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Environmental impact of fertilization with biogas digestates – Studies of inorganic and organic contaminants

Miljømessige konsekvenser av gjødsling med
biorest – Undersøkelser av uorganiske og
organiske forurensninger

Ivan Dragicevic

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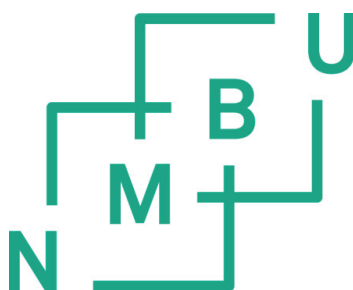
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Philosophiae Doctor (PhD) Thesis

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SUMMARY

Sustainable and safe energy and food production are important issues affected by climate change. Biogas production is among the technologies with a potential to reduce negative effects on the environment that increased energy production and consumption may have while making use of organic waste materials. Use of biogas to cover energy needs is increasing in Europe, but it is also followed by the production of an organic residue called biogas digestate. Biogas digestates have a potential as sustainable fertilizers in agricultural production due to a high content of plant-available nutrients.

However, in Norway many/some farmers have concerns about the use of digestates due to limited confidence mostly in their chemical safety and thus possible consequences for future crop production. The most common feedstock for biogas production in Norway are sewage sludge and food waste from households and industry.

The main goal of this thesis is to contribute to an increase in knowledge regarding environmental aspects of the use of biogas digestates as organic fertilizers in cereal production. The thesis addresses the safety of digestates with focus on potentially harmful compounds and their fate when applied on agricultural land in a field experiment (**Papers 2, 3 and 4**) and leaching studies (**Papers 1 and 2**). Both inorganic and organic pollutant groups were put in focus.

In **Paper 3**, the focus was on trace elements in digestates and their soil content and variability. In addition, we have also investigated a potential for uptake in grains when applying digestates at a fertilizing rate of 100 kg plant-available N ha⁻¹. The use of digestates as fertilizers was compared to cattle manure, mineral fertilizer and a control without fertilizer addition, with respect to the presence and distribution of the trace metals Cd, Cu, Zn, Ni, Cr, Mn and Mo, as well as Al in the soil and plant. The results of the study showed that despite an increase in total concentrations, there was no sign of increased plant uptake or increased mobility of these elements that would be considered to have an adverse effect to soil/water environment.

As the digestates applied in the field experiment had high concentrations of aluminium (Al) and chromium (Cr), Paper 2 concentrates on the fate of these two elements both in the field and in leaching studies simulating heavy rainfall events. Results showed an increase in total concentrations for both metals on plots fertilised with digestates, but also a significant increase of water-soluble Al. Modelling free metal concentrations under field conditions using WHAM 7.0 software, suggested that an environmental risk due to high Al mobility has to be

considered. Our study also showed that the use of digestates with high Al and Cr content was still comparable to the use of animal manure with respect to leaching.

Paper 1 focuses on copper (Cu), zinc (Zn) and nickel (Ni) leaching potential from digestates under simulated heavy rainfall conditions shortly after application. Besides using the two commercial digestates, we have also included three experimental digestates that were produced from animal manure alone, or different mixtures of animal manure and whey with or without fish industry waste. The mobility and release of Cu, Ni and Zn were investigated in three differently textured soils (sand, silt, loam). The effect of digestates was compared to that of fresh manure, mineral fertilizer and an untreated control. In loam and silt, the addition of commercial digestates with higher original Ni, Cu and Zn content showed less environmental impact in terms of leaching than that treated with experimental digestates with lower original metal content and less than when animal manure or mineral fertilizer was applied.

The final paper included in this dissertation is focused on organic pollutants, more precisely on polar organic pollutants and their presence in biogas digestates as well as their fate/concentration in the soil during the growing season (**Paper 4**). This study investigated the occurrence and fate of 21 micropollutants (POM), divided into perfluoroalkyl acids (PFAAs) as the most persistent organic contaminants and non-perfluoroalkyl compounds, mostly selected based on the screening studies and available methodological support. Overall, the concentration of these compounds in the two food waste based digestates was low compared to studies on other organic fertilisers. Use of biogas digestates at the selected fertilizer rate to grow cereal crops did not contribute significantly to the POM concentrations in soil. Measured concentrations of all POMs in soils after application of biogas digestates were consistent with theoretical estimates based on the concentrations determined in biogas digestates. Among the organic pollutants determined, only two (PFOA and 1-Methyl-1*H*-benzotriazole) were taken up into grains independent of fertilizer treatments, but concentrations were below 15 ppb.

In conclusion, the effects of applying biogas digestate as organic fertilizer were comparable to the more common use of animal manure, with respect to trace element mobility, and grain uptake. Although some digestates contained high amounts of metals, such as Cr or Ni, all the soils have shown a capacity to hinder metal leaching. Polar organic pollutants have been detected in both of the used digestates, but also in animal manure, and were, with two exceptions, not taken up into edible plant parts.

SAMMENDRAG

Bærekraftig og sikker mat- og energiproduksjon, er de to viktige områder som er påvirket av klimaendringene. Biogassproduksjon er en av de teknologiprosessene som har et potensiale til å redusere de negative effektene økt energi- og matforbruk har på miljøet fordi den bruker organisk avfall som en ressurs. Bruken av biogass for å imøtekomme dagens energibehov er økende i Europa, men med den produseres samtidig en økende mengde organisk avfall, en såkalt biorest. Denne bioresten har et potensiale som bærekraftig gjødselmiddel i agronomisk planteproduksjon fordi den har et høyt innhold av plantetilgjengelige næringsstoffer.

I Norge er imidlertid fremdeles en del bønder skeptiske til bruk av biorest som gjødsel. Skepsisen har sitt opphav i manglende kunnskap om kjemisk og mikrobielt innhold i biorest og dermed hvordan de vil påvirke planteproduksjonen i framtiden. De vanligste råstoffene for biogassproduksjon i Norge er kloakk-slam og organisk avfall fra husholdninger og industri.

Hovedmålet med denne avhandlingen er å bidra med økt kunnskap om miljøaspektene knyttet til bruk av biorest som organisk gjødsel i kornproduksjon. I avhandlingen blir sikkerhetsaspektet belyst gjennom et fokus på potensielt skadelige innholdsstoffer og deres skjebne når biorest blir tilført jordbruksjord i feltforsøk (**Artikkel 2, 3 og 4**) og i utvaskingsforsøk (**Artikkel 1 og 2**).

I **Artikkel 3** var fokuset på innholdet av spormetaller i biorestene, samt innhold i jord og hvordan det varierte. I tillegg ble potensialet knyttet til planteopptak og transport til korn undersøkt når biorest, tilsvarende 100 kg plantetilgjengelig N ha⁻¹ ble tilført som gjødsel. Forekomst og fordeling av spormetallene Cd, Cu, Zn, Ni, Cr, Mn og Mo, samt Al, i jord og planter i forsøksledd tilført biorest ble sammenlignet med ledd tilført husdyrgjødsel, mineralgjødsel og en kontroll uten gjødsel. Resultatene viste at selv om det var en økning i totalkonsentrasjonene var det ingen økning i plantenes metalloptak eller tegn på økt mobilitet og negative effekter på jord/vannsystemet.

Artikkel 1 fokuserer på Kobber (Cu), Zink (Zn) og Nikkel (Ni) og mulighetene for økt avrenning når kraftig regn kommer rett etter tilføsel av biorest. I tillegg til de to kommersielle biorestene ble tre eksperimentelle biorester tatt med i studiet. Disse var produsert fra husdyrgjødsel eller forskjellige blandinger av husdyrgjødsel og myseprotein med eller uten tilføsel av avfall fra fiskeindustrien. Mobiliteten og frigjøringen av Cu, Zn og Ni ble undersøkt i tre ulike jordarter (sand, silt, leire). Avrenningen fra leddene tilført biorest ble sammenlignet med leddene tilført husdyrgjødsel, mineralgjødsel og en ubehandlet kontroll. Jordsøylene med

silt og leire som ble tilført kommersiell biorest med et høyt metallinnhold hadde generelt lavere avrenning av Ni, Cu og Zn enn jordsøylene som fikk tilført husdyrgjødsel eller mineralgjødsel.

I den siste artikkelen i denne avhandlingen er det fokus på organiske forurensninger, på polare organiske forurensninger og deres tilstedeværelse i biorest og deres skjebne/konsentrasjon i jord over en vekstsesong (**Artikkel 4**). I dette studiet ble forekomsten av, og skjebnen til 21 mikro-forurensninger (POM), fordelt på grupper av perfluoralkyl-syrer (PFAA-er) og ikke-PFAA-er, hovedsakelig valgt på grunnlag av resultater fra screening og hvilke analysemetoden som var tilgjengelige. I det store og det hele viste resultatene at konsentrasjonen av de 21 valgte forurensningene var lav i alle de undersøkte biorestene sammenlignet med konsentrasjoner i andre organiske gjødselmidler. Bruk av biorest, i det valgte gjødselnivået, bidro ikke i noen betydelig grad til økte POM-konsentrasjonen i jorda. De målte konsentrasjonene i jord var sammenfallende med teoretiske beregninger gjort på bakgrunn av konsentrasjonen i biorestene tilført. Blant alle de undersøkte POM-ene var det kun PFOA og 1-Metyl-1H-Benzotriazol som ble funnet i kornet, men dette funnet var uten sammenheng med gjødslingen og lå under 15 ppb.

Det kan konkluderes med at tilførsel av biorest kan sammenlignes med bruk av andre, mer vanlige gjødselslag som f.eks. husdyrgjødsel, med hensyn på akkumulering og avrenning av spormetaller i jord. Selv om betydelige mengder av metaller som f.eks. Cr eller Ni, i noen tilfeller ble tilført, hadde alle jordartene brukt i forsøket en betydelig kapasitet til å akkumulere og dermed hindre avrenning av metaller. Polare organiske forurensninger ble målt i begge de kommersielle biorestene, men også i husdyrgjødsel, og ble med to unntak, ikke tatt opp av planter og videreført til spiselige plantedeler

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“The pursuit of PhD is enduring daring adventure.”

-Lailah Gifty Akita

LIST OF SUPPORTING PAPERS

Paper 1

Dragicevic Ivan, Eich-Greatorex Susanne, Sogn Aulstad Trine, Linjordet Roar, Krogstad, Tore
-Fate of copper, nickel and zinc after biogas digestate application to three different soil types,
Environmental Science and Pollution Research. Vol 24 (2017), 13095-13106.

Paper 2

Dragicevic Ivan, Eich-Greatorex Susanne, Sogn Aulstad Trine, Horn Svein Jarle, Krogstad
Tore
-Use of high metal-containing biogas digestates in cereal production – Mobility of chromium
and aluminium
Journal of Environmental Management, Vol 217:1 (2018), 12-22.

Paper 3

Dragicevic Ivan, Sogn Aulstad Trine, Eich-Greatorex Susanne
-Recycling of Biogas Digestates in Crop Production—Soil and Plant Trace Metal Content and
Variability
Frontiers in Sustainable Food Systems, Vol 2:45 (2018), 1-14.

Paper 4

Dragicevic Ivan, Posselt Malte, Sandblom Oskar, Eich-Greatorex, Susanne, Sogn Aulstad
Trine, Kallenborn P. Roland, Benskin P. Jonathan
-Occurrence of polar organic micropollutants in soil and grain following field application of
biogas digestates
To be submitted to: Science of Total Environment (2018)

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1. BACKGROUND KNOWLEDGE

1.1 Tackling increased energy demands while preserving the environment

Today, the daily requirement for energy is increasing all over the world, while the available resources can hardly cover the increasing energy needs. All societies call for the services of energy to meet basic human needs such as: food production, health, lighting, cooking, space comfort, mobility and communication (Intergovernmental Panel on Climate Change 2012). When trying to enhance the energy supply worldwide, the biggest challenge is to reduce emissions due to energy production and use in order to limit climate change effects. Only with careful consideration of both positive and negative effects of new energy sources, we can work on a sustainable future by using renewable resources.

While developed countries are striving to find new ways to supply their economies with energy, there are still over one billion people that lack access to electricity, and the majority of these communities are distributed in rural areas. This situation clearly indicates a need to put into action already available resources for energy production, such as organic wastes, since a large number of rural areas have such resources available. It is forecasted that the use of traditional biomass in rural areas of the world will in total increase for 100 million tons by the year 2030 (Kaygusuz 2012). Most of this increase is attributed to biomass use for energy production. This is seen as a good alternative to use of fossil fuels that are considered as a main driving force in the climate change.

Renewable energy may be considered as any type of energy source that is naturally replenished (not depleted), like solar, wind, geothermal or hydroelectric energy. Energy produced from biomass, including organic wastes, is also classified as renewable. The importance of renewable energy sources lies in the possibility that greenhouse gas (GHG) emissions can be reduced, thereby mitigating their climate change effects (Caro 2018). Replacement of fossil fuel based energy systems with resources that can be characterized as renewable and beneficiary to the environment can contribute to sustainable development and controlling GHG emissions.

1.2 Waste recycling and circular economy

Currently, the global economy produces more than one billion tons of waste per day. This waste consists of plastic, paper, organic residuals, corroded metals and a significant number of all kinds of by-products that can be classified as mixed waste (Hoornweg et al. 2013). In the 21st century, it is expected that rates of global waste generation will grow exponentially, driven by growth in population, wealth, and urbanization. In Fig. 1, expected increase of waste generation is given per region by the year 2025.

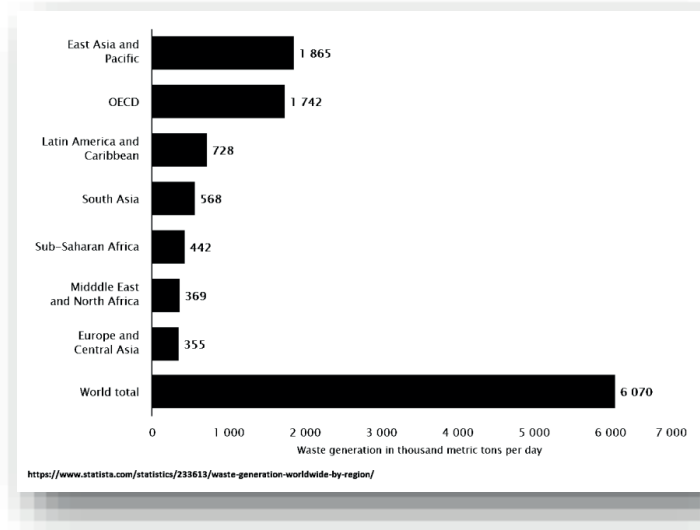


Figure 1. Regional waste generation worldwide by 2025 (Statista 2018).

The growing population will in general cause a significant increase in consumption and, as a result, the disposal of significant amounts of waste. Although waste management has become a part of everyday life for the local, national and international communities there is still need for additional effort and improvements (Guerrero et al. 2013). In recent years, there has been a lot of activity focused on improving waste management for both the industrial and the municipal sector. This has often been done by following the three main principles of waste management, i.e. to reduce the production of waste, to reuse the collected waste and to recycle the collected waste back to industrial production of energy or products. Still, even with all the actions taken to improve waste management today, there are still countries in which a lot of the collected waste ends up in landfills that are considered as a potential threat to the

environment. The principles of a circular economy include creating new products from waste materials that will have an economical value and that will contribute to preservation of the environment (Geisendorf & Pietrulla 2018). Waste management in a circular economy should have a role in utilizing the waste through recycling and thus lowering the adverse effects to the environment (Tisserant et al. 2017). Following this principle, the biogas process may serve as an example of waste utilization with the potential for both economic and environmental benefits.

1.3 Biogas – production, use and benefits

Anaerobic digestion of organic material by bacteria and archaea is the basis of biogas production. The process of anaerobic digestion is commonly used for biogas production based on different types of organic wastes such as animal manure or food waste. Biogas today usually refers to a mixture of mainly methane (CH_4 , 45-70 %) and carbon dioxide (CO_2 , 25-45 %) (Sørheim 2010). The produced gas needs to go through a purification and concentrating process, which can then lift the concentration of methane up to 98-99% and remove accompanying gases (Murphy 2018). This level of purity is mostly used for heating or transport purposes. In any case, it can be used instead of natural gas (propane-butane) and can be transported through the same gas line systems. There are four main waste streams treated by anaerobic digestion: (a) treatment of municipal sewage sludge, (b) treatment of industrial wastewater from agro-food and fermentation industries, (c) treatment of livestock waste, (d) treatment of the organic fraction of municipal solid waste, and co-digestion with different combinations of these. All of them have the same main goal, which is production of biogas.

Increasing need for sustainable energy and unstable markets with rapid changes in fossil fuel prices have led to an increase in utilization of biogas as a new source of the energy. Biogas production has increased considerably all over the world in the last decades. Compared to fossil fuels, application of AD technology has the potential to reduce GHG emissions (Abbasi et al. 2012).

In Europe, the production of biogas is increasing (Figure 2), and the leading countries are Germany and Italy. According to a report by the European Biogas Association (EBA), there were 17,439 biogas plants registered in Europe by the end of 2017 (EBA 2017). In the EU, Germany is the biggest biogas producer with around 50 % of all the biogas produced. In Germany alone, there was an increase from 4,000 to 8,000 farm-scale anaerobic bioreactors for the period 2010-2014 (Achinas et al. 2017; Weiland 2010).



<http://european-biogas.eu/2017/12/14/eba-statistical-report-2017-published-soon/>

Figure 2. Number of existing biogas plants in Europe with the number new plants installed from 2009 to 2016. EBA © 2018

According to the last report of the European Commission on the status of biogas, more than two thirds of the biogas in the EU is produced from different kind of energy crops and other biowaste, while one third is produced from sewage sludge and landfills (Kampan 2016). In Figure 3, the number of biogas plants per used feedstock in selected countries around the world is presented until the end of 2016.

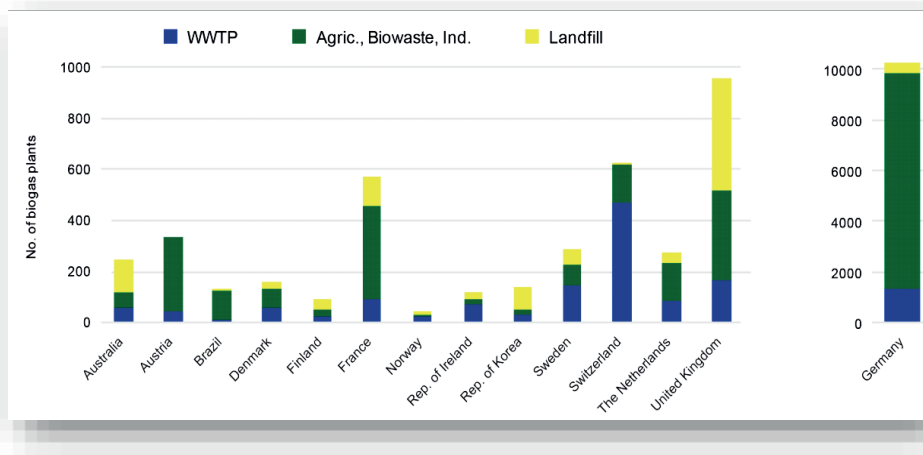


Figure 3. Number and type of biogas plants in selected countries (Murphy 2018).

Besides using biogas production to produce energy in Norway (biomethane), anaerobic digestion has been mostly used as a waste treatment method. Still, the produced biogas is today used for internal energy consumption of the biogas plant, municipality or a city (mainly heating and transport services). It is anticipated that the amount of energy produced as biogas in Norway will be 2,3 TWh by 2020 (Norwegian Environment Agency 2013).

For biogas production, the most important factor is the type of feedstock. In Norway, more than 50 % of the feedstock used for biogas production comes from sewage sludge, while food waste and other co-digestion feedstock are used less but with increasing trend (Stastical Agency of Norway 2015). Approximately 50 % of all the organic waste in Norway in 2015 was delivered to biogas companies for biogas production (Norwegian Environment Agency 2016). Still, there is currently only a low percentage (between 1-2 %) of the biogas potential exploited with regard to the use of animal manure as a feedstock. There are currently 6 small scale biogas plants in Norway that use animal manure as main feedstock (Pettersen 2017). As the trend in Europe is increasing towards the use of animal manure as easily available feedstock (EBA 2017), especially in co-digestion with other types of wastes or biomass, it can be expected that Norway will follow this path. Still, the latest biogas plants in Norway have been developed to use municipal wastes, such as food waste or sewage sludge.

Besides exploiting the benefits of biogas, the by-product of this technology, called biogas digestate, can be an efficient fertilizer for crop production. There are studies showing that digestates can replace common mineral fertilizers in crops production (Iocoli et al. 2019; Koszel & Lorencowicz 2015).

1.4 Biogas digestates – new means of organic fertilization

Common feedstock for biogas production includes manure, agricultural residues, sewage sludge, food wastes and landscape wastes (Drennan & DiStefano 2010; Möller & Müller 2012; Nkoa 2014). Production of biogas by anaerobic digestion is generally focused on the overall goal of optimizing the biomethane yields, but also trying to increase the fertilizer quality of the remaining by-product, digestate. By using different feedstock to produce the biogas (Fig. 4), it is natural that the chemical composition, important for the potential fertilizer use, will also be different in the produced biogas digestates. Variability can be significant, starting from basic parameters such as pH, levels of nitrogen (both organic and inorganic), total organic matter content, and C/N ratio, up to levels of trace metals, both essential and non-

essential to plant growth and development (Mangwandi et al. 2013; Risberg et al. 2017; Teglia et al. 2011a).

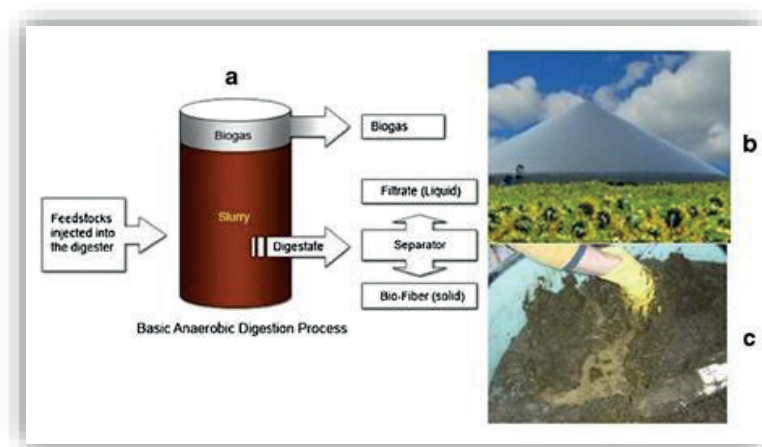


Figure 4. The basic anaerobic digestion process (a) and its two -products: biogas (b) and biogas digestate (c). Figure from (Nkoa 2014).

Most studies support the use of digestates as fertilizers, especially those that have a high fraction (more than 30 %) of mineral nitrogen available (Teglia et al. 2011b). The nutrient value of digestates, as previously mentioned, depends primarily on the nature of the feedstock and applied biogas production technology. Biogas digestates that go through a separation process (separation of solid and liquid fraction), show major differences in nutrient levels. For example, solid fractions of digestates produced from different kinds of manure usually have higher concentrations of phosphorus, as well as total and organic nitrogen, whereas liquid fractions are usually rich in plant-available nitrogen (Albuquerque et al. 2012). Based on this general assessment, the solid digestate material has potential as a soil amendment, while the liquid fraction may be more suitable as a fertilizer in crop production.

One of the challenges with the field application of biogas digestates is their chemical safety (Törnwall et al. 2017). In Norway, some farmers are still hesitant to use biogas digestates as fertilizers since long-term studies on effects are not available yet. The main concerns are related to the safety of the food grown on fields fertilized with biogas digestates, but also whether long-term application may lead to pollutant accumulation in soils. Details concerning potential inorganic and organic contaminants in digestates and other organic fertilizers will be given in Chapter 3 of this dissertation, where a special literature overview is provided.

For most of the studies referred to in this chapter, contaminant levels of digestates were within the respective nationally regulated limits for the use of organic products/wastes as fertilizers. Some results also show that the application of anaerobic digestates to agricultural land can have positive effects on soil properties (Abubaker et al. 2012). Regarding the fertilizer effects of anaerobic digestates, they were comparable to mineral fertilizers in many different scenarios with respect to crop yield (Herrmann et al. 2017; Siebielec et al. 2018; Sogn 2018). There is still need for research regarding other aspects of biogas digestates use in agriculture.

1.5 Norwegian regulatory system for the use of organic wastes in agriculture

The Norwegian regulation on the use of organic fertilizers (Norwegian Food Safety Authority 2006) takes into account all organic fertilizers intended for use in agriculture, including sewage sludge, compost, biogas digestate and similar products. The current regulation includes nutrient value, and chemical and hygienic limitations but with no direct limits on potential organic contaminants. The organic fertilizers may be classified in 4 classes with limitations for the use in agriculture or green areas. The classes and limitations are as follows:

- Class 0 – can be used for agricultural land, private gardens, parks and green area in general. Used amounts cannot be added over the defined need for nutrients necessary for plant growth.
- Class I: can be used for agricultural land, private gardens, and parks up to 40 tons dry matter per hectare per 10 years. It can also be used on other green areas where no plants will be grown for food consumption. The organic waste can be applied at a maximum thickness of 5 cm and must be mixed in with the soil.
- Class II: can be used for agricultural land, private gardens, parks up to 20 tons dry matter per hectare per 10 years. It can also be used on other green areas where no plants intended for food consumption will be grown. The organic waste can be applied at a max. thickness of 5 cm and must be mixed in with the soil.
- Class III: It can be used on green areas where no plants intended for food consumption will be grown. The organic waste can be applied at a max. thickness of 5 cm every 10 years and must be mixed in with the soil. Soil incorporation with machines must not exceed a depth of 15 cm.

The current limits on trace metals defined in the Norwegian regulation are given in Table 1.

Table 1. Maximum allowed concentrations of trace metals in organic fertilizers used in agricultural production (mg kg⁻¹ dry matter).

Trace metal	Class 0	Class I	Class II	Class III
Cadmium (Cd)	0.4	0.8	2	5
Lead (Pb)	40	60	80	200
Mercury (Hg)	0.2	0.6	3	5
Nickel (Ni)	20	30	50	80
Zink (Zn)	150	400	800	1500
Copper (Cu)	50	150	650	1000
Chromium (Cr)	50	60	100	150

Table 2 shows maximum allowed concentrations of trace metals in fertilized soils.

Table 2. Maximum allowed total soil trace metal concentration in soils fertilized with organic fertilizers Class I and II (Norwegian regulation).

Trace metal	Maximum allowed concentration in fertilized soil / mg kg ⁻¹ dry matter
Cadmium (Cd)	1
Lead (Pb)	50
Mercury (Hg)	1
Nickel (Ni)	30
Zink (Zn)	150
Copper (Cu)	50
Chromium (Cr)	100

The importance of limiting the maximum concentrations of trace metals in soils after organic fertilizer addition is mostly connected to possible long-term accumulation.

2. THESIS OBJECTIVE AND SCOPE OF RESEARCH

2.1 Thesis objective

The main objective of the thesis was to contribute to an increase of knowledge with regard to digestate quality that would provide more security for farmers by investigating the fate of possible harmful compounds in biogas digestates upon application on agricultural land. This has been mainly done through the following activities:

- Conducting laboratory, greenhouse and field experiments with sampling of digestates, soil, leachates and plant material.
- Analysis of the total content of trace elements and organic contaminants in biogas digestates, soils and grain and/or other plant material.
- Investigation of the mobility of trace elements from biogas digestates in soils.
- Comparison of measured concentrations of inorganic and organic contaminants in digestates and soil based on the difference in feedstock.
- Comparison of measured concentrations of inorganic and organic contaminants with soil background concentrations and Norwegian national regulations on organic fertilizer use and soil contamination.

2.2 Research questions

Based on the thesis objectives, four research questions were formulated:

- 1.* What are the levels of inorganic and organic contaminants in commercially produced biogas digestates and are they comparable to more common organic fertilizers (animal manure)?
- 2.* How do biogas digestates influence the soil chemistry (pH, DOC, trace metals) under usual agronomic conditions when cereal crops are grown in a Nordic climate?
- 3.* Does the use of biogas digestates influence the contaminant level in edible parts of the plant?
- 4.* What is the leaching potential of trace elements from different soils fertilized with biogas digestate in high precipitation incidents?

2.3 Scope of research

Generally, there is an increasing concern about potential entry of various contaminants into the human food chain by consuming products provided by agricultural production and food processing. In Norway, major focus is given to the production of secure and clean food, which is considered a selling argument for Norwegian produce compared to imported agricultural products

The quality of biogas digestates is dependent on the used feedstock and technological process. For this reason, it is important to emphasize that other resources used for production of biogas will result in different compositions of biogas digestates. All the analytical work was done on state of the art instruments in laboratories in Norway (NMBU, Ås) and Sweden (University of Stockholm, Stockholm).

An interdisciplinary research approach has been used to characterize the influence of digestates on soil/plant systems in both an agricultural and environmental context. The experimental and analytical methods were chosen to assess the used digestates with respect to their contaminant levels and potential for plant uptake upon their use.

3. APPLIED THEORY AND METHODOLOGY

3.1 Trace elements in soil and organic wastes, and their implications to soil environment

Trace element behaviour in soil

The behaviour of trace metals in soils depends on complex reactions, which result in different chemical forms of the trace metals in their cationic or oxyanionic forms, respectively. The natural state of the soil may be considered as an equilibrium that can be easily disrupted by different factors, such as pH changes, soil weathering, addition of fertilizers or irrigation (Kabata-Pendias 2011). Biogeochemistry of trace metals is generally controlled by as the following factors:

- Change in soil heterogeneity, which includes change in soil components (soil weathering),
- Variability of soil characteristics such as pH, soil carbon and nitrogen content, organic matter content, caused by changing environmental conditions (e.g., change of seasons, precipitation, drought),
- Transfer between the soil phases of different soil components/soil compounds and potential for soil accumulation.

Trace metals can be naturally present in soil, depending on the parent material, added by atmospheric deposition (e.g., volcanic activity, industrial activity, transport,) or by direct human activity (e.g., fertilization, addition of material). The behaviour of trace elements in soil is regulated by their specific chemical form. For example, some of them appear as positively charged cations (Cu, Ni, Zn), oxyanions (As, Mo, Cr), complexes or low-soluble salts. Their mobility in soil, besides the general factors mentioned earlier, is in particularly dependant on soil type, soil pH and soluble organic matter content. In addition, mentioned forms can also give an indication of the origin of a particular metal in the soil.

It is a well-known fact that levels of metals such as mineral elements (Ca, K, Mg and Na) and trace elements as micronutrients (Cu, Fe, Ni, Mn and Zn) are crucial for an agronomic use of the soil as a growth medium. An increasing and intensified use of soil resources for agricultural production, however, ought to include the monitoring of potentially problematic metals as well. These metals can may have adverse effects on the soil/plant environment if present in sufficient concentrations. The following trace metals are of particular concern: cadmium (Cd), arsenic (As), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni) etc. Most of

these metals are not essential for plant growth (except for Ni, and V in some cases). Transport of these metals into the food grown on these soils may ultimately imply health risks for those who consume it (Peralta-Videa et al. 2009). One of the challenges in agriculture today is society’s wish to reuse the nutrients contained in organic wastes as fertilizers for crop production. For example, the use of sewage sludge has been seen to contaminate the soil with Cd, but it has also been seen in some cases to lower uptake of Cd in edible parts of the plant (Khan et al. 2017). This suggests that the use of organic amendments could in some circumstances also reduce the amounts of Cd available for plant uptake (Figure 5).

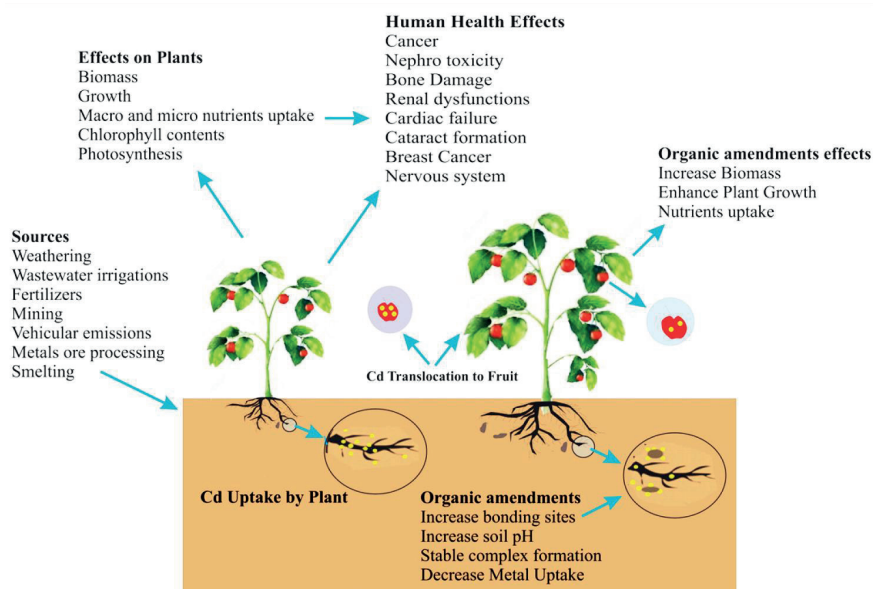


Figure 5. The sources of Cd, uptake by plants, effects on plant growth and human health and influence of organic amendments on Cd uptake by plants (Khan et al. 2017).

Figure 5 also shows that the addition of organic amendment can decrease the potential for a Cd plant uptake by increasing the bonding sites in the rhizosphere, by increasing the pH value, which in turn lowers Cd mobility, and in the end by forming a stable complex between Cd and high molecular weight organic acids. The mentioned examples suggests that pH and organic matter (including the dissolved organic carbon, DOC) can play a key role in the metal mobility. Understanding the complexity of trace metal presence and mobility would enable a more secure use of organic fertilizers.

In the following section a short overview of the research on trace metals added through animal manure or sewage sludge and their implication to the soil/water environments will be

presented. Presenting the research on the use of other organic wastes is important to evaluate the effect of using biogas digestates as fertilizers. The comparison is also useful since it addresses the same issues that the addition of trace metals has on agricultural plant production, such as soil trace metal levels (including accumulation) and potential for plant uptake.

Sewage sludge

Sewage sludge is the product of municipal and/or industrial wastewater treatment. Today, a common name for dewatered sewage sludge is biosolids. Sewage sludge is composed of both inorganic and organic materials, with often high concentrations of plant nutrients, but also containing potentially problematic trace elements and organic chemicals, and possibly pathogens (Studer et al. 2017). At present, the main method of sewage sludge disposal and comprehensive utilization is application unto agricultural land (Fytili & Zabaniotou 2008; Singh & Agrawal 2008). Long-term studies on sewage sludge application in cereal production have shown an increased plant uptake of several trace metals, both essential and non-essential for plants. For example, in wheat grains Hg and Zn uptake has been seen to increase with the use of sewage sludge, while concentration of metals such as Cu, Cr, Ni, Pb, and As were higher in straw parts of the plant (Jamali et al. 2009; Yang et al. 2018). Reuse of the nutrients contained in sewage sludge in agriculture still results in divided opinions and the legislation regulating its use is highly diverse in different regions of the world (Renner 2000). Since the diversity in production of sewage sludge is large, as it is for many other organic wastes, sewage sludge can have a large variations of different trace metals. This is especially seen if it is produced by mixing industrial with municipal waste water. In addition, this means also show why it is important the policies are in place that can regulate agricultural use of sewage sludge with defined limitations (Mosquera-Losada et al. 2010).

Manure

Manure is commonly used in different agricultural plant production systems. Manure may contribute to the fertility of the soil by adding organic matter and plant nutrients, and stimulating soil microorganisms. For animal manure, the variability in the plant macro- and micronutrient is high, since the origin of the manure can be from cattle, pig or poultry production.

Modern crop production is focused on both good yield and grain quality, but also on preservation of the environment. In general, manure often has a high content of Zn and Cu (Nicholson et al. 1999; Yang, X. P. et al. 2017), while sewage sludge can be rich in

contaminants such as Cd, Cr or Pb (Ashworth & Alloway 2004; Chen 2017). However, also animal manure can be a source of Cd in agriculture (Li et al. 2010).

