Distribution and transport of microplastics and metals in sewage sludge-amended agricultural soil

- A Comparative study between microplastics copper, cadmium, and nickel

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Keywords: Bioturbation, advective transport, earthworm, *Aporrectodea longa*

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Abstract

This thesis investigated the vertical transport of microplastics and metals in agricultural soil after sewage sludge application. Sewage sludge is commonly used in agriculture for adding nutrients and carbon to improve the soil quality. From a bio-based economy perspective, it is considered an important strategy to re-use this nutrient-rich by-product from wastewater treatment plants. However, the application of sewage sludge can also lead to unintentional spreading of unwanted elements to the soil, such as microplastics and metals. While extensive studies have been carried out to examine the environmental fate of metals in different soils, little is known about the emerging pollutant microplastics. Hence, the aim of this study was to investigate the distribution and transport of microplastics in direct comparison to more investigated metals. Moreover, this study compared microplastic and metal distributions in field measurements and in a laboratory-based process study where plastic and metal transport were studied under the influence of either earthworm bioturbation or simulated rainfall using intact soil cores, given that transferring results from controlled experiments to field conditions can often be difficult. The initial hypothesis was that there might be similarities between the transport of microplastics and particulate-bound metals, whereas dissolved metals were expected more mobile in the soil profile compared to microplastics. Secondly, bioturbation activity was suspected to contribute significantly to plastic transport. The distribution of microplastics showed spatial similarities to the distribution of copper and cadmium in depth profiles of agricultural soils after long-term sewage sludge application. Since copper and cadmium tend to bind to soil organic matter or clay particles, the results indicate that similar transport processes may affect microplastic transport in the field. Limited transport of microplastics was observed under controlled conditions in the laboratory in the presence of earthworms, but no significant metal transport could be detected in the intact soil columns. Overall, the results from the process study indicate that transport of microplastics and metals was limited compared to previous findings. The dense clay soil used in the intact soil columns and an apparent lack of macro-pores may have prevented advective transport and caused a high mechanical resistance preventing transport via earthworm mediated bioturbation. Thus, suggesting that translocation of MPs in clayey soil may not be as fast as earlier bioturbation studies have observed. This means that the MPs are likely accumulated within the upper part of the soil profile and retained within the soil. However, the generalisation of this finding is limited, since only one soil was examined, and the process study was short in time and consisted of few replicate soil columns. Continued research on this topic needs to be done to establish the transport processes and fate of MPs in terrestrial ecosystems.

Keywords: Bioturbation, advective transport, earthworm, *Aporrectodea longa*

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Abbreviations

ICP-SF/MS	Double focusing inductively coupled mass spectrometer
MP	Microplastic
NP	Nanoplastic
PE	Polyethylene
PET	Polyethylene terephthalate
Py-GC/MS	Pyrolysis gas chromatography mass spectrometry
SOM	Soil organic matter
SOC	Soil organic carbon
WWTP	Wastewater treatment plant

1. Introduction

Soil organic matter tremendously affects soil fertility. Increasing the carbon (C) content in soils both improves the soil structure (Börjesson *et al.* 2014)(Börjesson & Kätterer 2018) and adds charged particle surfaces that can help to retain nutrients (Essington 2005). The carbon content can be increased by, for example, applying organic amendments such as sewage sludge (Börjesson *et al*. 2014). Recycling of nutrients is a prerequisite for sustainability. The use of sewage sludge on agricultural land is considered an important and cheap strategy to reuse nutrients within a circular bio-based economy (Formas 2012). In Sweden, as an effort towards becoming a more bio-based society, an increasing share of sewage sludge is used in agriculture. In 2018, the agricultural use corresponded to approximately 39% of Sweden's total sewage sludge production (SCB 2020).

However, potentially problematic substances such as metals are often enriched within sewage sludge and thus unintentionally added to soils (Bergkvist *et al.* 2005). Regulations have been developed, both in Sweden and EU, to limit the input of metals to farmland by setting limit values for metal concentrations in the sewage sludge and in the sewage sludge-treated soil (SNFS 1994:2) (EU Council Directive 86/278/EEC). The limit values are, however, generally considered outdated and approximately 50 percent of the sewage sludge produced in Sweden follow stricter guidelines accordingly to the REVAQ certification system (Svenskt Vatten 2018). In addition, the declining metal emissions from society in general has resulted in drastically decreasing metal contents in the Swedish sewage sludge since the 1970s (Kirchmann *et al*. 2017).

Nevertheless, the increasing trend of using sewage sludge on agricultural soil has once again raised the debate for stricter regulations and new to the discussions this time is another emerging pollutant, namely small plastic particles (<5 mm) termed microplastics (MPs) (Hushållningssällskapet 2021). During the separation of liquid and solid fractions in the wastewater treatment plant (WWTP), MPs tend to be enriched in the solid sludge fraction that is later spread on arable land (Corradini *et al*. 2019).

Microplastics can have potentially adverse effects in agricultural soil, either directly through the release of toxic additives (De Souza Machado *et al.* 2017) or by imparting physical toxic effects on soil organisms, or indirectly through potential alterations of soil properties in turn affecting soil biota and plant growth (De Souza Machado *et al.* 2019). Yet, it is difficult to fully assess the risks of MPs and their potential effects on the agroecosystems, because little is known about MP loads, transport and fate in the terrestrial environment (Alimi *et al.* 2018). It is important to understand how different transport process may affect the fate of MPs, as it will determine whether the MPs are accumulated in the soil or transported from it, to possibly enter other environmental compartments. Thus, more knowledge about MPs transport and fate is required to make reliable predictions on the exposure rates of MPs in terrestrial ecosystems.

Controlled laboratory process studies indicate that MPs and nanoplastics (NPs) are potentially transported down in the soil profile through movement with water (Alimi *et al.* 2018) or soil biota, in particular by earthworms (Huerta Lwanga *et al.* 2017a; Rillig *et al.* 2017; Heinze *et al.* 2021). For predicting the long-term fate of MPs it is important to know which is the dominating transport mechanism, as movement with water or bioturbation may result in different behaviour of the MPs over time. However, field studies and comparative investigations of different transport processes in intact soils are still lacking. In contrast, there is extensive research made on metal transport and fate in the soil. Different sewage sludge-associated metals, like copper (Cu), are known to sorb strongly to particulate organic matter (Gustafsson *et al.* 2007). Consequently, their vertical distribution in soils and susceptibility to transport may be determined by similar factors that determine the fate of MPs. However, a direct comparison of the distribution and transport of MPs and metals in agricultural soil is so far lacking.

To fully assess MPs in the soil it is furthermore essential to connect findings made in laboratory-based studies to observations from the field, as it is often difficult to directly extrapolate results from controlled experiments to field conditions. Such comparative studies investigating MPs both in the field and under controlled laboratory conditions are currently lacking.

1.1 Aims

The main aim of this thesis was to investigate the distribution and transport of microplastics in a sewage sludge amended agricultural soil in direct comparison to the distribution and transport of typical sewage-sludge associated metals, both in controlled laboratory conditions as well as in the field.

The working hypotheses were 1) that MPs might behave similarly to particulate-bound metals such as Cu, and 2) that bioturbation activity was suspected to contribute significantly to plastic transport. This study was carried out in two main steps to verify these hypotheses. Firstly, the depth-dependent plastic and metal concentrations were measured and compared in depth profiles taken from agricultural soils that have received sewage sludge since 1996. Secondly, a transport process study was carried out, where MPs and a selection of metals, i.e., copper (Cu), cadmium (Cd) and nickel (Ni), were added to intact soil columns, enabling a direct comparison between their transport under the influence of simulated rainfall and bioturbation respectively, allowing to additionally make conclusions on the likely dominating mechanisms controlling the transport of plastics in these studies. The comparison of metals and plastics finally allows extrapolating conclusions from the controlled lab studies to the field.

2. Theoretical Background

2.1 Microplastics

2.1.1 Definition and sources of MPs in the terrestrial environment

The broad term microplastic (MP) can be used for describing plastic particles <5 mm (Ljung *et al.* 2018), there is no general consensus on the lower limit value for MPs, however, 1000 nm is commonly used (Horton *et al.* 2017). Smaller plastic particles (>1000 nm) are often referred to as nanoplastics (NP) (Alimi *et al.* 2018). Furthermore, microplastics can be sorted into primary and secondary MPs, depending on if the plastic was originally produced within the MP size range, or if degradation of plastic material has resulted in a plastic fragment that is within the MP size range (Ljung *et al.* 2018). MPs compose a heterogenous group of particles, as the shape, e.g. spherical, fragment or fiber (Ljung *et al.* 2018), and type of polymer can vary substantially. The most produced plastic polymers include polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), Polyethylene Terephthalate (PET) and polystyrene (PS) (Plastics Europe 2021). Which also reflects the most commonly MPs found as contaminants in the environment (Alimi *et al.* 2018).

Microplastics were first acknowledged in the marine environment and are consequently less studied in terrestrial ecosystems. No comprehensive monitoring of sources of MP emissions exists for Swedish soils to this date, the main sources for the marine environment in Sweden are however considered to be tire wear and artificial grass football fields (Magnusson *et al.* 2016). Agricultural soils are furthermore suspected to be exposed to major direct inputs of MPs through the widespread use of Sewage sludge on arable land (Nizzetto *et al.* 2016). Other diffuse and direct sources of MPs in arable land may be related to atmospheric deposition, or fragmented agricultural plastics, e.g., mulching films, seed coatings, or silage bales (Horton *et al.* 2017).

2.1.2 Mobility of MPs in soil

Most studies quantifying MP content in soil focus on the concentrations in the topsoil or the plough layer (Heinze *et al.* 2021). However, MPs as other particulate matter may be affected by different vertical and horizontal transport processes and might thus enter the deeper soil profile (Alimi *et al.* 2018) or translocate to nearby environmental compartments. For example, MPs have been found both in the top and sub-soil in a field study investigating spatial trends of MPs in a floodplain soil, thus indicating that vertical transport of MPs in the soil may occur under field conditions (Weber & Opp 2020). Furthermore, laboratory-based studies have shown that MPs are transported vertically in soil via Earthworm bioturbation (Huerta Lwanga *et al.* 2017b; Rillig *et al.* 2017; Yu *et al.* 2019; Heinze *et al.* 2021) and through advective driven transport (Yu *et al.* 2019; Alimi *et al.* 2018).

Both chemical and physical properties may influence the transport distances of MPs in soils. MPs are larger than mobile natural particles such as clays or organic matter. It is therefore considered that the size of MPs is constraining the advective transport (i.e., via percolating water) (Zhao *et al.* 2022), due to the generally much smaller size of pores and their limited continuity within the soil profile (Bradford *et al.* 2004). For smaller sized plastic particles, i.e., NPs, the advective transport is known to be a function of pH, soil texture and the nature and concentration of dissolved organic carbon (Cornelis *et al.* 2014). For structural soils like clay, the transport of particle-bound nutrients, such as phosphate, has been observed to depend primarily on the presence of macropores in the soil profile (Jarvis 2007). Similarly, for MPs, transport by preferential flow in earthworm burrows has been observed in one study using disturbed soil columns (Yu *et al.* 2019).

Biologically mediated transport, for example by earthworms, is thought to be less restricted by constraints in the soil structure than transport with water, as ingestion and excretion can be spatially separated. However, bioturbation dynamics are also affected by the agricultural management practice (Capowiez *et al.* 2009) and may be limited by compaction of the soil (Torppa & Taylor 2022).

2.2 Metals

2.2.1 Metal contaminants associated with sewage sludge

Municipal sewage sludge contains trace metals, e.g., Cu, Cd and Ni, that are emitted from society and accumulated in the particulate fraction of the sewage sludge (Kirchmann *et al.* 2017). Thus, the metal content may vary between different areas depending on inputs from the surroundings, as well as over time. As sewage sludge is used in agriculture, the metal contaminants are unintentionally spread to the soil and may pose risks to the environment and human health. Amongst the trace metals found in sewage sludge, Cd is of special interest as it is severely toxic for humans and may be highly mobile in the soil (Bergkvist *et al*. 2003). The main risks are associated with Cd leaching and potentially contaminating the groundwater or being taken up by the crops. Swedish limit values for Cu, Cd and Ni in agricultural soil where sewage sludge is used are: 50-140, 1-3, 30-37 mg kg^{-1} DM for the metals respectively (SNFS 1994:2).

2.2.2 Mobility of metals in soil

The term sorption will be used for describing immobilization and retention mechanisms of the metals in this thesis, as distinguishing between adsorption or absorption processes in the soil is often complicated (Essington 2005). Retention and mobility of metals in the soil is regulated by sorption processes that determine if the metals are accessible for plant uptake and susceptible to leaching. More mobile metals tend to pose increased risks to humans and the environment, especially if they are also more toxic intrinsically. Potential sorption surfaces for metal cations include oxides, clays and soil organic matter, and it is common that more than one type of surface can be important for the retention process of the metals (Gustafsson *et al*. 2007). In general, the stronger the metal ions' affinity for binding to a solid phase is, the less mobile they are in the soil profile (Essington 2005). However, if metals are sorbed to colloids, small mobile particles below 1 μm in size, such as clay minerals or organic matter, they can instead be mobilized and transported together with the colloids (Frimmel *et al.* 2007). For colloidal transport, the presence and continuity of macropores is important (Koestel & Larsbo 2014).

