
5,3	Umeå	*M. glareolus*	1	0	2	2
5,3	Umeå	*S. araneus*	1	0	1	1
5,3	Umeå	*M. glareolus*	9	0	1	1
5,3	Umeå	*S. araneus*	9	0	3	3
6,4	Umeå	*M. glareolus*	4	1	2	3
6,4	Umeå	*S. araneus*	4	0	2	2
6,4	Umeå	*M. glareolus*	10	0	1	1
6,4	Umeå	*S. araneus*	10	0	1	1
13,6	Himmerfjärden	*M. glareolus*	8	2	0	2
13,6	Himmerfjärden	*S. araneus*	8	0	3	3
13,6	Himmerfjärden	*M. glareolus*	12	0	1	1
13,6	Himmerfjärden	*S. araneus*	12	0	2	2
16,1	Umeå	*M. glareolus*	7	0	1	1
16,1	Umeå	*S. araneus*	7	0	2	2
16,1	Umeå	*M. glareolus*	2	1	1	2
16,1	Umeå	*S. araneus*	2	0	1	1
Control (K 1)	No sludge	*M. glareolus*	3	1	0	1
Control (K 1)	No sludge	*S. araneus*	3	0	3	3
Control (K 1)	No sludge	*M. glareolus*	11	0	0	0
Control (K 1)	No sludge	*S. araneus*	11	0	1	1

Table 2. 2006-fertilised plots, total collection of mammals.

Treatment (ton/Ha)	Sludge origin	Species	Plot	Male	Female	Number of mammals
12	Himmerfjärden	*M. glareolus*	A	2	3	5
12	Himmerfjärden	*S. araneus*	A	0	3	3
12	Himmerfjärden	*M. glareolus*	B	3	2	5
12	Himmerfjärden	*S. araneus*	B	1	4	5
12	Himmerfjärden	*M. glareolus*	C	13	2	15*
12	Himmerfjärden	*S. araneus*	C	2	6	8*
Control (K 2)	No sludge	*M. glareolus*	D	8	5	13*
Control (K 2)	No sludge	*S. araneus*	D	0	2	2
Control (K 2)	No sludge	*M. glareolus*	E	0	2	2
Control (K 2)	No sludge	*S. araneus*	E	0	1	1
Control (K 2)	No sludge	*M. glareolus*	F	6	2	8*
Control (K 2)	No sludge	*S. araneus*	F	0	2	2

*Five of the animals were randomly selected for analysis.

Table 3. 2003-fertilised plots, total collection of *A. subfuscus*.

Treatment (ton/Ha)	Sludge origin	Species	Plot	Total dw (g)	Number of snails
4,5	Himmerfjärden	*A. subfuscus*	5	0,397	10
4,5	Himmerfjärden	*A. subfuscus*	6	0,053	2
5,3	Umeå	*A. subfuscus*	1	0,36	6
5,3	Umeå	*A. subfuscus*	9	0,099	7
6,4	Umeå	*A. subfuscus*	4	0,234	8
6,4	Umeå	*A. subfuscus*	10	0,085	8
13,6	Himmerfjärden	*A. subfuscus*	8	0,058	6
13,6	Himmerfjärden	*A. subfuscus*	12	0,061	7
16,1	Umeå	*A. subfuscus*	7	0,114	9
16,1	Umeå	*A. subfuscus*	2	0,173	6
Control (K 2)	No sludge	*A. subfuscus*	3	0,146	3
Control (K 2)	No sludge	*A. subfuscus*	11	0,072	5

Table 4. 2006-fertilised plots, total collection of *A. subfuscus*.

Treatment (ton/Ha)	Sludge origin	Species	Plot	Total dw (g)	Number of snails
12	Himmerfjärden	*A. subfuscus*	A	0,241	12
12	Himmerfjärden	*A. subfuscus*	B	0,243	8
12	Himmerfjärden	*A. subfuscus*	C	0,345	25
Control (K 2)	No sludge	*A. subfuscus*	D	0,249	22
Control (K 2)	No sludge	*A. subfuscus*	E	0,292	17
Control (K 2)	No sludge	*A. subfuscus*	F	0,742	28

Table 5. Mean values from each treatment in the 2003-fertilised plots for *M. glareolus*. K 1 represents control plots from the 2003-fertilised plots and K 2 represents the control plots from the 2006-fertised plots.