Soil quality is an important factor for crop quality and yield, and will be affected negatively by accumulation of trace metals or other pollutants. Further, soil leaching studies for trace metals have shown high ecological risk factors with the application of organic wastes such as manure (Cambier et al. 2014). Pollutants may be applied to soil through organic or inorganic fertilizers and may bioaccumulate in soil organisms and plants (Antoniadis et al. 2017). Animal manure application to farmland is regarded as an important source of trace metals in soils used for agricultural production. Previous studies have shown that application of animal manure or manure composts containing high levels of trace metals can result in their excessive accumulation in soil, decreasing soil quality (Alvarenga et al. 2015; Cornu et al. 1999; Fang 2016). Trace metals may contribute to adverse health effects in humans and animals with both cancer and non-cancer effects. The freshwater ecotoxicity that is related to the use of animal manure worldwide has identified mercury (Hg) as the most potent for contamination that results in cancerous changes, while was seen Zn as the most potent in contamination that results with non-cancerous changes (Leclerc & Laurent 2017). Continuous application of manure to agricultural land is considered as one of the main issues regarding metal accumulation. A recent research study has shown that As, Hg, Cr, Cu, Zn and Mn can accumulate significantly in the soil after just four continuous years of animal manure application (Qian et al. 2018).

Biogas digestates

Research studies on metal content in biogas digestates and its potential implications for the use as fertilizer are still scarce. In a study from Switzerland it has been found that almost 2 % of the yearly deposition of Cd and 20 % of the yearly deposition of Pb to agricultural land comes from the use of composts and digestates (Kupper et al. 2014). Naturally, the content of potential contaminants in substrates used for the biogas process determines the content of these contaminants in the digestates (Tavazzi et al. 2013). However, the use of digestates as fertilizers on agricultural fields may present several challenges, e.g., mobility of potentially harmful elements or substances. Trace metals are expected to remain in the biogas digestates after biogas production (Iocoli et al. 2019; Koszel & Lorencowicz 2015). The sources of trace metals that can be found in digestates are almost limitless due to a large variety of available feedstock. By co-digestion of different substrates this variability may increase even more (Di Maria 2017). Among the trace metals, those essential to plants as micronutrients are Cu, Ni, Zn, Mo and Mn.

The main focus in many research studies is given to plant micronutrients such as Cu, Ni and Zn, including their presence and leaching from soils fertilized with different organic wastes (Hao 2008; Pivato et al. 2016). While these metals are important for plant growth, they can also cause adverse effects in the environment if they are present in high amounts. Another important aspect of digestate application is the effect of digestates on the availability of originally present metals in soil. Addition of the biogas digestates can for instance cause desorption of some naturally present soil elements. In general, Cu, Zn and Ni, are mobile in soil under common conditions for crop production in a Nordic climate, i.e., in a pH range between ~5 and 7. Their mobility can be considered as an advantage since they will be more available for plants during the growing season. On the other hand, higher mobility can also mean increased risk of leaching that can have an adverse effect for the surrounding soil environment or water recipients.

Environmental effects of added trace metals, such as uptake into plants and soil organisms or leaching to water recipients, depend on their mobility in the soil, which in turn is strongly affected by pH and dissolved organic carbon (DOC) content (Antoniadis & Alloway 2002a; Welikala et al. 2018). Applying organic fertilizers represents an addition of DOC and may influence the pH in the soil, and may thus increase mobility. Soil that is amended or fertilized with animal manure, sewage sludge or digestates would most increase the total metal concentrations in soil (Zhang et al. 2012). Studying the fate of trace metals under realistic conditions in the field can be an important tool for evaluating the potential environmental risks of digestate application, even though some initial studies indicate that leaching of trace metals from digestates is mostly below threshold values defined by national or international regulatory bodies (Tlustos et al. 2016; Toribio & Romanya 2006).

3.2 Presence and fate of organic micropollutants in organic wastes

Besides inorganic contaminants, biogas digestates can contain organic pollutants that can be introduced to the soil ecosystem by digestate application. For trace metals, today a large number of countries have developed quality standards that are meant to assure safe use of these fertilizers in agriculture, taking care of the trace metal burden in the soil over several years. Still, this is not the case for organic contaminants, since regulation is different from country to country. In the last few decades, the knowledge about organic pollutants in organic wastes has been expanding. One of the earliest comprehensive studies has been dealing with legacy pollutants such as polyaromatic hydrocarbons (PAH) or polychlorinated biphenyl (PCB)

(Brändli et al. 2007). Many commercial biogas plants utilize a mixture of source-separated organic waste from private households, catering, and food industry, and/or sewage sludge as feedstock, all of them aiming at a good biogas yield and high quality of the remaining biogas digestates (Möller & Müller 2012). Small-scale biogas plants in particular, also include animal manure that is a known source of different kinds of organic contaminants (Kay et al. 2005). The different types of feedstock may lead to the occurrence of a large range of organic pollutants in the digestates. Studies in Finland have shown that seasonal application of biogas digestates as fertilizers is equal to an annual soil burden of polychlorinated dibenzo-p-dioxins and furans coming from the atmosphere (Suominen et al. 2014). Earlier study on digestate contaminant levels conducted in Norway (Govasmark et al. 2011) focused mostly on legacy pollutants (PAHs, pesticides). The mentioned study has shown that chemical contamination of the food chain or the environment from agricultural use of digestates seemed to be low.

Today, research interest is increasing towards groups of compounds that have not been in focus previously. These compounds include perfluoroalkyl acids (PFAAs), antibiotics, personal care products, different endocrine disruptors etc. The risk for soil and plant contamination may be high especially in circumstances where there is little knowledge concerning the presence and potential spread of these compounds in the environment (Bourdat-Deschamps et al. 2017; Carballa et al. 2007; Gottschall et al. 2012; Narumiya et al. 2013).

In general, there is a lack of knowledge on polar organic contaminants, or more precisely polar organic micropollutants (POMs), in digestates or other organic fertilizers. The group of POMs in this dissertation refers to a wide range of chemicals, pharmaceuticals and personal care products, and their transformation products. Among them, perfluoroalkyl acids (PFAAs) are the most frequently detected POMs, owing to their environmental persistence and intensive production over the last seven decades. Perfluorinated compounds have been applied to a wide range of commercial products and processes but can also be synthesized in the environment from precursor substances (Martin et al. 2010). There are several studies that have reported the occurrence of PFAAs in substrates (e.g. sewage sludge) that can be used for biogas production (Noorlander et al. 2011; Yu et al. 2009).

A risk assessment done in Norway (Norwegian Scientific Committee for Food Safety (VKM) 2009) for the use of sewage sludge as organic fertilizer has identified perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) as substances of concern and prioritized them for further assessment in the future. Not long after the assessment in Norway, a report from Finland identified perfluoroalkyl substances (PFAS) as compounds of concern in biogas digestates (up to 900 µg/kg). In addition, several other groups of POMs, such as

pharmaceuticals and personal care products have been reported at concentrations of up to 2000 µg/kg in biogas digestates (Suominen 2013). For personal care products, it is known that they occur in high concentrations in sewage sludge/wastewater, probably making this one of the most challenging feedstock for biogas production (Carballa et al. 2007; Yang, Y. et al. 2017). An interesting question concerning the anaerobic digestion process is how would anaerobic bacterial activity affects the stability of POMs during the digestion process. Based on the current knowledge, the effect of the process can be characterized both as substance- and applied digestion process-specific. For examples, Narumiya et al. (2013) reported a decrease in triclosan levels in digested sludge during anaerobic treatment (Narumiya et al. 2013). Another POM, carbamazepine was seen to persist during this process of anaerobic digestion (Armstrong et al. 2017). Currently, there is little work available on the fate and behaviour of PFAAs during digestion, or their fate after field application by using digestates. Most of the literature refers to the screening studies mostly done on laboratory base experiments. Still, PFAA are highly persistent and are not expected to degrade (Yu et al. 2009; Zareitalabad et al. 2013). For cases where pollutant transformation can occur during digestion, challenges would multiply, since transformation products may have different ecotoxicological properties than their parent compounds. This means that it would be more difficult to monitor them due to the absence of chemically verified standards. The presence of POMs in digestates suggests that anaerobic digestion is not a reliable means of degrading organic pollutants.

The field application of digestates that contain different POMs is particularly challenging due to the mobility of POMs, which can lead to contamination of ground and/or surface water (Hua-Jie et al. 2014) or uptake in plants intended for human and/or animal consumption (Gottschall et al. 2012; Holling et al. 2012). The translocation of POMs from soil to plants is dependent on several factors such as soil type, soil organic matter, pH, irrigation levels, microbial community etc. (Pullagurala et al. 2018). Recent plant uptake studies have shown that the occurrence of PFAAs, antibiotics, personal care products and other micropollutants in both grains and different kinds of vegetables grown using biosolids, wastewater or animal manure under field conditions can be expected (Navarro et al. 2017; Pan & Chu 2017; Wu et al. 2015). This also includes vegetables grown in compost amended soils (Bizkarguenaga et al. 2016).

3.3 Applied methodology and used experimental set ups

3.3.1 Experimental set-ups

Field experiment

A randomized complete block experiment with different types of organic fertilizer was carried out on a loam soil at the field station of the Norwegian University of Life Sciences (59°39'52"N 10°47'40"E), with barley and oats as test crops in 2015 and 2016, respectively (Figure 6). The fertilizer treatments included two commercially produced biogas digestates, fresh animal manure from the Animal Production Experimental Centre at the Norwegian University of Life Sciences, mineral fertilizer, and control plots with no addition of any kind of fertilization. Details on digestates, manure and mineral fertilizer can be found in section 3.3.2. The fertilizer addition and sowing was done at the end of April 2015 and middle of May 2016, respectively.



Figure 6. Application of biogas digestates to field plots (field experiment 2015) and the look of randomized field block experiment after fertilizer application (Kjerringjordet, Ås, Norway).

The size of the testing area was 39 m × 24 m (0.094 ha), while each plot was 3 × 7 m (0.0021 ha). The plots were organized in triplicates for each treatment. The amounts of digestates and manure were determined based on their content of plant available nitrogen, and corresponded to the nitrogen applied as mineral N fertilizer, i.e. 100 kg N ha⁻¹.

Soil (both years) and plant samples (2015) for chemical analysis were collected from the different treatments once a month during the growing season. In 2016, grain samples were analysed after harvest. The results from the field experiment were used in Papers 2, 3 and 4.

Leaching potential of aluminium and chromium after harvest

Two potentially problematic elements with particular high concentrations in the digestates used in the field experiment were aluminium and chromium. Mobility and uptake into plants over the growing season was followed in the field experiment. In order to evaluate the potential of leaching of these elements, e.g., to water bodies after a growing season, a simple leaching study was conducted following a greenhouse pot experiment with different organic fertilizers for a cereal crop (Figure 7). The growth experiment was conducted in pots with a soil volume of 6.7 L in three differently textured soils, i.e. a sand, a silt and a loam. It consisted of the same treatments that were used in the field experiment and fertilization rates were again based on 100 kg plant available N ha⁻¹, calculated by considering a 20 cm deep top soil layer and thus a soil volume of 200 L per m² (for more details see Paper 2).



Figure 7. Greenhouse experiment with cereals grown in three different soils and fertilised with different digestates.

The water content in the soil was kept at 60% of the soils' water holding capacity throughout the growing season until the harvest. After the harvest, the pots were irrigated up to 100 % water holding capacity. After 24 hours, 1 L deionized water was added subsequently over 1 hour, and the leached solution was collected by means of a tube at the base of the pots, filtered and stored refrigerated for analysis. The results from this leaching study were used in Paper 2.

Leaching potential of trace elements after application of digestates

The potentially highest risk of leaching of both nutrients and possible contaminants from organic fertilizers may occur in the period shortly after application and incorporation into soil and before a plant cover is established. Heavy rainfall at this stage may lead to considerable losses both due to erosion and leaching through the pore system. The use of a leaching experiment in soil columns has provided a possibility to closely monitor metal leaching in different soil texture types during a 7 day-period of intensive irrigation. A high irrigation rate was used to simulate an extreme precipitation episode shortly after spreading digestates to an agricultural field. The column leaching study was designed, including the same three soil types as in the pot experiment. For this purpose, plexiglass columns of 24 mm in diameter and 30-cm length were used (Figure 8). Each column was filled with 15 cm of soil. The selected soil column length is approximated to the depth of digestate incorporation on agricultural fields (down to 10–15 cm). Treatments in the experiment were: untreated (control) soil (NN), mineral fertilizer (MF), experimental digestates EDIG1, EDIG2, EDIG3 and commercial digestates, CDIG1 and CDIG2. At the bottom of the column, a plastic filter covered with PVC pellets (about 2 cm in height) was placed to prevent the loss of soil from the columns. In order to provide an equal distribution of water, the top of each column was also covered with PVC pellets after fertilizer addition.



Figure 8. Set-up of the column leaching experiment

To avoid preferential flow through the columns as much as possible, the soils were sieved through a 2 mm mesh size, and then carefully filled into the columns trying to avoid air gaps. Prior to the experiment, the soil columns were saturated with deionized water for 24 h. All the

treatments were added after the 24 h column saturation. During the experiment, the columns were regularly irrigated using a peristaltic pump with deionized water at a flow rate of 1.3 ml h⁻¹ corresponding to high precipitation conditions. The leaching experiment was run for 7 days at room temperature. Based on the initial soil pH, the sand and loam soils were limed, prior to saturating the soils. The experiment was run in the absence of light to minimize algae growth as much as possible. Leachates were collected daily, filtered and immediately after collection stored at 4 °C until further analysis. The results of the column leaching experiment were used in Papers 1 and 2.

3.3.2 *Fertilizers and soils used in the research studies*

Fertilizers

Two municipally produced biogas digestates were investigated within the research work presented in this dissertation. They were collected from two biogas plants in the years 2015 and 2016. One of the digestates was produced at the Romerike biogas plant (RBA) owned by the City of Oslo (Figure 9), with a capacity of up to 50 000 tons of feedstock per year (Oslo EGE 2018).



Figure 9. Romerike biogas facility (RBA) (Oslo EGE 2018).

The feedstock used at this biogas plant consists of source-separated food waste from households of the Oslo area. Preparations for the biogas process include a step where materials such as glass, plastics, metal and potential stone material are removed. After the cleaning

process, food waste is mixed with water and exposed to thermic hydrolysis under high pressure and a temperature of 130 °C to reduce the particle size. This hydrolysis process also enables sterilization of the feedstock. After the thermic hydrolysis, the feedstock is pumped into large tanks where the anaerobic digestion takes place under mesophilic conditions, i.e. a bacterial community degrades the organic material at a temperature of 38 °C and produces biogas in the process. At RBA, the usual retention time in these digesters is 24 days. The digestates from RBA are marked with CDIG1, DIG1 or FWBD in Papers 1 to 4.

The other digestate was produced in the Frevar KF biogas plant near the city of Fredrikstad (Fig. 10) and owned by the same city. The capacity of this facility is 30 000 tons of feedstock per year (FREVAR KF 2018). The biogas production at the RBA and Frevar facility differ with respect to both feedstock and process temperature. Frevar uses a mixture of equal volumes (50:50) of sewage sludge and municipal food waste. The production process in the beginning is similar to the one in RBA where food waste is cleaned from most of the impurities (plastic, metal etc.) and then pumped into the receiving tanks.



Figure 10. FREVAR KF biogas facility (FREVAR KF 2018).

The sewage sludge originates from the municipal wastewater treatment plant nearby and is pumped into the same tank as the food waste. For the sterilization process, three tanks ensure that all of the mixed substrate stays at least 1 h at 70 °C before pumping the substrate to a digestion tank. The anaerobic digestion at Frevar is conducted under thermophilic conditions at temperatures around 60 °C, with a usual retention time of 10 to 14 days. The digestates from Frevar are marked as CDIG2, DIG2 or SS/FWBD in Papers 1 to 4.

For comparison with a commonly used organic fertilizer, all experiments include a treatment with fresh manure. The manure was cattle manure from the Animal Production Experimental Centre at the Norwegian University of Life Sciences in Ås. The fresh manure is referred to as AM in all papers.

The mineral fertilizer used in the studies was YaraMila NPK compound fertilizer 22-3-10 (Yara 2018). It contained 10% nitrate-N and 11.6% ammonium-N. It is a fertilizer specific for eastern Norway and is not exported. Mineral fertilizer was marked with MF in Papers 1 and 3.

For Paper 1, in addition to the commercially produced biogas digestates used, three experimentally produced digestates were included as organic fertilizers. These experimental digestates (EDIG) were produced by thermophilic anaerobic digestion at the Biogas Laboratory of the Norwegian University of Life Sciences and the Norwegian Institute of Bioeconomy Research. EDIG1 was a digestate produced using only animal manure as substrate, EDIG2 was a digestate from co-digestion of manure and whey permeate in a ratio v/v 230:70, and EDIG3 from co-digestion of manure and whey (v/v 120:280) with addition of fish ensilage.

Soils

The soils used in the leaching studies were a loam (Ås, 59°39'52"N 10°47'40"E), a sand (Norderås, 59°40'46"N 10°46'08"E) and a silt (Solør, 60°23'31"N 11°54'01"E). Soil texture was determined using the pipette method (Elonen 1971) and classified according to the United States Department of Agriculture (UN 2016; USDA). The loam was from the same area as the field experiment. The silt soil was collected from an agricultural field, while the sand soil was from a forest area. The soils were all sampled in the upper 0-20 cm horizon. Prior to use in the experiments (greenhouse and column leaching experiment), the soils were air dried and sieved through a 2 mm sieve. Basic characteristics of the soils used in the experiment are given in Table 3.

Table 3. Soil texture and selected chemical characteristics.

Soil type	pH	Tot. C	DOC	Sand	Silt	Clay
		%	mg L ⁻¹			
Loam	4.8 ± 0.15	2.42	29.7 ± 1.1	45	38	17
Sand	5.1 ± 0.25	0.37	15.5 ± 1.7	94	3	3
Silt	6.2 ± 0.3	1.81	23.5 ± 2.1	2	93	5

3.3.3 Inorganic analysis

General soil and digestates analysis

In all the research papers, several chemical and physical parameters of organic fertilizers, soils and/or plants have been determined. Prior to soil and fertilizer analysis, samples were dried, sieved and ground, and plant samples were dried and ground. In this section, only a short explanation will be given, while details on inorganic analysis can be found in the accompanying papers. The soil pH was determined using dry soil with a soil to water ratio of 1:2.5. A dry combustion method (Nelson D.W. 1982) was applied for determining the total carbon content of soils, digestates and manure, using a Leco Carbon Determinator EC12. The determination of NO₃⁻ and NH₄⁺ in digestates was done in fresh samples after extraction with 2M KCl by flow injection analysis using FIAstar 5000, FOSS. Concentration of DOC in collected leachates and soil, manure and digestates extracts (5 g of soil/digestates/manure with 25 mL deionized water) was determined using a Shimadzu TOC analyser. The soil extracts based on samples from the field experiment were additionally used for ion chromatography (IC) measurements of Cl⁻, NO₃⁻, SO₄²⁻ and F⁻ anions used in Paper 2.

Trace element analysis

Internal standards were added to all the samples prior to decomposition. For determination of total concentrations of trace elements, a subsample of 0.2 g soil was digested in concentrated ultrapure nitric acid (HNO₃) prior to ICP-MS analysis by stepwise heating up to 250 °C using a Milestone Ultraclave. Both total trace metal concentrations in soils, digestates and plants, and water-extractable concentrations in soils were determined using an Agilent ICP-MS 8800 TripleQ. The ICP-MS analysis was done with minor method validation (depending on the

sample matrix). The needed analytical quality control assurance was conducted by a systematic use of blanks (minimum 7 blanks per 30 samples) and analysis of a certified reference material for soil, organic waste (sewage sludge) and plant material. In addition, an in-house standard was used for control of the method stability for every measurement applied for different matrices. The laboratory/greenhouse experiments have also included the calculation of the amounts of metals added in the beginning of the experiments for comparing to those found in leachates (column leaching experiment). The analysis of water-soluble metal fractions was done in samples acquired by extracting 5 g of soil/digestates/manure with 25 mL deionized water.

Water-extractable concentrations of trace metals have been measured in different scenarios that included the investigation of metal leaching upon the use of organic wastes (Antoniadis & Alloway 2002a; Antoniadis & Alloway 2002b; Ashworth & Alloway 2004), and this fraction of metals was in also focus of the present work on digestates.

Investigation of presence of Cr⁶⁺

The commercial digestates had a high chromium content which would restrict their use in agriculture severely. The limits are set to avoid high concentrations of toxic Cr⁶⁺ but little is known about the chromium species that dominate in digestates and soils amended with digestates. In order to determine the presence of Cr⁶⁺ in digestates, an analytical approach has been developed based on several earlier studies on application of ICP-MS/MS and HPLC in determining Cr⁶⁺ (Cuello et al. 2016; Neubauer 2003; Prokisch et al. 1997). Concentrations of Cr⁶⁺ in digestates and mixtures were determined using an Agilent ICP-MS 8800 TripleQ. For analytical quality control, internal reference standard (animal manure) was used. All speciation samples were chromatographed in an Agilent HP1260 liquid chromatograph (Agilent Technologies Inc., USA) in tandem with ICP-MS/MS in time-resolved analysis mode (TRA). Chromium was speciated using two methods. Sampling was done over 12 months period with four different sampling points. A mixture of 1:1 digestate and water was used since it was necessary to reduce matrix effects on the speciation of Cr⁶⁺.

Method 1 was carried out using a Hamilton PRP-1 column (4.6 mm x 150 mm, 5 µm). The column was equilibrated with an aqueous mobile phase containing 1 mM tetrabutylammonium hydroxide, 0.6 mM EDTA and the pH adjusted to 9. These conditions were used for the separation of Cr⁺³ and Cr⁺⁶ peaks by isocratic elution at a flow rate of 1.5 ml min⁻¹. Method 2 was carried out using a Hamilton PRP-X100 column (2.1 mm x 250 mm, 5 µm). The column was equilibrated with an aqueous mobile phase containing 40 mM

ammonium carbonate and 0.2 mM EDTA. These conditions were used for the separation of Cr(III) and Cr(VI) ions by isocratic elution at a flow rate of 0.55 ml min⁻¹. The injection volume was 50 µL. A calibration curve with Cr(VI) concentration of 0, 1 and 5 µg L⁻¹ diluted in mobile phase was used. The LOD (limit of detection), LOQ (limit of quantification) and RL (reporting limits) were calculated from five method blank replicates.

3.3.4 Organic analysis

The first step of the organic analysis was an extraction procedure. Prior to extraction, organic fertilizer, grain and soil samples were dried and sieved. Two separate methods were used for extraction and analysis: one for PFAAs and the second for non-fluorinated micropollutants. Briefly, for PFAAs, 1 g of dry solid sample (i.e. soil, fertilizers, or grain) was spiked with surrogate standards and extracted using methanol. After centrifugation and supernatant collection, the extracts were evaporated to approx. 1mL volume size. The concentrated extract was cleaned up with activated carbon and extracts were stored at -20 °C prior to analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). Fertilizers, soil, and grain samples were extracted and analysed in triplicate.

Extraction of non-fluorinated POMs was carried out using 1 g of dry soil, organic fertilizer and grain samples. Samples were spiked with surrogate standards, dispersed with 1.5 mL of methanol, and then vortexed for 3 minutes and left in the fume hood for the methanol to evaporate overnight. Thereafter, the samples were supplemented with 20 mL of acetonitrile (ACN) sonicated in an ultrasonication bath, and then centrifuged at 2000 RPM. The supernatant was subsequently transferred to a clean 15mL tube. The ACN extraction was repeated and the extracts were combined. The resulting extract was placed in a Buchi evaporator and reduced to a volume of <1 mL. The final extraction volume was diluted with 4 mL of water and stored at -20 °C for 48 h prior to instrumental analysis.

Instrumental analysis of PFASs was carried out by liquid chromatography tandem mass spectrometry (LC-MS/MS) using a Waters Acquity UPLC coupled to a Xevo TQ-S triple quadrupole mass spectrometer. The instrument was operated in negative electrospray ionization, selected reaction monitoring (SRM) mode (see Paper 4 for details).

Non-fluorinated micropollutants were analyzed by LC-MS/MS using a Thermo Scientific Ultimate 3000 UHPLC system coupled to a Thermo Scientific Quantiva triple-

quadrupole mass spectrometer. The instrument was operated under polarity switching, selected reaction monitoring mode using an electrospray ionization source (see Paper 4 for details).

3.3.5 *WHAM modelling*

The specific process of speciation and binding of metals (Cr and Al) to DOC and the free metal concentration in soil water extracts, was done using WHAM (Winderemere Humic Aqueous Model) 7.0 model (Tipping et al. 2011). The parameters such as DOC, pH, and concentrations of dissolved anions (Cl^- , NO_3^- , SO_4^{2-} , and F^-) and dissolved cations (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Fe^{3+} , Al^{3+} and Cr^{3+}) have been analysed to provide the data inputs for WHAM 7.0. The equilibrium constants between the metal cations and anions were taken from the WHAM 7.0 default database (Tipping 1994). Concentration of humic substances was assumed to be 1.27 times the DOC concentration, representing 63.5 % of the DOC being active on average and presented as fulvic acid (FA) as suggested by Lofts and Tipping (Lofts & Tipping 2011). The humic substances (HS) binding constants were also taken from the WHAM 7.0 default database (Tipping 1994).

3.3.6 *Statistical analysis of the results*

Analysis of variance (one-way and two-way ANOVA) and Pearson correlations were carried out in Papers 1, 2 and 3. The one-way ANOVA was used to determine the effect of treatment on total metal concentrations in soil and grains (Paper 3), while two-way ANOVA was applied to several scenarios in papers 1 and 2. The statistical programs used were R Commander 3.2.3 and Sigma Plot 14.0. The confidence limit was 95% ($p < 0.05$).

In addition, principal component analysis (PCA) as a dimension reduction technique, has been used in Paper 3. The principal components (PC) were identified by the analysis of the correlation matrix for the data set regarding the water-extracted soil concentrations prepared from soils collected from the field experiment. The PCA analysis was used to identify factors underlying our set of variables (fertilizer treatments, sampling time) in order to determine relationships among them. Results were also used to investigate clustering of samples into groups.

4. MAIN RESULTS AND DISCUSSION

4.1 Trace metals presence in biogas digestates – implications for field application

The metals in focus of the study that is presented in Paper 3 were both plant micronutrients (i.e., Cu, Zn, Ni, Mn and Mo), and those that are not considered as plant nutrients (Cd, Cr and Al). However, both groups of metals can negatively influence plant development and soil quality (including runoff water) if present in excessive amounts. In the field study, the use of digestate as fertilizers has resulted in a significant increase in the total soil metal concentration for Ni, Zn, Cr and Cd. In addition, water-extractable concentrations in the soil have significantly increased for Ni, Zn and Cr.

Total metal concentration in digestates and manure used in field experiments

The total concentrations of trace metals and Al in digestates and manure used for the field experiment in 2015 and 2016 are given in Table 4. With the exception of Cr, Ni and Cd in DIG1 in 2016, trace metal concentrations were below limits set for use in agriculture (Class I). For the biogas digestate produced from equal mixtures of sewage sludge and food waste, DIG2, Al concentrations were high for both sampled years.

Table 4. Concentration of investigated metals (\pm standard deviation) for biogas digestates and animal manure from the years 2015 and 2016. Limits for organic fertilizer (class 1) are given for those metals that are a part of Norwegian regulation on organic fertilizer use (Mattilsynet 2006).

	Al	Cu	Zn	Cr	Cd	Mo	Mn	Ni
Treat.	g kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	g/kg	mg kg ⁻¹
Limit for organic fertilizer (class 1)	/	150	400	60	0.8	/	/	30
AM-15	0.4 \pm 0.08	70.6 \pm 2.1	163.3 \pm 1.1	51.4 \pm 4.7	0.12 \pm 0.08	8.5 \pm 0.94	0.19 \pm 0.05	36.6 \pm 3.2
DIG1-15	1.6 \pm 0.51	30.6 \pm 1.8	146.3 \pm 2.2	176.6 \pm 8.6	0.44 \pm 0.22	21.3 \pm 1.8	0.13 \pm 0.08	110.1 \pm 9.9
DIG2-15	15.2 \pm 2.8	88.1 \pm 4.3	256.2 \pm 2.4	156.6 \pm 7.7	0.43 \pm 0.06	21.1 \pm 2.3	0.11 \pm 0.04	106.6 \pm 6.9
AM-16	0.2 \pm 4.7	30.1 \pm 3.1	185.1 \pm 6.7	42.1 \pm 2.8	0.11 \pm 0.03	0.14 \pm 0.01	1.29 \pm 0.06	51.1 \pm 4.7
DIG1-16	1.7 \pm 1.2	39.5 \pm 2.8	245.2 \pm 10.1	165 \pm 6.8	1.1 \pm 0.07	5.1 \pm 1.43	1.7 \pm 0.1	135.5 \pm 9.3
DIG2-16	16.1 \pm 1.3	79.2 \pm 5.7	225.1 \pm 14.5	156 \pm 7.3	0.42 \pm 0.05	3.2 \pm 1.12	0.65 \pm 4.4	195.7 \pm 10.7

AM – animal manure, DIG1 – food waste based digestate, DIG2 – sewage sludge/food waste digestate

Total concentration of metals in soil

Total soil trace metal concentrations are given in Figure 11. Total metal concentrations in the MF treatment were almost identical to those in the control plots (NN).

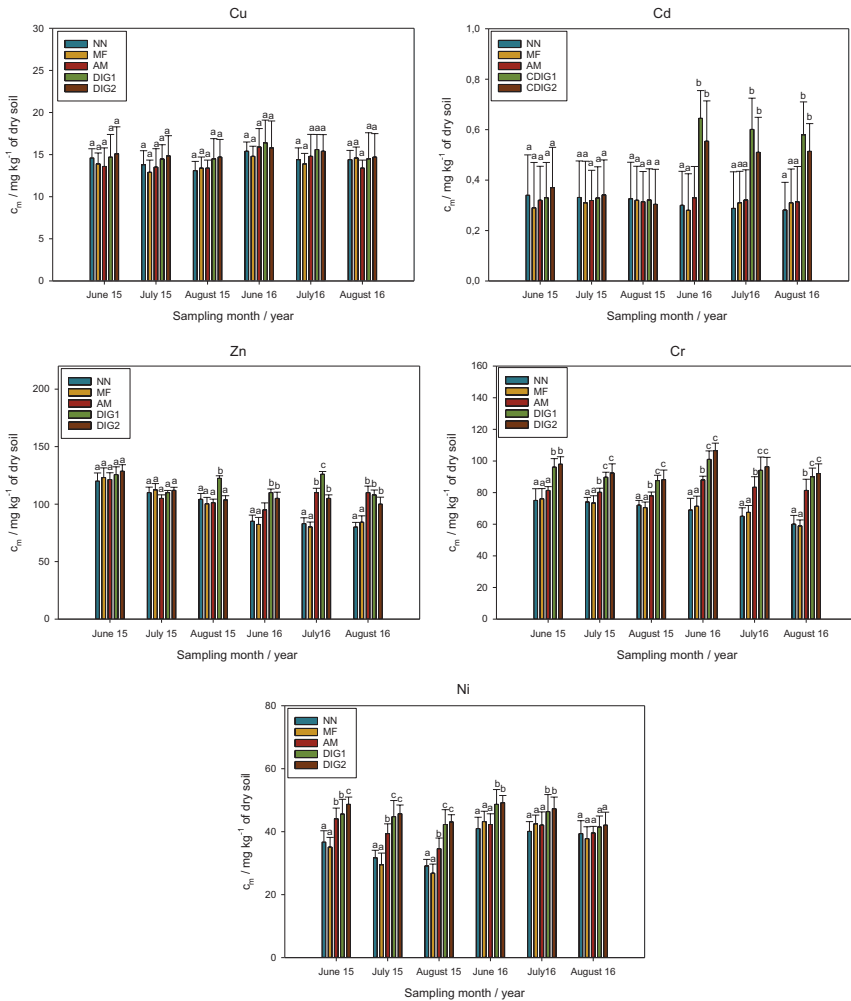


Figure 11. Total metal concentrations in soil samples taken during the growing seasons 2015 and 2016. The values are presented as means \pm SD. Different letters are showing significant differences between the treatments ($p < 0.05$) per sampling month.

Use of biogas digestates as fertilizers has significantly increased total soil content of Zn and Ni for both growing seasons. For Zn, addition of digestates has resulted in higher concentrations than the other treatments at the first sampling especially in 2016. For the majority of the sampling points, the soil concentrations in the organic fertilizers plots were significantly increased compared to the untreated soil (control). For the experimental year 2015, highest concentrations of Zn were found in the DIG1 and DIG2 plots. The Zn content in digestates and manure in 2016 especially was on a level that increased total soil concentration in the top 20 cm significantly (Fig. 11). For the north of Europe, the concentrations in soil are

on average in the range of 45-60 mg kg⁻¹, whereas the measured concentrations in this study were up to 130 mg kg⁻¹ and thus higher than average levels for growing cereal crops (Kabata-Pendias 2011). In a similar two-year field study in which Zn was added with the use of organic waste based fertilizer (composted municipal solid waste) and total Zn concentration in soils was below defined limits (Yuksel 2015). As mentioned above, the concentration of Ni in digestates was higher than allowed by the Norwegian regulations. The measured values for Ni in 2015 have been significantly different, while in 2016 this difference was smaller. were similar in 2016 b. In 2016, the highest concentrations were measured for the digestate treatments at the earlier sampling dates, whereas the manure treatment was on a similar level to the control (Fig. 11). Beneficial levels of total Ni in soil for crop growth are around 50 mg kg⁻¹. In our study, concentrations were below nationally defined threshold (i.e. below 100 mg kg⁻¹ in soil) and well within the most common levels (35-55 mg kg⁻¹) for cereal production in general (Brunetti et al. 2012; Kabata-Pendias 2011).

In the case of Cu, higher concentrations were found in the DIG2 treatment for the 2015 season. In 2016, however, the manure treatments had lower concentrations of Cu. The analysis has shown lower levels of Cu in fertilized soil plots than in the control plots. This could be attributed to a slightly higher potential for leaching than from the control plots at the first two sampling points because of an increased mobility due to the added fertilizers. Details can be found in Chapter 4.3. First signs of Cu toxicity can be expected at concentration of 60 mg kg⁻¹ and higher for growing plants (Kabata-Pendias 2011).

For Cr and Cd, the total soil concentrations were significantly higher in the plots treated with biogas digestates (Fig. 11) when compared to NN and MF plots. This is especially seen for the total concentrations of Cr and Cd concentrations in soil (season 2016). There were no differences in Cd concentrations between treatments at the first sampling date in 2015 (Fig. 11). Still, for 2016, concentrations at the first sampling were generally higher for Cd than in 2015, and concentrations increased more with time in the organic treatments than in the control soil. The Cd concentrations were higher for all treatments and at all sampling dates in 2016 compared to 2015. The maximum allowed concentration of Cd in agricultural soils treated with organic fertilizers such as sludge is usually set to 3 mg kg⁻¹ (Brunetti et al. 2012), and this limit was not exceeded in our experiment in the two growing seasons. For both digestate, the Cr concentration was above 150 mg Cr kg⁻¹ DM (Tab. 4). This was higher than the limits set by the Norwegian regulation on organic fertilizers used in agriculture (60 mg kg⁻¹ dry fertilizer, class I). The measured values of total Cr in soil were under the limits defined by the Norwegian regulation for soils treated with organic fertilizers, i.e. 100 mg kg⁻¹. Toxic values for total Cr

concentration in agricultural soils may vary between 150 and 400 mg/kg depending on the soil type but also on the forms of Cr found in the soils (Cr^{3+} or Cr^{6+}) (Kabata-Pendias & Pendias 2001; Kabata-Pendias 2011).

Water-soluble metal concentrations in soil

Water-soluble concentrations of Zn and Ni in the biogas digestate treatments were comparable to the concentrations measured in the animal manure treatment over the two growing seasons. (Fig. 12). This indicates that the digestates added as fertilizer affect Zn mobility in a similar way to animal manure. For the plots treated with biogas digestates, water-soluble Ni concentrations have significantly increased during both growing seasons. This may be due to an observed decrease in pH since Ni mobility is enhanced under acidic conditions (Zhu et al. 2011). The maximum water-soluble concentration of Ni measured was around $50 \mu\text{g L}^{-1}$. Although the measured value refers to the water extracts the Ni concentration was below The World Health Organization (WHO) where $70 \mu\text{g Ni L}^{-1}$ is a limit for drinking water (WHO 2006).