Cu, Cd and Ni are all examples of metals that are most typically found in one oxidation state in the soil: Cu^{2+}, Cd^{2+} and Ni^{2+} (Essington 2005). Out of the three metals that were focused on in this study, Cu binds strongest in soil (Eriksson 2001). According to the Irwing Williams order, the general affinity for binding to particles follows $Cu^{2+} > Ni^{2+} > Cd^{2+}$ (Essington 2005). In particular, Cu tends to be closely associated with soil organic matter and humic substances (Gustafsson *et al*. 2007), but it also tends to form complexes with oxides and clays. As some of these have variable charge sites, such as organic matter, sorption of Cu also depends on the pH (Gustafsson *et al*. 2007). Cd and Ni form relatively weak complexes, in comparison to Cu, and are thus more susceptible for leaching (Gustafsson *et al*. 2007). It has been shown that for Cd and Ni similar geochemical properties determined their maximum sorption capacities (Elbana & Selim 2019). Cd and Ni forms complexes to organic matter, and may also bind to oxides, Ni being bound to oxides is related to high pH (Gustafsson *et al*. 2007). Additionally, competition for sorption sites between ions may have implications for their solubility or retention (Gustafsson *et al*. 2007).

3. Materials and Methods

3.1 Overview

To understand the fate of contaminants associated with sewage sludge this thesis was split into two parts 1) a field study, where the vertical distribution of MPs and metals was quantified in agricultural depth profiles after long-term sewage sludge application, and 2) a laboratory-based transport process study, where soil columns were first spiked with indium-doped MPs and metals to then quantify the transport caused by earthworm bioturbation or simulated rainfall. The MP contents in the field study were acquired before this thesis and provided for comparison. An overview of the methods used for the different parts in this study is shown in Figure 1.

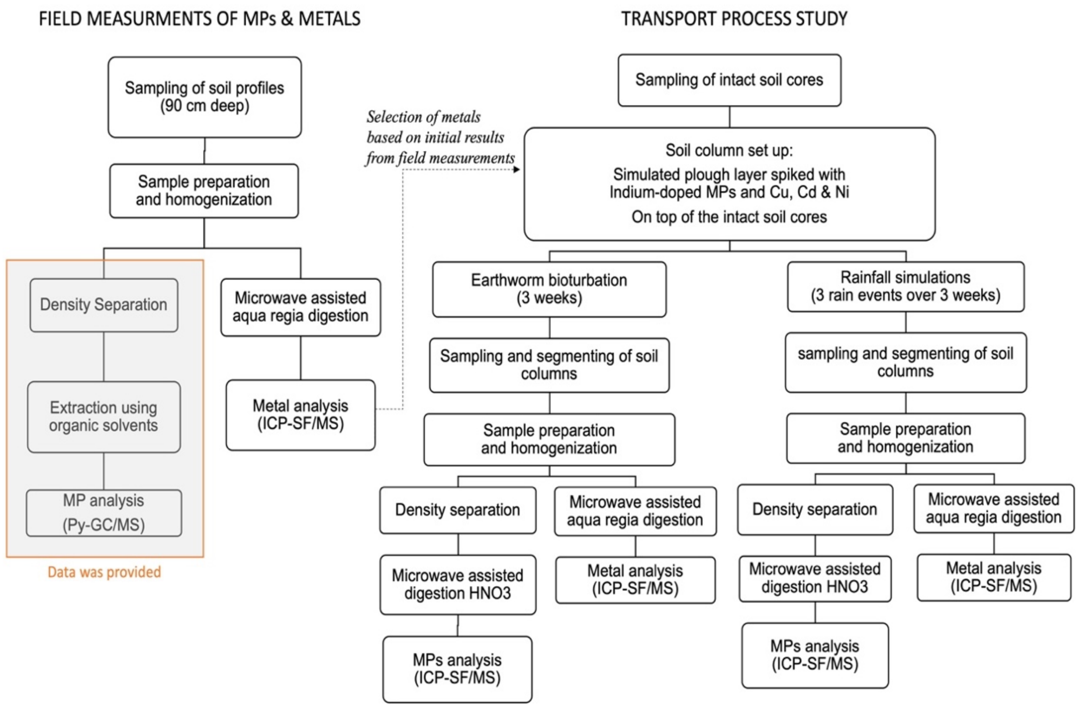

Figure 1: Overview of the methods used for quantifying microplastics and metals from the field samples and the general set up of the transport process study for quantifying the transport of metals and microplastics caused by earthworm bioturbation or simulated rainfall.

3.1.1 Field site description

The soil analysed in the field study and used for the transport process study was taken from an agricultural field at Lanna research station (SLU), situated in Skara in the western part of Sweden. Table 1 shows the most important properties of this soil. The soil can be classified as a Udertic Haploboroll and it has a high clay content, 43-45% in the topsoil and 56-61 in the subsoil (Eriksson *et al.* 2016).

Table 1: Selected soil properties of topsoil 0-20 cm, measurements of pH and C% are from 2018 and obtained from Lanna research station. Mineral fraction % are based on textural analysis from samples taken in 1996.

pH sewage sludge treatment (F)	pH control treatment (B)	C % sewage sludge treatment (F)	C % control treatment (B)	Clay %	Silt %	Sand %
6.0	6.4	2.34	1.99	43-45	13-18	1-6

For the field measurements, soils cores were taken from field plots belonging to a long-term agricultural experiment established in 1996. The long-term field trial has a randomized block design, comprising different fertilization treatments with four replicate field plots per treatment (Figure 2). Samples were taken from two of the treatments, one where sewage sludge was applied biennially (treatment F) and one managed with mineral fertilizers (treatment B) (Table. 2), of which the latter served as a control in this study, thus contrasting the sewage sludge application to conventional agricultural practice. For the past 26 years, the application of sewage sludge has followed a biennial cycle, corresponding to 13 application instances that took place after the autumn harvest and before ploughing. Prior to field application, the ash-free dry mass for the sewage sludge is determined (Kätterer *et al.* 2014) and then amounts corresponding to 8 tons per ha^{-1} are applied to the fields manually. Treated municipal sewage sludge has been used and the origin has varied over the years due to availability. Crop rotations on the long-term field trial have consisted of solely cereals, which are harvested each year.

Table 2: management practices for treatment F and B in the long-term field study at Lanna

Treatment	Management
B	Every year: 80 kg nitrate (N) ha-1 (applied as calcium nitrate Ca(NO3)2) Every second year: 40 kg phosphorous (P) and 30 kg potassium (K) ha-1
F	Every second year: 8 ton ash free dry mass of sewage sludge ha^{-1}

For the process study, soil samples were taken from a field located right adjacent to the long-term field trial at an approximate 10 m distance from the edge of the nearest field plot (Figure 2). Management practices regarding e.g., crop-rotation and ploughing were similar in this field as compared to the fields belonging to the long-term field trial.

Figure 2: Sampling sites for sewage sludge treatment, mineral fertilize treatments and the soil cores used in the transport process study. Map data: ©2022 Google

3.2 Field measurements of microplastics and metals

3.2.1 Sampling of soil

Samples were taken from each replicate field plot of the sewage sludge (F) and the mineral fertilizer (B) treatment. To capture spatial heterogeneity, eight soil cores of 2.5 cm in diameter and a maximum depth of 90 cm were taken for each field plot and used to create composite samples. The soil cores were segmented into depth layers according to 0-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90 cm. The uppermost depth segment was made thicker compared to the others to cover the full plough layer (0-20 cm). Corresponding depth segments from the soil cores were compiled into one composite sample per field plot and depth layer of approximately 400-500 g each. The equipment used for sampling was a metal soil corer aided by a tractor. The soil samples for the field study were taken in January 2021, prior to the start of this thesis project.

3.2.2 Sample preparation

The composite samples for each depth layer were air-dried and then homogenized manually using a porcelain mortar and pestle and sieved to remove the fraction >2 mm. The fraction below 2 mm was then analysed for their MP and metal content (see section 3. 4). The MP analysis was done prior to this work and data provided for this thesis, whereas the metal content was determined as part of this work as described in section 3.4.1.

3.3 Transport process study

3.3.1 General setup of process studies

A transport process study was set up in the laboratory to assess MP and metal transport under controlled conditions in intact soil columns. The study focused on two potential transport processes: advective flow and bioturbation. Soil columns were prepared by first taking intact soil cores from a field directly adjacent to the long-term field trials. Sewage sludge was sampled from a WWTP in Uppsala and was spiked with known concentrations of metal-labelled MPs and metals (Cd, Cu and Ni). The spiked sewage sludge was then mixed with soil sampled from the plough layer and added on top of each soil column, forming a simulated plough layer. Then, the soil columns were exposed to either rainfall simulations or earthworm bioturbation. After approximately 3 weeks of exposure, the soil cores were segmented into depth layers and the vertical distribution of MPs and metals was analysed. This way, it was possible to directly contrast the transport of MPs with different metals and assess the impact of water movement and bioturbation, respectively.

3.3.2 Sampling of intact soil cores

For the transport process study, intact soil cores of 10 cm diameter and 30 cm length (n=7) were taken from a field directly adjacent to the long-term field trials, within an area of approximately 2 × 2.5 m. The intact soil cores were sampled by first removing the top 20 cm of soil, corresponding to the depth of the plough layer. The intact soil cores were then taken from the subsoil at a depth of 20-50 cm (Figure 3). For sampling, polyvinyl chloride (PVC) columns (10 cm diameter, 64 cm length) equipped with a sharp metal ring were pressed into the soil using a hydraulic pump. The PVC column containing the intact soil core was then dug out manually. The topsoil (0-20 cm) was also collected within the same sampling area and stored separately. Sampling was done in March 2022.

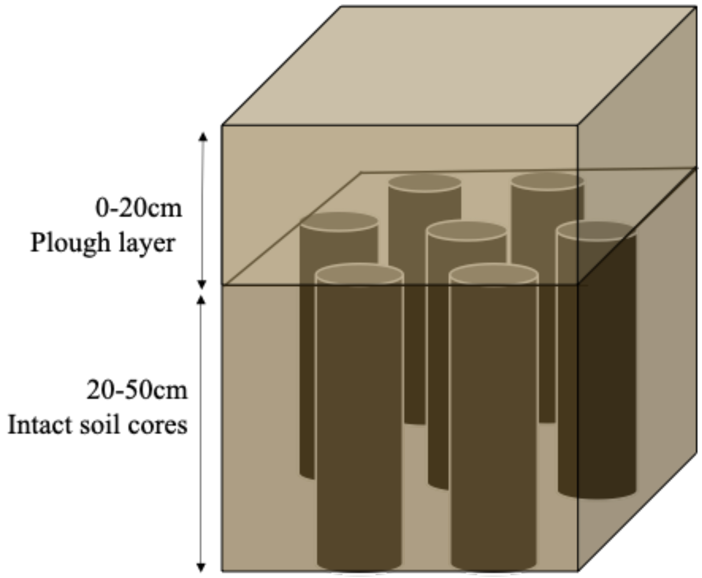

Figure 3: Schematic figure showing the sampling of intact soil cores for the transport process study. The plough layer was first dug out and then sampled separately.

3.3.3 Microplastic characteristics

Indium-doped (In) polyethylene terephthalate (PET) fragments with a size range between 125-250 µm were used to assess the transport of MP. The PET-fragments were synthesized by and obtained from Denise M. Mitrano (ETH Zürich). Using MPs doped with an In-tracer allowed to quantify the MPs transport in a simpler and more time efficient manner, as the concentration of In could be used as a proxy for the plastic fragments. The In-tracer accounted for 0.172 weight-% of the PET fragments. The MP were synthesized in a so called two-step emulsion polymerization procedure (Mitrano *et al*. 2019), in which the In-tracer was first incorporated into the core of the particle and then encapsulated by an outer shell made entirely out of PET-material. The metal-tracer has previously been confirmed to not leach from the plastic particles in relevant environmental samples nor did it affect the surface chemistry of the particle (Mitrano *et al*. 2019). Furthermore, the biodegradation of the In-doped fragments was considered negligible considering the short time span of the experiment, 3 weeks.

The size range of the MPs used in the transport process study are only slightly larger than the average size of MPs (median 71 um) found in a Swedish WWTP (Ryaverket, Göteborg) (Rasmussen *et al.* 2021), which has previously been used for sewage sludge in the long-term field study in Lanna (Börjesson *et al.* 2014).

PET was primarily chosen due to its availability in metal-doped form. While PET was not measured in the field samples, the size of the fragments was assumed to have a more decisive role rather than the plastic density regarding the vertical transport processes. Moreover, PET is a widely used plastic material (Plastics Europe 2021), which made it relevant to use in this study. Considering this, the metal-doped PET fragments were considered suitable for a first investigation.

3.3.4 Sewage sludge properties

Sewage sludge was applied in the transport process study to simulate field conditions similar to the sewage sludge (F) treatments in the long-term field trial. The sewage sludge was obtained from Uppsala vatten och avfall AB and sampled from a long-term storage site used for eliminating pathogens before application to the fields. Long-term storage, for at least six months, is a common hygienisation strategy accepted by the REVAQ-certification system. The sewage sludge used in this study was stored for nine months (From July 2021 on) before our sampling in April 2022. Dry weight for the sewage sludge was determined after the long-term storage (33 ± 4 %), by drying approximately 60 g WW of sewage sludge (n=3) at 105°C for 72 h. Metal contents of the sewage sludge were analysed prior to the long-term storage by Uppsala vatten och avfall AB (Table 3).