Elements	4,5 ton/ha (n=2)		5,3 ton/ha (n=3)		6,4 ton/ha (n=4)		13,6 ton/ha (n=3)		16,1 ton/ha (n=3)		K 1 (n=1)		K 2 (n=12)	
	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean
AL	2,7	0,94	9,8	6,4	3,7	2,09	1,9	0,27	3,9	1,58	3,6	*	6,4	3,53
B	3,1	0,15	4,4	1,2	2,7	0,59	3,2	0,15	4,5	0,73	4,1	*	2,6	0,21
CD	1,8	0,04	3,4	1,28	1,9	0,30	1,6	0,52	2,0	1	1,9	*	3,4	0,87
CO	0,1	0	0,1	0,01	0,1	0,03	0,1	0,02	0,1	0	0,1	*	0,1	0,01
CR	2,0	0,59	77	75	2,5	0,68	2,2	0,26	5,3	2,68	2,4	*	1,3	0,08
CU	18	0,65	17	1,42	16	1,66	17	0,39	19	0,58	17	*	18	0,57
FE	302	65	247	25	291	12,9	446	87	339	43	332	*	358	44
MN	7,2	0,32	15	4,43	7,0	1,44	9,9	2,06	8,5	1,65	9,1	*	8,3	0,85
MO	0,7	0,18	19	17	0,9	0,29	1,0	0,03	4,2	3,05	1,8	*	0,7	0,13
NI	0,5	0,15	78	74	1,2	0,79	0,6	0,18	13	12	2,8	*	0,4	0,17
PB	0,5	0,32	1,2	0,12	1,5	0,58	0,6	0,42	0,9	0,24	0,6	*	0,9	0,20
SE	2,4	0,42	2,8	0,34	2,6	0,33	2,9	0,23	2,7	0,15	2,4	*	2,0	0,12
SI	117	2	113	6,67	100	9,24	107	4,84	118	10,7	103	*	124	8,53
SN	4,6	1,59	4,9	1,6	2,4	0,91	1,5	0,56	2,9	0,74	1,5	*	2,3	0,33
V	0,2	0,04	0,2	0,02	0,1	0,03	0,2	0,03	0,2	0,02	0,1	*	0,2	0,01
ZN	89	3,1	88	7,61	76	9,76	81	3,75	90	1,2	92	*	93	3,93

Table 6. Mean values from each treatment in the 2003-fertilised plots for *S. araneus*. K 1 represents control plots from the 2003-fertilised plots and K 2 represents the control plots from the 2006-fertised plots.

Elements	4,5 ton/ha (n=6)		5,3 ton/ha (n=4)		6,4 ton/ha (n=3)		13,6 ton/ha (n=5)		16,1 ton/ha (n=3)		K 1 (n=4)		K 2 (n=5)	
	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean
AL	3,0	0,80	2,5	0,40	9,2	5	4,0	1,1	3,4	1,36	4,5	1,31	3,1	0,45
B	3,7	0,45	3,7	0,62	10,6	3,05	4,7	0,68	3,6	0,54	5,5	0,80	3,1	0,63
CD	12	5,85	2,2	0,23	0,7	0,07	1,3	0,32	1,6	0,53	6,1	2,07	4,9	0,83
CO	0,4	0,07	0,4	0,07	0,3	0,003	0,6	0,15	0,3	0,05	0,48	0,06	0,4	0,05
CR	5,0	2,97	1,9	0,23	3,5	0,57	1,4	0,24	1,0	0,23	10,0	7,15	0,7	0,19
CU	29	1,93	27	3,89	24	2,03	30	2,42	24	3,83	35	1,36	30	2,04
FE	448	66	422	137	357	41	298	39	338	93	541	86	431	72
MN	25	5,28	15	3,56	18	8,13	27	6,79	13	2,35	69	31,7	22	5,84
MO	0,6	0,18	0,6	0,24	0,5	0,45	0,7	0,22	0,4	0,32	1,6	0,89	0,1	0,06
NI	1,2	0,23	1,8	1,21	2,9	1	1,7	0,72	0,8	0,21	4,2	2,88	0,2	0,06
PB	3,7	0,83	1,5	0,34	1,1	0,44	1,7	0,25	1,9	0,37	6,6	1,26	3,8	1,02
SE	4,4	0,17	4,4	0,69	5,3	0,59	4,6	0,46	4,2	0,57	5,2	0,28	5,2	0,36
SI	123	6,98	122	7,51	166	14,5	121	12,7	113	9,83	139	17,7	132	9,24
SN	2,9	0,63	3,2	0,52	10,5	5,81	2,6	1,52	5,1	0,64	3,4	1,33	3,8	0,54
V	0,2	0,02	0,2	0,01	0,3	0,02	0,2	0,02	0,2	0,03	0,2	0,04	0,3	0,02
ZN	101	9,1	78	2,02	94	15,3	84	6,03	66	9,16	81	3,88	85	2,93

Table 7. Mean values from each treatment in the 2003-fertilised plots for *A. subfuscus*. K 1 represents control plots from the 2003-fertilised plots and K 2 represents the control plots from the 2006-fertised plots.