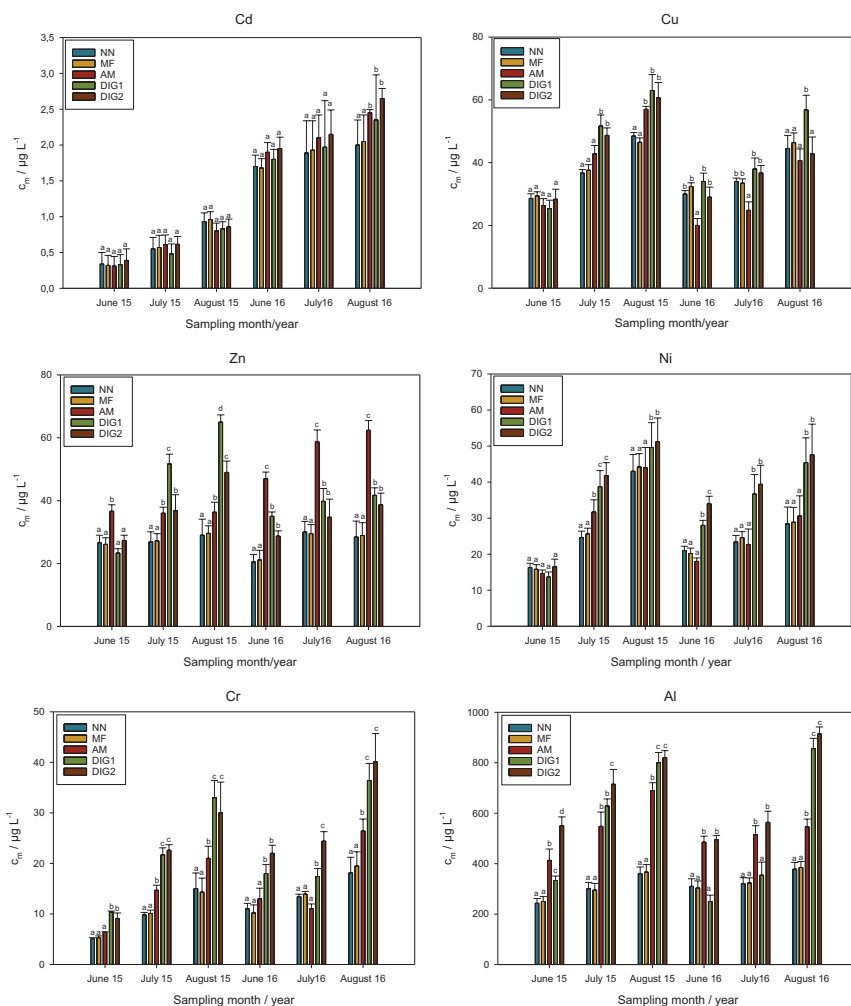


Figure 12. Selected water-soluble metal concentrations in soil (growing seasons 2015 and 2016). The results are presented as means \pm SD. Different letters indicate significant differences between treatments at each sampling time ($p < 0.05$). The extracts were prepared by adding 5 g of soil in 25 mL of deionized water.

Copper mobility, measured as water-extractable Cu, generally increased from June to August for all organic fertilizers, which was comparable to trends seen for Ni. Copper mobility is mostly influenced by changes in soil pH and DOC concentration due to the different treatments. An increase in dissolved organic carbon, i.e. the soluble phase of organically bound carbon is known to increase metal mobility both in soil solution and thus in the soil (Perez-Esteban et al. 2014).

For the trace elements that are not plant nutrients, water-extractable concentrations in the soil were also increased. Water-extractable Cd concentrations were low in all treatments in the 2015 season, as Cd additions with the organic fertilizers were low and little Cd was present in the soil before the beginning of the experiment. In studies with similar levels of Cd in manure, it has been seen that the addition of manure does not cause a significant change in the soil Cd content (Xu et al. 2015). Using the data from both growing seasons, Cd was more strongly correlated to the pH than to DOC, which is expected and also confirmed in a recent study (van der Sloot et al. 2017), where lower pH was seen to increase the water-extractable Cd concentrations in the soil. There was also a significant increase of water-soluble Cr concentrations during both growing seasons (Fig. 12) for digestate treatments when compared to control or mineral fertilizer plots. Most importantly, measured concentrations were below the limit of 50 $\mu\text{g L}^{-1}$ of total Cr in water, which is used as a limit for drinking water (WHO 2006).

Addition of the digestate with the high Al content, due to post treatment of sewage sludge with Al salts to fell phosphorus (DIG2), resulted in high concentrations of water-soluble Al at all sampling dates compared to the control, but the other two organic treatments showed similar levels either early in the growing season (manure) or at the later sampling dates (DIG1). Only DIG2 increased the total Al content of the soil. Aluminium availability in soil and soil solution is generally influenced by soil pH and DOC. In our study, both pH and DOC showed good correlations to water-extractable Al concentrations (Appendix, Paper 3). In general, Al does not occur in toxic forms in the soil at pH values suitable for cereal production (Wang et al. 2006).

Total metal concentrations in plant material

For the growing season 2015, total metal content in plants was determined at three times during the growing season. In 2016, only total trace metal content in grains at harvest was determined. Figure 13 shows the concentrations of trace metals in the plant material. Low concentrations in both total and water-soluble forms in the soil are reflected by low Cd levels in the plant material (Fig. 13), with a significant increase in Cd grain concentration only in the AM and DIG2 treatments. Still, the measured grain concentrations were below 0.1 mg kg^{-1} , which is considered as limit for safe value of Cd in grains (Kabata-Pendias 2011). Cd originating from sewage sludge and similar sources is controlled more strictly than that from mineral fertilizers, although the latter can also have a high Cd content (Pizzol et al. 2014). For Cu and Zn, a different uptake scenarios were seen, where total plant concentrations have significantly

increased for digestates treatments. Measured grain concentrations were in average for Cu approximately 0.2 mg kg^{-1} , while for Zn they were around 25 mg kg^{-1} .

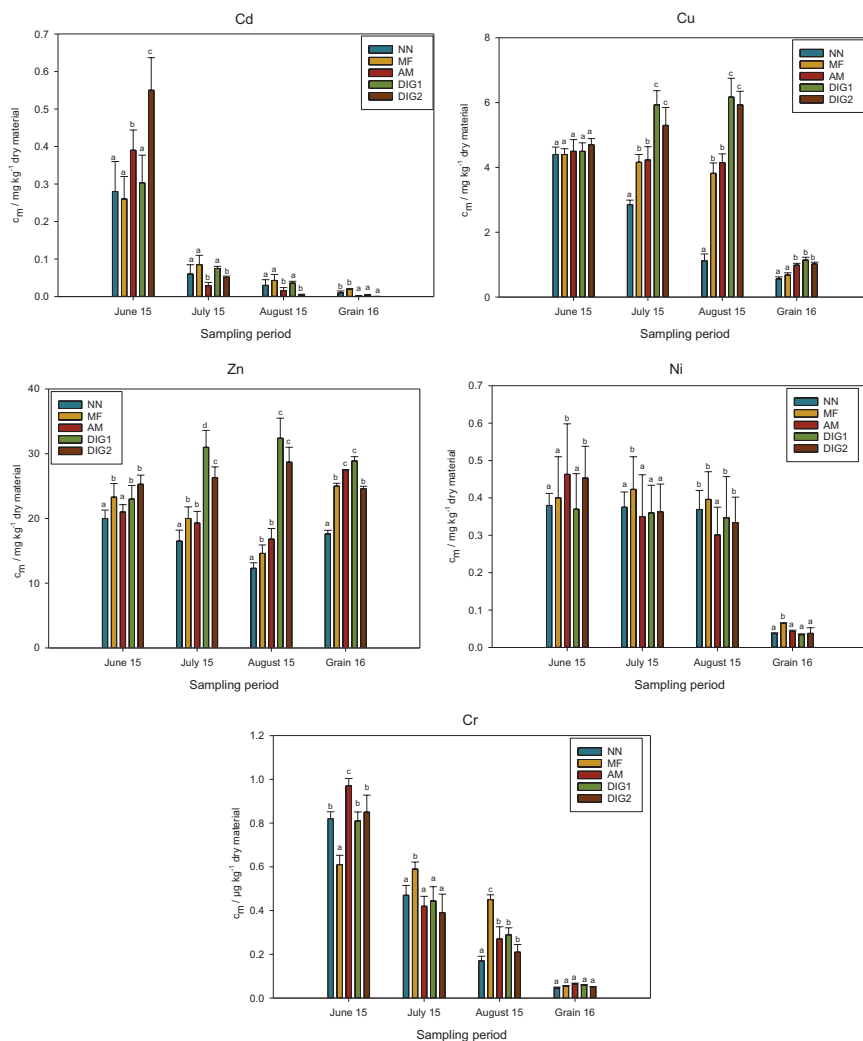


Figure 13. Total concentration of selected metals in aboveground plant parts sampled during growing season 2015, and in barley grains harvested in 2016. Different letters indicate significant differences between treatments at each sampling time ($p < 0.05$).

For the plant material sampled in 2015, there were no significant differences in Ni and Cr concentrations among the treatments throughout the growing season. Cr concentration in general, was lower than concentration of other metals detected in the plant material. This was partially expected based on the fact that Cr is not a plant nutrient. A significant increase in plant Zn concentration was seen in digestate treatments during the 2015 season compared to animal

manure or mineral fertilizer (Fig. 13). These findings reflect the higher availability of Zn as indicated by increased water-soluble concentrations. There were, however, no significant differences in Zn concentration in the grain samples between the digestate and MF or AM treatments. Added digestates had no significant influence on grain Ni concentration and the values found were comparable to the AM and MF treatments. Although there were significantly higher water-extractable Ni concentrations in the soil fertilized with digestates, no significant effect of the organic fertilizer treatments on plant Ni concentrations was found (Fig. 13). This suggests that the use of digestates when compared to animal manure results in the same Ni uptake in the edible part of the plant. The level of Ni concentrations in grains was comparable to study on organic fertilizers use conducted in Northern Europe (Hamner et al. 2013).

4.2 Mobility of Al and Cr in soils treated with biogas digestates – a modelling approach

Analysis of the biogas digestates used in the field experiments showed high concentration of several metals, among them Cr and Al. In Paper 2, the focus has been given to the presence and mobility of Cr and Al in three different experimental scenarios. Here the focus will be given to the results obtained from the modelling study. For assessment of the amounts of Al and Cr being complexed with dissolved organic matter (DOM), the WHAM model was applied to the water-extractable concentrations of Al and Cr measured in the field experiment. The data for water-soluble Al and Cr concentrations in soils, as well as model-estimated values of free Al and Cr concentrations are presented in Paper 2. The pH, DOC and cation and anion measurements are also available in Paper 2.

WHAM simulates an increase of free Al between June and August, especially in the CDIG2 treatment. The modelled values increased almost five times from June to August. The modelled free Cr concentrations also show an increase from June to August samples. However, the modelled values are extremely low compared to the water-soluble Cr concentrations.

The binding of Al and Cr to humic substances (i.e., fulvic acid, FA, as explained in Paper 2) was modelled and results are presented in Figure 14. The model simulated a higher amount of Al bound to FA in June than in August for the AM treatment. For both CDIG treatments, the model estimated a lower amount of Al bound to FA in August than in June (Figure 14).

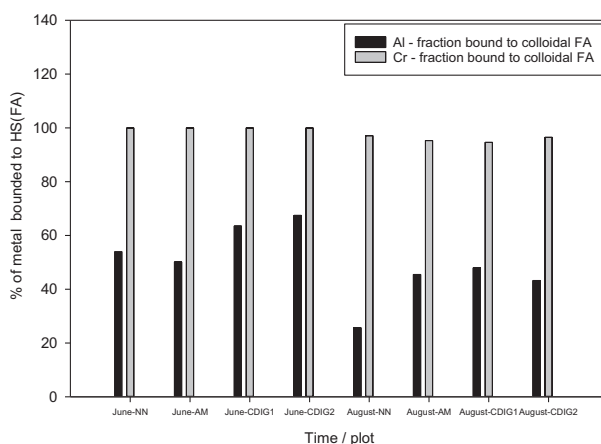


Figure 14. Modelled percentage of Al and Cr bounded to humic substance in water extracts of soil samples from June and August (field experiment).

WHAM simulated almost 100 % of the water-soluble Cr to be bound to FA, both in June and in August, being slightly lower in the August samples.

The highest concentration of water-soluble Al was found in August for the CDIG2 treatment, which was expected based on the fact that CDIG2 had highest content of both total and water-soluble Al for all the treatments. This can partially explain the increased mobility of Al with the use of CDIG2. Still, other factors, such as pH, DOC and irrigation control the mobility of Al (details in Paper 2). The WHAM modelling has confirmed that the controlling process for Al solubility were complexing reactions between Al and organic matter. A study by Roy and Couillard (Roy & Couillard 1998) with the use of sewage sludge as soil amendment has shown that pH and DOC control Al leaching from the soil. Modelling results have also shown changes in Al solubility between the June and August samples. Since WHAM 7.0 is not a dynamic model, it was applied separately on results obtained from June, and then on the results from August. The data from the beginning of the experiment are showing that water-soluble Al concentration increased from June to August, while the model estimated free inorganic Al concentration (Al_{free} concentration) also increased. Modelled results reflect also in Al bound to humic substances (FA) shown in Fig. 15, where a decrease in amount of Al bound to FA from June to August is apparent. Modelled free concentrations of Al are indicating that the majority of the soluble Al is complexed either with organic matter or with inorganic ligands. Complexing with organic material has been shown to control Al mobility in earlier studies (Tipping et al. 2002).

The modelled free Cr concentrations were generally very low, but increased somewhat from June to August. WHAM modelling also showed that water-soluble Cr is complexed with organic matter (FA) and that the change between June and August is small (Figure 16). Model results of Cr(III) complexation to DOC also support that soluble Cr(VI) forms are not favoured within the investigated experimental conditions. Measured values of Cr in the water extracts from the field experiment were well below the limit ($100 \mu\text{g L}^{-1}$) defined by the United States Environmental Protection Agency (EPA) for total Cr concentration in freshwater (EPA 2017). Initial total concentrations of Cr in digestates were almost twice as high as the values given in Norwegian national regulation on the use of organic wastes in agriculture, and significantly higher than the usual amounts seen in digestates or manure (Al Seadi & Lukehurst 2012; Kupper et al. 2014).

4.3 Leaching potential of trace metals from soils treated with biogas digestates

The greenhouse and column leaching experiment have been conducted under fully or partially controlled conditions and the discussion of these results will be used in order to address a possibility of two different scenarios of Al and Cr leaching by using biogas digestates. Besides studying Al and Cr leaching in greenhouse and column leaching experiment, the high precipitation event simulated using the column experiment has been used to investigate leaching patterns of Cu, Ni and Zn for three different soil types treated with five biogas digestates (two commercial and 3 experimental as explained in Chapter 3. Findings presented in this chapter are related to the research published in both Paper 1 and 2.

Leaching of Al and Cr after harvest

The added amounts of trace metals in the organic fertilizers were compared to the amounts leached after harvest in the greenhouse experiment. The highest amount of Al was added with the CDIG2 treatment due to sewage sludge treated with Al salts in the feedstock, while the lowest input was for AM (Tab. 4). Concentration of Al measured in leachates differed within the same treatment depending on soil type (Figure 15).

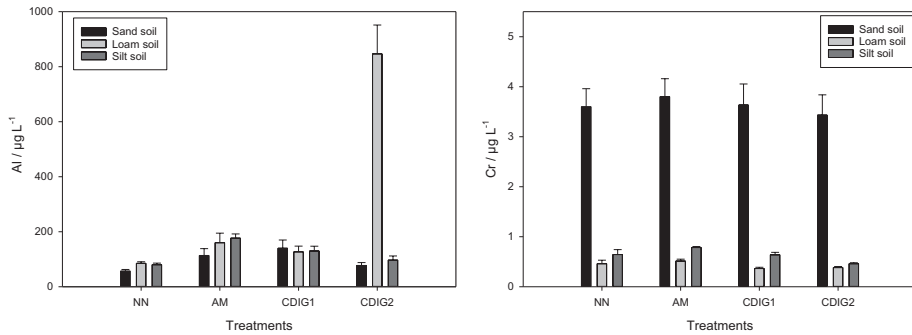


Figure 15. Concentration of Al and Cr in leachates obtained from the greenhouse experiment after harvest by irrigation.

For sand and silt soils, the digestate and animal manure applications resulted in Al concentrations below $200 \mu\text{g L}^{-1}$, which was comparable with levels of untreated soils (NN). A different pattern was seen for the loam soil, where very high amounts of Al were leached from the CDIG2 treatment. This was reflected in a lower pH and higher DOC concentration in the leachate which can increase Al mobility. An increase in DOC concentrations in soils by digestate addition would affect the soil/soil solution equilibrium and may lower the adsorption ability of trace metals in the loam.

For Cr, highest amounts were added with CDIG1 treatment, followed by the CDIG2. This pattern was also seen in the leachates from the pots containing sand, whereas neither loam nor silt showed any increase of Cr concentration in the leachates compared to the control (NN). Concentrations of Cr in leachate from the silt and the loam were around $1 \mu\text{g L}^{-1}$ (Figure 15), indicating a strong sorption in both soils.

Leaching of trace metals from soils treated with biogas digestates – high irrigation scenario

The metals that were in focus in this study were Al, Cr, Ni, Cu and Zn. The research presented in this chapter is available in detail in Papers 1 and 2.

Aluminium - In Fig. 16 leached percentages of Al relative to the input of water-soluble Al in the different treatments and soils are shown. Aluminium leached from the columns receiving digestates was well below 30 % of the added amounts, whereas higher percentages of Al added with manure were leached from all soil types. This suggests a different binding of Al and organic matter from manure, which could also imply a differences in organic matter

structure between manure and digestates. The highest absolute Al amounts leached were seen with the use of CDIG1 treatment in the loam, although the CDIG2 treatment added by far the highest amounts of Al in absolute terms. The soil type has been seen to affect significantly the amounts of Al that was leached, especially for AM treatment.

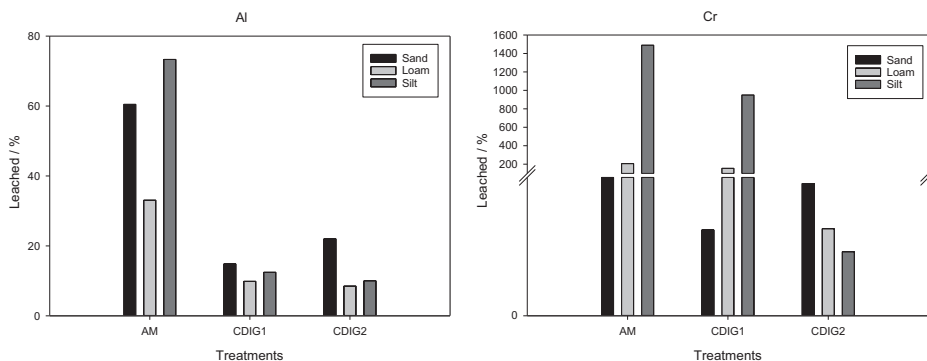


Figure 16. Percent of Al and Cr input leached from the soil columns. The amount leached in each treatment was corrected for the amounts leached from the control treatment (NN). The break for Cr figures is set at 100 %.

Highest leaching of Al occurred in the sand and silt. For the loam, an adsorption was seen, since the leached amounts for the digestate treatments were generally less around 10 % of the Al added for CDIG treatments. The use of animal manure resulted in higher Al leaching than the commercial digestates (Figure 16). The amounts of Al leached from sand and silt are around 75 % of the added amounts, which may be expected based on the low clay and organic matter content for these soils (lower sorption ability). Over the experimental period, a pH increase in the leachates was observed (see Paper 1), which may have been caused by a high concentration of DOC and a high irrigation rate inducing leaching of naturally present Al in the soils. Dissolved organic matter from biogas digestates has been seen to reduce the electrostatic binding of Al in soils (Karak et al. 2015), which would increase Al leaching. In the present study, however, this effect of digestates on Al binding was not obvious, as leaching from the respective treatments was very low in all soils. The higher leaching rates in the manure treatments, on the other hand, might be explained by such a process.

Chromium - Cr leaching has shown a different pattern than Al (Fig. 16). In general, higher percentages of Cr added were leached and in several cases, and considerably higher rates were seen for silt soil. Still, the highest leached amount of Cr was seen for CDIG1. What was interesting to see were the results obtained for AM treatment that represented the lowest input of Cr. The AM treatment has been seen to have highest leaching rate, especially in the

silt soil. The leaching of Cr, relative to the input was high for CDIG1 and AM in the loam and the silt soils. For the used the digestate treatments, application of CDIG1 resulted in the highest leached Cr amount during the experiment. The statistical analysis showed that Cr was significantly correlated to soil texture and DOC as factors for Cr leaching. The high irrigation rate and the low adsorption capacity of the sand resulted in low retention of the added Cr and almost equal input and output concentrations. In the silt, however, leaching of Cr was much higher than added amounts for the CDIG1 and AM treatments. This may have been due to enhanced mobility of naturally present Cr in silt induced by the addition of large amounts of DOC from the fertilizer treatments. On the other hand, this effect was not seen in the second digestate treatment. In the loam, only slightly higher leached amounts relative to the amount of Cr added were seen but also here, leaching from the CDIG2 treatment was considerably lower than from the other two organic fertilizer treatments. A recent study by Reijonen and Hartikainen (Reijonen & Hartikainen 2016) has shown that higher organic matter content in soils contributes to a lower capability of Cr oxidation, from Cr(III) to Cr(VI), in agricultural soils. This observation also agrees with a study of Gustafson and co-workers (Gustafsson et al. 2014) where it was seen that Cr(III) forms are dominant within the conditions of pH >4.0 and high DOC concentration.

Nickel – Leached amounts of Ni (Figure 17) show that there was no mobilization of native soil Ni in any of the soils treated with digestates on top of the amounts of Ni added to the columns. However, a high mobilization effect, with leached Ni amounts above added amounts, was visible in the AM treatment in the loam. The AM treatment caused higher Ni leaching than the commercial and experimental digestates in all three soils, although the total content of Ni in AM was lower than in the commercial digestates. Leaching of Ni from the biogas digestate treatments was highest in the sand and lower in the loam and silt soils, which was expected due to the low adsorption capacity of sandy soils. The percentages of leached amounts compared to amounts added varied from 35 % for CDIG2 to 259 % for animal manure in the loam. Figure 17 shows that the leaching rate of Ni from both experimental and commercial digestates was lower than from animal manure. For EDIG3 and CDIG1 treatments, relatively high leaching was recorded in the in all three soils. For silt, the highest leaching of Ni occurred in the CDIG1 treatment with around 70 % of added amounts, but also in the EDIG3 treatment as much as 65 % of the input was leached. Still, the highest percentage of Ni added was leached from AM treatments in all three soil types, (Figure 17).

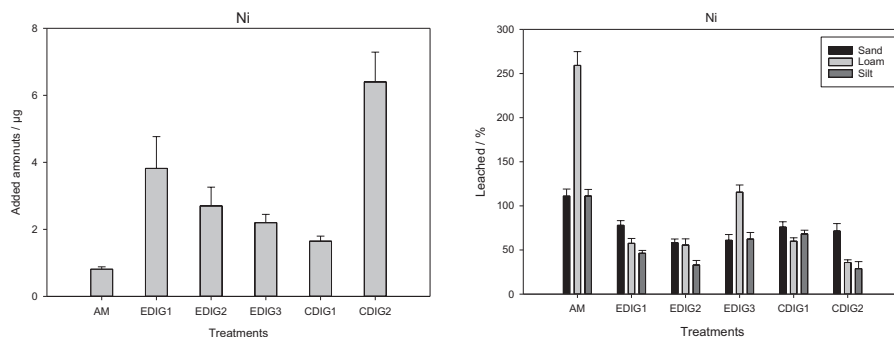


Figure 17. Added amounts of Ni based on water-soluble concentrations and added volumes, and percentage of Ni leached from the different treatments. The leaching values were corrected for the Ni concentrations leached from the control soils (NN).

Similar percentage of Ni leached compared to amounts added can also be seen in other studies that used de-watered organic residues (sewage sludge) in sandy loam soil (Antoniadis & Alloway 2002a; Cornu et al. 1999). Ni occurs as a positively charged ion in soil solution. Soil adsorption capacity for metal ions is mostly determined by the negatively charged surfaces of clay particles and SOM when the pH is high and thus the SOM functional groups deprotonated. Also infield experiments, pH was shown to have a dominant effect on Ni leaching in acid soils (Toribio & Romanya 2006). In our experiment, pH was negatively correlated to Ni leaching in the loam and silt, which can be explained by Ni being bound to negatively charged surfaces (oxides and oxide groups of clay minerals) when the pH value is high (details in Paper 1). The key role of pH has been seen also confirmed in a similar study (Covelo et al. 2007) that investigated the influence of the soil characteristics on Ni leaching. For a simple environmental assessment, the maximum leached concentration of Ni in the experiment was $30 \mu\text{g L}^{-1}$ (AM treatment in the loam), which can be to some extent compared to regulation of WHO for drinking water ($70 \mu\text{g L}^{-1}$). Still, it was rather high when compared to untreated soil ($6 \mu\text{g L}^{-1}$).

Copper –Added and leached amounts of Cu concentrations during the leaching experiment are shown in Figure 19. For all treatments, the total amount of Cu leached from sand and loam was lower than the input amounts, while significantly higher leaching of Cu was seen from the silt soil (Figure 18). In all soils, the highest Cu leaching in percent of added amounts was found in the CDIG1 treatment, which was the treatment with lowest amounts of Cu added. Still a different leaching pattern can be seen between the loam and silt soil regarding Cu leaching.

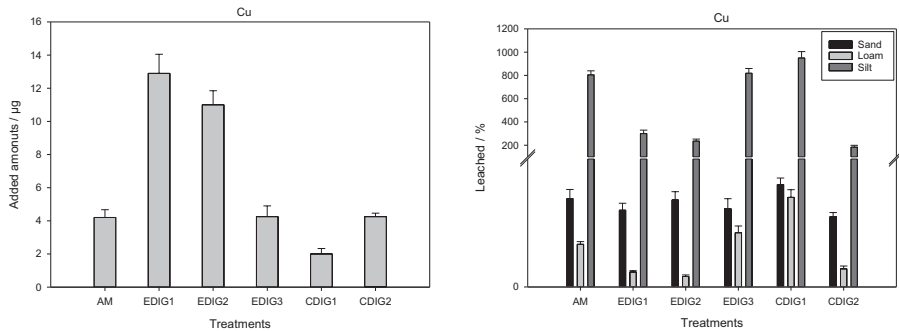


Figure 18. Added amounts of Cu and percentage of Cu leached from the soil columns (figure break set on 100%). The leaching values were corrected for the Cu concentrations leached from control soils (NN).

The amount of Cu that was applied to the soils via the EDIG1 and EDIG2 digestates was highest among all fertilizer treatments. However, in all the three soil types, the highest leaching rate of Cu added was found other treatments, i.e. EDIG3, CDIG1 and AM. Comparing the three soils, the lowest percentage of Cu added was leached from the loam, probably due to binding of Cu to negative surface charges on clay particles. Amounts of leached Cu were generally ranging far above the added amounts in silt soil, suggesting that the application of digestate or manure mobilized naturally occurring Cu in the soil. As in other studies (Perez-Novo 2008) the Cu concentration in leachates was also correlated to the pH in the loam and silt, but pH was not found to be a main factor causing the enhanced Cu leaching in the silt. Measured amounts of leached Cu for the silt are similar to those found in a study of Fang et al. where leaching was investigated by using organic fertilizers (composted sewage sludge) (Fang 2016). The mechanism responsible for Cu mobilization might be related to the structure of the added organic matter, where it seems that the part of soil Cu in a form of less soluble fraction, gets mobilized by forming stable complexes between Cu and DOC, thus inducing leaching from silt soil. Chemical reactivity of Cu and DOC was discussed by Romkens and Dolfing (Romkens & Dolfing 1998), who have shown the importance of DOC composition, and especially molecular size of organic matter, which can influence Cu complexing ability. The maximum leached concentrations of Cu were found in leachates from the silt, i.e. $185 \mu\text{g L}^{-1}$ in the AM and EDIG3 treatments, which is well below the WHO limit for drinking water of $2000 \mu\text{g Cu L}^{-1}$ (WHO 2011).

Zinc –The experimental digestates, particularly EDIG 1 and 2, represented the highest Zn input of all organic fertilizer treatments (Fig. 19). In the loam and silt, a retention of added

Zn was found in all the treatments except for EDIG 3, whereas in the sand, leaching of Zn was close to 100 % of added amounts in most cases.

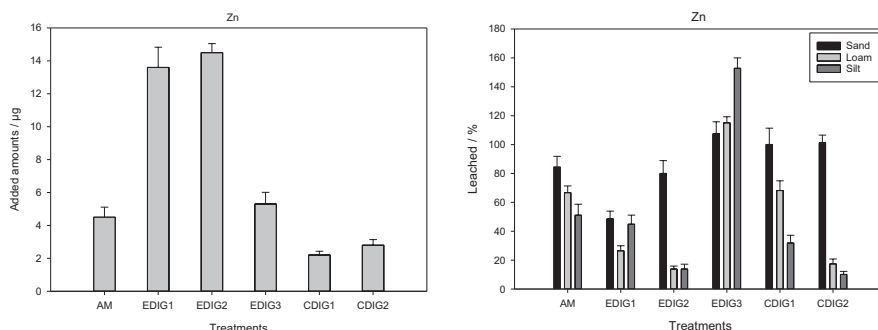


Figure 19. Added amounts of Zn based on water-soluble concentrations and applied volumes, and percentage of Zn leached from the soil columns in the different treatments. The leaching values were corrected for the Zn concentrations leached from control soils (NN).

For all three soils, addition of EDIG3 resulted in leaching of Zn, which was over 100 % of the input Zn, indicating a release of natural soil Zn (Figure 19). In general, leached amounts of Zn were lower than the added amounts or almost equal to the added amounts for sand soil. Leaching of Zn was high in the sand as may be expected. Leaching of Zn from the treatments with commercial digestates was comparable to the amounts leached from the AM treatment, and lower than in those from the experimental digestate treatments. For the sand, DOC was the main factor influencing Zn leaching. For the loam and silt soils, most of the Zn was leached in the beginning of the experiment, and pH seemed to be the dominant factor in controlling the release of Zn in these soils. Several research studies have shown the importance of the stability constants between organo-metallic complexes in controlling Zn mobility, especially at neutral soil pH (Ashworth 2004; Giusquiani et al. 1998; Zhang 2016). Zn has a lower ability to form complexes with organic matter compared to, e.g., Cu, because of filled *d*-orbitals in the atomic structure.

The highest measured concentration of Zn in leachates was $60 \mu\text{g L}^{-1}$. In the WHO drinking water guidelines, no adverse effect of high Zn concentrations is mentioned and thus no limit defined.

4.4 Occurrence of polar organic pollutants in biogas digestates

As presented in Chapter 3.2. there is still limited data available surrounding the presence of organic pollutants in digestates, especially polar organic micropollutants (POMs). The overall aim of the this study was to investigate 21 POMs (12 PFAAs, 5 pharmaceuticals, 3 benzotriazoles and an artificial sweetener) in two different biogas digestates both before and after field application and compare them to animal manure. Objectives in this study was first to quantify a range of POMs of emerging concern in digestates derived from 2 unique feedstocks; second, to assess the fate of these POMs in soils following field applications, and finally, to determine whether any POMs have the potential to translocate grain planted on the field.

Organic compounds included in the study

Based on a combination of environmental relevance, digestate screening, and the availability of analytical methods a 21 POMs have been investigated. The PFAAs included: linear and branched isomers of perfluorooctane sulfonic acid (l- and br-PFOS, respectively), perfluorohexane sulfonic acid (PFHxS), perfluorohexane alxyl acid (PFHxA), perfluoroheptanoic acid (PFHpA), , l- and br- isomers of perfluorooctanoic acid (l-PFOA and br-PFOA, respectively), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorodecanesulfonic acid (PFDS), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoate (PFTriDA) and perfluorotetradecanoic acid (PFTeDA). The 9 non-fluorinated POMs included: 1-H-benzotriazole, 1-methyl-1H-benzotriazole, 4-hydroxy-benzotirazole, acesulfame, acetaminophen, acridine, carbamazepine, oxazepam and venlafaxine.

Study site and sample collection

The experimental set up used in this study was the randomized block field experiment explained in Chapter 3.3. Soil was sampled from each treatment plots from June to August, in the first week of each of the sampling months, respectively. The soil samples were then dried (55 °C), sieved (< 2 mm) and stored at 4 °C prior to analysis. Oat grains were collected after the harvest sieved and stored in the same way as soil samples until further analysis.

Extraction procedure for fertilizer, soil and grain samples

Prior to extraction, organic fertilizers, grain and soil samples were dried (24 h, 105°C). Two separate methods were used for extraction and analysis: one for PFAAs and the second for non-fluorinated micropollutants. For PFAAs, the extraction procedure was a modified version of the Powely method (Powley et al. 2008). Briefly, 1 g of dry solid sample (i.e. soil, fertilizers, or grain) was spiked with surrogate standards and extracted two times with methanol. After centrifugation and supernatant collection, the extracts were evaporated to approximately 1mL. The concentrated extract was cleaned-up with activated carbon, centrifuged and supernatant were stored at -20 °C prior to analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). More details can be found in Paper 4.

Extraction of non-fluorinated POMs was carried out using 1 g of dry soil, organic fertilizer and grain samples. Samples were spiked with surrogate standards, dispersed with 1.5 mL of methanol, and then vortexed for 3 minutes and left in the fume hood for the methanol to evaporate overnight. Thereafter, the samples were supplemented with 20 mL of acetonitrile (ACN) sonicated for 15 minutes in an ultrasonication bath, and then centrifuged for 5 minutes at 2000 RPM. The supernatant was subsequently transferred to a clean 15mL tube. The ACN extraction was repeated and the extracts were combined. The resulting extract was placed in a Buchi evaporator and reduced to a volume of <1 mL. More details can be found in Paper 4.

Instrumental analysis of POMs

Instrumental analysis of PFASs was carried out by liquid chromatography tandem mass spectrometry (LC-MS/MS) using a Waters Acquity UPLC coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters Corp., Milford, MA, U.S.). The instrument was operated in negative electrospray ionization (ESI), selected reaction monitoring (SRM) mode (see SI for details). Quantification was performed by isotope dilution using a 5-point calibration curve (linear, 1/x weighting), which was run before and after samples. For most targets, analogous isotopically labelled internal standards were available.

Non-fluorinated micropollutants were analyzed by LC-MS/MS using a Thermo Scientific Ultimate 3000 UHPLC system coupled to a Thermo Scientific Quantiva triple-quadrupole mass spectrometer (Thermo Scientific, Dreieich, Germany). The instrument was operated under polarity switching, SRM mode using an ESI source. Details can be found elsewhere (Posselt et al. *In preparation*). For both of the method details please see Paper 4.

Quality control

Method accuracy and precision were good for most PFAAs in all matrices, with mean percent recoveries ranging from 92-129, 89-108, and from 93-120 in BDs, soil, and grain respectively. For non-fluorinated POMs, with the exception of acridine and 4-hydroxybenzotriazole, the mean percent recoveries ranged from 118-147, 110-231, and 121-180% in BDs, soil, and grain respectively. Acridine and 4-Hydroxybenzotriazole displayed lower recoveries, ranging from 40-83 and 4-62 %, respectively (see Paper 4 for details).

POMs in biogas digestates and animal manure.

A total of 12 PFAAs and 9 non-Total concentration of PFAAs (\pm standard deviation) were highest in SS/FWBD (9.53 \pm 0.20 ng/g) followed by FWBD (2.26 \pm 0.10 ng/g), and non-detectable in manure (Fig. 20). For both digestates, the PFAA homologue profile was dominated by PFOS (Σ isomers; 0.6-2.65 ng/g) followed by PFOA (approx. 0.50 ng/g). The seen difference in the PFAAs profiles between treatments is not surprising as sewage sludge is a known source of different PFAAs (Cervený et al. 2018; Eriksson et al. 2017; Paolini et al. 2018; Yu et al. 2009). The concentrations of PFAS reported in this study are significantly lower than those reported previously (3.4 to 35 $\mu\text{g kg}^{-1}$ d.w.) for a screening study on digestates (Brandli et al. 2007). For non-fluorinated POMs, 1-methyl-1*H*-benzotriazole (1M-1HBT, 9.82-2.42 ng/g) and acesulfame (0.2-1.62 ng/g) were the predominant substances detected in fertilizers.