Table 3: Selected sewage sludge properties
metal analysis performed with ALS (values obtained from Uppsala vatten och avfall AB, unpublished material)

Metal	Concentration (mg kg DW^{-1})
Cd	0.472 ± 0.047
Cu	278 ± 27.8
Ni	12.8 ± 1.28

3.3.5 Soil columns

The general setup of the soil columns was the same in both experiments, although aspects of the treatments varied between the rainfall- and bioturbation experiments. The setup was designed to simulate an input scenario of MPs and metals in a sewage sludge amended soil. For this purpose, a simulated plough layer (0-20 cm) was spiked with sewage sludge, metals, and MP before it was applied to the intact soil cores (20-50 cm). A schematic figure of the set up can be seen in Figure 4.

Based on initial results from the field measurements, three metals: Cd, Cu, and Ni were selected for further investigation in the transport process study. The spiking was done by first adding Cd, Cu, Ni and indium-doped PET fragments to the sewage sludge and then sequentially adding the spiked sewage sludge to ca. 2 kg per soil column (2053 ± 95 g topsoil per soil column) (Table. 4). The spiked soil layer was thoroughly mixed in both steps of the preparation. The total amount of sewage sludge added to each soil column was approximately 6.4 g DW, representing an application of ca. 8 tons DW ha^{-1}.

Table 4: Summary of metal and plastic additions to the simulated plough layer. Total additions are given as mean value (± standard deviation) based on 6 replicates (7 for sewage sludge). The added concentrations are shown as mg per kg dry soil, calculated based on the average weight of the simulated plough layer (1724 g). The background concentrations are estimates based on measurements of topsoil from Lanna (replicates=3, aqua regia digestions).

	Sewage sludge (DW)	MP[c]	Cd	Cu	Ni
Tot. addition (mg $column^{-1}$)	6390 ± 40	1000 ± 30	0.46	13.3	15.0
Addition to the PL[a] (mg kg^{-1} DW[b])	3706	580	0.3	7.7	8.7
Estimated bkgd. conc. (mg kg^{-1})	-	-	0.1	10.1	12.4
Total conc. in the PL[a] (mg kg^{-1} DW[b])	3706	580	0.4*	19.6*	21.2*

[a]plough layer, [b]dry weight, [c]microplastic

**Calculated based on bkgd. conc., estimated metal conc. in the sewage sludge + metal additions*

The concentration of PET-fragments added to the simulated plough layer were considerably higher than the measured MP concentrations in the field samples (see Table 4, and Results 4.1.4), such high concentrations were deemed necessary for ensuring detectability of low concentrations in the previously uncontaminated soil layers after transport. Regarding the metals Cd, Cu and Ni, concentrations added to the plough layer were dependent on 1) detectability of concentrations after transport based on the detection limits of the detection methods (<0.1, <5 and <3 µg/L for Cd, Cu and Ni respectively) and estimated background concentrations for the soil 2) toxicity risks affecting the earthworms 3) availability of standard solutions. The total concentration of Cd in the plough layer was more than two times higher than the maximum field background concentration (0.16 mg kg^{-1}), based on measurements from the mineral fertilizer (B) treatment (see Results Figure 6). In contrast, the total concentrations of Cu and Ni were lower in relation to their maximum background concentrations (21.3 and 31.7 mg kg^{-1}, respectively, see Results Figure 5 and 7), due to the availability of Cu and Ni standard solutions as well as in concern over toxicity risks for the earthworms. For example, effects on earthworms have been observed at Cu concentrations >32 mg kg^{-1} (Spurgeon *et al.* 1994).

For keeping the intact soil cores in place, the bottom of each PET column was first covered with a mesh net and then put into a bucket filled with sand and gravel to enable free water drainage. In order to have a system as closed as possible and thereby avoid plastic or metal contamination, a glass petri dish was placed on top of the column, whereas aeration was maintained through small holes drilled into the uppermost cm of the column walls. After preparation, the columns were carefully placed inside a climate chamber set to 13°C, 65% relative humidity and 12h of daylight. The experiment then ran for three weeks in total, before sampling (sections 3.3.7 and 3.4).

Three replicates were prepared for each experiment and one column was used as a control. The control was set up as the other treatments but without adding MPs and metals and only exposed to earthworms to ensure that potential negative impacts on the earthworms in the process studies were not related to added MPs or metals. One soil column in the rain simulation experiment was lost during the experiment, so only two replicate columns were analysed in this experiment.

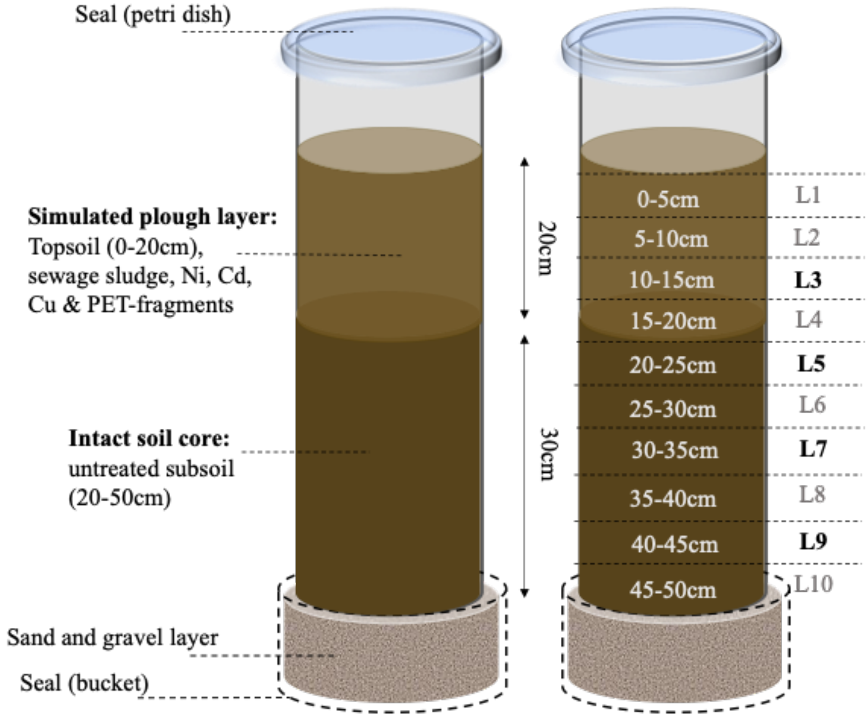

Figure 4: Schematic figure showing the soil column set-up for the process study. The right column is showing the different compartments of the soil columns and the left column is showing the segmentation used for sampling and analysis (sections 3.3.7 and 3.4).

3.3.6 Rainfall simulations

The effect of water movement on the transport of MPs and Cu, Cd and Ni inside the soil columns was tested by simulating three rainfall events. The experiment was run for 25 days in total and the rainfall events were performed on days 7, 12, and 18, with drying periods in-between. Columns were placed inside a fume hood for the rainfall simulation. The artificial rainwater was prepared using ultrapure water (Milli-Q, 18.3 Ω) according to Table 5.

Table 5: Salts added to ultrapure water (milli Q, 18.3 Ω) to form artificial rainwater, recipe from (Norrfors *et al.* 2021).

Salts	Conc. in rainwater (umol/L)
Sodium chloride, NaCl	10
Ammonium sulphate, $(NH_4)2SO_4$	5.3
Sodium nitrate, $NaNO_3$	5.9
Calcium chloride, $CaCl_2$	5.9

The artificial rainwater was dripped onto the soil surface inside the column over a rainfall simulation head using a peristaltic pump. The rainfall intensity was set to 16 mm h^{-1} and the duration time was 1 h, corresponding to a rainfall event with a return period of 2 years in the region of southwest Sweden (Olsson *et al.* 2017). The pump was calibrated before and after the rainfall events, in order to ensure constant rainfall.

3.3.7 Earthworm bioturbation

For investigating the effects of bioturbation on the transport of MPs, Cd, Cu and Ni, adult individuals of the earthworm species *Aporrectodea longa* were introduced to the soil columns for 21 days. The species selection was based on their expected burrowing behaviour, which is epi-anecic, i.e., it includes borrows both in the horizontal plane as well as deeper borrows in the vertical plane (Eisenhauer *et al.* 2008; Emmerling & Strunk 2012; Arrázola-Vásquez *et al.* 2022). In addition, Viketoft *et al*. (2021) found *Aporrectodea* to be the dominating genera in the long-term field trials at Lanna. The earthworms were collected from an agricultural field at SLU Ultuna and sorted on a species level and *A. longa* species were retained.

The earthworms were depurated for 48 h in petri dishes with moist filter paper before the start of the experiment. After depuration, the wet weight of the earthworms was 2.4 ± 0.2 g. The depurated earthworms were introduced to the soil surface on the day after the column setup, to allow the soil and spiked sewage sludge to first equilibrate overnight and thereby minimize potential negative impacts of reactive metals on the earthworms. Three earthworms were added to each of the columns, corresponding to an abundance of 382 individuals m^{-2}. This was approximately twice as high as the mean abundance of earthworms found in the sewage sludge treated plots in the long-term field trial at Lanna (ca 150 individuals m^{-2}) (Viketoft *et al.* 2021). Although the abundance was higher compared to the actual field conditions, the higher density of earthworms was chosen to enhance the chances of measuring bioturbation effects considering the short time span of the experiment.

The moisture level inside the columns was maintained throughout the experiment by repeated spray application of ultrapure water every 2-4 days. The respective evaporation loss in between the application instances was used for determining water additions.

3.3.8 Sampling & homogenization of soil samples from the process study

After three weeks, the soil columns were sampled by segmenting each column into 10 depth segments of equal thickness (5 cm), denoted as layer 1-10 as depicted in Figure 3. The PVC columns were opened on their horizontal plane by sawing them open on two sides with 180° in between the cuts, keeping the soil column inside intact. By first determining the intersect between the plough layer and the previously intact soil core, using each intact cores initial length, segmentation started from that point and outwards to the bottom and top of the soil column. Resulting in layer 1 and 10 potentially having deviating depths in the different columns. Sampling was performed by sequentially partitioning the segments from the rest of the soil core using a knife. The plough layer was sampled first, starting at the soil surface from Layer 1-4, after which, the lower layers were sampled from bottom up starting with Layer 10-5, this order was implemented to minimise the risk of cross-contamination. The bottom 0.5 cm of each column was discarded.

The columns from the rainfall simulation experiment were put into the freezer (-20°C) for at least 48 h before sampling. This step was adapted to stop the water movement inside the column as ponding was suspected. Freezing of rainfall columns was performed 18 days after the first rainfall event. The upper part of the soil columns (0-25 cm) was segmented by sawing, whereas the lower part (25-50 cm) was sampled with a knife after thawing.

Earthworms from the bioturbation columns were collected during the sampling. For assessing potential negative impacts of the experimental set up, earthworms were again rinsed, depurated for 48 h, and weighed.

Wet and dry weights for the soil samples were noted, dry weights were obtained from oven-dried samples (105 °C, for at least 3 days). Individual depth segments were then homogenized manually using a mortar and pestle. The samples were then sieved for homogenization with a 2 mm sieve to focus on the fine soil fraction. Each depth segment was then divided into subsamples by successive halving to ensure representative samples for metal and MP analyses. The vertical distribution of PET-fragments based on In, and the metals Cd, Cu and Ni was then analysed in 4 selected layers per column (Layer 3 (10-15 cm), Layer 5 (20-25 cm), Layer 7 (30-35 cm) and Layer 9 (40-45). Since only four of the ten depth layers per column were analysed one major drawback of the method was that a mass balance of the added contaminants could not be made.

3.4 Analytical methods for microplastic & metal detection

3.4.1 Metals

Metals were extracted from soil samples using a microwave-assisted *aqua regia* (1:3, HNO_3 65 % : HCl 37 %) digestion. The extractions followed an international standard method for analysing total concentrations of trace elements in soil (ISO 11466:1995). The detailed protocol for the metal extraction can be found in Appendix 1. Subsamples of soils for the metal detection corresponded to approximately 0.5 g each. For the laboratory process study, 3 replicates per depth segment and field sample were analysed. For the field study, 2 replicates were analysed per depth segment and field replicate in the sewage sludge (F) treatments, whereas 1 sample was analysed per depth segment and field replicate in the control (B) treatments (with exception for 0-20 and 20-30 cm, n=2).

The metals analysis was performed by ALS Global using a double focusing inductively coupled mass spectrometer (ICP-SF/MS). In the field study, cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn) and silver (Ag) were initially measured and used to pre-select metals to focus on in the laboratory study. Thus, only Cd, Cu and Ni were measured in the samples from the soil columns in the transport process study.

3.4.2 Microplastics

In the field study, concentrations of polyethylene (PE) were measured via pyrolysis gas chromatography mass spectrometry (Py-GC/MS). The analysis followed steps described by (Steinmetz *et al.* 2022). In short, homogenized soil samples (50 g) were pre-concentrated in a density separation (NaCl) step, the MPs collected in a filter and extracted with suitable organic solvents for analysis on the Py-GC/MS. This analysis was performed prior to the start of this thesis and data was provided for comparison.

For quantifying MPs in the transport process studies, one sample of 50 g soil was analysed from each of the selected depth segments for each soil column. Similar to the field study, samples were first pre-concentrated using density separation to ensure detection. However, as PET has a higher density (ca 1.4 g cm^{-3}) compared to PE (0.9 g cm^{-3}) a saturated sodium bromide (NaBr) solution with a target density of ≥1.5 g cm^{-3} was used instead. First the soil, then the NaBr solution (250 mL) were added to a density separation funnel and shaken in a horizontal shaker for 4 hours. Afterwards, material stuck on the glass walls was rinsed down with NaBr solution using a Pasteur glass pipette. The samples were then left for settling overnight (at least 16 h). Settled material was carefully released, while the supernatant containing the floating MPs was collected (80-100 mL), vacuum filtered and transferred into microwave digestion tubes over a glass funnel. Transferring was performed by carefully rinsing using ultrapure water (Milli Q, 18.3 Ω). The excessive water was evaporated slowly at 60°C in an oven until the sample was dry. Afterwards, the samples were extracted following a modified digestion protocol, using 1 mL hydrogen peroxide (H2O2) and 8 mL of HNO3 (65 % vol.).