Elements	4,5 ton/ha (n=3)		5,3 ton/ha (n=2)		6,4 ton/ha (n=4)		13,6 ton/ha (n=3)		16,1 ton/ha (n=4)		K 1 (n=4)		K 2 (n=12)	
	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean	Mean	SE mean
AL	101	30	474	76	376	15	309	121	159	8,95	212	50	124	8,07
B	5,0	0,54	7,8	2,19	4,8	0,33	6,7	1,27	5,2	0,73	8	1,22	6,1	0,95
CD	7,7	0,26	4,5	1,2	4,2	0,80	3,8	0,64	3,0	0,28	5,6	0,57	7,9	0,67
CO	0,8	0,54	2,3	0,46	1,6	0,2	1,8	0,48	0,8	0,10	1,1	0,37	0,5	0,10
CR	332	279	1133	257	828	107	809	316	359	52	484	174	139	23
CU	108	9,06	65	1,8	65	5,28	59	1,14	53	2,57	70	3,57	68	1,86
FE	2819	2101	9225	1775	6168	702	6963	2069	2790	338	4493	1693	1299	154
MN	12247	1580	6170	2390	2600	788	1800	183	1988	301	9625	4633	4357	498
MO	0,9	0,55	3,2	0,6	2,0	0,27	2,2	1,26	1,7	0,66	1,4	0,44	0,7	0,13
NI	8,4	6,36	25	1,45	15,6	2,48	19	3,23	10,9	2,33	12	3,84	3,9	0,56
PB	3,1	0,41	7,7	0,68	6,6	0,69	6,4	0,36	4,0	0,72	5,0	0,43	4,4	0,36
SE	1,6	0,06	1,3	0,07	1,2	0,13	1,4	0,46	1,0	0,11	1,3	0,16	1,4	0,10
SI	221	51,9	607	257	304	22	469	33	328	44	289	31	241	8,91
SN	2,8	1,18	5,9	2,15	2,9	0,38	6,2	1,92	3,5	0,60	3,5	0,63	2,6	0,25
V	1,0	0,59	4,8	0,56	3,2	0,1	3,2	1,22	1,7	0,26	1,6	0,42	0,9	0,06
ZN	422	19	376	6	329	2,9	372	30	348	42	312	28	336	4

Table 8. Mean values from treatments in the 2006-fertilised plots for *M. glareolus*.

Elements	12 ton/ha (n=15)		K 2 (n=12)	
	Mean	SE mean	Mean	SE mean
AL	3,3	0,60	6,4	3,53
B	2,3	0,13	2,6	0,21
CD	2,3	0,38	3,4	0,87
CO	0,1	0,01	0,1	0,01
CR	5,0	3,43	1,3	0,08
CU	19	0,63	18	0,57
FE	491	96	358	44
MN	11	1,62	8,3	0,85
MO	5,1	3,8	0,7	0,13
NI	17	15,6	0,4	0,17
PB	1,1	0,19	0,9	0,20
SE	2,4	0,19	2,0	0,12
SI	112	3,14	124	8,53
SN	2,1	0,15	2,3	0,33
V	0,2	0,01	0,2	0,01
ZN	90	1,99	93	3,93

Table 9. Mean values from treatments in the 2006-fertilised plots for *S. araneus*.

Elements	12 ton/ha (n=13)		K 2 (n=5)	
	Mean	SE mean	Mean	SE mean
AL	2,6	0,31	3,1	0,45
B	2,6	0,17	3,1	0,63
CD	10,5	3,64	4,9	0,83
CO	0,4	0,03	0,4	0,05
CR	0,9	0,09	0,7	0,19
CU	30	0,87	30	2,04
FE	350	31	431	72
MN	28	3,98	22	5,84
MO	0,4	0,07	0,1	0,06
NI	0,3	0,09	0,2	0,06
PB	3,0	0,32	3,8	1,02
SE	4,8	0,11	5,2	0,36
SI	119	3,65	132	9,24
SN	3,1	0,29	3,8	0,54
V	0,2	0,01	0,3	0,02
ZN	88	3,92	85	2,93

Table 10. Mean values from treatments in the 2006-fertilised plots for *A. subfuscus*.

Elements	12 ton/ha (n=12)		K 2 (n=12)	
	Mean	SE mean	Mean	SE mean
AL	324	45	124	8,07
B	5,7	0,20	6,1	0,95
CD	5,7	0,13	7,9	0,67
CO	0,6	0,01	0,5	0,10
CR	210	16	139	23
CU	70	1,12	68	1,86
FE	2268	32	1299	154
MN	9370	1318	4357	498
MO	0,6	0,09	0,7	0,13
NI	4,8	0,26	3,9	0,56
PB	3,1	0,17	4,4	0,36
SE	1,3	0,05	1,4	0,10
SI	291	6,17	241	8,91
SN	3,7	0,34	2,6	0,25
V	1,2	0,04	0,9	0,06
ZN	399	12,6	336	4,05

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