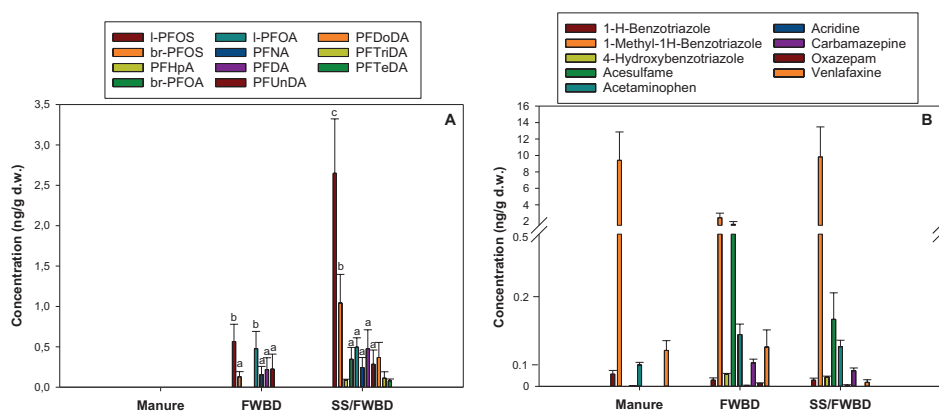


Figure 20. Concentrations of PFAAs (A) and non-fluorinated POMs (B) in biogas digestates and animal manure used for the field experiment. Different letters indicate significant difference between specific targets ($p < 0.05$) for each of the treatments. Error bars represent standard deviation.

The levels of PFOS and PFOA, which were dominant in PFAA profiles are consistent with prior measurements in organic wastes used in agriculture; however, concentrations are considerably lower than previously reported. For example, Suominen et al. (2014) investigated PFAAs in BDs produced from different mixtures of municipal sewage sludge, sorted municipal biowaste, food industry by-products, and animal manure and reported Σ PFAA concentrations ranging from 1-170 ng/g dry weight with profiles dominated by PFOS (Suominen et al. 2014). Despite the low concentrations in the present work, the occurrence of differing PFAA profiles between the two BDs along with their absence in manure, indicates that the substrate used for the production of biogas plays an important role in influencing the contaminant burden. While both BDs contained low levels of PFAAs, overall, the FWBD appears to be more desirable in terms of lower PFAA concentrations. This is perhaps unsurprising considering food waste in general contains lower levels of PFAAs relative to sewage sludge (Allred et al. 2015; Sepulvado et al. 2011).

For the benzotriazoles, as a non-fluorinated POMs, it has been confirmed that they can be pose a potential environmental problem in Norway mainly because of their persistence in soils and sediments (Breedveld et al. 2003). In our study, reported concentrations of benzotriazoles in BDs are comparable to those found in earlier studies of biosolids application to agricultural soils (Hua-Jie et al. 2014). The source of these corrosion and flame inhibitors is unclear, but may arise from the use of anticorrosive covering in biogas facilities or storage tanks. The remaining non-fluorinated POMs are regularly consumed by humans, and wastewater is identified as main pollution source for the aqueous environment (Wang et al. 2016; Wu et al. 2015). Thus, there occurrence in the SS/FWBD is not surprising. For other POMs, acetaminophen and venlafaxine, concentrations were comparable to previous measurements in digested organic material and fertilized soils, respectively (Martínez-Piernas et al. 2018; Verlicchi & Zambello 2015) while carbamazepine levels (0.05-0.07 ng/g) were orders of magnitude lower than concentrations in organic wastes or biosolids (140-260 ng/g) (Muñoz et al. 2009).

POMs in soils fertilized with biogas digestates and animal manure

Soil samples taken from the field experiment have revealed low and consistent concentrations (i.e. < a few ppb dry weight) of up to 8 PFAAs and a single non-fluorinated POM (1M-1HBT) in both treated and control plots. Recorded small differences in the pollutant profiles and

concentrations among plots, and the limited change in concentration during the experiment suggested that the BDs had only minor impact on the contaminant loadings.

There are several studies that have reported occurrence of POMs in soil following application of organic fertilizer. One of the earlier studies on the field application of municipal biosolids (dewatered sewage sludge) has shown that venlafaxine was not measured 2 months after the application to the soil (Gottschall et al. 2012), consistent with the absence of this substance in the presented field study. The highest concentration of 1-Methyl-1*H*-Benzotriazole in soil was approx. 2 ng g⁻¹ dw. Measured concentrations in this study are comparable to a study that included a soil type with similar characteristics (clay content) as ours treated with biosolids (Hua-Jie et al. 2014). Nevertheless, in the same study application rate was 60 t ha⁻¹ of the agricultural land and sampling was done independent of the growing season (12 months) with the same depth of organic waste soil incorporation (20 cm). Benzotriazoles are known as hydrophobic compounds and it was expected that they would sorb onto soil particles, which would then influence the soil/plant distribution and making them more persistent in the soil (Lai et al. 2014).

POM Crop uptake

For all POMs that were in focus of this study, only PFOA and 1-methyl-1*H*-benzotriazole (Fig. 21) were detectable in grain (0.05-0.13 ng/g and 8-10 ng/g). Still, the measured concentrations were not significantly different among fertilized plots for either of the substances. For example, PFOA's concentrations were low when compared to prior measurements in grain reported for field and greenhouse studies involving dewatered sewage sludge (Blaine et al. 2013; Navarro et al. 2017) and oat grain (Stahl et al. 2009). Regarding the 1-methyl-1*H*-benzotriazole, to the best of our knowledge this is the first detection of this substance in cereal grains (oats). Earlier studies on uptake of benzotriazoles in other types of plants (lettuce and strawberries) have also indicated uptake of these compounds in the fully-grown edible plant parts (LeFevre et al. 2017). The mentioned study has included the use of a treated wastewater for growing plants, which means that even applied methods of wastewater treatment cannot guarantee the removal of benzotriazoles. This could also explain why benzotriazoles were detected in the SS/FWBD where treatment of wastewater (sewage sludge production) is a part of the biogas production process.

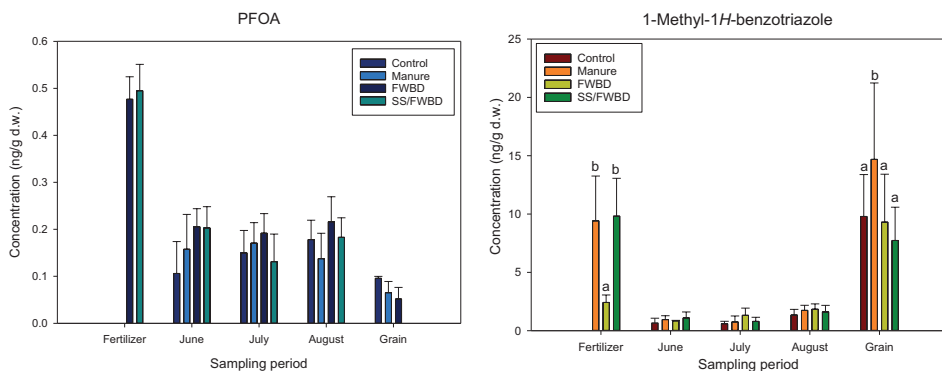


Figure 21. Concentration of PFOA and 1-Methyl-1H-benzotriazole in biogas digestates (fertilizer), soil samples and grains from the field experiment. The letter *a* presents no significant difference, while letter *b* shows significant difference between the treatments for a specific target ($p < 0.05$). Error bars represent standard deviation.

Still, detection of 1-Methyl-1H-benzotriazole in control plots and in FWBD indicates a possibility of local sources of these compounds. This may be attributed to the storage of digestates in the tanks protected with anticorrosive coating containing benzotriazoles, especially in the case of FWBD. The presence of the bezotriazoles in control plots can originate from the field preparation for the growing season, since the field experiment has been conducted for two years with the same treatments before the sampling for the current study was done.

4.5 Presence of Cr(VI) in biogas digestates

Due to the detected high concentration of Cr in the biogas digestates, a special study on Cr speciation in digestates has been conducted in close cooperation with the Romerike biogas facility (RBF). Details may be available but not accessible to everybody due to the used language (Norwegian) in a scientific report on Cr⁶⁺ content in biogas digestates (Dragicevic 2018).

Chromium (Cr) is a potentially toxic trace metal, which does not have any essential metabolic function in plants. Chromium occurs in different chemical forms (primarily as chromite (Cr(III)) and chromate (Cr(VI)) in soil which vary markedly in terms of their biogeochemical behaviour. Chromium behaviour in soil, its soil-plant transfer and accumulation in different plant parts vary with its chemical form, plant type and soil physical-chemical properties (discussed later). Addition of biogas digestates as organic fertilizers with

high Cr content, may affect -plant growth but may also have implications on surrounding water bodies. Plants tolerate Cr toxicity via various defence mechanisms such as complexation by organic ligands. Results presented in Tab. 5 (details in Chapter 3.3.3), show that Cr⁶⁺ concentrations in the biogas digestate samples were below the method quantification limit (MQL) for the two used analytical methods, and are thus not regarded to be of importance according to the regulations in Norway for the tested samples.

Table 5. Results of Cr⁶⁺ speciation analysis (digestate and water mixture) by using method 1 and 2 with method quantification limits.

Sample name and time of sampling	Method 1 - Cr ⁶⁺	Method 2 - Cr ⁶⁺	
	µg/l	µg/l	
Digestate (April 2016)	<MQL	<MQL	
Digestate (May 2016)	<MQL	<MQL	
Digestate (September 2016)	<MQL	<MQL	
Digestate (April 2017)	<MQL	<MQL	
Analytical parameter			
Method 1 quantification limit, MQL (µg L ⁻¹)		0.70	0.30
Method 2 quantification limit, MQL (µg L ⁻¹)		0.18	3.29

In the Tab. 6 results on the Cr⁶⁺ recoveries are presented for all sampling points and digestate samples. This test (spiking test) was done in order to investigate is there any contribution from digestates to the added Cr⁶⁺ (using certified reference material).

Table 6. Recoveries for spiked digestates samples (spiked with CRM of Cr⁶⁺).

Sample name and time of sampling	Cr ⁶⁺ , spiked amounts	Cr ⁶⁺ , recovery
	µg/l	%
Digestate (April 2016)	2.55	98.8
Digestate (May 2016)	2.30	91.8
Digestate (September 2016)	2.28	91.1
Digestater (April 2017)	2.40	96.0

The results presented in the Tab. 6 for the spiking test indicated that there is no increase in Cr^{6+} concentration after the addition of CRM, which suggests that the chromium in the digestate mixtures is dominantly present in less mobile forms (such as Cr^{+3}).

5. CONCLUSIONS

Application of biogas digestates as organic fertilizers in crop production was investigated in the context of potential environmental hazards regarding inorganic and organic contamination. The research has been carried out by using column leaching experiment in the laboratory, as well as greenhouse and field experiments which have enabled us to acquire new knowledge on the safety of biogas digestate use in crop production

The field study (Papers 2 and 3) has shown that fertilization with biogas digestates, including one with a high metal content (Cr, Ni and Al), did not result in a critical increase in concentration of the investigated metals in the soil. Analysis of plants grown in a loam soil treated with digestates has shown that trace metal uptake did not increase. In most cases, trace metal and DOC concentrations, and pH that were measured in samples from digestate plots did not differ significantly from those from manure plots. Based on these observations, the benefits of biogas digestates fertilization in cereal crops were judged to outweigh the potential risks related to the trace metal levels. However, as the field study was conducted in the last two growing seasons of a three-year field experiment, further research will be necessary to fully assess the potential risks of soil metal accumulation after repeated use of the digestates as fertilizers.

Special focus has also been given to the mobility and leaching of trace metals (Cr, Ni, Zn, Cu) and Al from differently textured soils fertilized with biogas digestates. In all the experimental set ups, i.e. from the high irrigation column experiment to the greenhouse and field experiments, leached concentrations of trace elements did not exceed the limits set by Norwegian or international regulation for fresh water. Although total soil metal concentrations have increased with the use of digestates as fertilizers, the water-extractable concentrations were on a level that adverse effects should not be expected. As may be expected, an important role in controlling metal leaching from digestates can be attributed to soil texture, pH and DOC. However, some differences between digestates and fresh manure suggest that also the quality of the (dissolved) organic matter may have an impact. The findings on the leaching of trace metals can support an increased use of digestates as fertilizers in the future in order to reuse nutrients available from organic residues and to replace conventional mineral fertilizers. Thus, the energy demand connected to the production of conventional fertilizers may be reduced. Still, an important issue to be considered in future research is speciation of the metals present in digestates. Metal speciation would enable a full assessment of the dominant chemical forms of metals upon the use of biogas digestate as fertilizers in agronomic plant production.

The study presented in Paper 4 focused on the presence and disposition of 21 organic compounds that were found to be present in low concentrations for all digestates, animal manure as well as in treated soil. From the 21 compounds investigated, only PFOA and 1-Methy-H-benzotriazole were detected in grains, but this could not be related to the use of any specific fertilizer. While differences have been seen in the pollutant profiles of the digestates depending on the type of feedstock that was used for the biogas production, their use did not affect the levels of the organic contaminants studied in edible plant parts. This area of research is currently lacking comprehensive studies that would enable a clearer link between the used feedstock and the resulting digestate contamination.

Knowledge on the quality and safety of biogas digestates used in agricultural food production is necessary to address concerns that the actual users (farmers) of digestates have. The papers presented have contributed to increased knowledge by addressing two aspects of digestate safety; i.e. potential inorganic and organic contamination. Future research may address challenges such as digestates heterogeneity (variability in composition), quality of the organic matter and speciation of metals that are present in digestates. In addition, research related to other groups of organic contaminants, as well as the efficiency of the anaerobic digestion process to degrade possible organic contaminants will also be important in order to ensure safe use of biogas digestates in agriculture.

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7. APPENDICES

PAPER 1

Fate of copper, nickel and zinc after biogas digestate application to three different soil types

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Abstract Soil application of organic residues from anaerobic digestion of municipal food waste and/or sewage sludge may introduce considerable amounts of heavy metals into the environment. In a column leaching experiment, mobility and release of Cu, Ni and Zn were investigated in three contrasting soils (sand, silt, loam) fertilized with biogas digestates of different origin. The effect of commercial digestates, based on food waste and sewage sludge, was compared to that of experimental digestates based on animal manure and whey permeate with or without fish ensilage, as well as untreated manure, mineral fertilizer and an untreated control. Manure and digestates were added to the columns as fresh material at equal amounts of available nitrogen. The experiment simulated high-intensity rainfall over a period of 7 days. In general, soil treated with the commercial digestates with higher original metal content showed less environmental impact in terms of Ni, Cu and Zn leaching than that treated with experimental digestates with lower original metal content and less than when animal manure or mineral fertilizer was applied. Although effects of digestate application on metal mobility in soils were seen in conditions of extreme precipitation, the leached concentrations of metals were below limitations

published by the WHO but still significantly higher than that measured for control soils.

Keywords Metal leaching · Biogas digestates · Nickel · Copper · Zinc

Introduction

Production of methane from organic waste by anaerobic digestion (biogas) represents one of the growing industrial sectors in Western Europe (Hijazi et al. 2016), with the overall goal of a green and sustainable production of energy for factories, transport, heating systems, etc. Anaerobic digestion (AD) leads to the production of biogas digestates, which can then go through treatment processing (separation) resulting in different fractions, such as liquid, solid or liquid-solid mixtures. Digestate is considered as a liquid-solid mixture type of organic by-product. During the AD, digestates are produced in significant amounts and therefore, a need for an adequate disposal of this by-product is clear (Eriksson et al. 2016). Today, the produced digestates are predominately used in agriculture as soil amendment and/or fertilizers. Based on their content of nutrients such as nitrogen (N), phosphorus (P), potassium (K) and other essential macro- as well as micronutrients, digestates might present a valuable resource to the agricultural industry. However, the use of digestates as fertilizers on agricultural fields may present several challenges, e.g., with respect to availability of nutrients but also with respect to mobility of potentially harmful elements or substances. For instance, high contents of heavy metals in digestate used as fertilizer may lead to problems due to either plant uptake or leaching. Heavy metals are known for their toxic effect (Ran et al. 2016; Toth et al. 2016) and potential accumulation both in living organisms and in the environment (Pivato et al. 2016;

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Tervahautaa et al. 2016). Among the heavy metals, copper (Cu), nickel (Ni), and zinc (Zn) are of particular interest because they also are essential plant nutrients.

Several studies are dealing with potential high Cu, Ni and Zn leaching from digestates or bio solids added to soils (Ashworth and Alloway 2004; Fang et al. 2016a; Hao et al. 2008; Pivato et al. 2016). Still, an important aspect is the effect of digestates on the availability of originally present metals in soil, which, for instance, can cause additional desorption of these elements or accumulation. One of the main issues in approaching the problem of possible elevated concentrations of heavy metals in biogas digestates is in their high variability of metal content, which depends on the substrate origin and used biogas technology (Tavazzi et al. 2013). Utilization of household waste or sewage sludge as feedstuff in biogas production can potentially result in digestates that could serve as a source of high concentrations of Cu, Zn and Ni. Soil that is amended or fertilized with digestates may then reach values that are higher than the background concentrations (Zhang et al. 2012). Therefore, studying the fate of heavy metals from biogas digestates in conditions of realistic agricultural field application is important in ensuring the reduction of potential environmental risks.

Among factors influencing metal leaching, the pH value is of importance. Several studies show that low pH values in the range of 4.5 to 5.5 contribute to higher metal mobility in organic waste-amended soils (McBride et al. 1997; Robertson et al. 1982). On the other hand also, more alkaline pH conditions have resulted in high leaching of metals due to complexation by dissolved organic matter (DOM) (Sauve et al. 2000). With its high amounts of DOM, a digestate by itself represents ideal surroundings for metal mobilization and complexation (Perez-Novo et al. 2008; Wu et al. 2012), which in turn can contribute to enhanced leaching upon field application. Binding of metals in soils depends on the soil type, which includes content of soil organic matter (SOM), clay minerals, Fe oxides, and other precipitation reactions that might occur in the soil equilibrium especially at higher pH values. Soil organic matter is of particular importance since it strongly influences the general metal equilibrium between soil and soil pore water (Gustafsson et al. 2003).

Some studies indicate that leaching of heavy metals from organic by-products such as digestates or sewage sludge is mostly below threshold values defined by the national or international regulatory bodies (Toribio and Romana 2006). However, especially in the first weeks after application when little or no plant cover and thus plant uptake exists, heavy rainfall events may cause considerable leaching and have adverse effect to the environment through runoff or ground water pollution.

In this study, the main objective was to investigate the effect of different types of biogas digestates on nickel, copper and zinc leaching under high precipitation conditions for three

different soil types. We hypothesized that leaching of Cu, Ni and Zn under high precipitation conditions will increase in the order animal manure - experimental digestates - commercial digestates. A second hypothesis was that leached amounts of Cu, Ni and Zn from soils amended with digestates would not exceed the maximum allowed concentrations given by the regulation for drinking water of the World Health Organization (WHO 2011). High concentration of dissolved organic carbon in soils amended with digestates was further expected to increase metal leaching in soils.

Materials and methods

Soils from three different sites in the southeast of Norway were used in a column leaching experiment.

Soils

The soils were loam (Ås, 59° 39' 52" N; 10° 47' 40" E), sand (Norderås, 59° 40' 46" N; 10° 46' 08" E) and silt (Solør, 60° 23' 31" N; 11° 54' 01" E). Soil texture was determined using data provided by the US Department of Agriculture (UN 2016; USDA 2014). The selected loam and silt soils are used for agricultural plant production, while the sand soil is from a forest area. The soils were all sampled in the upper 0–20-cm horizon. The soils were air-dried and sieved through a <2-mm sieve prior to use in the experiment. Basic characteristics of the soils used in the experiment are given in Table 1.

Soil treatments

In a leaching experiment, experimentally and commercially produced digestates in fresh form without any separation were added to soil. Experimental digestate samples (EDIG) were produced by thermophilic anaerobic digestion at the Biogas laboratory of the Norwegian University of Life Sciences and the Norwegian Institute of Bioeconomy Research. EDIG1 was a digestate produced using only animal manure as substrate, EDIG2 was produced from the mixed substrate manure and whey permeate in a ratio v/v 230:70 and EDIG3 was also produced from mixed substrate manure/whey (v/v 120:280) with addition of fish ensilage. Although elevated concentrations of heavy metals were not detected in the experimental digestates, they have been included in the study to investigate their influence on the leaching of metals naturally present in soils in regards to their substrate composition. The commercial digestates (CDIG) were produced at two Norwegian biogas companies that are using different mixture of substrates. Commercial digestate CDIG1 is produced based on pretreated (steam explosion) food waste as substrate, while CDIG2 is produced using a combination of food waste and sewage sludge (ratio 50:50) as substrate for the anaerobic digestion

Table 1 Soil texture and selected chemical characteristics. The Ni, Cu and Zn content is given in milligrams per kilogram of dry soil and water-soluble metal concentrations before treatment are given in micrograms per litre

Soil type	pH	Tot. C %	DOC mg/L	Sand %	Silt	Clay	Ni _{tot} mg/kg	Cu _{tot} mg/kg	Zn _{tot} mg/kg	Ni _{ws} µg/L	Cu _{ws} µg/L	Zn _{ws} µg/L
Loam	4.8 ± 0.15	2.42	29.7 ± 1.1	45	38	17	24.0 ± 0.1	10.3 ± 0.1	78.6 ± 2.1	7.2 ± 0.2	4.5 ± 0.25	17.3 ± 1.5
Sand	5.1 ± 0.25	0.37	15.5 ± 1.7	94	3	3	12.9 ± 0.1	2.4 ± 0.1	54.5 ± 0.1	2.2 ± 0.2	0.92 ± 0.02	12.0 ± 1.0
Silt	6.2 ± 0.3	1.81	23.5 ± 2.1	2	93	5	9.5 ± 0.1	7.9 ± 0.1	31.3 ± 0.1	11.7 ± 0.6	10.0 ± 1.0	30.3 ± 1.5

Tot. C total carbon, DOC dissolved organic carbon, tot total concentration determined in soil, ws water-soluble concentration determined by extraction of 5 g soil/25 mL of deionized water

process. All experimental digestates had a total metal concentration below the threshold values for class I given for organic fertilizers in Norway, which are for Ni 30 mg/kg, for Cu 150 mg/kg and for Zn 400 mg/kg of dry matter (Agriculture 2006). The commercial digestates had elevated concentration of Ni, while the Cu and Zn concentrations were below the threshold values defined for class I, II and III in the Norwegian regulation on organic fertilizers. Based on the regulated and allowed amounts of these metals in organic fertilizers, the commercial digestates are considered to have potentially more adverse effect on to the soil/soil solution equilibrium than the experimental digestates.

The different digestate treatments in the leaching experiment were compared to treatments with manure, and with mineral fertilizer, as well as a control without any fertilization. The animal manure (AM) that is used in the experiment is cattle manure produced at the University farm at the Norwegian University of Life Sciences. The mineral fertilizer treatment received N, P and K at a rate of 120 kg/ha, respectively, and all other essential nutrients to cover the needs of a cereal crop. The manure and digestates were stored at 4 °C prior to use. Basic characteristics of digestates and manure are given in Table 2. The amounts added in the different treatments were based on inorganic N content and were equivalent to a dose of 120 kg N/ha, a common amount for cereal production in Norway.

The total content of Cu, Ni and Zn in manure and digestates is given in Table 3. Besides analysing the total concentrations, the water-soluble fraction was analysed and measured

concentrations were taken as reference values for the calculation of added amounts to the soil columns. Further explanation is given in the “Chemical analysis” section.

Experimental setup

For the leaching experiment, plexiglass columns of 24 mm in diameter and 30-cm length were used. Each column was filled with 15 cm of soil. The selected soil column length is approximated to the depth of digestate incorporation on agricultural fields (down to 10–20 cm). Column diameter used in the experiment was selected based on the established experimental setup in our research group (Krogstad and Rakic 2016; Pavlović et al. 2016). Selected diameter length has been taken in to account within results discussion. At the bottom of the column, a plastic filter covered with PVC pellets (about 2 cm in height) was placed to prevent the loss of soil from the columns. In order to provide an equal distribution of water, the top of each column was also covered with PVC pellets after treatment addition. To avoid positive preferential flows through the columns, the soils were prepared as explained in paragraph 2.1 (sieving <2 mm) and then filled to columns without air gaps enabling an equal level of homogeneity. Prior to the experiment, they were saturated with deionized water for 24 h. Volume of deionized water needed for the saturation was for sand average volume 20.6 mL, for loam it was 27.5 mL and for silt it was 32.8 mL. During the experiment, the columns were regularly irrigated using a peristaltic pump with deionized water at a flow rate of 1.3 ml/h,

Table 2 Biogas digestates, animal manure and mineral fertilizer characteristics with amounts used for the column experiment

Treat.	pH	Dry matter %	Tot. C %	DOC mg/L	NO ₃ ⁻ -N mg/kg	NH ₄ ⁺ -N g/kg	Added amounts to columns mL
AM	7.3	6.6 ± 0.52	41.4	5879.1 ± 11	56.0 ± 1.5	21.1 ± 1.1	2.9
EDIG1	7.7	4.73 ± 0.81	35.1	3379.6 ± 12	68.9 ± 2.2	20.0 ± 1.7	4.2
EDIG2	7.6	4.64 ± 0.54	38.2	3338.1 ± 8	54.6 ± 1.9	29.2 ± 1.3	2.9
EDIG3	7.0	4.54 ± 0.27	37.8	10,489.1 ± 16	58.1 ± 2.4	16.3 ± 1.0	5.4
CDIG1	7.5	3.1 ± 0.36	39.7	3899.5 ± 5	95.1 ± 1.2	37.4 ± 1.8	3.5
CDIG2	7.1	1.6 ± 0.36	31.8	3630.3 ± 9	176.8 ± 3.1	55.1 ± 2.1	4.4

* Concentration of NO₃-N and NH₄-N was measured in wet sample

Table 3 Total content of Ni, Cu and Zn (mg/kg dry matter), water-soluble concentrations (expressed both in $\mu\text{g/L}$ and mg/kg dry matter) in biogas digestates and animal manure and percentage of water-soluble concentration expressed as a part of the total metal concentration

Treat.	Ni _{hot} mg/kg	Cu _{hot} mg/kg	Zn _{hot} mg/kg	Ni _{ws} $\mu\text{g/L}$	Cu _{ws} $\mu\text{g/L}$	Zn _{ws} $\mu\text{g/L}$	Ni _{ws} expressed per mass of dry matter mg/kg	% Ni _{ws} from Ni _{hot} %	Cu _{ws} expressed per mass of dry matter mg/kg	% Cu _{ws} from Cu _{hot} %	Zn _{ws} expressed per mass of dry matter mg/kg	% Zn _{ws} from Zn _{hot} %
AM	36.6 ± 3.5	71.3 ± 5.7	163.3 ± 12.0	280 ± 10	1450 ± 50	1557 ± 51	4.2	11.5	21.9	30.7	23.6	14.4
EDIG1	24.5 ± 0.1	63.5 ± 2.1	320.2 ± 15.0	910 ± 10	3083 ± 29	3250 ± 50	19.2	78.4	61.5	96.8	68.7	21.4
EDIG2	26.5 ± 0.1	65.5 ± 0.1	305.1 ± 10.0	928 ± 10	3021 ± 31	5010 ± 10	20.0	75.5	65.0	99.2	108.0	35.4
EDIG3	22.0 ± 1.7	43.5 ± 0.1	215.2 ± 10.0	400 ± 10	787 ± 8	980 ± 26	8.8	40.0	17.3	39.8	21.6	10.0
CDIG1	109.0 ± 11.5	30.6 ± 0.1	146.3 ± 6.1	472 ± 8	580 ± 7	617 ± 6	15.2	14.0	18.7	61.1	20.0	13.7
CDIG2	106.6 ± 5.8	92.3 ± 2.1	256.2 ± 6.0	1650 ± 6	967 ± 9	637 ± 7	103.1	97.0	60.4	65.4	39.8	15.5

for total concentration determined in digestate, _{ws} water-soluble concentration determined by extraction of 5 g digestate/25 mL of deionized water

corresponding to high precipitation conditions. The leaching experiment was run for 7 days at room temperature. Based on initial soil pH, the sand and loam soils were lined with 0.1 and 0.2 g of CaCO_3 per column, respectively, prior to saturating the soils. The experiment was run in the absence of light to minimize as much as possible algae growth in the selected experimental conditions. Leachates were collected daily, filtered and immediately after collection stored at 4 °C until further analysis.

Chemical analysis

Soil pH was determined in a soil to water solution ratio of 1:2.5. The concentration of total carbon (Tot. C) in soil, digestate and manure was determined by a dry combustion method (Nelson and Sommers 1982) on a Leco Carbon Determinator EC12. The amount of dissolved organic carbon (DOC) in leachates and in water-soluble fractions from soils, manure and digestates were determined using a Shimadzu TOC analyser. The analysis was done promptly after acquiring the filtered samples. The same filtered samples were used for nitrate (NO_3^- -N) and ammonium (NH_4^+ -N) analysis by flow injection analysis using FIAstar 5000, FOSS. For the determination of total concentration of metals, soil samples, digestates and manure were digested in concentrated ultrapure nitric acid (HNO_3) by stepwise heating up to 250 °C using a Milestone Ultra Clave for 90 min. For water-soluble metal concentrations, 5 g of soil/digestate/manure were extracted with 25 ml of water (Antoniadis and Alloway 2002a). Both total and water-soluble concentrations of Cu, Ni and Zn in soil and amendments were determined using an Agilent ICP-MS 8800 TripleQ. For the purpose of analytical quality control, both soil and digestate certified reference materials were used. Similar methods as for soil, digestate and manure samples were used for the leachates analysis using adjustments with regard to different sample matrix. The filtered samples (leachates and water-soluble fractions) were prepared in 10% ultrapure nitric acid prior to analysis.

Statistical analysis

Dissolved organic carbon and pH have been found to be the factors mostly influencing leached concentrations of metals in similar leaching studies (Antoniadis and Alloway 2002b; Toribio and Romana 2006) and they were for that reason taken into account for correlation analysis. Results on metal concentration from the present leaching experiment were analysed statistically using Pearson correlation. Analysis of variance (two-way ANOVA) was carried out to determine the effect of treatment and soil on metal concentration in leachates. In the experimental set up, each combination of treatment and soil was replicated three times ($n = 3$). Analysis also included the interactions between soil and

treatments. The statistical programs used were Sigma plot version 13 (regression analysis) and R statistics package 3.2.3 (II type ANOVA analysis). The confidence limit was 95% ($p < 0.05$).

Results

pH and DOC

The pH of the soil leachates varied over the course of the leaching experiment and depended on soil type and treatment (Fig. 1). The pH differences between treatments were most profound in the beginning of the experiment, while values stabilized with increasing experimental duration independent of the amounts of added water. This is especially visible in leachate from the sand and silt. In leachates from columns containing sand and experimental digestates (EDIG), pH was stable in the range of 6.5 and 7.5, while commercial digestates (CDIG) led to wider pH range in leachates with values varying from 4.3 to 6.5. Experimental and commercial digestate leachates from loam had a similar pH during the experiment, ranging from 4.0 to 6.0, with only CDIG1 showing a significant increase in the first 3 days. Experimental digestates in silt showed similar behaviour as in sand, with values mostly measured in the range of 6.0 to 7.8, while only CDIG2 resulted in leachates with a pH lower than 6.0 on day 1 of the experiment. In silt, the extreme irrigation conditions did not cause a significant shift in pH values of leachates for the majority of used treatments. Control soils (NN) and soils treated with mineral fertilizer (MF) had generally the lowest pH values in leachates during the experiment.

When digestates (both experimental and commercial) are compared to the manure (AM), they had a similar effect on the pH in all three soils. The NN and MF columns had significantly lower value of pH in leachates compared to digestates, and the difference was most profound in sand soil.

Figure 2 shows the DOC leaching through the soils. Generally, the peak of the leached DOC concentration was reached at day 2 of the experimental period for all digestate

treatments in all soil types. Application of experimental digestates resulted in the highest DOC leaching. After the initial increase on the second day, the effluents showed a gradual reduction in DOC concentration with time and a flattening of the concentration curve.

In all three soils, highest leaching of DOC was measured for the EDIG3. In loam soil, CDIG1 resulted in higher leaching than CDIG2. In silt, the lowest leaching among digestates was recorded for EDIG1 and CDIG2. Subtracting the amounts leached from the NN column (control soils), shows that the addition of digestates increased the total amounts of DOC leached. At the end of leaching experiment, the treatments with digestate addition caused a slight accumulation of organic matter in soil. This can be seen in the case of loam and silt soils where the values measured in leachates after day 7 of the experiment were ranging from 40 to 80 mg/L. For the control- and mineral fertilizer-treated soils, the DOC concentrations were generally below 20 mg/L during the entire experiment. However, relative to the animal manure digestates have caused a higher leaching of DOC only in sand.

Leaching of nickel, copper and zinc

The leaching patterns for mineral fertilizer are not shown because the leachate concentrations were much higher than those that were measured for the other treatments. All the metals added as mineral fertilizer were eluted in the first 2 days of the experiment and the leached concentrations were consistent with the added amounts.

Nickel

At the beginning of the leaching experiment, eluted Ni concentrations were highest in the loam (Fig. 3). In particular, relatively high Ni leachate concentrations were observed for the EDIG3 treatment, which peaked at around 80–85 $\mu\text{g/L}$ at day 1, but almost similar concentrations were also recorded for other experimental and commercial digestates in this soil. At days 3 and 4, there is a plateau in Ni concentrations and further irrigation after day 4 had almost no effect on Ni leaching in the

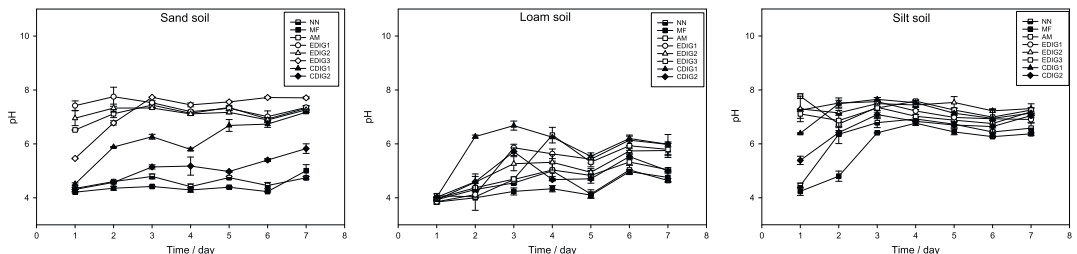


Fig. 1 Changes of pH in leachates from the soil columns without and with the addition of mineral fertilizer, manure and digestates during the 7-day experiment (measured in triplicates). Error bars refer to the standard error

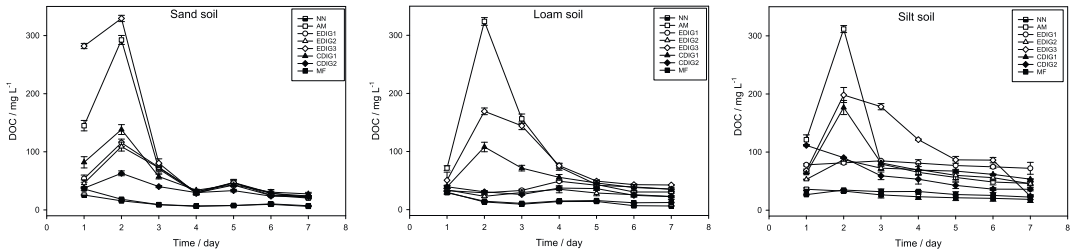


Fig. 2 Changes of DOC concentrations in leachates from sand, loam and silt soils with and without the addition of mineral fertilizer, manure and digestates during the 7-day experiment (measured in triplicates). *Error bars* refer to the standard error

loam soil. Leached amount of Ni from loam was 35% for CDIG2 and as high as 259% for animal manure of added amounts calculated based on the determined water-soluble concentration for digestates and manure. Figure 4 clearly shows that the leaching of Ni from both experimental and commercial digestates was lower than from animal manure.