Indium concentrations served as a proxy for the PET-fragments. The In-detection analysis of extracts was performed similarly to the other metals by ALS Global using a double focusing inductively coupled mass spectrometer (ICP-SF/MS).

3.4.3 Quality control

Before acid digestions, all laboratory equipment was treated in an acid bath (1 vol. % HNO_3) for at least 12 hours and then rinsed with ultrapure water (Milli-Q, 18.3 Ω). For the provided field measurements of MPs precautions regarding usage of any plastic material were taken both in the field and in the laboratory.

For assessing the quality of the digestion methods spike recoveries (n=3) and procedural blanks (n=3) were included in all the prepared digestion batches. Cd, Cu, Ni, and In spike concentrations corresponded to 0.5 mg L^{-1} in the diluted acid extract and were prepared from stock solutions (999 ± 2 mg L^{-1} diluted in 3 % HCl). The spike recoveries were then measured after digestion in absence of soil (See appendix 2).

Results from the spike recoveries in the field measurements showed a recovery percentage that ranged between 97-119 % for Cu, Cd and Ni. For the spike recoveries in the transport process study, recovery percentages were 102 ± 4 % for Cu, 103 ± 3 % for Ni, and 123 ± 50 % for Cd, however one of the Cd spike recoveries was likely an error due to duplicate pipetting and without that sample the recovery percentages of Cd were 109 ± 4 %. The general trend shows recoveries slightly over 100% and it is likely that dilution or pipetting steps of the digestion method contributed to a slight overestimation of the metal concentrations in this study.

For measuring the recovery of In from the PET-fragments, approximately 15 mg of the In-doped plastics were included in the MPs digestion (n = 3). Recoveries were 73 ± 3 %. This was lower than expected, compared to initial trials (unpublished material). The spike recoveries with In stock solution (999 ± 2 mg L^{-1}) had recoveries of 74 ± 3 % (n = 3). Thus, a systematic underestimation of In is suspected to also affect the recovery for In incorporated in MPs. Moreover, the recovery of MPs after density separation for one sample resulted in a recovery of 62 %. Considering that this was only 10% less than the direct recovery, we accepted the potential underestimation as feasible for the context of this study. Tables showing the results from procedural blanks and spiked recoveries can be found in appendix 2.

3.5 Statistical analyses

Two tailed t-tests (Microsoft Excel) were used for comparing the F-treatment with the B-treatment in the field measurements and for comparing rainfall and bioturbation treatments in the transport process study. Equal variance was assumed and a significance level of 0.05 was used.

4. Results and discussion

4.1 Field measurements of microplastics and metals in agricultural soil after long term sewage sludge application

Quantification of metal- and MP concentrations in the depth profiles of the long-term field trial allowed for making comparisons between the sewage sludge treatment (F) and the control treatment with conventional mineral fertilizer (B). Initially, six metals associated with sewage sludge were analysed in the soil samples. Three metals: Cu, Cd and Ni where then pre-selected for this thesis, based on the results of their vertical distribution trends. Results showing the vertical distributions of the remaining metals (chromium, lead and zink) are presented visually in Appendix 3.

4.1.1 Vertical distribution of copper

When sewage sludge was applied (treatment F), Cu contents of the soil were significantly higher ($p > 0.05$) than when mineral fertilizer was applied (treatment B) and most of the Cu was accumulated within the top 0-40 cm of the soil (Figure 5). This is in line with findings by Börjesson *et al.* (2014), who found that the levels of Cu were higher in the sewage sludge treated soils in the same long-term field trial (0-20 cm). Moreover, the accumulation of Cu observed in depth layers 20-40 cm indicates that Cu has been translocated beyond the plough layer.

Figure 5 shows that the two treatments have contrasting depth-dependent trends. Concentrations of Cu in the sewage sludge treatment (F) are decreasing with depth, whereas Cu concentrations in the control treatment (B) are increasing with depth. Furthermore, the concentration of Cu below 60 cm seems to be higher for the control treatment than the sewage sludge treatment. This observed pattern was also statistically significant for depths 70-80 cm (F treatment = 17.0 ± 1.6 mg kg^{-1}, B treatment = 19.5 ± 0.9 mg kg^{-1}, $p = 0.002$).

A possible explanation for the contrasting trends in the two treatments may be related to the fact that organic matter has also been added to the soil via the application of sewage sludge. Börjesson *et al.* (2014) measured soil organic matter (SOM) contents in both the sewage sludge and mineral fertilizer treatments and found that SOM (measured as total C concentrations) contents were significantly higher after sewage sludge applications. Because Cu has a high affinity for organic material it is likely that the retention of Cu in the upper soil layers of the sewage sludge treatment are related to the high SOM content. Similarly, the translocation of Cu to lower soil layers observed in the mineral fertilizer treatment could be explained by lower SOM content in the topsoil layers, as compared to the sewage sludge treatment, resulting in fewer sorption sites for Cu. As a result, dissolved Cu would be more mobile in the soil profile.

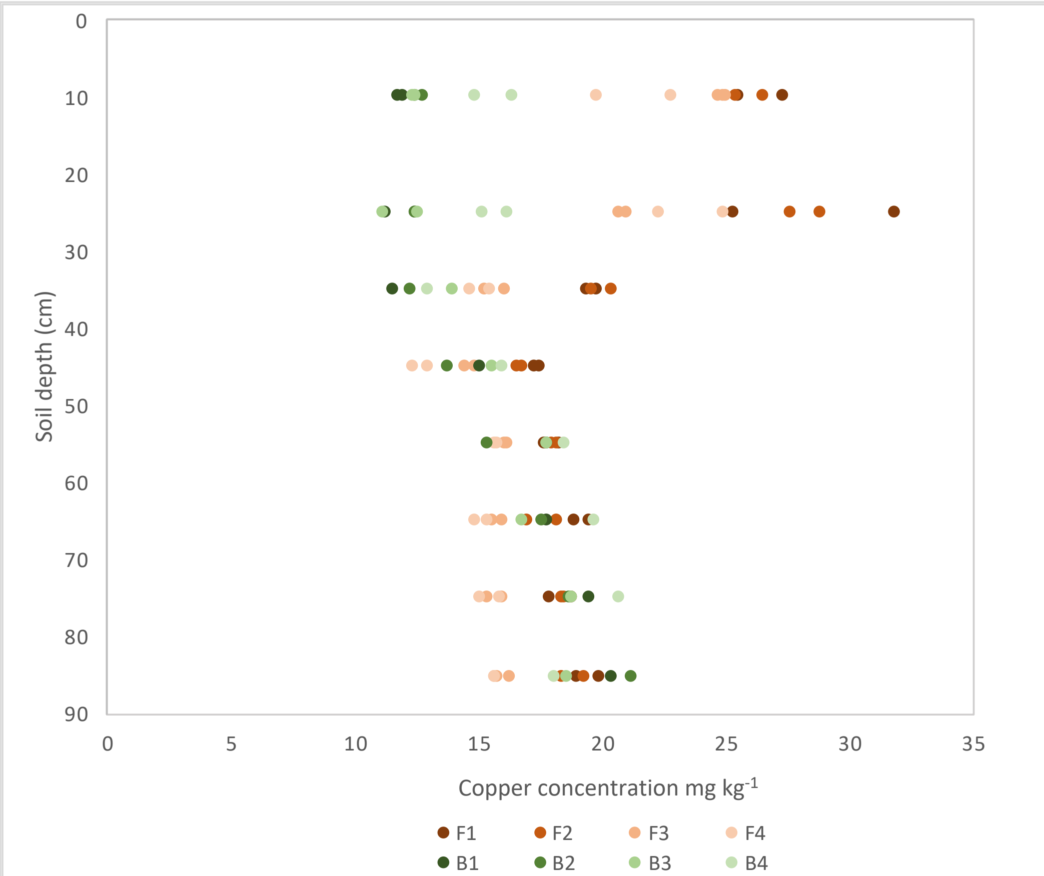

Figure 5: Copper distribution in the depth profile. Cadmium distribution in the depth profile. F = sewage sludge treated soil, B = soil treated with mineral fertilizer. Samples taken 2021.

4.1.2 Vertical distribution of cadmium

The results showed no significant differences between Cd concentrations in the sewage sludge and the control treatments. However, Figure 6 clearly shows that there were higher concentrations of Cd in the topsoil layers for both treatments and a steep decline from layer 30-40 to 40-50 cm. For both treatments, the concentrations in the upper soil layers (0-40 cm) were significantly higher ($p<0.05$) than the concentrations in the lower soil layers (40-90 cm). It is common with elevated Cd levels found in the topsoil of Swedish agricultural soils, with the average concentration being around 60 % higher in the plough layer as compared to the subsoil (0.23 and 0.14 mg kg^{-1}, respectively) (Eriksson 2009). A major part of the elevated Cd content in the topsoil is generally due to the continuous anthropogenic inputs of Cd over time (Eriksson 2009). Common input pathways of Cd to the soil, other than sewage sludge, are phosphate-fertilizers and atmospheric deposition (Bergkvist *et al.* 2003).

Cd inputs through P-fertilizers and sewage sludge might explain the elevated levels of Cd found in the topsoil for the B- and F-treatments respectively. The average concentration of Cd measured in the plough layer corresponds to approximately 326 g Cd ha^{-1}. The concentration of Cd impurities in P-fertilizers after year 1997 can be estimated to approximately 39 mg Cd kg^{-1} P (Bergkvist *et al.* 2003), corresponding to a total input of 20 g Cd ha^{-1} in the B-treatment plots during the long term field trial (from 1996-2021). For the F-treatment, the concentration of Cd in the sewage sludge could be estimated to approximately 1 mg Cd kg^{-1} DW (Davidsson 2013), corresponding to a total input of 120 g Cd ha^{-1} (from 1996-2021). Although the estimated input from sewage sludge was 6 times higher than that of the P-fertilizers, there was no significant difference between the measured Cd concentrations in the B- and F-treatment plots. The reason for this is currently unknown, however it is possible that the actual Cd content in the sewage sludge was lower than the estimated 1 mg Cd kg^{-1} DW. In addition, these calculations did not include the potential outputs of Cd, i.e., leaching or uptake by plants.

Furthermore, historical inputs of Cd might also have contributed to the elevated Cd levels found in the topsoil. Before year 1997 P-fertilizers could contain Cd concentrations up to 173 mg Cd kg^{-1} P (Bergkvist *et al.* 2003). Thus, depending on the agricultural use of the fields before the start of the long-term field trial, it is possible that Cd inputs through P-fertilizers might have been an important pathway to this soil historically. The atmospheric deposition of Cd is estimated to have contributed to approximately 43 g ha^{-1} on Swedish agricultural soil during the 1900s (Eriksson 2009). Both the historical inputs and the estimated inputs through P-fertilizers and sewage sludge during the field trial gives a relatively high increase in Cd concentration, which can in part explain the elevated Cd levels observed in the topsoil in both treatments, as compared to the deeper soil layers.

Inputs of Cd through sewage sludge, P-fertilizers and atmospheric deposition enter the soil surface and are then ploughed into the soil (0-20cm). However, the accumulation of Cd observed in the depth layers below the plough layer (20-40 cm), indicate that Cd might have been subject to transport processes. It has earlier been shown by Börjesson *et al.* (2014) that the SOM content was higher in the sewage sludge treatment than in the control treatment, yet there were no significant differences seen between the retention of Cd in the sewage sludge and mineral fertilizer treatments. A possible explanation for this might be that Cd predominantly sorbs to the mineral fraction in this soil. Expandable minerals, such as smectite or vermiculite, is the dominant mineral clay fraction found in the long term field trials (Eriksson *et al.* 2016), and may constitute sorption sites for Cd. However, more detailed analysis of the soil properties at the site would be required to draw more definite conclusions regarding the potential sorption sites for the metals.

Cadmium was examined in a similar long-term field trial as has been investigated in this thesis: Bergkvist *et al.* (2003) examined Cd distribution as an effect of long-term (1956-1997) biennial application of sewage sludge in a clay loam soil from a field trial at SLU Ultuna, Sweden. In contrast to Lanna, the total Cd concentration at the Ultuna site had increased by 500 % in the top 20 cm of the soil profile since the start of the field trial in 1958. Similarly, to results from this study, the Cd budget of the sewage sludge treatment in Ultuna indicated a translocation of Cd (ca 7 % of the tot. input) from the topsoil to below the plough layer (20-37 cm depths) (Bergkvist *et al.* 2003). Moreover, Bergkvist *et al.* (2003) found that the solubility of Cd was higher in the sewage sludge treated soil, in comparison to control treatments. Which could not be observed in Lanna, probably due to considerably lower concentrations of Cd in the topsoil compared to the Ultuna soil.