Relatively high leaching was recorded in the EDIG3 and CDIG1 treatments in all three soils. In silt, the highest leaching of Ni occurred not only in the CDIG1 treatment with around 70% of added amount but also in the EDIG3 treatment as much as 65% of the input was leached. However, Ni was generally leached above 50%, but reaching 100% in case of AM for all three soil types, which means that addition of animal manure caused higher leaching of Ni than the digestates (Fig. 4). This was unexpected since most of the digestates had a higher content of Ni (water-soluble and total concentrations) than AM (Table 3).

Generally, a high negative correlation between pH and Ni concentration in leachate was found in loam (Fig. 5) while in the silt soil, a strong positive correlation has been observed for the Ni concentration and pH value, particularly for CDIG1 ($R = 0.847$, $p = 0.0162$). In sand, Ni was strongly positively correlated with DOC (Fig. 5).

Copper

Variations in Cu concentrations during the leaching experiment are shown in Fig. 6. The leaching pattern for the control indicates that small quantities of soil Cu were leached

naturally in all three soils. The Cu leaching was faster in sand than in loam and silt, as most of the Cu was leached during the first 3 days in sand. In both sand and loam, the highest Cu leaching was found in the CDIG1 treatment.

In all treatments, the total amount of Cu leached from sand and loam was lower than the input, while significantly higher leaching of Cu was seen from the silt soil (Fig. 7). In the silt, the highest leaching of Cu was for CDIG1, which was the treatment with lowest amounts of Cu added (calculated based on Cu_{ws} from Tab. 3).

The average Cu concentration in the total solution acquired after the 7 days of experiment was found to be highest for EDIG1 treatment (202 $\mu\text{g/L}$) in silt soil. In general, both digestates and animal manure resulted in increased Cu leaching from the silt, significantly higher than the added amounts.

In sand, the leachate concentration of Cu after addition of digestates was positively correlated with DOC and the best correlation was found for CDIG1 (Fig. 8). In loam, the correlation between Cu concentration in the leachate and pH was found to be significant only with EDIG1 ($R = 0.897$, $p = 0.006$) and EDIG3 ($R = 0.813$, $p = 0.02$).

In the silt, significant positive correlation between the leachate Cu concentration and pH was found in the EDIG3 treatment ($R = 0.936$, $p = 0.001$).

Zinc

In loam and silt, high zinc concentrations were found in leachate during the first 2 to 3 days (Fig. 9). In sand, the leaching

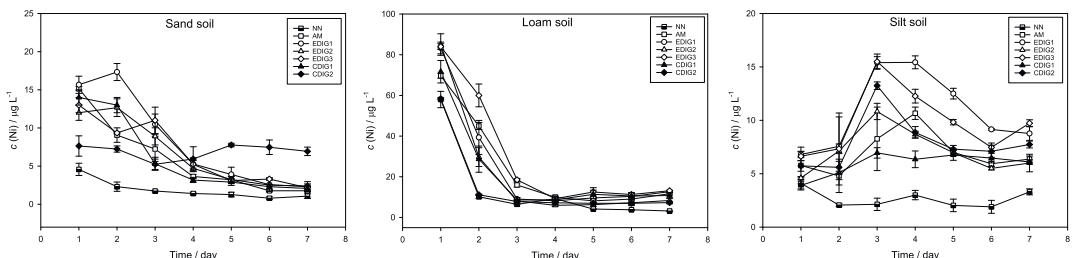


Fig. 3 Changes in concentration of Ni in leachates from sand, loam and silt. *Error bars* refer to the standard error

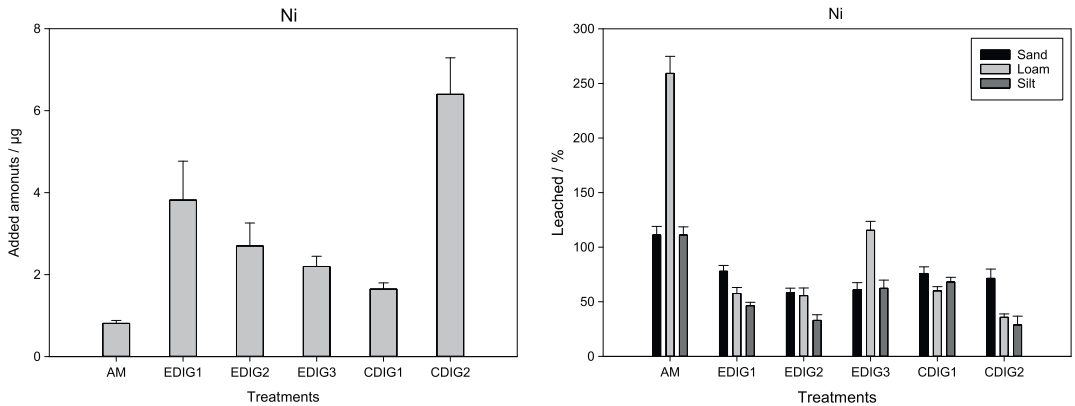


Fig. 4 Added amounts of Ni based on water-soluble concentrations and added volumes, and percentage of Ni leached from the different treatments. The leaching values were corrected for the Ni concentrations leached from the control soils (NN)

patterns were more variable over the course of the experiment and among treatments.

The experimental digestates, and particularly EDIG 1 and 2 represented the highest Zn input (Table 3). In loam and silt, retention of added Zn was found in all the treatments except for EDIG 3 (Fig. 10).

In all three soils, application of EDIG3 resulted in leaching of Zn, which was over 100% of the input Zn, indicating a release of natural soil Zn (Fig. 10).

In general, significant negative correlation was found between Zn and pH in loam and silt soils (Fig. 11), especially strong for the EDIG1 and CDIG2 treatments. In sand, a strong positive correlation was found between the leachate Zn concentrations and DOC, stronger than with pH for most digestate treatments, with particularly good correlations for the CDIG1 ($R = 0.847, p = 0.01$) and EDIG3 treatments ($R = 0.899, p = 0.005$).

In all the treatments, correlations explain between 60 and 90% of the variation in data, but only the most significant were presented in Figs. 5, 8 and 11.

In Table 4, results of the two-way ANOVA analysis are presented in terms of how the factors soil and treatment, as well as the interaction between soil and treatment explain the variation in leached metal concentrations.

For all three metals, soil type significantly influences the concentration in leachate. In the case of Ni and Cu, digestate treatments did not influence metal concentrations, while the Zn concentration in leachates was significantly influenced by treatment ($p < 0.003$). The combined effect of soil and treatment significantly influenced the leachate concentration for all three metals.

Discussion

The digestates used in this study were generally rich in dissolved organic matter and leaching of DOC was similar for all the organic amendment treatments, including animal manure, but differed between soil types. The higher leaching of DOC in sand can be directly coupled to the sandy texture and rapid water transport in the sand. A similar trend was observed by several authors (Ashworth and Alloway 2004; Toribio and Romanya 2006) studying leaching in acid soils after surface-application of organic fertilizers where the sandy soil texture was proven to be one of the main factors affecting the high leaching rate. The sorption of applied organic matter is generally low in the pH interval measured in leachate (Figs. 1 and 2), which corresponds with some recent research studies on

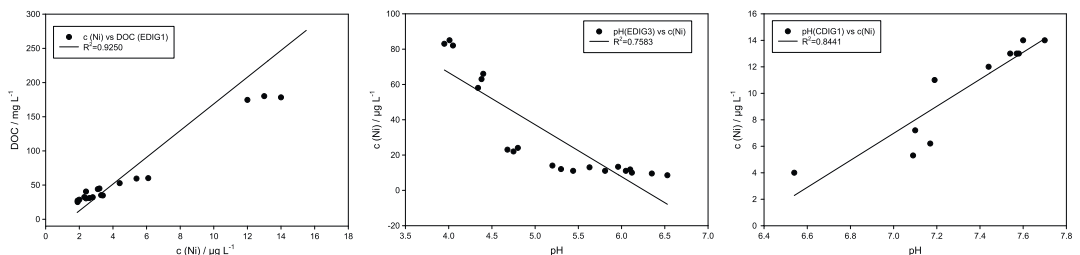


Fig. 5 Linear correlations of Ni concentration with DOC (sand) and pH (loam and silt) during the leaching experiment for selected treatments

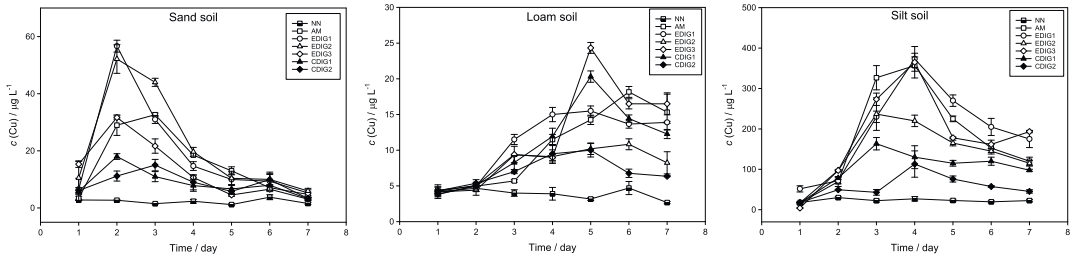


Fig. 6 Changes in Cu concentration in leachate from sand, loam and silt during the 7-day experimental period. Error bars refer to the standard error

DOC kinetics through amended soils (Sorrenti and Toselli 2016). At the pH values measured in animal manure and digestates used in the experiment (Table 3), the free carboxyl and hydroxyl groups of the organic matter are deprotonated, inhibiting their interaction with the negatively charged soil particles. At the high irrigation rate used in this experiment, the possibility of organic matter adsorption, as it moves through the soil, is thus limited. For the AM treatment, leachate from the loam and silt showed high DOC concentrations, actually higher than the digestate treatments (Fig. 2). Although diameter of used columns has been 2.4 cm, the results obtained both for DOC and pH were comparable to similar leaching studies (Antoniadis and Alloway 2002a; Ashworth and Alloway 2004).

Nickel

In general, influence of digestate on metal leaching in selected soils was considered under extremely high precipitation conditions, which can be described as a worst-case scenario when digestates are applied but the plants have not grown yet. The leaching budget of Ni (Fig. 4) shows that there was no mobilization of native soil Ni in any of the soils treated with digestates on top of the amounts of Ni added to the columns.

However, a high mobilization effect, with leached Ni amounts above added amounts, was visible in the AM treatment. The AM treatment caused higher Ni leaching than the commercial and experimental digestates in all three soils, although the total content of Ni in AM was lower than in the commercial digestates. Leaching of Ni from the biogas digestates was highest in the sand soil and lower in the loam and silt, which was expected due to the low adsorption capacity of sandy soils. Similar Ni leaching behaviour, as found for digestates in this study, can be found in other studies that used dewatered organic by-products in sandy loam soil (Antoniadis and Alloway 2002a; Cornu et al. 1999). No significant differences in Ni leaching were found between experimental and commercial digestates. Ni occurs as a positively charged ion in soil solution. Soil adsorption capacity for metal ions is mostly determined by the negatively charged surfaces of clay particles and SOM when the pH is high and thus the SOM functional groups deprotonated. A high positive correlation between Ni concentration and DOC was seen in the sand (Fig. 5 most likely due to a low adsorption capacity and rapid water movement through the sand). Still, at larger scale studies, such as field experiments, pH has a dominant effect on Ni leaching in acid soils (Toribio and Romanya 2006). In our experiment, pH is negatively correlated to Ni leaching

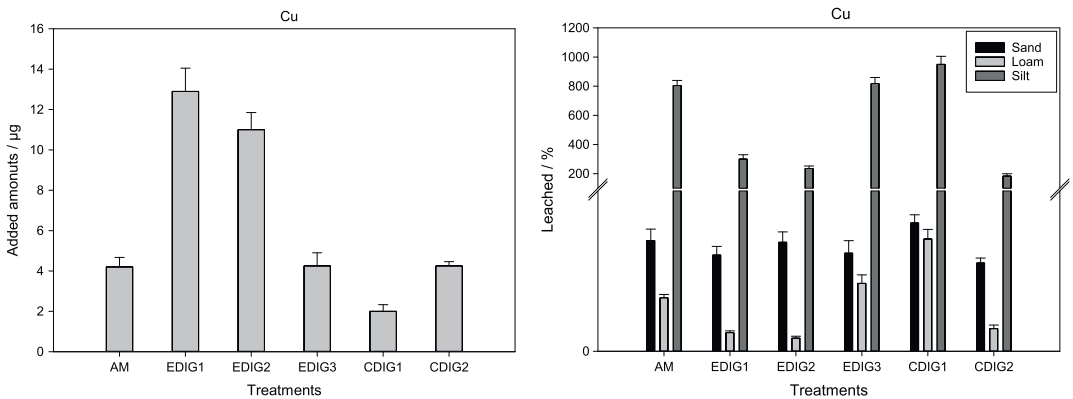


Fig. 7 Added amounts of Cu and percentage of Cu leached from the soil columns (figure break set on 100%). The leaching values were corrected for the Cu concentrations leached from control soils (NN)

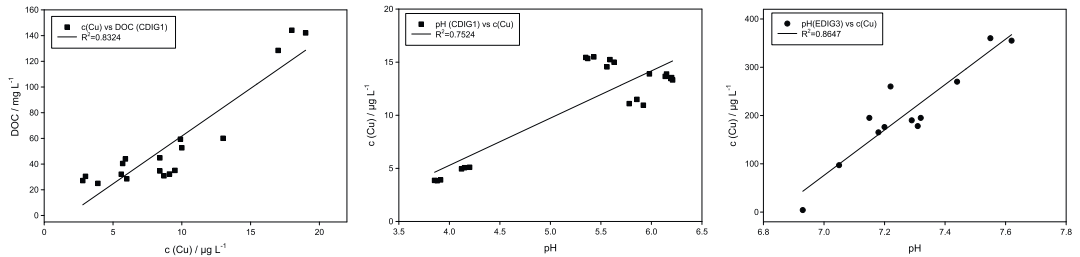


Fig. 8 Linear correlations of Cu concentration with DOC (sand) and pH (loam and silt) during the leaching experiment for selected treatments

(Fig. 5) in the loam and silt which can be explained by Ni being bound to negatively charged surfaces (oxides and oxide groups of clay minerals) when pH is high. When pH decreases, the hydroxide groups on oxides get protonated and Ni is mobilized. That pH is the factor mainly influencing the soil Ni mobility in loam and silt type of soils was shown in a similar study (Covelo et al. 2007) that has investigated several different soil types and influence the soil characteristics on Ni leaching. Although the two-way ANOVA analysis (Table 4) has not shown significant influence of treatments as factors in leaching of Ni, interaction between treatments and soil had a significant influence ($p < 0.001$) on Ni leaching, which means that the soil as a factor affected more Ni leaching.

Copper

Soil type significantly influenced Cu leaching. The generally lowest leaching of Cu was found in the loam. The leached amounts of Cu in the sand were approximately equal to the amounts added, suggesting that rapid water flow and Cu complex bound to the dissolved organic matter are the main processes determining the relatively high Cu leaching rate (Fig. 8). In the silt soil, the amounts of leached Cu were generally ranging far above the added amounts, suggesting that the application of digestate or manure mobilized naturally occurring Cu in the soil. The highest amount of Cu was applied to the soils via the EDIG1 and EDIG2 digestates. However, in all the three soil types, the highest leaching of Cu was found for the treatments EDIG3, CDIG1 and AM. Thus, there is no significant difference between the digestate

treatments and animal manure. In loam, soil retention of applied Cu was seen in EDIG1 and 2, as well as in the CDIG2 treatments. This retention is most probably due to binding of Cu to the negative surface charges on the clay particles. The total concentration of Cu in the silt is in the same order of magnitude as in the sand and loam, but the water-soluble part is higher in the silt. This may cause a different leaching pattern in the loam compared to the silt. Mobility of Cu in soil is closely related to the content of organic matter because Cu specifically makes stable complexes with organic matter. Organic matter originating from both digestate and animal manure apparently mobilized this Cu in the silt soil. The DOC release is slightly higher in the silt than in the loam, which can additionally influence desorption of the Cu in silt. Cu concentration was also correlated to pH in the loam and silt (Fig. 9), but pH was not found to be a main factor causing the enhanced Cu leaching in the silt. Usually, lower pH values are more favourable to enhanced Cu leaching (Perez-Novio et al. 2008), but that was not the case in this experiment. For the pH values in the basic range, Cu is usually complexed with organic matter in soil. The amounts of leached Cu in the silt are similar to those found in the study of Fang et al. (2016a, b). These authors imply that both DOC and particulate organic carbon are increasing DOC-bound species in the liquid phase, which can significantly increase Cu leaching in the pH range of 6 to 8 in low-clay soils. The mechanism might be that added organic matter mobilizes soil Cu or the insoluble fraction of Cu, which then forms stable complexes between Cu and DOC, thus inducing leaching from silt soil. In our study, there is reason to believe that the water-soluble part of the native

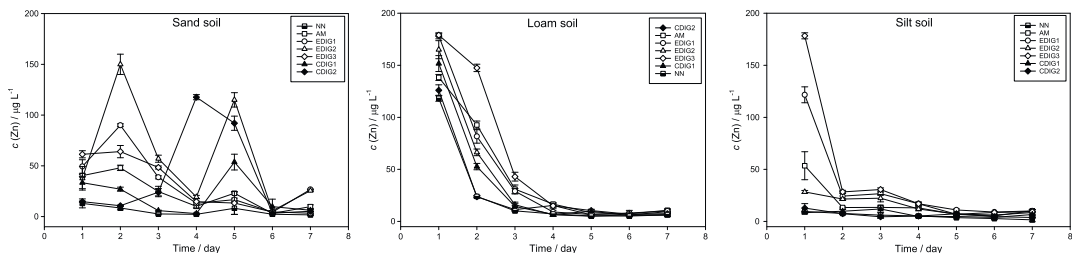


Fig. 9 Changes in concentration of Zn in leachates from sand, loam and silt. Error bars refer to the standard error

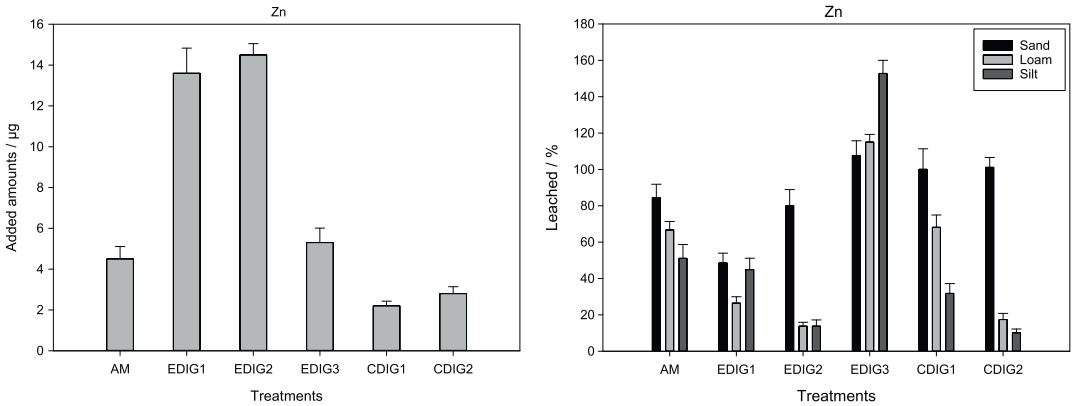


Fig. 10 Added amounts of Zn based on water-soluble concentrations and applied volumes, and percentage of Zn leached from the soil columns in the different treatments. The leaching values were corrected for the Zn concentrations leached from control soils (NN)

soil Cu concentrations are leached with the DOC since high water-soluble Cu concentrations were found in the silt. Organic matter effects on Cu mobility need additional clarification in relation to the DOC quality and composition. Reactivity of Cu and DOC were discussed by Romkens and Dolfing (1998) and they have shown the importance of DOC composition with a clear indication that molecular size of organic matter influences Cu complexing ability.

Zinc

Due to the rapid water transport, the leaching of Zn was high in the sand and usually more than 80% of added Zn was leached (Fig. 10). The highest leaching in all three soil types was measured for the EDIG3 treatment. Leaching of Zn from the treatments with commercial digestate was comparable to the amounts measured in the AM treatment and lower than in the experimental digestate treatments. In the sand, DOC appears to be the main factor influencing Zn leaching. The strong influence of DOC on Zn leaching is in accordance with previously published studies of increased Zn soil leaching in the presence of organic matter in sandy soils (Hao et al. 2008; Wong et al. 2007). For the loam and silt soils, most of the Zn is

leached in the beginning of the experiment, and pH seems to be the dominant factor in controlling the release of Zn. Generally, the Zn leaching from experimental and commercial digestate application is less than 70% of the added amounts, which indicates some Zn retention in soil. Several studies (Ashworth and Alloway 2004; Giusquiani et al. 1998; Zhang et al. 2016) have emphasized the importance of the stability constants between organo-metallic complexes controlling the Zn mobility, especially at neutral to basic soil pH. Usually, Zn has a lower ability to form complexes with organic matter compared to, e.g. Cu, because of filled *d*-orbitals in the atomic structure.

In general, leaching of Ni, Cu and Zn in this study and its dependency on DOC and pH follows some recent research studies, which included different leaching experiments and modelling investigating the interaction of waste products and soils (Fang et al. 2016a, b; Gwenzi et al. 2016). The maximum leached concentration of Ni in the experiment was measured in the loam soil for the AM treatment and it was 30 µg/L which is still below the limit (70 µg/L), but still rather high when compared to the untreated soil (6 µg/L). The maximum leached concentrations of Cu were found in the silt, i.e. 185 µg/L in the AM and EDIG3 treatments, which is still well

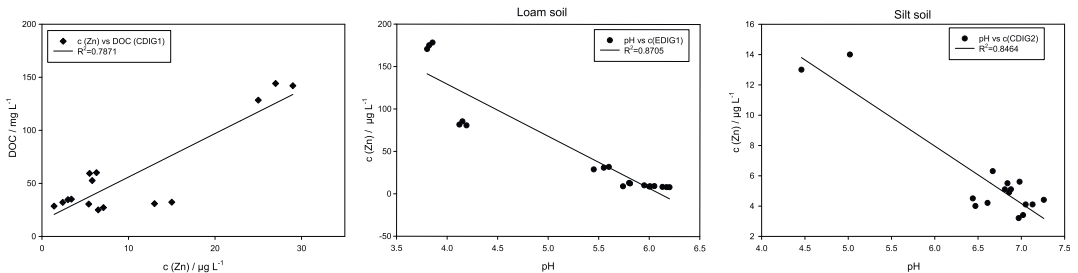


Fig. 11 Linear correlations of Zn concentration with DOC (sand) and pH (loam and silt) during the leaching experiment for selected treatments

Table 4 Results of two-way ANOVA analysis with given factor of significance for the amounts of metals leached during the 7-day experiment

Factors	Ni	Cu	Zn
Soil	$p < 0.001$	$p < 0.001$	$p = 0.003$
Treatment	$p = 0.1245$	$p = 0.2891$	$p < 0.001$
Soil × treatment	$p < 0.001$	$p < 0.001$	$p < 0.001$

below the WHO limit of 2000 µg/L (WHO 2011). The highest measured concentration of Zn in the leaching experiment was 60 µg/L. In the WHO guidelines, there is no defined adverse effect of high Zn concentrations that is mentioned and thus no limit defined.

Conclusions

With a focus on the high concentration of DOC in soils amended with digestates and its potential, as hypostasized, to increase Cu, Ni and Zn leaching in the order animal manure - experimental digestates - commercial digestates, presented results have not contributed to the confirmation of our main assumption. While experimental and commercial digestates have leached less relative to the added amounts, animal manure has showed increase in leached Ni when relative to added amounts. Both digestate- and animal manure-derived Cu was readily leached from sand, but not from loam. Still, in silt soil, the leaching of Cu was over the added amounts. The Zn leaching was lowest for commercial digestates and highest for experimental digestates, generally showing a high retention rate especially in loam and silt soils. However, it cannot be excluded that DOC (or soil-adsorbed organic carbon) may affect transport and mobility of the metals Ni, Cu and Zn under field conditions.

The concentration of Cu, Ni and Zn from soils amended with digestates did not exceed values given by regulation for drinking water of the WHO (2011). In general, application of digestate did not result in higher leaching of Ni, Cu and Zn than use of very common animal manure, and the concentrations were below critical loads for water quality published by the WHO.

With respect to the Cu, Ni and Zn, a low risk of soil leaching was observed when digestates are compared to animal manure, which shows agricultural potential of digestates as fertilizers. This research and similar ones are aiming to show that the use of digestates can be stimulated in the future, replacing the conventional fertilizers, such as mineral-based ones and by doing so, lowering the negative environmental effect that conventional fertilization has. Effect of high concentration of Ni in commercial digestates has been compensated by the soil characteristics (through sorption) showing a

low leaching effect in high irrigation conditions. Still, the concentration measured for Ni, need to be taken in to consideration in field conditions.

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PAPER 2



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Research article

Use of high metal-containing biogas digestates in cereal production – Mobility of chromium and aluminium

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ABSTRACT

Biogas digestate use as organic fertilizer has been widely promoted in recent years as a part of the global agenda on recycling waste and new sustainable energy production. Although many studies have confirmed positive effects of digestates on soil fertility, there is still lack of information on the potential adverse effects of digestates on natural soil heavy metal content, metal leaching and leaching of other pollutants. We have investigated the release of aluminium (Al) and chromium (Cr) from different soils treated with commercial digestates high in mentioned potentially problematic metals in a field experiment, while a greenhouse and a laboratory column experiment were used to address mobility of these metals in two other scenarios. Results obtained from the field experiment showed an increase in total concentrations for both investigated metals on plots treated with digestates as well as a significant increase of water-soluble Al concentrations. Factors that were found to be mostly affecting the metal mobility were dissolved organic carbon (DOC), pH and type of soil. Metal binding and free metal concentrations were modelled using the WHAM 7.0 software. Results indicated that the use of digestates with high metal content are comparable to use of animal manure with respect to metal leaching. Data obtained through chemical modelling for the samples from the field experiment suggested that an environmental risk from higher metal mobility has to be considered for Al. In the greenhouse experiment, measured concentrations of leached Cr at the end of the growing season were low for all treatments, while the concentration of leached Al from digestates was higher. The high irrigation column leaching experiment showed an increased leaching rate of Cr with addition of digestates.

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1. Introduction

For the past few decades, both environmental scientists and policy makers are struggling between addressing the climate change and renewable energy issues and, at the same time supporting the focus on increased food production. Industrial production of biogas through the anaerobic fermentation of different organic material represents just a small part of the 21st century's green industry agenda (Gissen et al., 2014; Hijazi et al., 2016). The importance of biogas industry contribution to the circular economy and environmentally friendly energy production has resulted in increase of the number of biogas plants in Europe where digestates are produced as by-products. According to the report of the

European Biogas Association (EBA) there was 17,439 biogas plants registered in Europe by the end of 2017 (EBA, 2017). For most of the biogas production facilities, there is a considerable amount of biogas digestates produced and regarded as waste that needs sustainable handling and reuse. Increased interest for the research of numerous aspects of biogas digestates have been supported by the European Union through financial support of projects with budgets ranging from 0.5 up to 1.5 million EUR (Up2Europe, 2018). Agronomical use of biogas digestates is today seen as an additional advantage of the biogas industry, since considerable amounts of digestates are made available as fertilizer, thus facilitating an organic waste disposal (Alvarenga et al., 2015) with additional benefits to both companies and farmers. Digestates are often used in agronomic plant production, in either their liquid or a dewatered solid form, and their increasing use has been widely accepted. There are efforts made continuously to use digestates as a basis for manufacturing other types of fertilizers that can be used for more

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specific agronomic production. In Norway one of the best examples of integrated approach to the commercial use of digestates is the Minorga fertilizer company, that is producing several fertilizing products by using biogas digestates acquired from the neighboring biogas plant (Minorga, 2012). Still, problems related to the use of digestates are similar to the use of other type of organic waste based fertilizers. One of the main challenges is the establishment of the fair certification system that would facilitate objective assessment of any organic based fertilizer prior its use. The European directive 2008/98/EC (EC, 2008), also known as Waste framework directive, only provides a frame for the national regulators to define the methodology, processes and limitations for organic waste handling and use in agricultural production. One of the ways to systematically test the quality of biogas digestates is the use of bio certification schemes applied in the United Kingdom (EA, 2014). In addition, implementation of similar certification standards such as PAS100:2014 (BSI, 2014), has supported a fast development of the digestate market in England and Wales and their use as organic fertilizer in agriculture. Norwegian national regulations in general are covering the common heavy metals and set limits to their total concentration in fertilizers (Mattilsynet, 2006). In general, all the organic fertilizers, which are exceeding these limits are usually not allowed to be used in agronomic production, especially organic food production in Norway. Fertilization by using digestates can serve as an input of different heavy metals and can result in soil and environment contamination (Fang et al., 2016; Studer et al., 2017) especially in cases where levels of metals are exceeding the defined regulations. In general, amounts of heavy metals and other pollutants are dependent on the type of substrate used for the biogas production process.

Metals such as nickel (Ni), cadmium (Cd), arsenic (As), mercury (Hg), but also chromium (Cr) and aluminum (Al), have an adverse effect on the environment if their concentrations are elevated in either fertilizers, soil or ground water systems. If the concentrations of heavy metals are elevated in biogas digestates, the risk of soil pollution and leaching and thus contamination of groundwater is increasing. Since digestates are rich in dissolved organic matter (DOM), this factor can enhance the metal mobility and by doing so, increase the release of the added and naturally present metals to the environment, especially to surface and groundwater systems. This study is focused on the potential use of high metal-containing digestates (exceeding the regulated values) for cereal production with a focus on mobility and release of Al and Cr when digestates are used as fertilizers in a usual agronomic practice.

Biogas digestates can contain high concentrations of Al because of the use of precipitating agent (Drosg et al., 2015), such as aluminium sulphate, $Al_2(SO_4)_3$ usually applied for waste water processing and production of sewage sludge. One of the biogas plants involved in this study uses aluminium sulphate as explained earlier. As aluminium silicates are a major constituent of the soil forming rocks, Al is typically present in soil as insoluble Al compounds and the potential threat from leaching of Al is usually low. Still under highly acidic conditions in soil, some of the Al is solubilized (Zhaohuai et al., 1995) as positively charged cations, particularly as Al^{3+} , $Al(OH)_2^+$ and $AlOH^{2+}$. These compounds can become mobile and cause significant problems for both plant and soils (Ziola-Frankowska and Frankowski, 2017). In addition, digestates contain high concentrations of dissolved organic carbon (DOC), which can mobilize soil Al. Application of digestates on agronomic soils may influence the soil pH as well and further enhance the rate of Al leaching. In agricultural soils, the soluble Al forms, especially Al^{3+} , can limit plant growth by affecting the root growth and in extreme cases, they can be toxic to terrestrial plants (Kochian, 1995; Samac and Tesfaye, 2003). If Al is released from soil, it can be leached to groundwater, lakes and rivers, where it can

cause severe damage to biological organisms, especially in its mobile form.

High concentration of Cr in biogas digestates is not common (Kupper et al., 2014), but in this specific study both of the used commercial digestates had a total Cr content higher than the limitations (100 mg/kg dry digestate) defined in the Norwegian regulation for organic fertilizers for class 0, I, II and III of organic fertilizers (Mattilsynet, 2006). The behavior of Cr in soil depends on soil properties such as pH, organic matter content, cation exchange capacity and soil redox condition (Banks et al., 2006). Since Cr mobilization can cause adverse effects to the environment, especially for aquatic organisms in the form of Cr(VI), the retention of Cr in soil is important, and it can be controlled by mechanisms such as sorption, precipitation and nucleation (Bradl, 2004). High concentration of DOM in digestates may affect Cr mobility of naturally present soil Cr and it can also influence the Cr redox equilibrium (primarily Cr(III)/Cr(VI)) and therefore limit the role of soil as a sink (Kyziol et al., 2006).

Main hypothesis of this study is that high metal-containing digestates will have an adverse effect on to the soil environment and further to the groundwater system. Mobility of Al and Cr were investigated in different soils fertilized with high metal-containing biogas digestates, estimating the potential for the use of these type of digestates with a focus on metal release from agricultural soils by using a field experiment. The results obtained with digestates were compared to animal manure and unfertilized soils (controls). Use of animal manure and control soils for comparison has facilitated in assessing the influence of digestate application and factors such as pH, DOC and soil texture on the release of investigated metals. Beside the field experiment, a leaching study in a greenhouse experiment and high irrigation column leaching experiment were also used to additionally investigate the mobility of mentioned metals in two different scenarios. Measured values of Al and Cr were assessed using the latest regulation on maximum allowed metal concentrations for aquatic organisms and fresh waters.

2. Materials and methods

2.1. Biogas digestates and soils

In the field, greenhouse and column experiments, two digestates were used. The commercial digestates (CDIG) were produced at two Norwegian biogas plants, which use different mixtures of substrates and different technological process. The CDIG1 was produced from with application of pretreatment (steam explosion) of food waste. The second used digestate, CDIG2, was produced using a mixture of municipally collected food waste and sewage sludge (ratio 50:50) as substrate for the anaerobic digestion process. The total concentrations of Al and Cr in digestates and manure are given in Table 1. Besides the total concentrations, also the water-soluble concentrations were analyzed as explained in paragraph 2.4 and 2.5.

Beside the use of treatments with different digestates a treatment with manure (animal manure, AM) and a control treatment without addition of organic or mineral fertilization was used for the comparison purposes. The AM treatment was a cattle manure from the University farm produced in Ås (Norway). Before chemical analysis all the samples were stored at 4 °C. Selected characteristics of digestates and manure are given in Table 2. Calculated and added amounts of different treatments were applied after using the data for inorganic nitrogen (N) content and were equivalent to a dose of 100 kg N ha⁻¹, which is a common amount of N fertilization for cereal production in Norway.

For the purpose of this study, soils from the upper layer (0–20 cm) from three different sites in Norway (southeast part)

Table 1
Total content of Al and Cr (mg of metal per kg dry matter) and water-soluble concentrations for biogas digestates and animal manure (per L and per dry matter of digestate/animal manure). Values behind ± are given as standard deviation.

Treat.	Al _{tot} mg/kg	Cr _{tot} mg/kg	Al _{ws} µg L ⁻¹	Al _{ws} expressed per dry weight mg/kg	Cr _{ws} µg L ⁻¹	Cr _{ws} expressed per dry weight mg/kg
AM	420 ± 4.7	42 ± 2.8	95.5 ± 10	1.44	10.1 ± 2.1	0.15
CDIG1	1700 ± 12	176 ± 6.8	360 ± 15	11.61	49.6 ± 3.3	1.60
CDIG2	15 000 ± 35	156 ± 7.3	8500 ± 58	531.25	64.7 ± 5.4	4.04

Table 2
Biogas digestates and animal manure characteristics with amounts used for column, greenhouse (pot) and field experiment. The total carbon (Tot. C) was measured in dry sample. Values behind ± are given as standard deviation.

Treat.	pH	Dry matter %	Tot. C %	DOC mg/L	NO ₃ ⁻ -N mg/kg	NH ₄ ⁻ -N g/kg	Added amounts to columns mL	Added amounts to pots mL	Added amounts per field plots (0.0021 ha) L
AM	7.3	6.6 ± 0.52	41.4	5879.1 ± 11	56.0 ± 1.5	21.1 ± 1.1	2.9	290	150
CDIG1	7.5	3.1 ± 0.36	39.7	3899.5 ± 5	95.1 ± 1.2	37.4 ± 1.8	3.5	350	183
CDIG2	7.1	1.6 ± 0.36	31.8	3630 ± 9	176.8 ± 3.1	55.1 ± 2.1	4.4	440	232

*Concentration of NO₃⁻-N and NH₄⁻-N were measured in wet sample; DOC – dissolved organic carbon tot-total concentration, ws-water soluble concentration determined by extraction of 5 g digestate (and animal manure)/25 mL of deionized water, total carbon (Tot. C) was measured in dry sample.

were used. The soils were a loam (Ås, 59°39'52"N 10°47'40"E), a sand (Norderås, 59°40'46"N 10°46'08"E) and a silt (Solør, 60°23'31"N 11°54'01"E). Soil texture was determined by the pipette method (Elonen, 1971). For the field experiment, a loam was used, while all three soils were used in greenhouse and column experiment. The most common use of loam and silt soils is mainly within agricultural plant production, while the sand is taken from forest area. For greenhouse and column experiments soils were air dried and sieved through a <2 mm sieve. Selected characteristics of the soils used in the experiments are given in Table 3.

2.2. Experimental setup

2.2.1. Field experiment

In the field, a randomized complete block experiment was carried out with barley (*Hordeum vulgare* L.) as a test crop. The fertilizer addition and sowing of seeds was done in May, while experimental sampling was done during the growing season every 30 days. The size of the testing area was 39 m × 24 m (0.094 ha), while each plot was 3 × 7 m (0.0021 ha). The plots were organized in triplicates for each treatment; control, animal manure and the two commercial digestates. For the purpose of this study soil was sampled from the testing field at two stages during the growing season, i.e. in June and August. The amounts of digestates and manure corresponded to the N applied as mineral N fertilizer, using the results from Table 2.

2.2.2. Greenhouse and column leaching experiment

In order to analyse the risk of Al and Cr leaching from digestates at the end of a growing season, a simple leaching study was

conducted in a greenhouse (pot) experiment following the harvest of wheat (*Triticum aestivum* L.) that had been grown as a test crop in a greenhouse. The soil volume in each pot (soil volume of 6.7 L) was approx. 100 times bigger than the amount of soils used in a column leaching experiment. Three different soils, i.e. sand, silt and loam (Table 3) were used as growth media. The experiment consisted of the treatments that were used in greenhouse and column leaching experiment. The amounts of manure and digestates were adjusted based on their content of inorganic N (Table 2). The loam and sand were limed with addition of 20 and 10 g lime (CaCO₃)/pot, respectively. The water content in the soil was kept at 60% of the soils' water holding capacity throughout the growing season until the harvest when the plants were cut at a height of approximately 5 cm. After the harvest, the pots were filled up to 100% water holding capacity. After 24 h, 1 L deionized water was added subsequently divided in to four doses of 250 mL over 1 h and the leached solution was collected by means of a tube at the base of the pots, filtered and stored at 4 °C until further analysis.

For the soil leaching experiment, plexiglass columns of 24 mm in diameter and 30 cm long were used. Each column was filled with 15 cm of dried and sieved soil. The selected height is related to the usual depth of digestate incorporation on agricultural fields (ca 15 cm). Soil volume in the columns was approximately 100 times smaller than the soil volume used in the greenhouse experiment. Before the start of the leaching experiment, the soil columns were saturated with deionized water. Based on their initial pH, the sand and loam soils were limed with 0.1 and 0.2 g of CaCO₃ per column, respectively, prior to the water saturation. The soil columns were covered by aluminium foil to reduce the exposure to light in order to minimize biological activity in the soils. Treatments used in the

Table 3
Soil texture and some chemical characteristics (pH, total carbon and dissolved organic carbon). The total concentration of Al and Cr in soil is given in mg of metal per kg of dry soil. The concentration of water-soluble Al and Cr are given in µg L⁻¹. The concentrations are measured before the addition of treatments. Values behind ± are given as standard deviation.

Soil type	pH	Tot. C %	DOC	Sand	Silt	Clay	Al _{tot}	Cr _{tot}	Al _{ws}	Cr _{ws}
			mg L ⁻¹	%			mg/kg	mg/kg	µg L ⁻¹	µg L ⁻¹
Loam	4.8 ± 0.4	2.42	29.7 ± 1.3	45	38	17	36 ± 2.1	62 ± 2.2	1.5 ± 0.4	3.1 ± 0.6
Sand	5.1 ± 0.6	0.37	15.5 ± 0.9	94	3	3	14 ± 1.5	28 ± 1.8	0.1 ± 0.5	0.3 ± 0.2
Silt	6.2 ± 0.7	1.81	23.5 ± 1.5	2	93	5	26 ± 1.2	31 ± 2.0	0.7 ± 0.3	0.2 ± 0.1

* – in dry soil, tot-total concentration, ws-water soluble concentration determined by extraction of 5 g soil/25 mL of deionized water.

column leaching experiment were the same as for field and greenhouse experiment.

The leaching experiment was run for 7 days at room temperature with 24 h sampling intervals. During the experiment, the soil columns were regularly irrigated using a peristaltic pump with deionized water at a flow rate of 1.3 mL h^{-1} , representing high precipitation conditions. For each of the three soils, leachate was sampled in the four treatments, with three replicates each, totalling twelve columns per soil type. Leachates were filtered immediately after collection and stored for further analysis.

2.3. Selected chemical parameters of digestates, soils and soil extracts

The soil pH was determined using a dry soil with soil to water ratio of 1:2.5. Dry combustion method was applied in determining the soil, digestates and manure total carbon content using a Leco Carbon Determinator EC12 (Nelson and Sommers, 1982). All the instrumental analysis was done shortly after collecting the filtered (Milipore, $0.45 \mu\text{m}$) samples. Concentration of DOC from leachates and soil extracts, manure and digestates was determined using Shimadzu TOC analyser. The soil extracts from the field experiment were additionally used for ion chromatography (IC) measurements of Cl^- , NO_3^- , SO_4^{2-} and F^- anions needed for the WHAM modelling. The determination of NO_3^- and NH_4^+ in digestates was done in fresh samples after extraction with 2M KCl by flow injection analysis using FIAstar 5000, FOSS.

2.4. Sample decomposition, extraction and ICP-MS metal analysis

Prior to digestion soil, digestate and manure samples were dried (24 h, 105°C) sieved and grinded. Digestion of 0.2–0.3 g of sample was done in concentrated ultrapure nitric acid (HNO_3) prior to ICP-MS analysis by stepwise (90 min) heating up to 250°C using a Milestone Ultraclave. For the analysis of Al and Cr concentrations in soils, digestates and water extracts Agilent ICP-MS 8800 TripleQ was used. The ICP-MS analysis was done with minor method validation (depending on the sample matrix) and certified reference materials and reference materials were used for quality control of the applied analytical methods. The analysis of water-soluble metal fractions was done in samples acquired by extracting 5 g of soil/digestates/manure with 25 mL deionized water (Ashworth and Alloway, 2004). The filtered samples (water extracts of soils, treatments and leachates) were prepared in 10% ultrapure nitric acid prior to analysis.

2.5. WHAM modelling

Speciation and binding of metals to DOM and the free metal concentration in soil water extracts, was done using WHAM (Winderemere Humic Aqueous Model) 7.0 model (Tipping et al., 2011). The parameters such as DOC, pH, and concentrations of dissolved anions (Cl^- , NO_3^- , SO_4^{2-} , and F^-) and dissolved cations (Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Fe^{3+} , Al^{3+} and Cr^{3+}) have been analyzed to provide the data inputs for WHAM 7.0. The equilibrium constants between the metal cations and anions were taken from the WHAM 7.0 default database (Tipping, 1994). Concentration of humic substances was assumed to be 1.27 times the DOC concentration, representing 63.5% of the DOC being active on average and presented as fulvic acid (FA) as suggested by Lofts and Tipping (Lofts and Tipping, 2011). The humic substances (HS) binding constants were also taken from the WHAM 7.0 default database (Tipping, 1994).

2.6. Statistics

The factors DOC, pH, soil type and treatment have been well known to affect the mobility of metals in similar research studies (Kulikowska et al., 2015; Sanders and Adams, 1987). Analysis of variance (two-way ANOVA) was carried out to determine the effect of the mentioned factors on metal concentration in all three experiments. For the experiments, each combination of treatment and soil was replicated three times ($n = 3$). Analysis also included different interactions such as soil and treatment, soil and DOC etc. The statistical program used for the ANOVA analysis was R statistics package version 3.2.3. The confidence limit was 95% ($p < 0.05$).

3. Results

The soils in the three experiments had a natural total Al concentration ranging from 14 to 36 mg/kg (Table 3), but the water-soluble Al concentrations were quite low (not more than $1.5 \mu\text{g L}^{-1}$, Table 3). In case of Cr, untreated soils had a total Cr content ranging from 28 mg/kg in the sand and up to 62 mg/kg in the loam. The water-soluble Cr concentrations for untreated soils were also low, ranging from 0.2 to $3.1 \mu\text{g L}^{-1}$.

3.1. Field experiment

There was no significant change in soil total Al concentrations between June and August samples in any of the treatments, but the plots receiving digestates or animal manure had slightly higher total concentration of Al than the control plots (Fig. 1). However, the difference was not high. For the water-soluble concentrations of Al, there was a significant change between June and August in all the treatments when compared to control treatments. The highest added amount of water-soluble Al per plot was in the CDIG2 treatment (Tables 1 and 2). In June, the highest water-soluble concentration of Al was recorded for CDIG2, being over $800 \mu\text{g L}^{-1}$. Results of statistical analysis presented in Table 4 showed a change in significance of selected factors influencing the Al concentration, especially for DOC (increasing the significance level) and pH (decreasing the significance level) between June and August samples.

The total Cr concentration is significantly higher in the plots receiving CDIG than in the AM and control plots (Fig. 1). There are no significant changes in total Cr concentration for any of the plots from June to August, while the water-extractable concentrations are increasing significantly from June to August in all the treatments. The water-soluble Cr concentrations in August are about two times higher for the plots treated with CDIG and two times higher for AM relative to the control treatment (NN) (Fig. 1). Presented statistical results (Table 4) are showing higher significance of factor DOC in August than in June sample, while at the same time significance level has decreased for pH from June to August. Influence of treatments on metal leaching remains to be significant in both sampling points.

3.1.1. WHAM 7.0 modelling results

In order to better understand and assess the speciation and amounts of Al and Cr being complexed with DOM, the WHAM model was applied to the water-extractable concentrations of Al and Cr measured in the field experiment as described in paragraph 2.5. The pH and DOC values used for the modelling are given in Table A1, Appendix 1. To model the binding to humic substances and free concentration of the selected metals, in general WHAM needs other variables. In addition to the water-soluble metal concentration (Table 5), data for NO_3^- , SO_4^{2-} , Cl^- and F^- concentration (and DOC concentration) was also given as inputs in order to

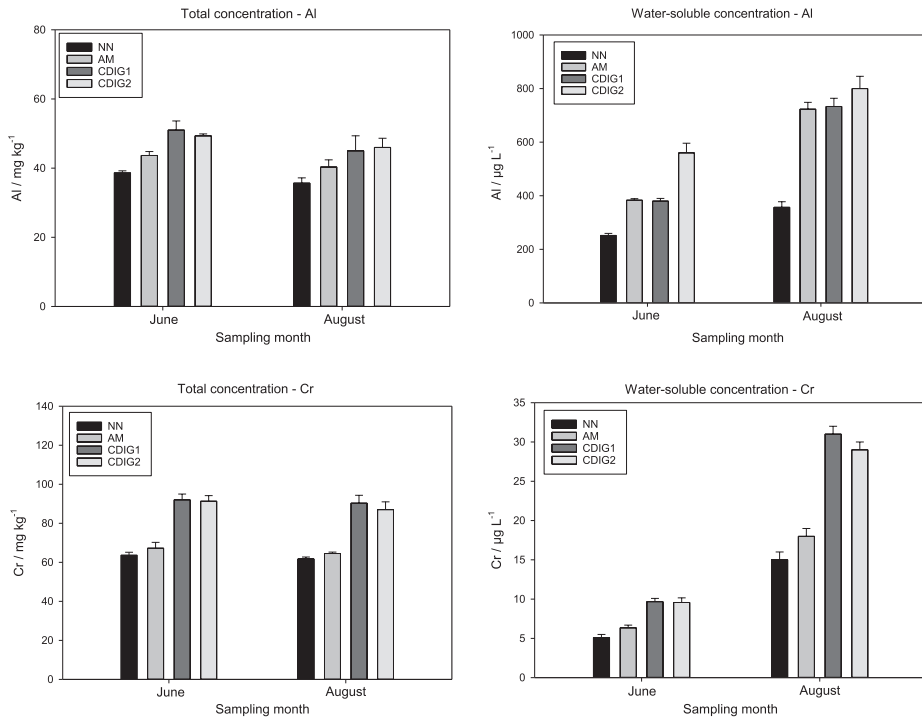


Fig. 1. Total and water-soluble concentrations of Al and Cr in soil sampled during the growing season in June and August from the field plots.

Table 4

Results of two-way ANOVA analysis with given factor of significance for the concentration of Al and Cr measured in extracted soil samples for June and August. The n.s. refers to non-significant value of factor/interaction.

Factors	Al-June	Al-August	Cr-June	Cr-August
Treatment	$p < 0.005$	$p < 0.005$	$p < 0.005$	$p < 0.005$
DOC	$p < 0.05$	$p < 0.005$	$p < 0.05$	$p < 0.005$
pH	$p < 0.05$	n.s.	$p < 0.005$	$p < 0.05$
Treatment \times DOC	n.s.	n.s.	n.s.	n.s.
Treatment \times pH	n.s.	n.s.	n.s.	n.s.

minimize the demand for using estimates from the literature (Table A2, Appendix 1). Furthermore, the concentration of Fe^{3+} was given as input to the model due to the influence of Fe^{3+} on metal and organic matter complexing through competitive reactions. The Fe^{3+} concentrations are also given in Tab. A2, Appendix 1).

The data for water-soluble Al and Cr concentrations in soils, as well as model-estimated values of free Al and Cr concentrations are presented in Table 5.

From the figures in Table 5 it is clearly seen that the modelled

free Al concentrations are significantly lower than the measured water soluble Al concentrations. WHAM simulates an increase of free Al between June and August, especially in the CDIG2 treatment. The modelled values increased almost five times from June to August. The modelled free Cr concentrations also show an increase from June to August samples. However, the modelled values are extremely low compared to the water-soluble Cr concentrations.

Binding of Al and Cr to humic substances (i.e., fulvic acid, FA, as explained in paragraph 2.4) was modelled and results are presented in Fig. 2. WHAM simulated a higher amount of Al bound to FA in June than in August for the AM treatment. For both CDIG treatments, WHAM estimated a lower amount of Al bound to FA in August than in June (Fig. 2).

WHAM simulated almost 100% of the water-soluble Cr to be bound to FA, both in June and in August, being slightly lower in the August samples.

3.2. Greenhouse experiment

In the greenhouse experiment (pot experiment), metal

Table 5

Measured water-soluble concentration of Al and Cr in soil samples from the field experiment (ws) and WHAM 7.0 estimated values of free Al and Cr concentration (free).

Plot	Al_{ws} $\mu\text{g L}^{-1}$		Cr_{ws} $\mu\text{g L}^{-1}$		$^*\text{Al}_{\text{free}}$ ng L^{-1}		$^*\text{Cr}_{\text{free}}$ pg L^{-1}	
	Jun.	Aug.	Jun.	Aug.	Jun.	Aug.	Jun.	Aug.
NN	251 ± 13	356 ± 11	5.0 ± 0.62	15.0 ± 0.85	0.42	6.1	0.03	8.6
AM	383 ± 18	723 ± 16	6.30 ± 1.0	18.0 ± 0.81	0.29	10.8	0.02	4.5
CDIG1	380 ± 11	733 ± 21	9.60 ± 0.73	31.0 ± 0.69	0.64	17.3	0.32	14.7
CDIG2	560 ± 0.46	800 ± 17	9.56 ± 0.87	29.0 ± 0.74	8.0	33.5	2.05	16.6

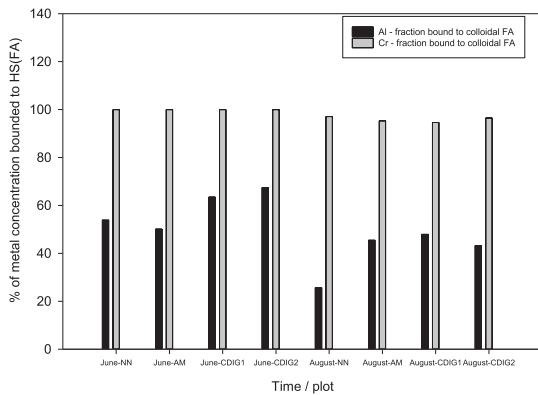


Fig. 2. Modelled percentage of Al and Cr bounded to humic substance in water extracts of soil samples from June and August (field experiment).

concentration was determined in the leachates as explained in Materials and methods. Comparison was made on the presence of the selected metals in the leachates against the added amounts through digestates and animal manure. To monitor the contribution of natural presence of Al and Cr in the soils, the concentrations in leachates from the untreated soils (control soils, NN) were also measured.

The added amounts of Al in digestates and animal manure treatments were calculated using the data on water-soluble concentrations (Table 1) and volume of added treatments (Table 2). The highest amount of Al was added with the CDIG2 treatment, while the lowest input value was for AM. Concentration of Al measured in leachates obtained from the pots was within the same treatment also different depending on soil type (Fig. 3).

In sand and silt, the digestate and animal manure applications resulted in Al concentrations below $200 \mu\text{g L}^{-1}$. In the loam soil, a significantly higher concentration of Al was seen only in the CDIG2 treatment (Fig. 3). Measured Al concentration for control soils (NN) varied from 60 to $80 \mu\text{g L}^{-1}$, depending on the type of soil. The results of the ANOVA analysis presented in Table 6 for Al are showing a high factor of significance for all the factors considered in the statistical analysis, except for the interaction between soil and DOC. Still, the highest degrees of significance were found for DOC, and for the interaction between treatment and soil.

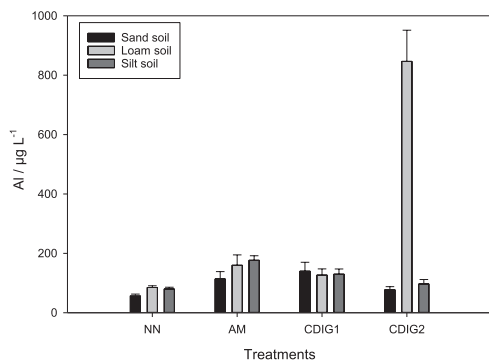


Table 6
Results of two-way ANOVA analysis with given degree of significance for the concentration of Al and Cr measured in leachates obtained from the pot experiment. The n.s. refers to non-significant value of factor/interaction.

Factors	Al	Cr
Soil	$p < 0.05$	$p < 0.005$
Treatment	$p < 0.05$	n.s.
DOC	$p < 0.005$	$p < 0.005$
Soil \times DOC	n.s.	n.s.
Treatment \times soil	$p < 0.005$	$p < 0.005$

Added amounts for Cr were highest for the CDIG1 treatment, followed by the CDIG2. Leachates from the pots containing sand show highest concentration of water-soluble Cr from the CDIG1 treatment, followed by CDIG2 (Fig. 3). In the loam and silt, no significant increase of Cr concentration was found in the digestate or animal manure treatments relative to the control treatment (NN). In the loam and the silt, the Cr concentrations measured in leachates from the different treatments were around $1 \mu\text{g L}^{-1}$. In general, digestates and animal manure had low Cr water-soluble concentrations (Table 1). The significance of soil type as a factor for the Cr concentration is high (Table 6). Besides soil type, the DOC has also significantly influences the Cr concentration in leachates obtained from the pot experiment. The interaction between treatment and soil also significantly influenced the Cr concentration.

3.3. Column leaching experiment

The use of a soil column leaching experiment enabled a scenario of close monitoring of metal leaching during the 7 days of intensive irrigation. The high irrigation scenario was supposed to simulate an extreme precipitation episode closely after spreading digestates in the spring and before plants are established.

In Fig. 4 leaching profiles and leached percentages of Al relative to the input of water-soluble Al in the different treatments and soils are shown. It can be seen that the highest leaching rate of Al was in the AM treatment for all three soils. The leaching of Al from the columns receiving digestates was below 30% of the added amounts. Still the highest calculated total leached concentration during the experiment for Al was with the use of CDIG1 treatment in the loam (1.44 mg L^{-1}). Within each treatment, the leached amounts of Al differed significantly depending on soil type, especially for AM.

The highest leaching of Al was seen for sand and silt. For the loam, an effect of adsorption was seen, as the leached amounts for all the digestates treatments were generally less than 35% of the Al

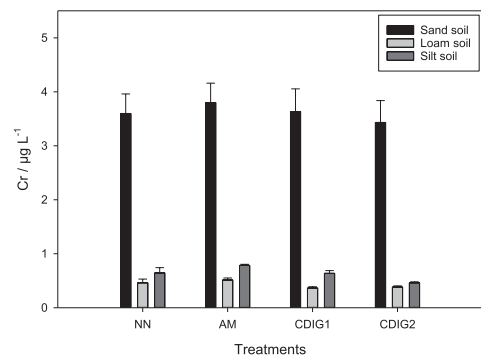


Fig. 3. Concentration of Al and Cr in leachates obtained after harvest by irrigation.

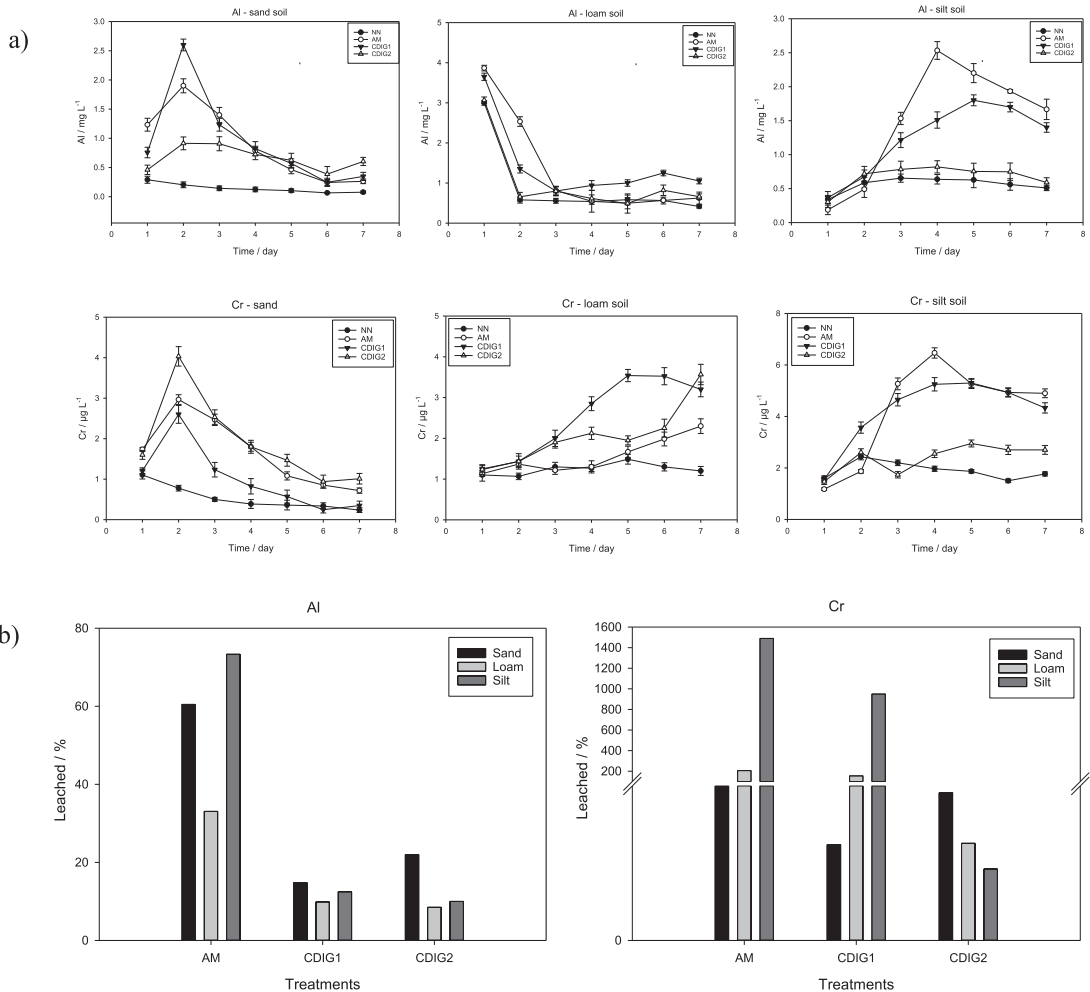


Fig. 4. a) Changes in concentration of Al and Cr in leachates from sand, loam and silt during the experiment. Error bars refer to the standard error. b) Percent of Al and Cr input leached from the soil columns. The amount leached in each treatment was corrected for the amounts leached from the control treatment (NN). The break for Cr figures is set at 100%.

added. Application of animal manure, a treatment used for comparison, i.e. a sort of control in this experiment, show higher Al leaching than when commercial digestates were applied (Fig. 4). The results of the statistical analysis for the observed Al leaching from the column experiment show that the factors treatment and DOC have a combined significant influence on the leached concentration of Al (Table 7).

The leaching of Cr during the experiment and calculated leached amounts of Cr based on the input of water-soluble Cr and added volumes of different treatments are also shown in Fig. 4. The highest leached amount of Cr was seen for CDIG1. The AM treatment represented the lowest input of Cr but had the highest leaching rate, especially in the silt (14 times above the added amount). The concentration of Cr leached from the silt were 6.1 μg L⁻¹ for CDIG1, and 4.4 μg L⁻¹ for AM. The leaching of Cr, relative to the input was high, especially for AM and CDIG1 in the

Table 7
Results of a two-way ANOVA analysis with given factor of significance for the concentration of Al and Cr measured in leachates obtained from column leaching experiment. The n.s. refers to non-significant value of factor/interaction.

Factors	Al	Cr
Soil	<i>p</i> = 0.289	<i>p</i> < 0.005
Treatment	n.s.	n.s.
DOC	<i>p</i> < 0.005	<i>p</i> < 0.005
Soil × DOC	n.s.	n.s.
Treatment × DOC	n.s.	n.s.

loam and the silt. Among the digestate treatments, application of CDIG1 gave the highest leached Cr amount during the experiment. Based on water-soluble concentrations of added Cr, it is clearly shown (Fig. 4b) that most of the treatments resulted in a Cr leaching

higher (>100%) than the added amounts. According to the results of the statistical analysis, a significant influence of soil and DOC as factors was seen for the Cr leaching (Table 7).

4. Discussion

The field experiment was conducted based on the available N corresponding to the agricultural use of biogas digestates as fertilizers in cereal production in Norway. The soil column and pot experiment have been conducted under fully or partially controlled conditions and the discussion of these results will be used in order to address a possibility of two different scenarios of Al and Cr leaching by using high metal-containing biogas digestates. The direct comparison between the results obtained from filed experiment and from pot/column leaching experiment is not possible based on the proposed experimental set-ups.

4.1. Aluminium

In the field experiment, Al mobility has increased with addition of both biogas digestates and animal manure. The water-soluble concentrations, measured in samples from the field experiment, are below as values defined as maximum allowed for aquatic life forms, where concentration of soluble Al is considered to be extremely acute (EPA, 2017). Release of Al and potential adverse effects on plant growth and health of aquatic organisms should be considered only in extreme field conditions (radical acidification of soil).

Between June and August, only a minor change in soil total Al concentration was observed. However, in the same soil, the water-soluble Al concentrations increased from June to August (Fig. 1). Guibaud and co-workers (Guibaud et al., 1996) confirmed an influence of DOM on Al release by inducing Al leaching from the upper soil horizons. Still, values measured in this field study are significantly higher than the values usually measured in similar studies. The highest concentration of water-soluble Al was found in August for the CDIG2 treatment, although the AM and CDIG1 treatments were not significantly lower, which shows that the total concentration can not be main determining factor in Al mobility. The CDIG2 has a significantly higher content of both total and water-soluble Al than AM and CDIG1 (Table 1). In addition, the part of the water-soluble concentration of Al expressed per kg dry matter was highest for CDIG2 treatment (Table 1). These facts can partially explain the increased mobility of Al with the use of CDIG2. However, other factors, such as pH, DOC and irrigation also control the mobility of Al. The results of ANOVA (Table 4) confirm the significance of pH and DOC ($p < 0.05$) as factors affecting the Al concentration, especially for the June samples. The controlling process for Al solubility is complexing reactions between Al and organic matter since the pH values were too high for Al to be fully soluble in a form of Al^{3+} . Earlier study by Roy and Couillard (Roy and Couillard, 1998) with the use of sewage sludge as soil amendment has shown that pH and DOC control Al leaching from the soil. Modelling results have also shown changes in Al solubility between the June and August samples. The modelled results suggest an increase in free inorganic Al concentration (Al_{free} concentration) as well, from June to August. WHAM 7.0 is not a dynamic model, but was here first applied on results obtained from June, and then on the results from August (Table 5). The input data is showing that water-soluble Al concentration increased from June to August, while the model estimated free inorganic Al concentration (Al_{free} concentration) also increased (Table 5). This result reflects also in the modelled Al bounding to humic substances (FA) shown in Fig. 2, where a decrease in amount of Al bounded to FA from June to August is apparent. The relationship between the Al water-soluble

concentrations and modelled free concentration are indicating that the majority of the soluble Al is complexed either with organic matter or with other type of inorganic ligands. Al speciation in soil pore water is a complex problem and has been partially studied earlier (Tipping et al., 2002). Specific forms of Al in soil and soil pore water are of particular importance, especially from an agricultural and environmental point of view, since the different soluble forms can have adverse effect on the plant growth and aquatic organisms (Singha et al., 2017). Still, appearance of soluble Al forms in low pH soils are usually treated as an extreme field condition when compared to the usual agronomical practice.

In the pot experiment, increased concentration of Al in leachates was found in the pots treated with CDIGs (Fig. 3), and can be attributed to lower pH and higher DOC concentration. The statistical analysis (Table 6) confirmed a significant influence of DOC on the Al concentration ($p < 0.005$). Soil was also seen to be significant factor ($p < 0.05$), which was expected based on the soil texture. Still, in leachates from the loam a significant increase in the Al concentration relative to sand and silt was found, especially in the CDIG2 treatment. The pots with loam were limed and previous study by Filep and co-workers (Filep et al., 2003) has shown that the liming may increase the DOC concentration with time. Such an increase in the DOC concentration could affect the soil/soil pore water equilibrium and thereby lowering the adsorption ability in the loam.

In the high irrigation column leaching experiment, the amounts of Al leached from sand and silt soils are close to 100% of the added amounts, which was expected based on the low clay and organic matter content. The Al-leaching from the loam can be explained by the content of clay (approximately 20%) in the loam (Table 3). In the soil column experiment, the AM treatment resulted in higher Al leaching rates than CDIGs, although the application of CDIGs represented a higher input of total and water-soluble Al (Table 1). The reason for the higher Al leaching rate from the AM treatment may be the concentration and composition of DOC in this treatment (Table 2). In the case where pH has changed during the experiment (from mildly acidic to neutral), high concentration of DOC and high irrigation rate could have induced leaching of naturally present Al in the soils, which could be the case in the AM treatment. DOC from biogas digestates may reduce the electrostatic binding of Al in soils (Karak et al., 2015), but this did not seem to be the case here since the increased leaching came only from the AM treatment. The results of the ANOVA statistical analysis (Table 7) indicate that the DOC has primarily influenced the Al concentration ($p < 0.005$) in the column leaching experiment. In the silt, the low clay content may also play an important role in the increased Al leaching.

4.2. Chromium

The amounts of Cr measured in the leachates and water extracts from field experiment were well below the limit ($100 \mu\text{g L}^{-1}$) defined by the United States Environmental Protection Agency (EPA) for total Cr concentration in freshwater (EPA, 2017). Initial total concentrations of Cr in digestates were almost two times higher than the values given in regulation by the state and significantly higher than the usual amounts seen in digestates or manure (Al Seadi and Lukehurst, 2012; Kupper et al., 2014). Results have not shown higher mobility or leachability of water-soluble Cr.

The total concentrations of Cr in the soil of the field experiment did not change significantly from June to August. Still, plots treated with CDIGs had elevated total concentration of Cr relative to the control plots. The increase in total concentrations of Cr in soil (Fig. 1) was expected based on the amount of Cr in the CDIGs that were added. In general, concentrations from all the sampled plots were about 3 times higher in the August samples compared to their

concentrations in June. This significant increase from June to August indicates that factors and processes during the growing season have affected the mobility of water-soluble Cr. The role of DOC in Cr soil mobility and change in its oxidation state, i.e. the relation between Cr(III) and Cr(VI), is still a subject of research. For the purpose of this study it is important to comment on the general possibility of redox conversion of Cr(III) to Cr(VI) in agricultural soils. Recently Reijonen and Hartikainen (Reijonen and Hartikainen, 2016) have shown that higher organic matter content in soils contributes to a lower capability of Cr oxidation, from Cr(III) to Cr(VI), in agricultural soils. An earlier study of Gustafsson and co-workers (Gustafsson et al., 2014) suggests the Cr(III) sorption is mainly to be determined by soil pH and DOC concentration, more precisely that at $\text{pH} > 4.0$ and high DOC concentration, the soluble Cr(III) concentrations in soil water suspensions can increase. Results from the statistical analysis (Table 4) show that DOC, pH and treatment significantly influence the Cr concentration in water extracts from soil samples taken from the field plots in both June and August. The WHAM-simulated free Cr concentrations were generally very low (Table 5), but increased somewhat from June to August. Thus, the model results suggest that most of the water-soluble Cr is complexed with organic matter (FA) and that the change between June and August is small (Fig. 1). Model results of Cr(III) complexation to DOC also support the fact that soluble Cr(VI) forms are not favoured within the investigated experimental conditions. It is also interesting to notice that a recent study by Choppalaa and co-workers (Choppalaa et al., 2016) has shown that redox state of Cr was significantly affected by the addition of animal manure as fertilizer and this addition has increased the rate of reduction of Cr(VI) to Cr(III). Still, results of our study show that the addition of animal manure within usual fertilizing conditions can enhance mobility of naturally present Cr in soil. Thus, it is difficult to expect significant adverse effect of Cr due to biogas digestate application at common field conditions.

In the pot experiment, concentrations of Cr in leachate from the silt and the loam were around $1 \mu\text{g L}^{-1}$ (Fig. 3), indicating a strong sorption effect in both soils. Results of the ANOVA statistical analysis (Table 6) indicated that soil and DOC ($p < 0.005$) are the main factors affecting the Cr concentration. In the sand, low sorption is assumed as higher concentrations of Cr were measured in leachate from treated soils, which corresponds to the low sorption ability of sand. The results of low Cr concentration in leachates from loam and silt soil can be a result of mineralization of organic matter, which would lower the Cr mobility.

The addition of biogas digestates has changed the leached concentration and mobility of Cr in all the soils in the column experiment. The highest leached concentrations of Cr were measured after addition of the commercial digestates in the field and pot experiment, but in the soil column experiment, the Cr leaching rate was higher when animal manure was added. The high irrigation rate and the low adsorption capacity of the sand can be considered as main factors responsible for the low retention of the added Cr and almost equal input and output concentrations. In the silt, increased leaching of Cr was measured for the CDIG and AM treatments. In the AM treatment, 14 times more Cr was leached from the silt than added, while for the CDIG1 treatment about 10 times the amount added was leached. The increased leaching may be explained by the enhanced mobility of naturally present Cr in silt induced by the addition of large amounts of DOC in the organic fertilizer treatments. In the loam, only slightly enhanced leached amounts relative to the amount of Cr added were seen. The weak mobilization of natural Cr in the loam may be explained by the pH values ($\text{pH} 4.7\text{--}5.9$) that could have lowered the effect of DOC to Cr mobility. However, in the silt where the Cr leaching was highest, the pH was higher than in sand and loam making the DOC more

available to influence the natural soil/soil pore water equilibrium. Results of this study confirm the importance of connection between the soluble Cr concentrations and DOC that acts as an important driving force behind Cr soil leaching at higher pH values, which is important when high metal-containing digestates are used. The influence of DOC is also visible from ANOVA results, where DOC was found to be a dominant factor controlling the leaching of Cr in the column experiment (Table 7).

Although this study has not included other types of soil amendments, there is a growing interest in the application of biochar as soil improver, which also includes those biochars produced from biogas digestates (Hung et al., 2017). Since biochar is pyrolysis product produced from biomass, the presence of heavy metals cannot be disregarded as a potential environmental issue. There are several studies showing positive effects of biochar to soil nitrogen, phosphorus and carbon content and to soil bacterial activity (Anderson et al., 2011; Prayogo et al., 2014). Still, there are also studies indicating that the addition of biochar can increase the mobility of heavy metals in soil (Liang et al., 2017). This means that the production and application of other digestate products needs to be carefully regulated. There are also organic waste treatments and products that can serve as means to suppress plant diseases or the decrease of soil quality characteristics, such as inactivated compost (Fuchs, 2010), which is today one of the fastest growing areas of household food waste processing. Use of organic waste products as fertilizers presents a unique opportunity and contribution for a better waste management both locally and globally depending on the type of waste and applied treatment processes.

5. Conclusions

This study has been mainly focused on the investigation of Al and Cr mobility when high metal-containing digestates are applied on the agricultural field with the purpose of growing cereal crops. By using different experimental set-ups Al and Cr release was investigated using different soils amended with biogas digestates in different scenarios.