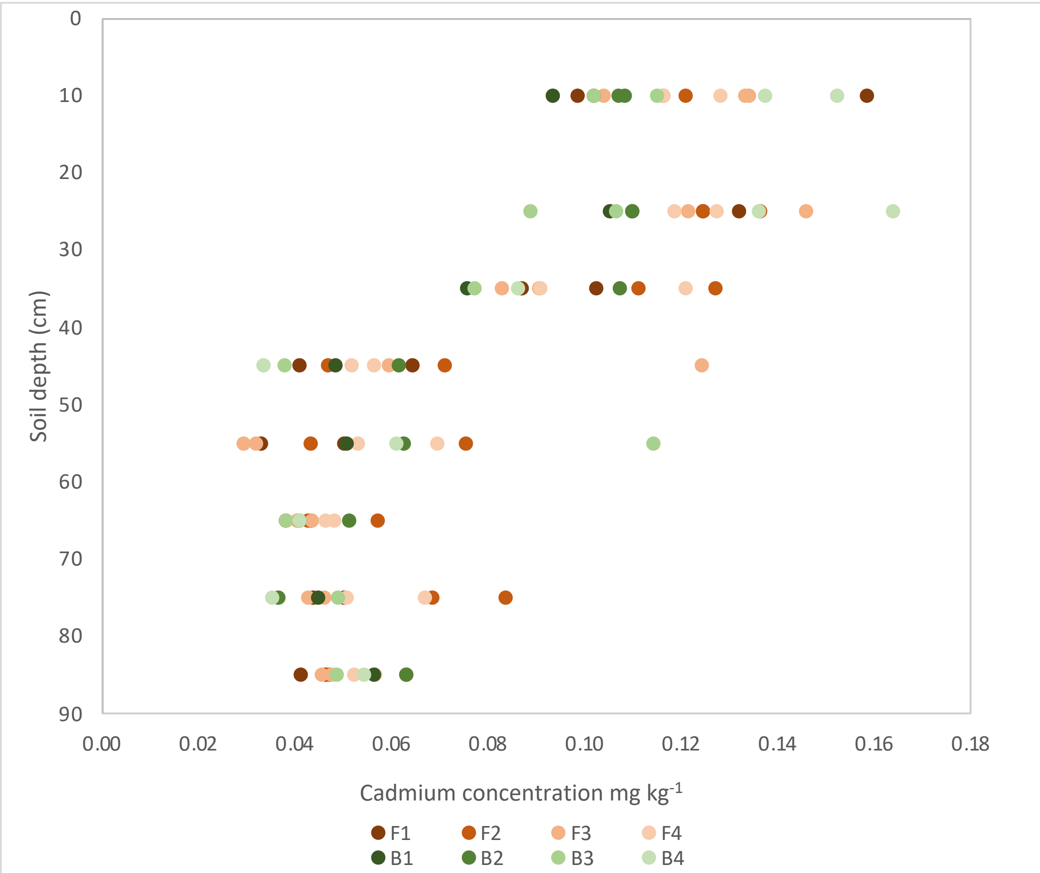

Figure 6: Cadmium distribution in the depth profile. Values on Y-axis refers to the depth segments 0-20, 20-30, 30-40 etc. F = sewage sludge treated soil, B = soil treated with conventional mineral fertilizer. Samples taken 2021.

4.1.3 Vertical distribution of nickel

Ni concentrations in the sewage sludge treatment were significantly lower in some depth layers (0-30 cm, 50-60 cm, 80-90 cm) than in the respective depths in the control treatment. However, without any clear depth-dependent trend. It seems possible that these results could be an effect of Ni being unintentionally added to the control treatments, e.g., impurities from the mineral fertilizers (Senesi & Polemio 1981).

Ni concentrations in both the sewage sludge treatment and the control treatment are lower in the upper soil layers from 0-30 cm, compared to the deeper soil layers > 30 cm (Figure 7). These results might indicate that Ni has been translocated downwards and retained in the deeper soil layers of the soil profile, a depth-dependent trend that is contrasting with the distribution found for Cd and Cu. However, the depth dependent distribution of Ni could also be a result of natural occurring differences in the Ni-content in the soil at different depths, depending on initial Ni concentrations in the clay minerals. The average Ni concentration in sewage sludge from Swedish WWTPs (year 2000) was approximately 20 mg Ni kg DW (n=48) (Eriksson 2001), corresponding to a total input of approximately 2000 g ha^{-1} (1996-2021). The estimated total increase of Ni in the soil via sewage sludge would then be 0.8 mg Ni kg soil. This estimate is a relatively small fraction of the measured concentrations in the soil (Figure 7), indicating that the Ni distribution is likely a result of naturally occurring Ni deriving from the clay minerals.

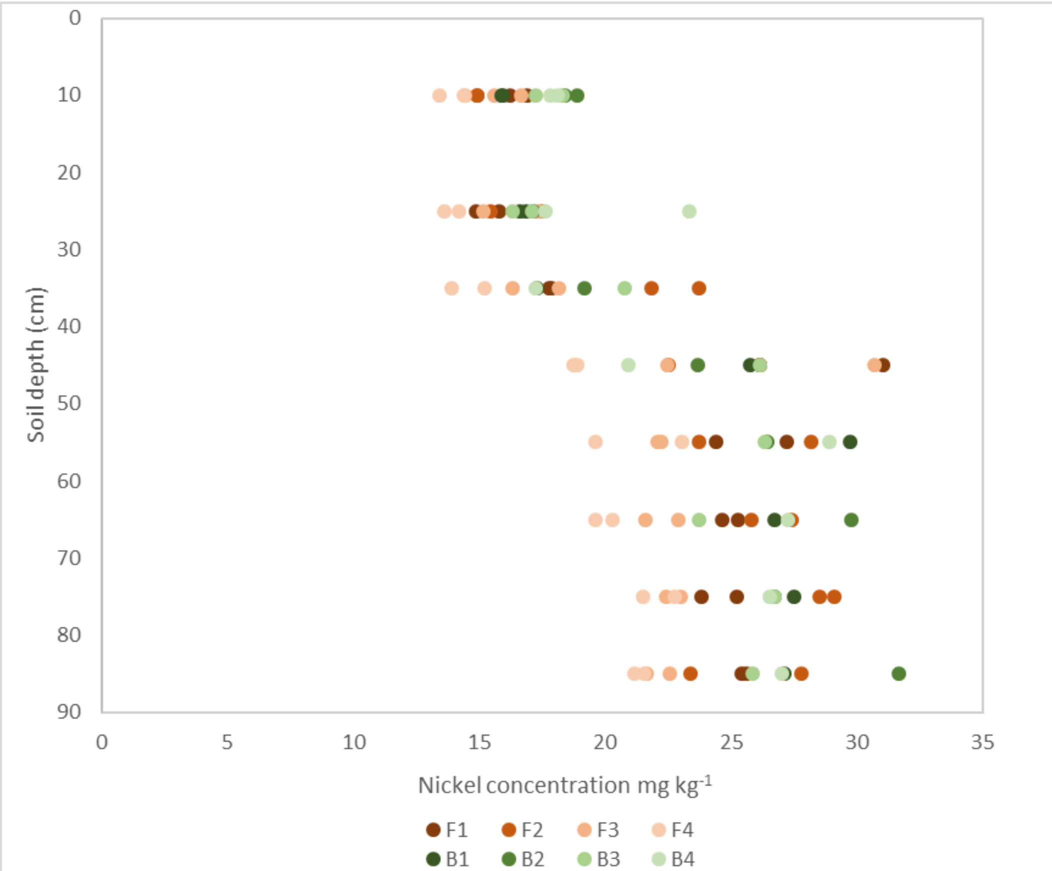

Figure 7: Nickel distribution in the depth profile, F = sewage sludge treated soil, B = soil treated with mineral fertilizer. Samples taken 2021.

4.1.4 Vertical distribution of polyethylene

The most striking results from the field measurements of PE was that when sewage sludge was applied significantly higher ($p < 0.05$) concentrations were found, as compared to when mineral fertilizers were used. Accumulation of MPs after successive sewage sludge application has also been observed in previous studies (Corradini *et al.* 2019; van den Berg *et al.* 2020). As suspected, the highest concentrations were found within the plough layer 0-20 cm. However, PE MPs were also observed in soil layers below the plough layer, i.e., 20-40 cm in the sewage sludge treatment. This observation indicates that transport processes have effects on the vertical distribution of MPs.

For the sewage sludge treatments, a steep decline in the MP concentrations could be observed after 40 cm depth in the soil profile (Figure 8). This depth dependent distribution trend could also be observed in the control treatment, where higher concentrations were detected above 40 cm depth than below. These results indicate that the vertical transport of MPs is primarily limited to a depth of 40 cm. One theory could be that the denser clayey subsoil is limiting the transport caused by either water or earthworm movements in the soil. This theory is discussed in more detail in following sections.

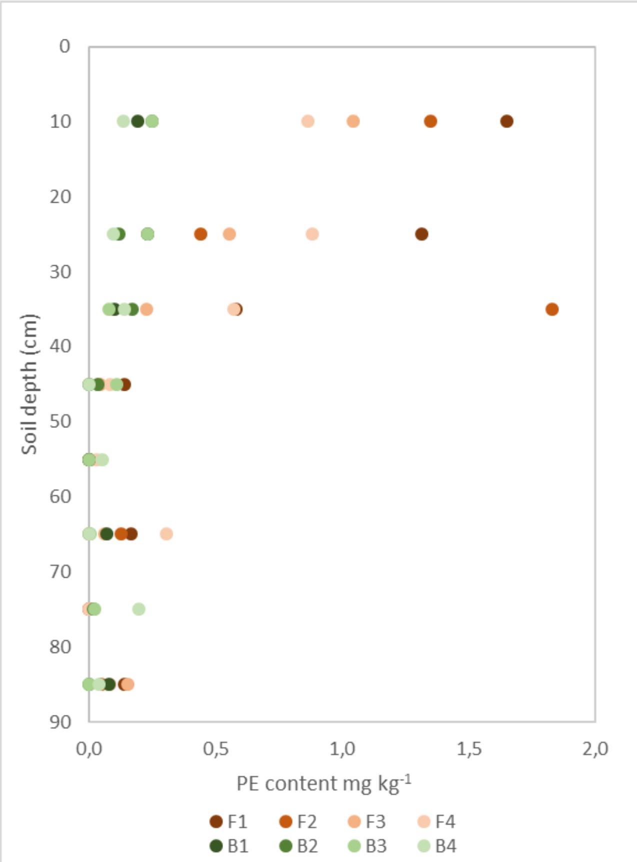

Figure 8: Polyethylene content in depth profile from the long-term fertilization experiment. F = sewage sludge treatment, B = mineral fertilizer treatment. Samples taken 2021.

4.1.5 Comparison between microplastics and metals and their vertical distribution based on the field measurements

Comparing the results from the field measurements reveal that MPs were distributed similarly to Cu and Cd in the depth profiles, whereas Ni showed an opposite distribution trend. Most of the Cu, Cd and MPs were accumulated within the uppermost 0-40 cm of the soil profile, indicating that the observed transport below the plough layer (>20 cm) was limited to a certain degree for Cu, Cd and MPs, but not for Ni. These observations may support the initial hypothesis that MPs would behave somewhat similarly as particulate-bound metals, because we argued that Cu was mostly bound to organic colloids and Cd bound to smectite clays. While the division between dissolved and particulate bound metals was not quantified in the field measurements, it was initially assumed that dissolved metals would move more easily through the soil profile than particulate bound metals and thereby likely show a distribution trend similar to the one observed for Ni. In contrast, the vertical distribution patterns for Cu and Cd indicate that they may have been sorbed to particulate matter in the upper soil layers (0-40 cm), thus becoming less mobile in the soil profile as compared to Ni. It was apparent from the field measurements that there must have been some transport processes affecting, at least Cu and MPs, as they were found below 20 cm depth in concentrations higher than the control treatment. We infer that the processes that may have caused this spatial pattern are bioturbation or advective transport with water.

If the observed transport was advection-driven, Cu were likely transported as DOC-complexes. Kätterer *et al.* (2014) investigated soil organic carbon (SOC) in the same field trial and found relatively high advective transport of SOC in the sewage sludge treatment (3–7 mm yr^{-1}). Interestingly, their study also argued that the organic amendments (sewage sludge, manure, and compost) were translocated to a maximum depth of 40 cm, supporting the theory that Cu may have been co-transported with the organic matter. Moreover, Kätterer *et al.* (2014) argued that the observed SOC translocation could be explained by transport via macropores in the clay soil. If the observed transport was indeed advection-driven, it is possible that a lack of macropores below 40 cm could explain the limited transport of the colloidal bound Cu, as well as the MPs who would also be physically retained because of their size. The transport of Cd could be explained by a similar restriction of colloidal transport of Cd-clay complexes. In addition, indications of frequent macropore flow have been observed in lysimeter studies, as well as in measurements of the hydraulic conductivity of Lanna soil (Andersson *et al.* 2013), supporting the theory that advective-driven transport in macropores may occur at the site.

Bioturbation driven transport would likely result in distribution patterns in the soil profile that would be similar for all particulate bound material, as the fate of particle associated contaminants is indifferent of contaminant chemistry as long as the earthworms are able to ingest the particles and the contaminants do not affect them. It is therefore possible that the observed translocation of Cu and Cd was due to bioturbation processes. The same theory could also be applied on the MPs.

It is likely that bioturbation is more intense in the upper parts of the soil profile where more organic material is available, and the soil is less dense. Food availability and high mechanical resistance of the dense clay soil may have limited transport in the deeper soil layers. It has been shown that with higher soil resistance the burrowing activity of earthworms can be significantly reduced (Arrázola-Vásquez *et al.* 2022). Agricultural management practices, may pose effects on the population of earthworms available in the soil, e.g., it has been shown that populations might be reduced significantly due to compaction of the soil caused by heavy vehicles (Keller *et al.* 2017). Furthermore, ploughing, may have effects on the species composition of earthworms, with less deep burrowing (Anecic) species observed in conventional ploughing systems, compared to soils with reduced- or no ploughing (Capowiez *et al.* 2009). The porosity and connectivity of macropores in the soil was also lower after conventional ploughing, compare to reduced or no ploughing (Capowiez *et al.* 2009). As a result, it has been shown that bioturbation was 17 times higher under no ploughing practices, compared to when conventional ploughing was used (Torppa & Taylor 2022). Thus, it is possible that little transport through bioturbation takes place at depths greater than 40 cm in the soil profile at Lanna, as a consequence of unfavourable habitat conditions. At the same time, the higher abundance of earthworms found in the Sewage sludge treatments at Lanna (Viketoft *et al*. 2021) may promote transport dynamics by bioturbation within the less dense part of the soil profile.