The main hypothesis that high metal containing digestates, when used as fertilizer in crop production, will cause significant problems to the environment and in particular affect Al and Cr release to soil pore water and further to the recipient, was not fully confirmed. The findings of this study have also indicated that the total concentration of metals in organic fertilizers, usually used to set limits to the use of organic waste by-products, needs to be discussed in future regulation changes. From the observed changes during the field experiment, it seems that the use of digestates is not correlated with potential adverse effects for the soil environment. Clear beneficial effects visible through the agricultural use of these organic waste by-products outweigh potential risks that were first hypothesized in regards to the Al and Cr release. In general, both commercial digestates in this study can be treated similar to the use of animal manure.

The concentrations of Al and Cr measured in soils and soil extracts were in most of the samples below the maximum values allowed for metal concentrations with acute or terminal effect to aquatic organisms in fresh waters. More detailed and specific metal speciation studies are required in order to fully assess the dominant chemical forms present when using biogas digestate as fertilizers in agronomic plant production.

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Appendix 1

Table A1

Measured pH and DOC values from the field experiment water extracted soil samples from treated plots for June and August.

Plot	pH		DOC/ mg L ⁻¹	
	Jun.	Aug.	Jun.	Aug.
NN	5.96 ± 0.11	5.03 ± 0.16	25.43 ± 0.83	29.38 ± 0.86
AM	5.90 ± 0.15	5.13 ± 0.18	52.73 ± 1.41	48.74 ± 0.90
CDIG1	5.44 ± 0.13	4.99 ± 0.22	46.36 ± 1.02	53.14 ± 1.67
CDIG2	5.12 ± 0.17	4.98 ± 0.14	50.73 ± 1.96	54.69 ± 1.01

Table A2

Measured concentrations of Fe³⁺, NO₃⁻, SO₄²⁻, Cl⁻ and F⁻ from the field experiment water extracted soil samples from treated plots for June and August.

Plot	Fe ³⁺ / mg L ⁻¹		NO ₃ ⁻ / mg L ⁻¹		SO ₄ ²⁻ / mg L ⁻¹		Cl ⁻ / mg L ⁻¹		F ⁻ / mg L ⁻¹	
	Jun.	Aug.	Jun.	Aug.	Jun.	Aug.	Jun.	Aug.	Jun.	Aug.
NN	3.23 ± 0.24	2.45 ± 0.17	3.86 ± 0.43	5.93 ± 0.71	3.02 ± 0.78	2.29 ± 0.20	1.36 ± 0.02	1.06 ± 0.11	0.76 ± 0.09	0.75 ± 0.12
AM	4.43 ± 0.37	2.76 ± 0.23	7.86 ± 0.16	8.70 ± 0.35	4.05 ± 0.47	4.04 ± 0.24	4.13 ± 0.33	2.72 ± 0.11	1.51 ± 0.40	1.04 ± 0.34
CDIG1	2.93 ± 0.31	4.25 ± 0.37	10.02 ± 0.73	9.99 ± 0.27	3.80 ± 0.64	3.45 ± 0.33	5.43 ± 0.34	2.62 ± 0.34	1.50 ± 0.16	0.93 ± 0.19
CDIG2	6.81 ± 0.46	3.50 ± 0.41	19.80 ± 1.30	8.58 ± 0.80	5.63 ± 0.67	4.01 ± 0.58	2.80 ± 0.35	1.40 ± 0.32	0.79 ± 0.16	0.72 ± 0.16

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PAPER 3



Recycling of Biogas Digestates in Crop Production—Soil and Plant Trace Metal Content and Variability

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Production of biogas and utilization of the resulting digestates as fertilizers has been increasing in Europe in the last few decades. Depending on the feedstock used for the anaerobic digestion process, these organic fertilizers may be a source of different pollutants, such as trace metals. When applied to soils, digestates may influence the natural metal content and enhance the release of metals to the environment since they can be rich in different trace metals and are usually rich in dissolved organic matter. This study focused on investigating metal presence, seasonal variability of their soluble forms and crop uptake in a 2-year field experiment, using two different biogas digestates as fertilizers. The use of digestates as fertilizers was compared to cattle manure, mineral fertilizer and a control without fertilizer addition, with respect to the presence and distribution of the trace metals Cd, Cu, Zn, Ni, Cr, Mn, and Mo, as well as Al in the soil and plant. The results of the study showed that both biogas digestates have caused a 10–20% increase in the total soil concentration of Ni, Cd and Cr compared to control plots without fertilizer addition. Application of biogas digestates had only a minor effect on metal uptake in plants. Overall, the selected application rate of 100 kg/ha of plant available nitrogen has had little effect on plant metal uptake and crop quality and the use of biogas digestates was comparable to the use of animal manure.

Keywords: organic waste, fertilizer, cadmium, chromium, nickel, zinc

INTRODUCTION

Over the last few decades, the use of different organic residues as fertilizers has increased. For instance, anaerobic digestion for biogas production results in large amounts of liquid digestate, which contains high amounts of nutrients, such as nitrogen, potassium and phosphorus, and micronutrients in plant-available forms. The fertilization benefit of digestates are well documented (Möller and Müller, 2012; Nkoa, 2014) and long-term application of digestates has been seen to improve soil quality, stimulate crop yields, and even influence positively on the soil bacteria growth (Abubaker et al., 2012).

The most common substrates for biogas production are animal manures (Fantozzi and Buratti, 2009), food waste collected from the municipal households and sewage sludge (Maragkaki et al., 2018) or co-digestion of several substrates. Most of the studies have focused on the use of manure or sewage-sludge based digestates with an emphasis on their nutrient and fertilizer value (Ni et al., 2017; Yun et al., 2018). There are few studies with digestates based on source-separated food waste focused on the optimization of digestate quality as fertilizer through co-digestion

with other materials, such as sewage sludge (Borowski et al., 2018). Recent findings have shown that food-waste based digestates can be used for growing vegetables and mushrooms, especially when anaerobic digestion is combined with hydroponics (Stoknes et al., 2016).

Modern crop production is focused on both good yield and grain quality, but also on preservation of the environment. There are studies showing that both animal manure (Nicholson et al., 1999; Yang et al., 2017) and sewage sludge (Ashworth and Alloway, 2004; Chen et al., 2017) contain trace metals in variable concentrations. Still, sewage sludge application to agricultural land is strictly regulated because of the trace elements build up in soil, in particular elements such as cadmium, mercury, lead, and chromium. Further, soil leaching studies for trace metals have showed high ecological risk factors with the application of organic wastes such as compost or manure (Cambier et al., 2014). Pollutants may be applied to soil through inorganic or organic fertilizers and may bioaccumulate in soil organisms and plants (Antoniadis et al., 2017a). The organic matter quality and structure in biogas digestates is dependent on the biogas production feedstock and the applied technology, which means that it can have an effect on the trace metal availability. Soil quality is an important factor for crop quality and yield, and will be affected negatively by accumulation of trace metals or other pollutants. One challenge with the field application of biogas digestates is their chemical safety (Törnwall et al., 2017). Naturally, the content of potential contaminants in substrates used for the biogas process determines the content of these contaminants in the digestates. Metal content in biogas digestates and its potential implications for the use as fertilizer has been a subject of increasing interest in recent years (Kupper et al., 2014; Parraga-Aguado et al., 2017). Environmental effects of added trace metals, such as uptake into plants and soil organisms or leaching to water recipients, depends on their mobility in the soil, which in turn is strongly affected by pH and dissolved organic carbon (DOC) content (Antoniadis and Alloway, 2002; Welikala et al., 2018). For instance, Cu, Zn, Cd, and Ni, are generally mobile in soil under common conditions for crop production in a Nordic climate, i.e., in a pH range between ~6 and 7. On the other hand, As, Sb, and Hg may precipitate in insoluble forms under these conditions. Applying organic fertilizers represents an addition of DOC and may influence the pH in the soil and may thus increase the risk of plant uptake or leaching to water recipients.

In the field experiment described in this study, two digestates based on food waste were used, one digested alone and one co-digested with sewage sludge. The study has focused on the presence and availability/water extractability of the trace metals Cd, Cu, Zn, Ni, Cr, Mn, and Mo, as well as Al in soil after applying biogas digestates and animal manure in a field experiment conducted over two growing seasons. Aluminum was included because one of the digestates studied contains large amounts of Al from phosphate removal in sewage sludge with Al salts. The main hypothesis of this study was that the application of biogas digestates as organic fertilizers would increase the mobility and thus plant availability of trace metals in soil. Results of the study were used to calculate a contamination factor

for each metal based on the concentrations from the soil top layer.

MATERIALS AND METHODS

Organic Fertilizers

For the field experiment, two commercially produced biogas digestates and cattle manure were used as organic fertilizers. The digestates (DIG) were produced at two municipal biogas plants in the southeastern part of Norway. The respective companies use different feedstock mixtures and different technologies for biogas production. The digestate marked as DIG1 is derived from pretreated (steam-exploded) source-separated household and industry food waste as substrate for the anaerobic digestion process. The second digestate, DIG2, was produced using a mixture of source-separated food waste and sewage sludge (ratio 50:50) as substrate.

The manure was a cattle slurry from the farm at the Norwegian University of Life Sciences in Ås (Norway). Before chemical analysis, all the samples were stored at 4°C, in the case of NH₄ and NO₃ analysis no longer than 48 h. Selected characteristics of digestates and manure used in both growing seasons are given in **Table 1**.

Field Experiment

The field experiment was located at Ås, southeastern Norway (59°39'52"N 10°47'40"E) on a loam soil (13% sand, 57% silt, and 30% clay, as determined by the pipette method; Elonen, 1971). The soil was an epistagnic Retisol according to the World Reference Base for Soil Resources (IUSS working group WRB, 2015). It had a pH value of approximately 6.0, a total organic matter content of 4.2% and total nitrogen content of 0.21% prior to the experiment. The field experiment was started in 2014.

The experiment was organized as a randomized complete block design with three replicates, growing barley (*Hordeum vulgare* L.) in 2015 and oats (*Avena sativa* L.) in 2016 as test crops. The field was spring-plowed and harrowed before adding the different fertilizers in April 2015 and mid-May 2016, respectively. The fertilizers were incorporated into the soil by harrowing, and fields were sown within 48 h after application of fertilizers. The size of the experimental area was 39 m × 24 m (0.094 ha), and the size of each treatment plot was 3 × 7 m (0.0021 ha).

Treatments consisted of a control without fertilizer addition (NN), mineral fertilizer (MF), animal manure (AM), and the two digestates. The mineral fertilizer used was an NPK fertilizer with an NPK ratio of 22:3:10. The applied rate per hectare was 100 kg N, 14 kg P, and 45 kg K. The amounts of digestates and manure were determined based on plant-available N NO₃-N and NH₄-N, **Table 1** measured in the samples and corresponded to 100 kg N ha⁻¹. In a recent study it was found that the content of inorganic nitrogen in digestates was a good estimation of the fertilizer value (Sogn et al., 2018). In Norway, farmers are currently obliged to register N amounts but not P addition from organic fertilizers such as manure.

Table 2 shows total amounts and the main nutrients N, P, and K applied with organic fertilizers in kg ha⁻¹.

TABLE 1 | Biogas digestates and animal manure characteristics for 2015 and 2016.

Treat.	pH	Dry matter (%)	Tot. C (%)	Tot N* (%)	DOC (mg/L)	NO ₃ -N (mg/L)	NH ₄ ⁺ -N (mg/L)
AM-15	7.3	6.6 ± 0.52	41.4	2.08	5879 ± 55	56.0 ± 1.5	1395 ± 20.04
DIG1-15	7.5	3.1 ± 0.36	39.7	5.71	3899 ± 5	95.1 ± 1.2	1142 ± 43.3
DIG2-15	7.1	1.6 ± 0.36	31.8	4.19	3630 ± 9	176.8 ± 3.1	904 ± 43.9
AM-16	6.9	7.8 ± 0.58	47.4	1.76	6856 ± 88	4.15 ± 0.71	969 ± 42.5
DIG1-16	7.2	4.2 ± 0.62	37.7	5.24	4125 ± 27	7.82 ± 0.46	1403 ± 11.2
DIG2-16	7.3	2.0 ± 0.46	33.4	2.55	3987 ± 34	7.43 ± 0.40	1401 ± 82.8

Total carbon (Tot. C) was measured in a dried sample. Values behind ± are standard deviation. *Calculated based on the Tot N values from dry fertilizer samples (55°C).

TABLE 2 | Amounts of nitrogen (N), phosphorus (P), potassium (K), and total carbon (Tot. C) added in kg per hectare for both growing seasons.

Treat.	N* (kg/ha)	P (kg/ha)	K (kg/ha)	Tot. C (kg/ha)	Added amounts (t ha ⁻¹)
MF-15	100	14	45	0	0.45
AM-15	100	47.6	145.4	303.1	71.4
DIG1-15	100	19.8	101.1	87.2	87.1
DIG2-15	100	18.2	12.2	36.1	110.4
MF-16	100	14	45	0	0.45
AM-16	100	33.4	25.6	264.1	110.9
DIG1-16	100	43.9	146.4	137.9	70.9
DIG2-16	100	5.9	33.1	73.8	70.9

*Plant available N.

The average yields for the 2015 seasons were 1.87 t ha⁻¹ NN (control plot), 5.31 t ha⁻¹ MF, 4.96 t ha⁻¹ AM, 6.18 t ha⁻¹ DIG1 and 5.64 t ha⁻¹ DIG2. For 2016 season, the yields were 1.2 t ha⁻¹ for NN (control plot), 4.0 t ha⁻¹ for MF, 2.2 t ha⁻¹ for AM, 3.9 t ha⁻¹ for DIG1 and 4.0 t ha⁻¹ for DIG2 plots. The data from 2016 was used for calculating total metal content in oat grains.

Sample Preparation and Chemical Analysis

In general, the metal content in digestates depends on substrate and applied technology for biogas production. The trace metals investigated in this study are either under Norwegian or EU regulation for application of organic fertilizers, or selected based on previous research findings. Aluminum was included in the analysis because of its high content in the sewage sludge used for the production of DIG2, which in turn is due to removal of phosphorus with Al-salts before the digestion process.

The soil pH was determined by using dried soil with a soil to water ratio of 1:2.5. Total carbon content in dried soil, digestate, and manure samples was determined by a dry combustion method using a Leco Carbon Determinator EC12 (Nelson and Sommers, 1982). Water-soluble concentrations of metals were determined by extracting 5 g of soil/fresh digestates/fresh manure with 25 mL deionized water (Ashworth and Alloway, 2004). Soil water extractions are considered to be good in assessing the plant nutrient availability (van der Paauw, 1971; Sonneveld, 1990). Water-soluble concentrations of metals were analyzed within 48 h after preparing and filtering (Milipore, 0.45 μm) the extracts. Water extracts of manure and digestates were also analyzed for

DOC by using a Shimadzu TOC analyzer. The concentration of NO₃-N and NH₄-N in the organic fertilizers was determined in fresh samples by extraction with 2 M KCl and flow injection analysis using FIAstar 5000, FOSS.

For trace metal analysis, soil was sampled from the plow layer (0–20 cm) of each plot once a month from June to August. In 2015, plant material was collected at the same time, whereas in 2016, only grain samples were taken at harvest. The plant samples were dried at 55°C for 5 days and then stored before sample preparation and analysis. Prior to total metal analysis, the soil, digestate, and manure samples were dried (24 h, 105°C), sieved and ground. A sample of 0.2–0.3 g was digested in concentrated ultrapure nitric acid (HNO₃) prior to ICP-MS analysis by stepwise (90 min) heating up to 250°C using a Milestone Ultraclave. For the analysis of metal concentrations in soils, digestates, plants, and extracts, an Agilent ICP-MS 8800 TripleQ was used. The ICP-MS analysis has followed minor method validation (depending on the sample matrix) while certified reference materials and reference materials were used for quality control of the applied analytical methods. The filtered samples (extracts of soils and fertilizers) were prepared in 10 % ultrapure nitric acid prior to analysis.

Statistics and Calculations

Analysis of variance (one-way and two-way ANOVA) was carried out to determine the effect of treatment on total metal concentrations in soil and grains. Two-way ANOVA was applied to the water extract concentrations after digestate application with treatment and sampling time as factors. Different means were separated by *t*-test. Pearson correlation was carried out to determine the pH and DOC correlation to the water-soluble metal concentration in soil. The statistical programs used were R Commander 3.2.3 and Sigma Plot 14.0. The confidence limit was 95% ($p < 0.05$).

Principal component analysis (PCA) is a dimension reduction technique, which may be used to demonstrate potential source and spatial distribution of metals in field experiments (Zhang et al., 2018). In the present study, the principal components (PC) were identified by the analysis of the correlation matrix for the data set regarding the water-extracted soil concentrations prepared from soils collected from the field experiment. The PCA analysis was used to identify factors underlying our set of variables (fertilizer treatments, sampling time) in order to determine relationships among them. Results were also used to

investigated clustering of samples into groups based on their correlations.

A soil contamination factor (Antoniadis et al., 2017b; Shaheen et al., 2017) was calculated in order to evaluate the potential of long-term accumulation of investigated metals by applying the selected rates of fertilizers. The contamination factor, *CF*, was determined by the following formula (1):

$$CF = \frac{C_i}{C_n} \quad (1)$$

Where, *c_i* represents the mean total concentration of the metal in soil of the organic fertilizer treatment, and *c_n* that of the unfertilized control.

RESULTS

Total Metal Concentration in Digestates and Manure

The total concentrations of trace metals and Al in digestates and manure used in 2015 and 2016 are given in **Table 3**. With the exception of Cr, Ni, and Cd (DIG1 2016), trace metal concentrations were below limits set for use in agriculture (Class I). Norwegian regulation in regards to the use of organic fertilizers categorizes all organic wastes in four classes (class 0, 1, 2, and 3). For comparison we have used class 1, since only class 0 and 1 are allowing the use of organic wastes with no or minimum limitations (Authority, 2006). Nickel concentrations in both digestates were higher in 2016 than in 2015. The Cr concentrations, although high, were not increased between 2015 and 2016. Due to the technology used, Al concentration were high in DIG2 in both years. Lead (Pb) concentrations ranged from 0.5 to 6.0 mg kg⁻¹ (data not shown), which was well below regulation values (Class I fertilizers, 60 mg kg⁻¹).

Total Concentration of Metals in Soil

Total metal concentrations in the MF treatment were almost identical to those in the control plots (NN), and results from both NN and MF are presented. Total soil trace metal concentrations are given in **Figure A1**.

When compared to control plots, total soil concentrations of Ni, Cr, Cd, Cu, Zn were significantly increased in the digestate

treatments. For Cr, there was a significant decrease from June to August in both seasons. For Ni, the same significant decrease can be seen. For Zn, results from 2016 showed significant increase in total Zn concentration with biogas digestates used as treatments.

Soil pH and DOC

Generally, pH was higher in NN and AM plots than in the digestate plots at all samplings, with the largest differences in the beginning of the season. The results from MF plots are not included in **Figures 1, 2** since the values were not significantly different from the NN plots. For both growing seasons, the soil pH from control plots (NN) was in the range 5.8–6.0. Soil pH varied over the course of both growing seasons and depended on the treatment type (**Figure 1**). The pH decreased in both growing seasons from levels around 6.0 to the lowest 5.1. The lowest values for 2015 was DIG1 and for 2016 DIG2 in respect to August samples.

In 2016, a significantly lower pH was found for DIG2 compared to the other treatments at all sampling points.

The addition of the organic fertilizers significantly increased DOC concentrations in the soil for the duration of the growing season. In both growing seasons, the effect was most pronounced in the AM treatment (**Figure 2**).

Water-Soluble Metal Concentrations in Soil

In general, water-soluble concentrations of the trace metals increased in all treatments over the course of the growing season, both in 2015 and 2016 (**Figure 3**). Concentrations were similar in both years with the exception of Cd, where concentrations were higher in all treatments and at all sampling dates in 2016 compared to 2015. As no significant differences in water-soluble concentrations were observed between treatments for Mn and Mo, the data is not included in **Figure 3**, but concentrations followed the same overall pattern with increasing values over the growing season.

There were no differences in Cd, Cu, and Ni concentrations between treatments at the first sampling date in 2015 and in the case of Cd, differences between treatments remained low throughout the season. In 2016, concentrations at the first sampling were generally higher for Cd than in 2016, and concentrations increased more with time in the organic

TABLE 3 | Concentration of investigated metals (± standard deviation) for biogas digestates 241 and animal manure from the years 2015 and 2016.

Treat.	Al (g/kg)	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cr (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Mo (mg kg ⁻¹)	Mn (g/kg)	Ni (mg kg ⁻¹)
Limit for organic fertilizer (class 1)	/	150	400	60	0.8	/	/	30
AM-15	0.4 ± 0.08	70.6 ± 2.1	163.3 ± 1.1	51.4 ± 4.7	0.12 ± 0.08	8.5 ± 0.94	0.19 ± 0.05	36.6 ± 3.2
DIG1-15	1.6 ± 0.51	30.6 ± 1.8	146.3 ± 2.2	176.6 ± 8.6	0.44 ± 0.22	21.3 ± 1.8	0.13 ± 0.08	110.1 ± 9.9
DIG2-15	15.2 ± 2.8	88.1 ± 4.3	256.2 ± 2.4	156.6 ± 7.7	0.43 ± 0.06	21.1 ± 2.3	0.11 ± 0.04	106.6 ± 6.9
AM-16	0.2 ± 4.7	30.1 ± 3.1	185.1 ± 6.7	42.1 ± 2.8	0.11 ± 0.03	0.14 ± 0.01	1.29 ± 0.06	51.1 ± 4.7
DIG1-16	1.7 ± 1.2	39.5 ± 2.8	245.2 ± 10.1	165 ± 6.8	1.1 ± 0.07	5.1 ± 1.43	1.7 ± 0.08a	135.5 ± 9.3
DIG2-16	16.1 ± 1.3	79.2 ± 5.7	225.1 ± 14.5	156 ± 7.3	0.42 ± 0.05	3.2 ± 1.12	0.65 ± 4.4	195.7 ± 10.7

Limit for organic fertilizer (class 1) are given for those metals that are a part of Norwegian regulation on organic waste use in the same units as for treatments. AM, animal manure; DIG1, food waste based digestate; DIG2, sewage sludge/food waste digestate.

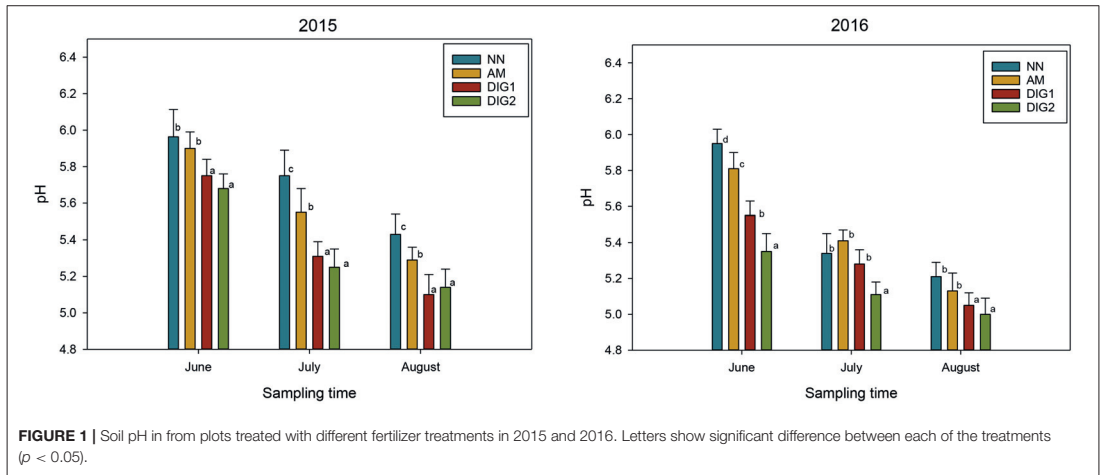


FIGURE 1 | Soil pH in from plots treated with different fertilizer treatments in 2015 and 2016. Letters show significant difference between each of the treatments ($p < 0.05$).

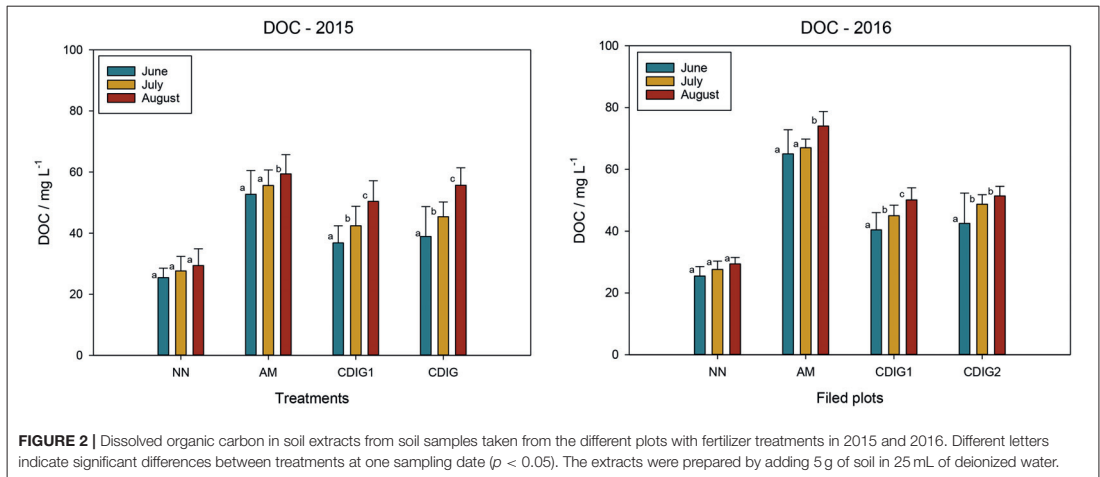


FIGURE 2 | Dissolved organic carbon in soil extracts from soil samples taken from the different plots with fertilizer treatments in 2015 and 2016. Different letters indicate significant differences between treatments at one sampling date ($p < 0.05$). The extracts were prepared by adding 5 g of soil in 25 mL of deionized water.

treatments than in the control. For Cu, higher concentrations were found in the organic and especially in the digestate treatments at the 2nd and 3rd sampling date in 2015. In 2016, however, the manure treatments had lower concentrations even than the control at the first two sampling dates and only DIG1 addition resulted in higher concentrations than the control. The pattern for Ni was similar in 2015 and 2016 with highest concentrations in both digestate treatments at the last two sampling dates, whereas the manure treatment was on a similar level to the control.

Zn, Cr, and Al already showed significant differences in concentrations at the first sampling date. In the case of Zn, the manure treatment showed higher concentrations than the other treatments both at the first sampling date in 2015 and 2016. For most dates, the concentrations in the organic

treatments were significantly increased compared to the control. In July and August 2015, highest concentrations were found in the DIG1 treatment, whereas in 2016, the manure treatment remained the one with highest Zn concentrations throughout the season. Cr concentrations were highest in the two digestate treatments at all sampling dates but also generally also enhanced in the manure treatment compared to the control. Addition of the digestate with the high Al content (DIG2) resulted in higher concentrations of water-soluble Al at all sampling dates compared to the control, but the other two organic treatments showed similar levels either early in the growing season (manure) or at the later sampling dates (DIG1). In the latter case, Al concentrations were at the same low level as in the control early in June but increased strongly until August.

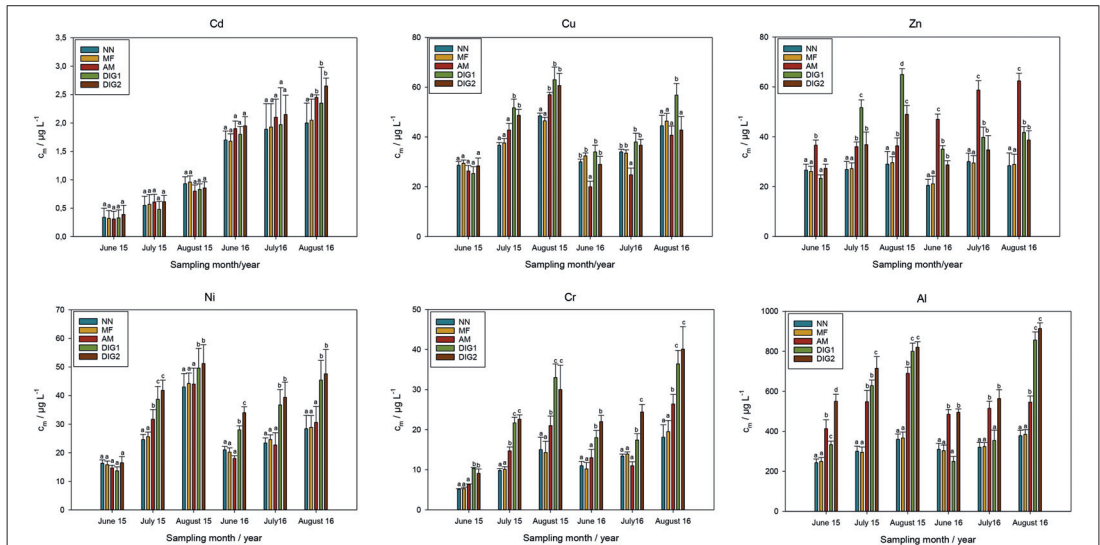


FIGURE 3 | Selected water-soluble metal concentrations in soil extracts (growing seasons 2015 and 2016). The results are presented as means \pm SD. Different letters indicate significant differences between treatments at each sampling time ($p < 0.05$). The extracts were prepared by adding 5 g of soil in 25 mL of deionized water.

Concentrations of the investigated metals from June to August sampling were in general negatively correlated with pH, with the exception of Zn, and positively correlated with DOC, with R -values above 0.75.

Additional statistical consideration of the results from the section of water-soluble metal concentration was used to assess the influence of the addition of different treatments, pH and DOC. The results of two-way ANOVA analysis are given in **Table 4**.

Water-soluble concentrations of Cu, Cr, and Ni were significantly affected by the factor treatment. Between the factors treatment and sampling time, the F -value is indicating that the sampling time had a more significant role especially for Cd, Cu, and Ni.

The PCA analysis was performed on the entire data set of measured concentrations from June to August for the 2016 season. In **Table 5**, the results for component loadings and eigenvalues of each PC are presented. PC1 had an eigenvalue >2 whereas PC2 had a value below 1 (0.8). Since only PCs with eigenvalues equal to or above 1 can be taken into consideration, only PC1 can be used to explain the variability.

The data structure and component loadings of the first two components, PC1 and PC2 are shown by use of a biplot (**Figure 4**). The first two components describe approximately 97% of the variance (PC1 85%, PC2 12%).

For all the metals, PC1 values were positive. Loading values were above 0.4 for all metals except for Zn. Most of the treatments formed a cluster, except for the DIG1 in July and August (**Figure 4**). The clustering of treatments is strongest for June and July sampling while for August the clustering was more dispersed.

Clustering in this type of experimental setup usually implies that used treatments have a similar effect on the metal concentration variability.

Total Metal Concentrations in Plant Material

In 2015, plants metal content was determined at three times during the growing season at the same time as the soil samples were taken. In 2016, grain metal content was analyzed at harvest. Total concentrations of metals in plant material are given in **Figure 5**. For Cd, during the growing season there was no significant difference between the treatments, where a concentration dilution effect is seen from June to August. Grain concentrations from 2016 were below 0.1 mg kg^{-1} for all treatments. A different uptake scenario was seen for the plant nutrients Cu and Zn, where total plant concentrations are significantly affected only by the digestates treatments (DIG1 and DIG2). Grain concentrations for Cu were around 0.2 mg kg^{-1} while for Zn they were around 25 mg kg^{-1} .

For treatments in season 2015, there was no significant difference in Ni concentrations during the growing season. There were no significant differences in Cr concentration for the used treatments in the sampled plant material for each of the sampling points (season 2015). The same dilution effect as seen for Cd can be also seen for Cr. In general, Cr concentration was lower than concentration of other metals detected in the plant material. The highest concentration of Cr in plant material was found in the plots treated with AM. Still, the grain concentrations in 2016 were very low (below $0.2 \mu\text{g kg}^{-1}$ of dry plant material).

TABLE 4 | Results of two-way ANOVA analysis with defined factor of significance for the concentration of selected metals measured in soil extracts using treatments and sampling time as factors and their interactions.

Factor	Cd	F-value	Cu	F-value	Cr	F-value	Ni	F-value	Zn	F-value
Sampling time	$p < 0.001$	2577.9	$p < 0.001$	1609.1	$p < 0.001$	702.6	$p < 0.001$	2062.6	$p < 0.001$	447.1
Treatment	$p < 0.01$	27.2	$p < 0.001$	85.1	$p < 0.001$	231.7	$p < 0.001$	97.9	$p < 0.001$	327.9
Sampling time × treatment	$p < 0.01$	26.2	$p < 0.01$	32.1	$p < 0.01$	21.6	$p < 0.01$	34.3	$p < 0.01$	152.6

TABLE 5 | Component loadings and eigenvalues for each PC of the selected data set.

Component	PC1	PC2	PC3	PC4	PC5
Cd	0.419002	0.608772	-0.4680404	0.4843647	0.0127055
Cr	0.464706	-0.238089	0.6608461	0.5338479	0.0751664
Cu	0.479098	0.073563	0.0186379	-0.5075744	0.7120911
Ni	0.472417	0.221168	0.2051211	-0.4705589	-0.6814733
Zn	0.394678	-0.719987	-0.5493618	0.0366013	-0.1506948
Eigenvalues	2.0623	0.8092	0.27239	0.22688	0.11939

Calculated amounts of trace metals taken up per hectare combine the concentrations in grains given in **Figure 5** with the yield (section Field Experiment, year 2016) of the different treatments. Thus, it can be seen (on a per hectare basis) what proportion of the trace metals added with the fertilizers is removed by harvesting.

The highest concentration was seen for Zn, while the lowest was seen for Cd, Ni, and Cr. After Zn, Cu was taken up and transported to the grains in highest amounts.

Contamination Factors for Soil Treated With Biogas Digestates

The potential metal contamination effect was assessed by using data from the total soil concentration from both growing seasons for the calculation of the contamination factor. Calculated contamination factors (*CF*) are given in **Figure 7**, using the formula from section Statistics and Calculations.

The general classification of the level of contamination available from the literature (Antoniadis et al., 2017a) usually divides *CF* into three classes, $CF < 1$, low degree, $1 \leq CF < 3$, moderate contamination and $3 \leq CF < 6$ with high degree of contamination. For 2015 within the experimental sampling the increase can be seen for Ni ($CF = 1.05$), Cr ($CF = 1.2$), and Al ($CF = 1.3$) for DIG2 treatment (**Figure 6**). In the calculation of *CF* value from 2016 there is increase for Cd ($CF = 1.4$) and Cr ($CF = 1.2$) for both DIG treatments. **Figure 6** shows that the rest of the investigated trace metals have a low or moderate level of contamination.