The transport of metals and MPs to layers just below the plough layer may, in addition, be explained by a reduction of the bulk density and a rise of the soil surface, as discussed by (Bergkvist & Jarvis 2004). They found that after long-term sewage sludge application, bi-annually for 39 years, the topsoil in the field trials had expanded by 8 cm, due to the absence of heavy machinery and addition of organic material, allowing for the plough layer moving upwards in the soil profile. It is possible that a similar expansion of the plough layer has taken place also in the long-term field trial in Lanna. Kätterer *et al* (2014) found that the bulk density in the sewage sludge treatment was significantly lower than in the mineral fertilizer treatment. However, it is not likely that this expansion of the soil is accountable for all of the accumulated concentrations of metals and MPs found at greater depths, e.g., 30-40 cm. Moreover, for Cd no differences between the sewage sludge and the mineral fertilizer treatment could be seen and for both treatments most Cd was concentrated between 0-40 cm, indicating that transport processes rather than surface elevation of the sewage sludge treatment is responsible for the observed spatial patterns.

4.2 Transport process study

4.2.1 Burrowing behaviour of the earthworms in the Bioturbation experiment

Earthworm activity could visually be confirmed down to approximately layer 7 or 8 in the bioturbation replicates, as well as the control column (Figure 9). The upper soil layers 0-20 cm showed clear signs of extensive earthworm activity, e.g., burrows and casts. A declining earthworm activity was then observed with increasing depth, down to approximately layer 7 (30-35 cm) or 8 (35-40 cm) in the replicate columns. At greater depths (>40 cm) there were no signs of earthworm activity. In addition, by visual inspection during sampling it could be observed that replicate 1 showed more signs of earthworm activity in comparison to replicate 2 and 3.

Figure 9: The photography shows signs of burrowing activity in the plough layer and the subsoil layers, the photo is of bioturbation replicate 1. The marking is located approximately at the top cm of layer 5 (20-25 cm), i.e., the first layer below the simulated plough layer.

The individual weights of the earthworms were measured before and after the experiment. During the time of the experiment the mean weights of the earthworms decreased slightly (losing on average between 0.3 - 0.5 g) in all the replicates soil columns, including the control column. Moreover, two individuals of the earthworms were found still and rolled up as shown in Figure 10 at the time point of sampling. This type of behaviour is observed when earthworms are in a state of diapause (Kiyasudeen *et al.* 2016), and it gives an indication that the soil conditions in this experiment were not ideal for this species, possibly a too low soil moisture level (Kiyasudeen *et al.* 2016), or high clay contents causing high mechanical resistance (Arrázola-Vásquez *et al.* 2022). Spike concentrations of metals or MPs was not suspected to be the cause of this behaviour because one of the rolled-up individuals was found inside the control column.

Figure 10: Rolled up earthworm detected in the control column.

4.2.2 Metal distributions in the soil columns after bioturbation and rainfall treatments

The total concentrations of Cu, Cd and Ni were quantified at four different depth layers in the soil columns after bioturbation or simulated rainfall treatments (Figure 11, 12 and 13). To assess if there was transport of the metals caused by either bioturbation or rainfall, metal concentrations in the transport columns were contrasted to the control column. Results showed that the concentrations in the investigated layers below the plough layer were not significantly higher in the transport columns as compared to the control column. This indicates that transport to the lower layers by bioturbation and rainfall was very limited and most of the metals were retained in the plough layer to which they were initially spiked. Furthermore, this theory is also supported by the average metal concentrations in layer 3, which are just slightly lower than estimates made for the initial concentrations in the plough layer, approximately 19.6, 0.4, 21.2 mg kg^{-1} for Cu, Cd and Ni respectively (concentrations in layer 3 in the control column + spike concentrations).

The main purpose of studying Cu, Cd and Ni in the soil columns after bioturbation and simulated rainfall was to see if there were any differences between the two treatments for any of the metals. While the differences were very small, significantly higher mean concentrations of Cu were detected in layer 5 (20-25 cm) in the columns after bioturbation treatment compared to the rainfall treatment (11.61 ± 0.48 mg kg^{-1} for bioturbation and 11.06 ± 0.31 mg kg^{-1} for simulated rainfall, $p=0.03$). Similarly, in layer 7 (30-35 cm) the Cd concentrations were significantly higher after the bioturbation treatment than after simulated rainfall (0.09 ± 0.02 mg kg^{-1} and 0.07 ± 0.02 mg kg^{-1}, $p=0.05$). It is difficult to relate these differences directly to the transport processes in this study, because of the high concentrations of the metals in the control column as discussed above. However, it seems possible that bioturbation might have caused more transport of Cu and Cd below the plough layer as compared to the rainfall treatment. For Ni, no significant differences could be seen when comparing the concentrations in the bioturbation and rainfall columns.

Contradictory to what was observed in the field measurements (section 4.1), the results of the process study showed that the transport of metals was more limited compared to the field study. There are several possible factors related to the experimental setup in this study that could explain the limited transport of metals. First, the time span of the experiment was only three weeks, as compared to the field measurements where the samples were analysed after over 20 years of sewage sludge application. It is possible that more transport could have been detected after a longer time span. Second, considerably lower spike concentrations of the metals were added to the simulated plough layers, in comparison to the high concentrations of the deeper layers in the control column, as can be seen in Figure 11, 12 and 13. Contradictory to what was initially planned for, concentrations of Cu and Ni in the control column were in some depth layers even significantly higher than the concentrations in the transport columns, indicating a higher background metal concentrations and more spatial variation than initially expected. The spike concentrations that were added to the soil columns would thus require some adjustments to obtain detectable transport results for especially Cu and Ni. It is not possible to draw any strong conclusions from these results, rather more studies would be required to assess which factors are limiting regarding the transport of metals in this type of soil.

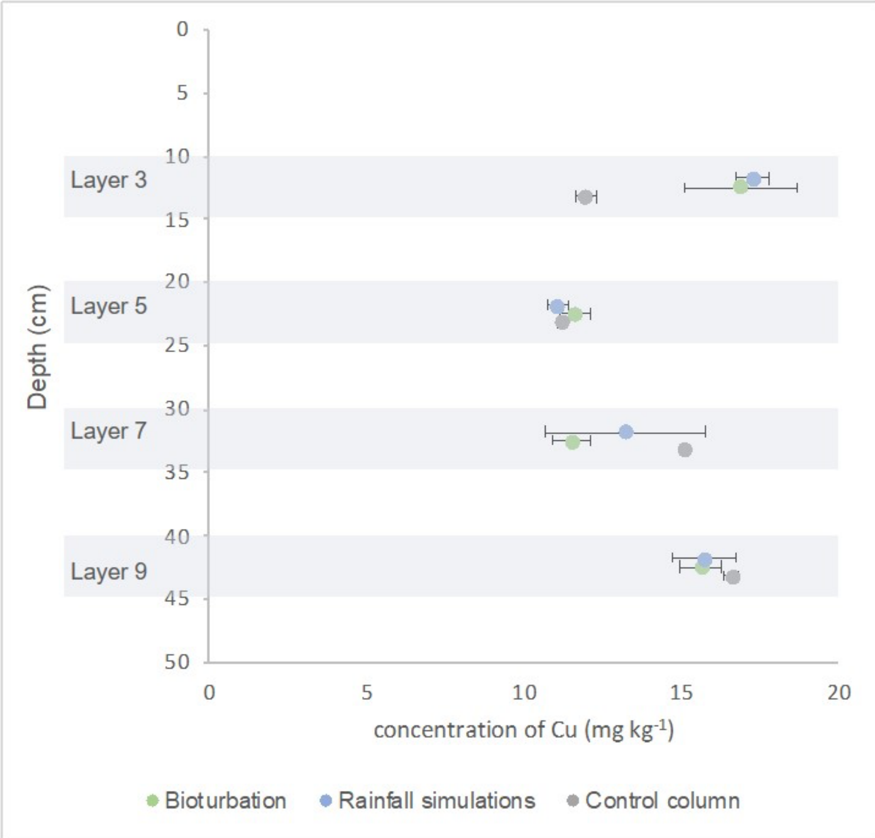

Figure 11: The vertical distribution of copper in the soil columns after simulated rainfall (blue) or bioturbation (green) treatments, in comparison to the control column (grey). Concentrations are given as mean values ± standard deviations (n of replicate columns for bioturbation = 3, rainfall = 2, control = 1). Note that the y-coordinates for the data points are not the exact depths as the quantification of metals was performed in 5cm thick depth segments (depicted as layer 3, 5, 7 and 9).

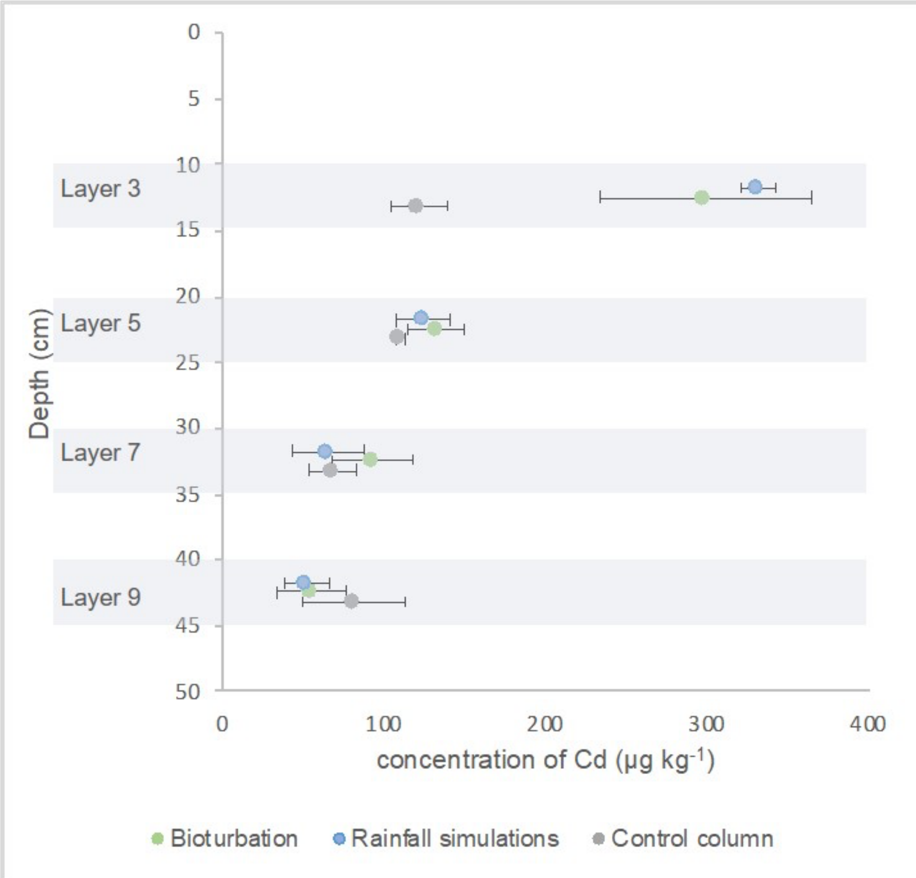

Figure 12: The vertical distribution of cadmium in the soil columns after simulated rainfall (blue) or bioturbation (green) treatments, in comparison to the control column (grey). Concentrations are given as mean values ± standard deviations (n of replicate columns for bioturbation = 3, rainfall = 2, control = 1). Note that the y-coordinates for the data points are not the exact depths as the quantification of metals was performed in 5cm thick depth segments (depicted as layer 3, 5, 7 and 9).

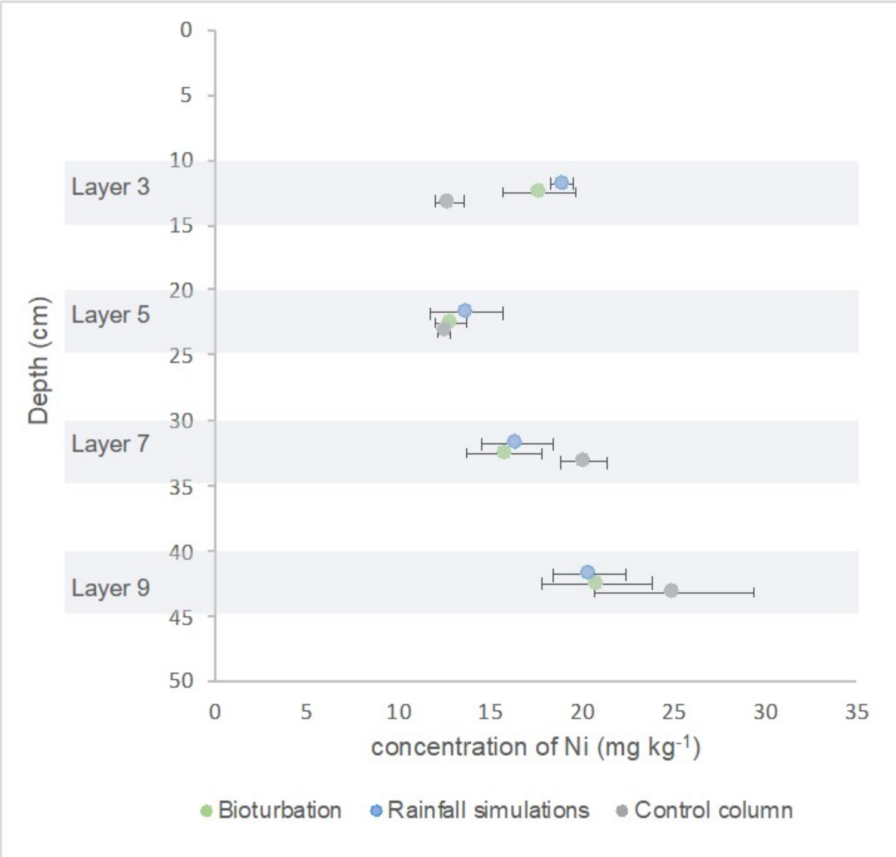

Figure 13: The vertical distribution of nickel in the soil columns after simulated rainfall (blue) or bioturbation (green) treatments, in comparison to the control column (grey). Concentrations are given as mean values ± standard deviations (n of replicate columns for bioturbation = 3, rainfall = 2, control = 1). Note that the y-coordinates for the data points are not the exact depths as the quantification of metals was performed in 5cm thick depth segments (depicted as layer 3, 5, 7 and 9).

4.2.3 Transport of PET-fragments due to bioturbation and simulated rainfall treatments

Most of the MPs in the process study were detected within the simulated plough layer in all the soil columns after exposure to rainfall or bioturbation (Table 7, see results for layer 3). The mean concentrations of PET detected in the plough layer corresponds to 37 ± 18 % and 46 ± 1 % of the initial spike concentrations in the bioturbation and rainfall columns respectively. Assuming that the plough layer was homogenous at the start of the experiment, the concentrations in the plough layer were suspected to be higher considering also the low transport of MPs to deeper soil layers. These rather low recovery percentages can in part be explained by the analytical challenges of the detection method discussed in section 3.4.3. The systematic underestimation of recoveries of MP via digestion suggests that all the detected MP concentrations in the soil columns are likely underestimated. For the results, this means that if the detected concentrations are corrected for potential underestimation the average recovery percentages in layer 3 would instead be approximately 51 % and 64 % of the initial spike concentrations, for the bioturbation and rainfall columns respectively. There is thus still a large fraction of the initial input that was not recovered in the soil columns. Since it was not possible to do a full mass balance, as only a selection of depth segments could be analysed, it is difficult to assess if the MP were possibly accumulated elsewhere in the columns.

MP concentrations in the investigated layers below the plough layer were overall low in comparison to the total input. Approximately 8, 4 and 1 % of the initial spike concentrations could be detected in layer 5 (20-25 cm) for the bioturbation replicates 1, 2 and 3 respectively. For the simulated rainfall columns, these values were 2 % and 0.1 % of the initial spike concentration for replicate 1 and 2 respectively. For deeper layers concentrations were even lower. Nevertheless, the results indicate that transport of MP was occurring in the soil columns to a limited extent. Furthermore, it appears that more MPs were transported by earthworm bioturbation than through the simulated rainfall, although the observed trend was statistically non-significant.

Table 6: Vertical distribution of MP in the soil columns after bioturbation or rainfall simulation. Concentrations of PET-fragments are based on measured indium concentrations.

Tot. amounts of PET-fragments in quantified soil layers (mg kg^{-1})					
Depth layer (Soil depth)	Bioturbation 1	Bioturbation 2	Bioturbation 3	Rainfall sim. 1	Rainfall sim. 2
3 (10-15 cm)	259.8	288.7	99.9	263.2	272.2
5 (20-25 cm)	43.8	24.3	5.4	12.1	0.6
7 (30-35 cm)	0.11	1.24	0.02	0.05	0.01
9 (40-45 cm)	0.06	0.04	0.01	0.03	0.03

In contrast to earlier findings of MP- and NP transport caused by earthworm bioturbation (Huerta Lwanga *et al.* 2017a; Rillig *et al.* 2017; Heinze *et al.* 2021) the transport of MPs observed in this study appeared more limited. However, there may be several reasons for this related to the experimental setup in this study. The most striking difference was that earlier studies examined the transport of MPs in re-packed soil columns (Huerta Lwanga *et al.* 2017a; Rillig *et al.* 2017; Heinze *et al.* 2021), whereas the subsoil in this study initially consisted of an intact soil core. It can therefore be argued that the intact cores used in this experiment pose conditions closer to the actual field conditions at the investigated site, and thus would give a more realistic indication of the transfer rates that may be expected in a dense clay soil. Arrázola-Vásquez *et al*. (2022) observed that burrowing rates of *A. longa* decreased with increasing mechanical resistance, expressed in bulk density. This means for my results that the observed low transport of MPs may be explained by the high density of the clay soil in the soil columns.

Moreover, the previous studies used shorter soil columns, e.g., Heinze *et al*. (2021) and Huerta Lwanga *et al.* (2017) investigated bioturbation in 30 cm long columns, it is possible that the limited volume of soil may have forced the earthworms to be more active in the given space, as compared to in this study. In addition, it has been suggested that Earthworm ingestion and transport of MPs may be dependent on particle size (Rillig *et al*. 2017). Rillig *et al*. (2017) found that smaller plastic particles (710–850 µm) were transported to a greater extent than bigger particles (1700–2800 µm). Although the particle sizes used in this study was smaller than that of Rillig *et al*. (2017), the MPs in this study were considerably larger than the NPs (256 nm) investigated by Heinze *et al.* (2021). Lastly, another species of earthworm was used in the other studies (Huerta Lwanga *et al.* 2017a; Rillig *et al.* 2017; Heinze *et al.* 2021), namely *Lumbricus terrestris.* There are different ecological categories of earthworms and In contrast to *A. longa,* who is considered an epi-anecic species that makes burrows both in the horizontal and vertical plane (Arrázola-Vásquez *et al*. 2022), *L. terrestris* is considered an anecic species that primarily makes deep vertical burrows (Rillig *et al*. 2017). Thus, *L. terrestris* potentially transport MPs and NPs more efficiently than *A. longa.* Overall, the implications of these results shows that more studies are required to establish what determines the rate of MP relocation in the soil.

Closer inspection of Table 7 shows that replicate 3 in the bioturbation experiment had considerably lower MP concentrations detected in all the depth layers, as compared to the other bioturbation replicates. For the concentrations detected in the simulated plough layer, differences between the replicates could be explained by unintentional MP hotspots being created during spiking of the plough layer. The variable concentrations observed in the investigated layers below the plough layer in different replicates are likely an effect of deviating earthworm bioturbation activity between the columns. The soil in replicate one, for instance, was moister, and it showed more signs of burrowing activity in comparison to the other two replicates which might explain the higher MP concentrations detected in layer 5 (20-25cm) in replicate 1.

Earthworm activity was visually apparent down to approximately 40 cm in the columns. This corresponds well with the distribution trend in the field measurements, where most MP were accumulated within 0-40 cm. If transport of MPs is bioturbation-driven, it is possible that less earthworm activity in deeper soil layers results in a transport that is limited to a certain degree. Moreover, the low earthworm activity observed below 40 cm in the soil columns is also an indication that earthworms may avoid highly compacted deeper soil layers, as discussed above, which in the field would result in more shallow burrow systems, in turn resulting in less macropores in the deeper soil in general. Such deficit in macropores might also prevent the advective transport of MPs.

An apparent lack of macro-pores in the soil below the simulated plough layer in the soil columns may likely have prevented advective transport of MPs. The bucket placed beneath each soil column was checked for drainage water after each rainfall event, as the bucket was dry on every one of these occasions it was assumed that the artificial rainwater was retained somewhere in the soil column. It was apparent at the time point of sampling that there was approximately 6-8 cm of water ponding on top of the denser subsoil, i.e., right at the lower boarder of the simulated plough layer at approximately 12-20 cm depth (Figure 12). It is therefore possible that elevated concentrations of MPs might be present in this soil layer due to ponding. However, since only a selection of depth layers were analysed in this study it is difficult to assess if MPs where indeed accumulated at this depth.

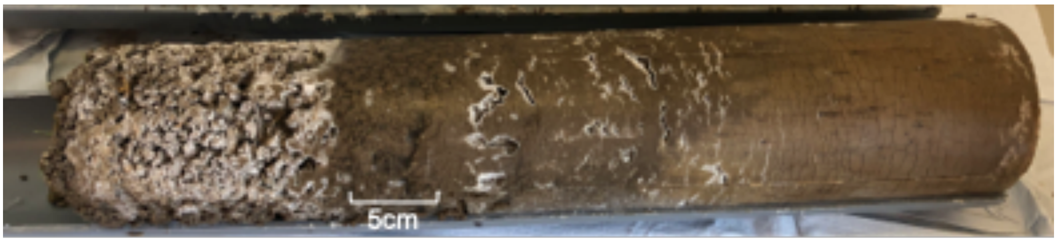

Figure 14: One of the frozen rainfall columns at the time point of sampling. The 5cm of ponding rainwater is marked on the picture. The ponding water was located at the lower boarder of the simulated plough layer, on top of the denser subsoil.

4.2.4 Comparison of bioturbation- and rainfall-driven transport of microplastics and metals

In contrast to the initial hypothesis and results from the field measurements, the transport process study was unable to demonstrate similarities between the transport of MPs and particulate-bound metals. For Cu, Cd and Ni no significant transport was detected, whereas for MPs transport was observed, although to a limited extent. While no significant transport of metals could be detected in this study, it is, however, still possible that transport of metals took place in the columns. The background concentrations of metals in the control column made it difficult to relate the detected metal concentrations in the transport columns directly to transport processes. In contrast, due to the sample preparation procedure used for detecting MPs it was possible to pre-concentrate the sample, i.e. through density separation, thereby analysing a larger sample volume and removing potential effects from background concentrations of In from the extracts. This factor may explain why it was possible to detect significant concentrations of MPs beyond the plough layer, but not metals.

Earthworm bioturbation was hypothesised to contribute more to the transport of MPs than advection. This trend was observed for MPs in the transport study, although it was not significant. The metal distribution also indicated that bioturbation may have caused more transport than advection. Significantly higher concentrations of Cu and Cd were detected in some layers of the bioturbation columns compared to the rainfall columns, however not following a clear depth dependent trend. It was, furthermore, not possible to relate these elevated levels to transport via bioturbation due to the high background concentrations.

Since no significant results could be found that supports, or rejects, the hypothesis that earthworm bioturbation is a driving mechanism for relocation of MPs, as well as metals, further investigation would be required to assess which mechanisms that controls the vertical transport of MPs. In addition, the comparison between the two transport mechanisms must be interpreted with caution since it is difficult to make a direct comparison between the two transport mechanisms. For example, one may argue that the rain simulations were more intense than the bioturbation experiment, as it is rather unlikely that a 2-year rainfall event takes place three times over three weeks time. To further investigate field conditions and possible effect on MPs, a similar transport study could be moved out to the field.

4.3 Conclusions and outlook

The present thesis aimed to gain further knowledge on the vertical transport of MPs, by comparing its distribution and transport to that of metals. To my knowledge, this is the first occasion that intact soil cores have been used to investigate transport of MPs, and furthermore, it is among the first studies investigating MPs in comparison to metals. The major implications of this thesis are:

1. It was initially hypothesised that bioturbation would significantly contribute to the transport of MPs. Conversely, the observed transport of MPs in the process study appeared more limited in comparison to previous findings. Thus, suggesting that MPs are primarily accumulating within the upper part of the soil profile and are retained within the soil.

2. While the laboratory-based process study did not confirm the hypothesis that MPs would behave similar as particulate-bound metals, similarities between the distribution of Cu, Cd and MPs were observed in the field study, thus indicating that MPs may indeed be transported similarly as particulate-bound metals. This finding may help to further develop predictions for MP-fate in the terrestrial environment, which can be useful for exposure and risk assessment.

3. It is, however, apparent that MPs are transported in the soil over time based on measurements from the field study. The praxis to only measure MP concentrations in the topsoil could thus potentially lead to a systematic underestimation of the total plastic load in the soil. Therefore, when monitoring agricultural soil, e.g., after sewage sludge amendments, it is important to also include soil depths below the plough layer.

Several limitations of the current study need to be stressed when formulating these implications. The process study was a limited pilot study using intact clay columns to assess the transport under conditions mimicking real field conditions. However, it was shown that the number of replicates in this study may not have been sufficient to obtain robust statistics, in part because of high insecurities of the measuring methods for MPs and the spatial variation of the background metal concentrations. In addition, less transport was observed in this transport study compared to similar studies. Additional work is required to assess which parameters limited the transport of MPs, e.g., by investigating different soils. Finally, in natural systems no transport process is isolated from the other, there are still many unanswered questions regarding the potential effects of earthworm burrows and other types of macropores on advective transport via preferential flow. Further studies are thus required to analyse the full effect of macropores on the transport of MPs.

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Popular science summary

Recycling of nutrients is a prerequisite for sustainable agriculture. As an effort towards becoming a more bio-based society, an increasing share of sewage sludge is used in agriculture in Sweden. Sewage sludge is a by-product from the wastewater treatment plants that contains valuable nutrients and organic carbon. However, the usage of sewage sludge as a fertilizer on agricultural soil is controversial. Potentially problematic substances such as metals and microplastics are often enriched within the sewage sludge and applied to the soil unintentionally. To assess the potential impacts of different sewage sludge associated contaminants it is important to understand their fate after entering the soil, as their distribution in the soil will for example determine the exposure levels for crops and soil organisms.

While there is substantial research made on how metals are transported in soil, there is currently very little known about the emerging pollutant microplastics. To gain knowledge on the fate of microplastics in soil, this thesis project compared the vertical distribution of microplastics to that of metals, to assess potential similarities or differences. The metals that were selected for this study are typically associated with sewage sludge: copper, cadmium, and nickel.

In the first part of this study, we analysed an agricultural soil after 26 years of sewage sludge application. Similarities were found between the distribution of microplastics and the two metals copper and cadmium, which were all accumulated within the uppermost 40 cm of soil. This finding may help to further develop predictions for microplastics in the field, which can be useful in exposure and risk assessments.

In the second part of this study, we looked at transport of microplastics and metals under more controlled laboratory conditions. Two different transport processes were analysed, transport caused by rainfall and by the burrowing activity of earthworms. Overall, very limited transport could be observed for both metals and microplastics in the laboratory study. The limited transport can in part be explained by the dense clay soil used in this study. The results suggested that transport of microplastics in clayey soil might not be as fast as earlier studies have observed. This means that microplastics in the field are likely accumulated within the upper part of the soil profile and retained within the soil. However, more studies are required before we can make any general conclusion on the fate of microplastics in soil.

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I would like to give my warmest thanks to my supervisors Geert Cornelis and Wiebke Mareile Heinze for all their support and encouragement along the way and for the opportunity for me to work with this project, thank you. I would also like to thank the people at Lanna research station and Uppsala vatten och avfall AB for providing me with the soil and sewage sludge. I would like to thank Denise M. Mitrano at ETH Zürich for the opportunity to work with metal-doped microplastics. Finally, I want to thank my opponent Inga Haalck and my examiner Jon Petter Gustafsson for your insightful questions and comments.

Appendix 1

Protocol for microwave assisted aqua regia digestion

Soil samples were prepared by weighing in approximately 0.5 g of dry homogenized soil that was then transferred to 80 ml PFTE liners. To ensure the quality of the digestion method three replicates of procedural blanks and spiked recoveries (0.25µl of metal standard solutions 1000 µg/L) were also prepared for each digestion batch. The acids, HNO_3 65 %, and HCl 37 %, were then added to the liners to obtain a volumetric ratio of 1:3. The acids were added sequentially, starting with 1.25 ml HNO_3 that was added directly on the sample, after which 3.75 ml of HCL was added by carefully rinsing the walls of the liners. Directly after the acids were added, the liner was closed tightly with a lid and cap, before it was placed inside the microwave rotor. When all samples were prepared the rotor was placed inside the microwave. The microwave used for the digestions was an ETHOS Easy Advanced Microwave Digestion System (Milestone) equipped with a Maxi-44 rotor. The programme was set to reach 180°C after 20 minutes and stay at that temperature for 10 minutes, after which the samples were cooled down to at least 30°C. After digestion the liners were carefully opened inside a fume hood. The digests were then transferred to 50 ml volumetric flasks over a funnel containing a filter paper. Transferring was done by first rinsing the lid and cap of the liner, the digest was then poured into the volumetric flask, after which the liner was rinsed repeatedly (at least three times) and lastly the filter paper and funnel was rinsed into the volumetric flask. The volumetric flask was then filled up to the 50 ml mark and the dilution was transferred to 50 ml centrifuge tubes that were then stored in the fridge until analysis.

Appendix 2

Supporting information of spike recoveries and procedural blanks.

Table appendix 1: Spiked metal recoveries from the field study. Prepared with 0.25µl metal standard solution (1000 µg/L) (n. of replicates=15).

	Cadmium	Chromium	Copper	Lead	Nickel	Zinc	Silver
Recovery %	101	119	110	97	113	99	113
Stdev %	4	4	4	2	6	4	4

Table appendix 2: Results from procedural blanks for the digestion batches in the field study

SAMPLE	Cadmium µg/L	Chromium µg/L	Copper µg/L	Lead µg/L	Nickel µg/L	Zinc µg/L	Silver µg/L
A-PB1	<0.1	7.23	<5	0.72	3.88	<10	<3
A-PB2	<0.1	6.92	<5	1.65	3.84	255	<3
A-PB3	<0.1	7.74	<5	1.25	4.08	10.8	<3
B-PB1	<0.1	2.31	<5	<0.5	<3	<10	<3
B-PB2	<0.1	2.06	<5	<0.5	<3	<10	<3
B-PB3	<0.1	4.15	<5	<0.5	<3	<10	<3
C-PB1	<0.1	0.70	<5	<0.5	<3	<10	<3
C-PB2	<0.1	<0.5	<5	<0.5	<3	<10	<3
C-PB3	<0.1	0.91	<5	<0.5	<3	12.6	<3
D-PB1	<0.1	0.73	<5	<0.5	<3	15.6	<3
D-PB2	<0.1	<0.5	<5	<0.5	<3	15.4	<3
D-PB3	<0.1	<0.5	<5	<0.5	<3	<10	<3
E-PB1	<0.1	<0.5	<5	<0.5	<3	<10	<3
E-PB2	<0.1	<0.5	<5	<0.5	<3	19.1	<3
E-PB3	<0.1	0.97	<5	<0.5	<3	<10	<3
F-PB1	<0.1	<0.5	<5	<0.5	<3	<10	<3
F-PB2	<0.1	<0.5	<5	<0.5	<3	<10	<3
F-PB3	<0.1	<0.5	<5	<0.5	<3	<10	<3

Table appendix 3: Results from spiked metal recoveries added to the digestions in the transport provess study (n. of replicates=11).

	Cd	Cu	Ni
Recovery %	109	102	103
Sdev %	4	4	3

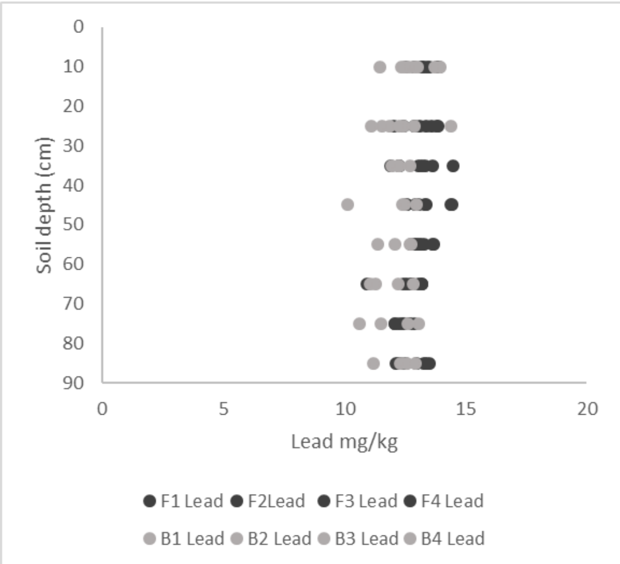
Table appendix 4: Results from indium recoveries, from spiked recoveries and indium-doped PET-fragments in the transport process study (n of replicates=3, except for the PET-fragments after density separation with only one sample).

	In[a]	PET-fragments[b]	PET-fragments after Density seperation[c]
recovery %	74	73	61
Stdev %	3	3	-

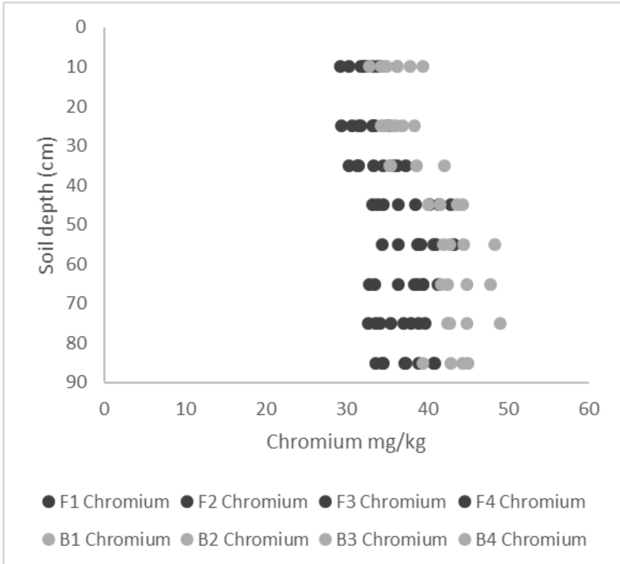
[a]Indium recovery from standard solution (0.25 µl of 1000 mg In/L)
[b]Indium recovery from PET-fragments after digestion
[c]Indium recovery from PET-fragments after density separation and digestion.

The results from all the procedural blanks in the digest batches from the transport process study were below detection limit for all metals (Cd, Cu, Ni and In).

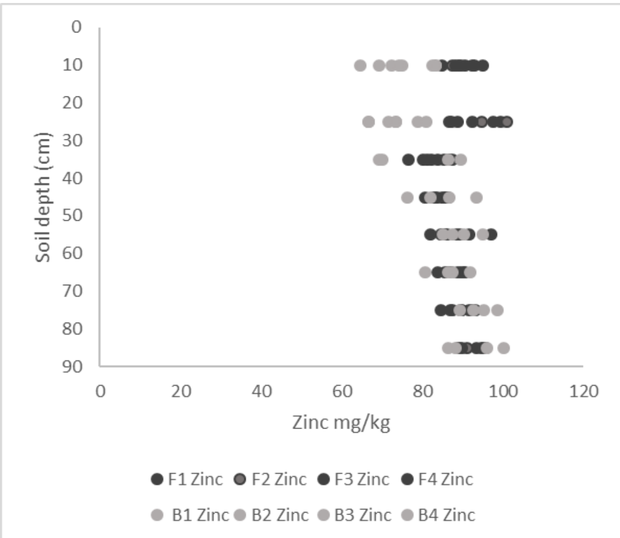
Appendix 3



a)



b)



c)

Figure appendix 1 a) b) c): Vertical distributions of metals (Lead, Chromium and Zinc) from a long-term field trial in Lanna, the metals are not investigated in detail in this thesis. Sewage sludge treated soils (F 1-4), Soils treated with mineral fertilizers (B 1-4).

Appendix 4

Supporting information: results of Cu, Cd and Ni contents from the field measurements analysing agricultural depth profiles in the long-term field experiment at Lanna.

Table appendix 5: Copper content in the depth profiles from field trials where F-treatment = sewage sludge, B- treatment = mineral fertilizers. Mean values are based on concentrations measured in the replicate field plots (mean ± standard deviations n: 3x4). Differences between the treatments were tested and P-values are provided from two tailed t-tests assuming equal variance. Samples were taken 2021.

Soil depth (cm)	F-treatments Cu content (mg/kg)	B-treatments Cu content (mg/kg)	P-value
0-20	24.7 ± 2.2	13.2 ± 1.6	1,8E-09
20-30	25.4 ± 4.0	13.1 ± 1.9	4,9E-06
30-40	17.6 ± 2.4	12.8 ± 1.0	3,5E-03
40-50	15.4 ± 2.0	15.1 ± 0.9	0,80
50-60	17.2 ± 1.1	17.4 ± 1.4	0,84
60–70	17.2 ± 1.8	18.0 ± 1.2	0,43
70–80	17.0 ± 1.6	19.5 ± 0.9	0,02
80–90	17.5 ± 1.8	19.6 ± 1.5	0,08

Table appendix 6: Cadmium content in the depth profiles from field trials where F-treatment = sewage sludge, B- treatment = mineral fertilizers. Mean values are based on concentrations measured in the replicate field plots (mean ± standard deviations n: 3x4). Differences between the treatments were tested and P-values are provided from two tailed t-tests assuming equal variance. Samples were taken 2021.

Soil depth (cm)	F-treatments Cd content (mg/kg)	B-treatments Cd content (mg/kg)	P-value
0–20	0.12 ± 0.02	0.11 ± 0.02	0.36
20–30	0.13 ± 0.01	0.12 ± 0.03	0.24
30–40	0.10 ± 0.02	0.09 ± 0.02	0.16
40–50	0.06 ± 0.03	0.05 ± 0.01	0.20
50–60	0.05 ± 0.02	0.07 ± 0.03	0.10
60–70	0.05 ± 0.01	0.04 ± 0.01	0.56
70–80	0.06 ± 0.02	0.04 ± 0.01	0.08
80–90	0.05 ± 0.01	0.06 ± 0.01	0.20

Table appendix 7: Nickel content in the depth profiles from field trials where F-treatment = sewage sludge, B- treatment = mineral fertilizers. Mean values are based on concentrations measured in the replicate field plots (mean ± standard deviations n: 3x4). Differences between the treatments were tested and P-values are provided from two tailed t-tests assuming equal variance. Samples were taken 2021.

Soil depth (cm)	F-treatments Ni content (mg/kg)	B-treatments Ni content (mg/kg)	P-value
0-20	15.3 ± 1.2	17.6 ± 1.1	0.001
20-30	15.5 ± 1.4	17.9 ± 2.5	0.034
30-40	18.1 ± 3.3	18.6 ± 1.7	0.771
40-50	24.6 ± 4.8	24.1 ± 2.4	0.858
50-60	23.8 ± 2.8	27.9 ± 1.7	0.026
60-70	23.4 ± 2.8	26.9 ± 2.5	0.065
70-80	24.5 ± 2.9	26.9 ± 0.4	0.145
80-90	23.7 ± 2.4	27.9 ± 2.6	0.017

Appendix 5

Photography of soil columns, replicate 1, 2 and 3, after bioturbation treatment.

Figure appendix 2: bioturbation replicate 1

Figure appendix 3: bioturbation replicate 2

Figure appendix 4: bioturbation replicate 3

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