DISCUSSION

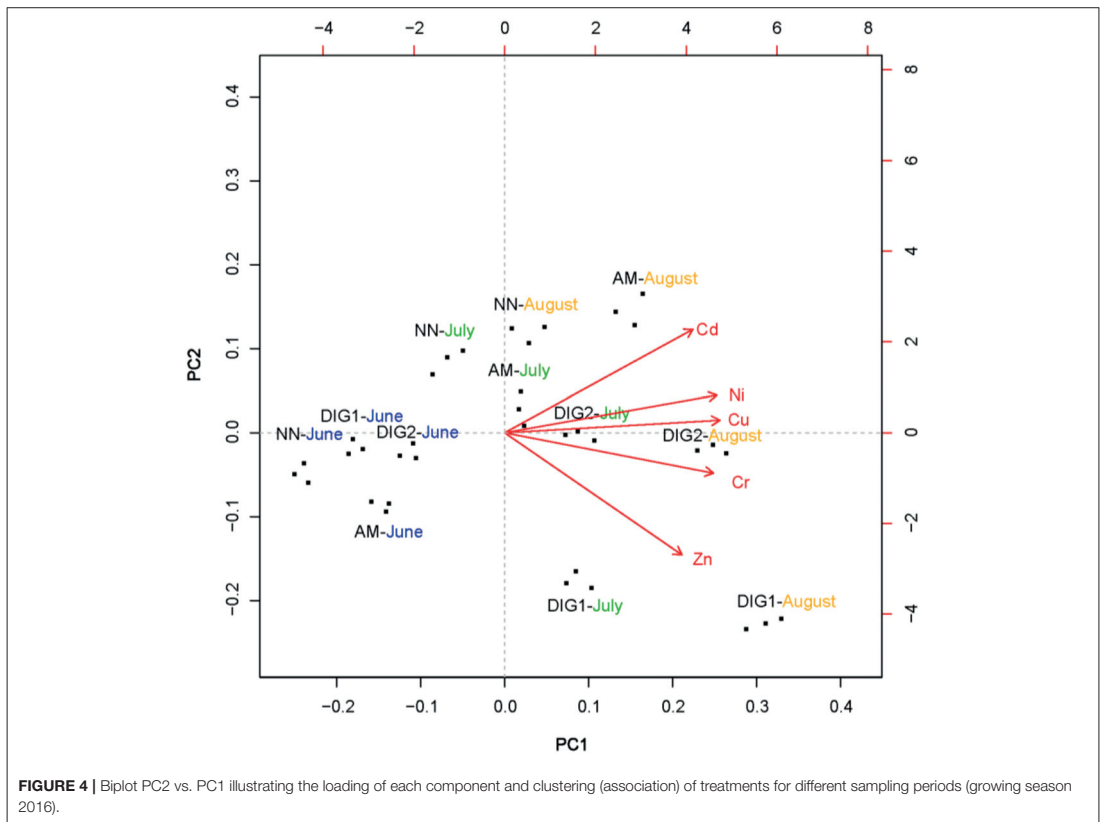
The trace metals in focus in this study were both plant micronutrients (i.e., Cu, Zn, Ni, Mn, and Mo), and those that are in general not considered to be important or even harmful for plant growth (Cd, Cr, and Al). However, both groups of metals can negatively influence both plant development and soil quality

(including runoff water) if present in excessive amounts. The addition of digestate has resulted in a significant increase in the total soil metal concentration, especially in the case of Ni and Zn, but also Cr and Cd. The water-extractable concentrations in the soil have significantly increased for Ni, Zn, and Cr with addition of digestate or manure in both growing seasons. A decrease in pH had an important role in the general increase of the water-soluble concentration of cationic forms of investigated metals. Still, the significant change in the pH and the addition of metals with biogas digestates did not influence metal uptake in plant material from 2015 or in the grain samples from 2016. The PCA has shown that the water-extractable concentrations of trace metals from plots treated digestate are similar to the ones measured for animal manure. In addition, clustering of specific sample points during one growing season as presented in PCA results (biplot) also contributes to the claim that digestates exhibit similar effects when compared to AM.

Influence of Biogas Digestates Fertilization on Trace Metals as Plant Micronutrients

Application of biogas digestates as fertilizers for both growing seasons has significantly increased Zn and Ni total soil content, while Cu, Mn, and Mo were not significantly increased (**Figure A1**).

Measured levels of total soil Zn are a result of the digestates/manure Zn content, which was in a range 150–250 mg kg⁻¹ (**Table 3**). Under Mediterranean conditions, suggested adequate Zn content is in a range of 100–300 mg kg⁻¹ soil for cereal production (Brunetti et al., 2012). Still, for the north of Europe the values are usually in range of 47–61 mg/kg, which means that the measured concentrations are within the suitable levels for growing cereal crops (Kabata-Pendias, 2011). A similar effect of Zn addition was seen in a published 2-year field study with the use of organic waste based fertilizer (composted municipal solid waste) where measured values of total Zn concentration in soils were also under 300 mg kg⁻¹



and comparable to our experimental results (Yuksel, 2015). The addition of DIG1 treatment significantly increased the total Zn content especially in growing season 2016. Still, measured values for AM treatment were comparable to the values of DIG1. The concentration of Ni in digestates was high. This resulted in significantly higher total Ni soil concentrations (Figure A1) when compared to the NN or MF plots. Suitable levels for total Ni concentration in soil is around 50 mg kg⁻¹ for a normal agricultural activity. The levels in our study were below a toxicity threshold (above 100 mg kg⁻¹ in soil) and well within the suitable levels (35–55 mg kg⁻¹) for crop production in general (Kabata-Pendias, 2011; Brunetti et al., 2012).

The observed changes for Zn and Ni water-soluble concentrations for DIG treatments were comparable to the levels measured for the AM treatment, especially in the case of Zn. Zn water-extractable concentration in the soil were not significantly higher than the ones measured for AM in season 2016 (Figure 3). The mentioned comparison clearly shows that the Zn added through digestates shows similar behavior as the Zn added through AM. Water-soluble Ni concentrations have significantly increased during both growing seasons in the

digestate treatments. This may be due to the decrease in pH, as Ni mobility is enhanced under acidic conditions (Zhu et al., 2011). Kim et al. (2015) also confirmed that low pH can increase the release of naturally present Ni from soil.

The Zn concentration in the plant material collected during the 2015 season was significantly higher in the digestate treatments than in all other treatments (Figure 5). This reflects the higher availability of Zn as indicated by increased water-soluble concentrations. There were no significant differences in Zn concentration in the grain samples between the digestate and MF or AM treatments. Added digestate treatments had no significant influence on grain Ni concentration and the values were comparable to AM or MF treatments. Despite differences for Ni concentrations in soil-water extracts between treatments, no significant effect of the organic fertilizer treatments on plant Ni concentrations was found (Figure 5). The level of Ni concentrations in grains was comparable to that found in other studies with organic fertilizers conducted in Northern Europe (Hamner et al., 2013). Still, the long-term effect of the Zn or Ni accumulation in soil should not be disregarded. Currently, Zn is marked as one of the three metals mostly

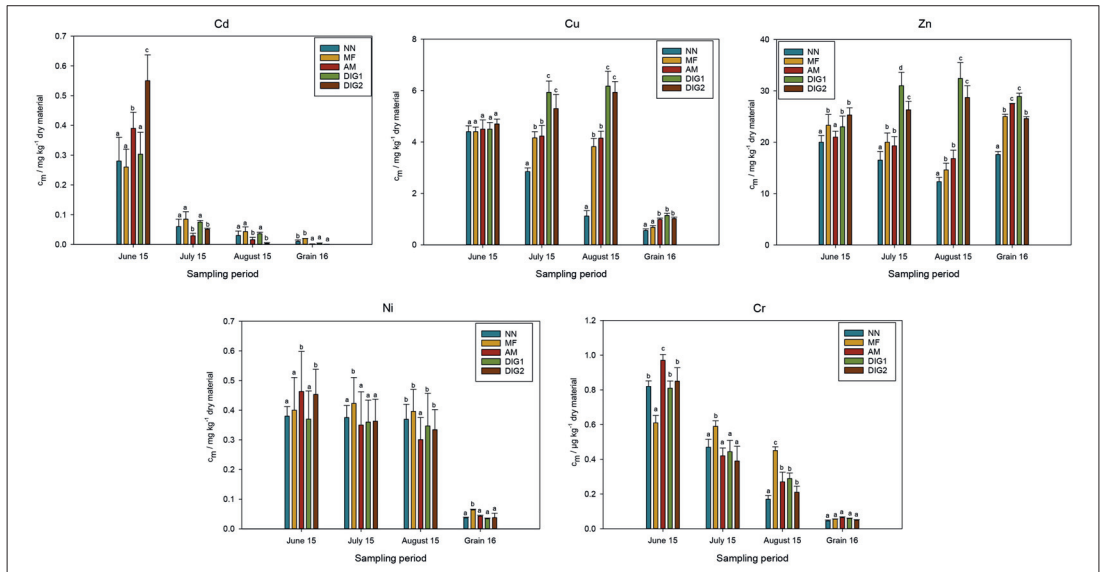


FIGURE 5 | Total concentration of selected metals in aboveground plant parts sampled during growing season 2015, and in barley grains harvested in 2016. Letters are showing significance of differences between the treatments ($p < 0.05$).

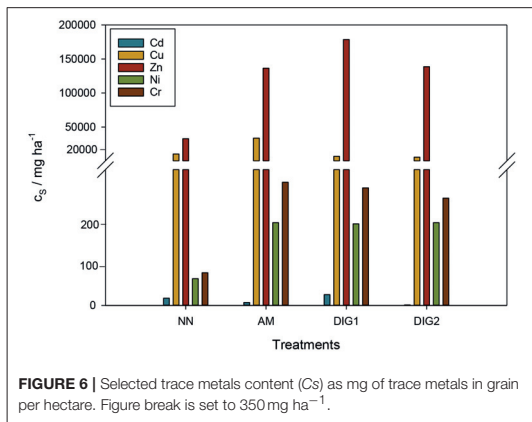


FIGURE 6 | Selected trace metals content (Cs) as mg of trace metals in grain per hectare. Figure break is set to 350 mg ha^{-1} .

contributing to the global environmental pollution through the use of animal manure fertilizing practices (Leclerc and Laurent, 2017). The soil contamination factors for Zn and Ni were below or at the moderate levels for the 2015 season and at moderate contamination level for the 2016 season, which implies a moderate increase in the risk of trace element accumulation. The maximum water-extractable concentration of Ni from soil was around $50 \mu\text{g L}^{-1}$, which is below the $70 \mu\text{g L}^{-1}$ defined by the World Health Organization (WHO) drinkingwater regulation (World Health Organization, 2006).

Addition of digestates has not increased significantly the total Cu, Mn, and Mo concentration. The Cu, Mn, and Mo are important micronutrients needed for normal plant growth and development, and change in soil concentration may have a positive effect on plant growth. The recommended values of Cu total soil concentration for cereal growth is ranging between 20 and 40 mg kg^{-1} , while toxic effects are dependent on the soil type and are usually seen between 60 and 125 mg kg^{-1} (Kabata-Pendias, 2011). Values measured in both growing seasons are approximately 10 times lower than the upper toxic limit for Cu in soil for all used treatments, which implies a lower concentration of Cu in soil than recommended. The reference values of Mn for soils used in agriculture in Norway is $\sim 6 \text{ mg kg}^{-1}$, where values measured in our studies was around 2 mg kg^{-1} , showing that the addition of biogas digestates has no significant effect on the total Mn concentration in soil. For total Mo concentration in soil the reference value in soils is $\sim 2 \text{ mg kg}^{-1}$, which is not different than the values measured in our 2-year experiment ($1.2\text{--}1.4 \text{ mg kg}^{-1}$). Based on the total concentrations it is clear that the addition of both biogas digestates had no significant influence on the reference values of Mo in soil used for plant production (Kabata-Pendias, 2011; Brunetti et al., 2012).

Copper mobility, measured as water-extractable Cu, generally increased from June to August for all organic fertilizers. Copper mobility is mostly influenced by the changes in soil pH and DOC concentration due to the different treatments. Dissolved organic carbon, i.e., the soluble phase of organically bound carbon is known to affect metal mobility both in soil solution and thus in the soil (Pérez-Esteban et al., 2014). Addition of biogas digestate

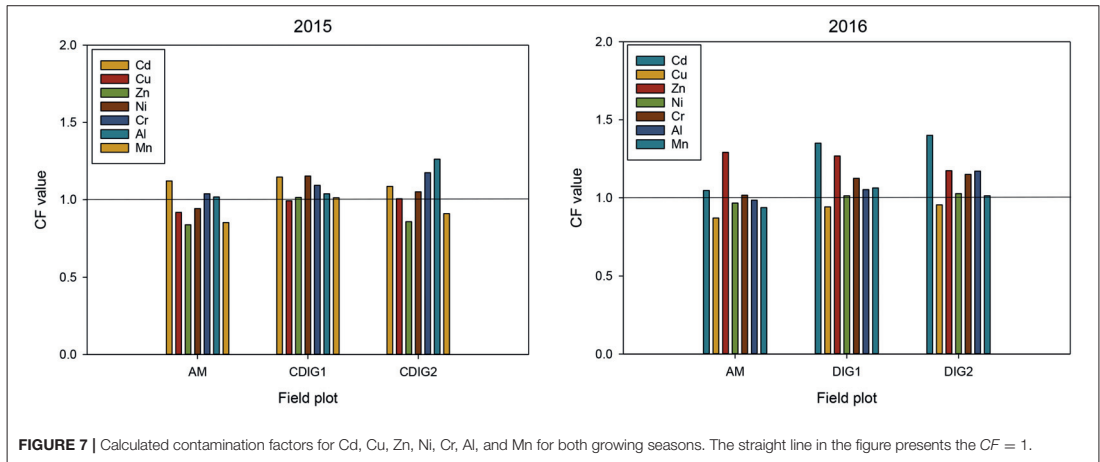


FIGURE 7 | Calculated contamination factors for Cd, Cu, Zn, Ni, Cr, Al, and Mn for both growing seasons. The straight line in the figure presents the $CF = 1$.

has increased the amounts of DOC in soil water extracts. In 2016, a significant increase in DOC was seen in the AM treatment in June. This follows the total amounts added with manure that year. In 2016, a higher volume of manure was added (233 L per plot) than in 2015 because of a lower N concentration. An increase in DOC concentration over the growing season with the use of organic fertilizers (composts) has also been confirmed in a recent study (Manninen et al., 2018). The rather low water-soluble concentration of Mo in our study may be explained by the fact that the strongest adsorption of Mo to the soil occurs at around pH 5 (Smith et al., 1997), which is not different from the pH values measured in the samples from our field experiment in August. Despite a pH change from 6.2 to 5.1 from June to August, no significant changes in water-extractable Mo concentrations were observed.

The total Cu concentrations in plants were significantly increased in the digestates treatments especially at the sampling in August 2015 (Figure 5) when compared to control plots, but grain Cu concentrations at harvest (year 2016) were not significantly increased. The contamination factor was below 1 for Cu, Mn, and Mo for all treatments (Figure 7), which implies a low risk of soil contamination.

Influence of Biogas Digestates Fertilization on Trace Metals Not Essential to Plants

For this group of trace metals (Cr, Cd, and Al), the total soil concentration was significantly higher in the plots treated with biogas digestates (Figure A1) when compared to NN and MF plots. Use of both digestates significantly increased total Cr and Cd concentrations in soil (season 2016), while only DIG2 increased the total Al content of the soil. This is due to the high concentration of Cr and Al, but also Cd (2016) in the digestates. Cr concentration in the digestates was above 150 mg Cr/kg DM (Table 3), which was higher than the limits set by the Norwegian regulation on organic fertilizers used in agriculture (60 mg/kg

dry fertilizer, class I). Slightly higher values were also seen for Cd (Table 3), while Al content is not regulated for organic fertilizers in Norway. The Cd content in organic wastes vs. that in mineral fertilizers is an important issue of discussion. The maximum allowed total concentration of Cd in soils treated with organic waste such as sludge was 3 mg/kg (Brunetti et al., 2012), and this limit was not been exceeded in the two growing seasons. Toxic values for total Cr concentration in agricultural soils may vary between 150 and 400 mg/kg depending on the soil type (Kabata-Pendias and Pendias, 2001; Kabata-Pendias, 2011). In both seasons, total Cr concentration did not exceed 100 mg kg⁻¹.

The water-soluble Cd concentrations were low in all treatments in the first season, as Cd additions with the organic fertilizers were low and little Cd was present in the soil initially. It is important to mention that the field experiment was started with the same treatments already in 2014, while the samples were taken and analyzed in the next 2 years (2015 and 2016). This may be important in evaluating potential Cd accumulation in the soils treated with digestates. Research studies with similar levels of Cd in organic fertilizers have also shown that the addition of organic fertilizers (manure) does not cause a significant change in the soil Cd content (Xu et al., 2015). In both growing seasons, Cd was strongly correlated to the pH then to DOC, which is expected and also confirmed in a recent study (van der Sloot et al., 2017), where lower pH was seen to increase the Cd soil water-extracted concentrations. (Van Zwieten et al., 2013) reported that application of organic fertilizers (poultry litter) with pH above 7 has resulted in soil pH decrease during the growing season (Van Zwieten et al., 2013), which has also seen for our experimental set up. There is a significant increase of Cr water-soluble concentrations during both growing seasons (Figure 3) for digestate treatments when compared to NN and MF. Still, measured concentrations were below the limit of 50 µg L⁻¹ of total Cr, which is a recommended value for fresh waters (World Health Organization, 2006). The values measured in both seasons were comparable to those found in a similar field experiment

reported by Wierzbowska et al. where values were also below 50 $\mu\text{g L}^{-1}$ (Wierzbowska et al., 2016). In the pH range measured for both growing seasons, Cr(III) is expected to be the predominant form of Cr present in the soil solution (Bradl, 2004; Choppala et al., 2018). The same authors are also suggest that lower pH values (below 6.5) considered as a major factor that is increasing the level of Cr(III) sorption to soil particles. This can be used to explain the low water-extractable Cr concentrations found in our study (Figure 3).

Aluminum availability in soil and soil pore water is generally influenced by soil pH and DOC. In our study, both pH and DOC showed good correlations to water-extractable Al concentrations. The high total Al concentration in DIG2 was due to the sewage treatment process where Al salts were used for precipitation of P from sewage water prior to anaerobic digestion. Aluminum phosphates are poorly soluble, and this is clearly reflected in similar levels of water-extractable Al in DIG2 and even though no Al salts were added in the process for DIG1. In general, Al does not occur in toxic forms in the soil at pH values suitable for cereal production (Wang et al., 2006).

Low concentrations in both total and water-soluble forms have resulted in low Cd levels in the plant material (Figure 5), with a significant increase in Cd grain concentration only in the AM and DIG2 treatments. The amounts of Cd taken up by grains calculated per hectare were very low for all treatments (Figure 6). Cd originating from sewage sludge and similar sources is controlled more strictly than that from mineral fertilizers, although the latter can have a high Cd content (Pizzol et al., 2014). Digestate treatments had no significant effect on plant Cd or Cr concentrations and measured concentrations were comparable with AM or MF. The contamination factor for Cd, Cr, and Al though moderate, were highest from all the CFs measured in this study. The contamination factor for Al is of little relevance unless pH levels decrease below the adequate range (below pH 4) for cereal production, which could firstly affect the barely production.

In our field experiment with cereals, biogas digestates were applied at the same fertilization rates (100 kg/ha available N). At these rates, there was little difference in the effects of the applied fertilizers on soil or crop metal concentrations. Concentrations measured in the cereal grains were well below maximum allowed values found in human exposure studies with cereal crops (Huang et al., 2008). Still, research on accumulation rates over a longer period of application may give valuable insight into soil processes that the use of organic fertilizers can induce in soil. Our field experiment was only conducted for 3 years, and

measurements only exist for the last 2 years. Longer time series will be necessary for assessing the plant uptake of trace metals after repeated additions of digestates.

CONCLUSIONS

In our study, we have reported for the first time results from field experiment conducted through two growing seasons to investigate the application of two different commercial biogas digestates based on food waste and food waste/sewage sludge mixture. The main hypothesis that the application of biogas digestates as organic fertilizers would increase the mobility and thus plant availability of trace metals in soil was not entirely confirmed. While total concentrations of Ni, Cd, Zn, and Cr were increased in soil upon application of digestates, their water-extracted concentrations were below the defined limits established by WHO. In addition, total Cu, Mn, and Mo concentrations were not significantly increased in the soil. Both pH and DOC were important factors in determining the mobility of the trace elements, in most cases increasing the water-soluble concentrations of trace elements from June to August. Plant concentrations did not indicate an increased uptake of trace metals into the cereal crops due to digestate application. Based on these results, the use of digestates can be compared to the use of animal manure or mineral fertilizers with respect to trace metal accumulation in soil and grain uptake. Still, a period of 2 years is too short to conclude on potential trace metal accumulation in soils due to the use of digestates as fertilizers.

AUTHOR CONTRIBUTIONS

In regards to the work done regarding this manuscript, ID main tasks were sampling during the field experiment, sample analytical lab work, statistical considerations, manuscript draft, and final version preparation. SE-G main tasks were planning and organizing the field experiment, experiment yield measurements, help with the sampling and work on the draft and manuscript final version, while TS main tasks were planning the field experiment and work on the draft and final version of manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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APPENDIX

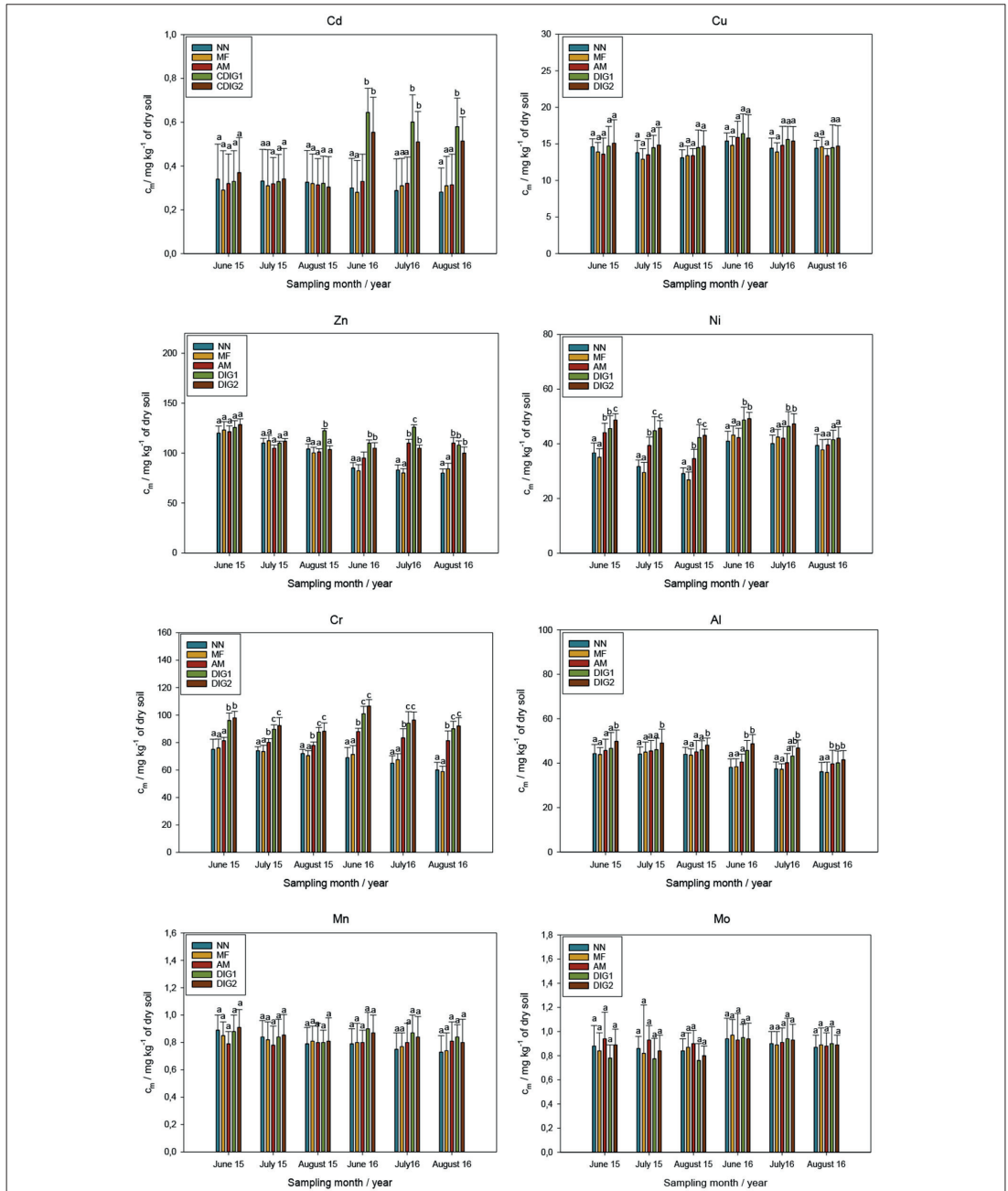


FIGURE A1 | Variability of total metal concentrations in soil samples taken during the growing seasons 2015 and 2016. The values are presented as means ± SD. Letters are showing significance of differences between the treatments ($p < 0.05$)

PAPER 4

1 **OCCURRENCE OF POLAR ORGANIC MICROPOLLUTANTS IN SOIL**
2 **AND GRAIN FOLLOWING FIELD APPLICATION OF BIOGAS**
3 **DIGESTATES**

4
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24 **ABSTRACT**

25 The use of biogas digestate as an organic fertilizer has been widely promoted over the last
26 few decades as part of global initiatives on recycling waste and new sustainable energy production.
27 While many studies have confirmed the positive effects of digestates on soil fertility and structure,
28 there remains a lack of information on the potential risk(s) associated with land application of
29 typical food waste- or mixed sewage sludge/food waste-based digestates. The present study
30 investigated the occurrence and fate of 22 polar organic micropollutants (POMs), including 12
31 perfluoroalkyl acids (PFAAs), 5 pharmaceuticals, 3 benzotriazoles, and an artificial sweetener
32 following field application of two different commercial biogas digestates: one food waste-based
33 and one based on a mixture of food waste and sewage sludge. POM concentrations across all BDs
34 were low compared to other studies. At an application rate of 100 kg plant-available nitrogen ha⁻¹,
35 POM soil concentrations following application of all BDs as fertilizer were low (0.1-2.0 ng/g).
36 Using these particular biogas digestates to grow cereal crops does not contribute significantly to
37 the background POM concentrations in soil, consistent with theoretical estimates based on
38 concentrations in BDs. Of the POMs investigated, only PFOA and 1-Methyl-1H-benzotriazole
39 were detectable in grains, but this appeared to be unrelated to fertilizer treatment and showed
40 little fluctuation over 3-month sampling period. Overall, these results point to the benefits of using
41 BDs as agricultural fertilizer due to a relatively low concentrations of POMs.

42 **Keywords:** perfluorinated compounds, pharmaceuticals, organic waste, soil, organic fertilizers

43 **1. INTRODUCTION**

44 Climate change, renewable energy, and food production have been arguably the most
45 challenging and urgent problems faced by environmental scientists and policy makers over the last
46 few decades. Industrial production of biogas through the anaerobic fermentation of organic
47 material represents just a small part of the green industry agenda of the 21st century (Gissén et al.
48 2014; Hijazi et al. 2016). The importance of the biogas industry's contribution to the circular
49 economy and environmentally friendly energy production has resulted in an increase in the number
50 of biogas plants in Europe. According to a report by the European Biogas Association (EBA) there
51 were 17,439 biogas plants registered in Europe by the end of 2017 (EBA 2018). For two of the
52 largest producers, Italy and Germany, the quantity of digestate produced is estimated at ~30 and
53 70 million tonnes/year, respectively (German biogas association 2018; Italian biogas association
54 2018). This material is largely regarded as waste. Consequently, sustainable solutions for disposal
55 and/or reuse of biogas digestate are needed.

56 Using biogas digestate as fertiliser is attractive due to its high concentrations of nutrients
57 (Möller & Müller 2012) and some organic matter, which may collectively improve the quality of
58 soil. Many commercial biogas plants utilize a mixture of source-separated organic waste from
59 private households, catering, and food industry, and/or sewage sludge as feedstock. Farm-scale
60 biogas plants in particular, also include animal manure. The different types of feedstock may lead
61 to the occurrence of both inorganic and organic pollutants in the digestates. To date, most studies
62 investigating contaminants in digestates have focused on legacy persistent organic pollutants
63 (POPs). One of the earliest studies showed that digestion tends to increase the concentrations of
64 organic compounds such as polychlorinated biphenyls (PCBs) and polycyclic aromatic
65 hydrocarbons (PAHs) in digestates (Brandli et al. 2007). Studies in Finland have shown that

66 seasonal application of biogas digestates as fertilizers is equal to an annual soil burden of
67 polychlorinated dibenzo-p-dioxins and furans coming from the atmosphere (Suominen et al. 2014).

68 Comparatively fewer data are available on the occurrence of polar organic micropollutants
69 (POMs) in digestates. POMs constitute a wide range of performance chemicals, pharmaceuticals
70 and personal care products, and their transformation products. Perfluoroalkyl acids (PFAAs), for
71 example, are among the most frequently detected POMs, owing to their extreme environmental
72 persistence and extensive production over the last 70 years. PFAAs have been applied to a wide
73 range of commercial products and processes but can also form from transformation of precursor
74 substances (i.e. PFAA-precursors) (Martin et al. 2010). Numerous studies have reported the
75 occurrence of PFAAs in substrates (e.g. wastewater treatment plant water/sludge) that are usually
76 used for biogas production (Noorlander et al. 2011; Yu et al. 2009). A preliminary risk assessment
77 in Norway (Norwegian Scientific Committee for Food Safety (VKM) 2009) for the use sewage
78 sludge as organic fertilizer identified perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic
79 acid (PFOS) as substances of concern and prioritized for further assessment. Likewise, a report
80 from Finland identified perfluoroalkyl substances (PFAS) concentrations of up to 50-900 µg/kg in
81 digestates. Other POMs, such as pharmaceuticals and personal care products (PPCPs) have been
82 reported at concentrations of up to 2000 µg/kg in biogas digestates (Suominen 2013). PPCPs are
83 known to occur at high concentrations in sewage sludge/waste water, making this one of the most
84 challenging substrates for biogas production (Carballa et al. 2007; Yang et al. 2017).

85 The stability of POMs during the digestion process is both substance- and process-specific.
86 While Narumiya et al. (2013) reported a decrease in triclosan levels in digested sludge during
87 anaerobic treatment (Narumiya et al. 2013), other POMs, such as carbamazepine, tend to persist
88 during this process (Armstrong et al. 2017). While there is little work on the fate and behavior of

89 PFAAs during digestion, PFAA are highly persistent and not expected to degrade (Yu et al. 2009;
90 Zareitalabad et al. 2013). In cases where POM transformation occurs during digestion, additional
91 challenges exist, since transformation products may have different toxicological properties than
92 their parent compounds and are more difficult to monitor due to the absence of authentic standards.
93 Overall, the presence of POMs in digestates suggests that the anaerobic digestion which occurs
94 during biogas production is not a reliable means of degrading POMs.

95 The field application of fertilizers containing POMs is of particular concern due to the
96 mobility of POMs, which can lead to contamination of ground and/or surface water (Hua-Jie et al.
97 2014) or uptake in plants intended for human and/or animal consumption (Gottschall et al. 2012;
98 Holling et al. 2012). The translocation of POMs from soil to plants is dependent on several factors
99 such as soil type and soil organic matter (Pullagurala et al. 2018). Recent plant uptake studies have
100 reported the occurrence of PFAAs, antibiotics, personal care products and other micropollutants
101 in both grains and different kind of vegetables grown using bio-solids, waste water or animal
102 manure in field conditions (Navarro et al. 2017; Pan & Chu 2017; Wu et al. 2015), but also in
103 vegetables grown in compost amended soils (Bizkarguenaga et al. 2016).

104 Given the limited data available surrounding the presence of POMs in biogas digestates,
105 the overall aim of the present study was to investigate 21 POMs (12 PFAAs, 5 pharmaceuticals, 3
106 benzotriazoles and an artificial sweetener) in two different biogas digestates both before and after
107 field application. Our objectives were 3-fold: first, to quantify a range of POMs of emerging
108 concern in digestates derived from 2 unique feedstocks; second, to assess the fate of these POMs
109 in soils following field applications, and finally, to determine whether any POMs have the potential
110 to translocate grain planted on the field.

111

112 2. MATERIALS AND METHODS

113 *2.1 Targets of interest*

114 The 21 POMs investigated here were selected based on a combination of environmental
115 relevance, digestate screening, and the availability of analytical methods. The PFAAs included:
116 linear and branched isomers of perfluorooctane sulfonic acid (l- and br-PFOS, respectively),
117 perfluorohexane sulfonic acid (PFHxS), perfluorohexane alxyl acid (PFHxA), perfluoroheptanoic
118 acid (PFHpA), , l- and br- isomers of perfluorooctanoic acid (l-PFOA and br-PFOA, respectively),
119 perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluorodecanesulfonic acid
120 (PFDS), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA),
121 perfluorotridecanoate (PFTriDA) and perfluorotetradecanoic acid (PFTeDA). The 9 non-
122 fluorinated POMs included: 1-H-benzotriazole, 1-methyl-1H-benzotriazole, 4-hydroxy-
123 benzotirazole, acesulfame, acetaminophen, acridine, carbamazepine, oxazepam and venlafaxine.

124

125 *2.2 Organic fertilizers used in field experiment*

126 Two commercial biogas digestates (BD) and an animal manure were used as fertilizer for
127 the field experiment. Selected characteristics of digestates and manure are provided in Table 1.
128 The BDs were produced at two municipal biogas plants in southeastern Norway. Each company
129 used different mixtures of substrates and process technology for biogas production. The food
130 waste-based biogas digestate (FWBD) was produced as a by-product of biogas production using
131 pre-treated (thermic hydrolysis), source-separated food waste as substrate for the anaerobic
132 digestion process. This is usually done within 24 days under mesophilic conditions with
133 temperature at 38 °C. The second digestate was based on a 50:50 mixture of municipal sewage
134 sludge and municipal food waste (SS/FWBD). Sewage sludge is produced in a separate waste

135 water facility and then transferred into the same tank where food waste was put after the
 136 homogenization process. For the sterilization process mixed substrate stays at least 1 h at 70 °C
 137 before pumping the substrate to digestion tank. The anaerobic digestion is then conducted under
 138 the thermophilic conditions at temperature around 60 °C. The animal manure was a cattle manure
 139 from the University farm at NMBU in Ås (Norway).

140

141 **Table 1.** Biogas digestates and animal manure basic fertilizing characteristics with amounts used
 142 for field experiment. The total carbon (Tot. C) was measured in dry sample. Values behind ± are
 143 given as standard deviation.

Treat.	pH	Dry matter %	Tot. C %	DOC mg L ⁻¹	NO ₃ -N mg/kg	NH ₄ ⁺ -N g/kg	Added amounts per field plots (0.0021 ha) L
AM	7.3	6.6 ± 0.5	41.4	5879 ± 11	56.0 ± 1.5	21.1 ± 1.1	150
FWBD	7.5	3.1 ± 0.4	39.7	3899 ± 5	95.1 ± 1.2	37.4 ± 1.8	183
SS/FWBD	7.1	1.6 ± 0.4	31.8	3630 ± 9	176.8 ± 3.1	55.1 ± 2.1	232

144 *concentration of NO₃-N and NH₄-N were measured in wet sample; DOC – dissolved organic carbon *tot*-total concentration, total
 145 carbon (Tot. C) was measured in dry sample.

146

147 2.3 Study site and sample collection

148 The field experiment was localized at 59°39'50.4"N 10°45'32.3"E in the municipality of
 149 Ås, southeastern Norway. The field was spring-ploughed and harrowed prior to addition of
 150 fertilizers in mid-May. The fertilizers were spread unto the surface and incorporated into the soil
 151 by harrowing within 24 hours, after which the fields were sown. The experiment was organized as
 152 a randomized complete block design with three blocks and a plot size of 3 × 7 m (0.0021 ha). The
 153 treatments consisted of a control without fertilizer addition, AM and the two BDs, each replicated
 154 3 times. The quantity of each treatment applied to the field plot was normalized to an inorganic
 155 nitrogen (N) content of 100 kg N ha⁻¹, which is common for cereal production in Norway (Table
 156 1). In 2016, oats (*Avena sativa* L.) were grown as a test crop.

157

158 Soil was sampled from each treatment plot from June to August, in the first week of each
159 of the sampling months, respectively. The soil samples were then dried (55 °C), sieved (< 2 mm)
160 and stored at 4 °C prior to analysis. Oat grains were collected after the harvest sieved and stored
161 in the same way as soil samples until further analysis.

162 The soil at the field site was a loam (13 % sand, 57 % silt and 30 % clay), as determined
163 by the pipette method (Elonen 1971). It was classified as an Epistagnic Retisol according to the
164 World Reference Base for Soil Resources (IUSS working group WRB 2015). Prior to the
165 experimental season 2016, the pH, total carbon, and dissolved organic carbon content in the soil
166 were 5.4 ± 0.4 , 2.42% (dry), and $29.7 \pm 1.3 \text{ mg L}^{-1}$, respectively.

167

168 2.4 Standards and reagents

169 LC/MS grade water was purchased from VWR (Darmstadt, Germany), methanol (MeOH) from
170 Merck KGaA (Darmstadt, Germany) and acetic acid (HAc, $\geq 99.7\%$) from Sigma-Aldrich
171 (Darmstadt, Germany). Native and isotope-substituted internal standards were purchased from
172 either Toronto Research Chemicals Inc., (North York, Canada), Sigma-Aldrich (Darmstadt,
173 Germany) or Wellington Labs (Guelph, ON, Canada). A complete list of standards and suppliers
174 is provided in the supporting information (SI).

175

176 2.5 Analysis of basic chemical parameters of digestates, animal manure and soil

177 The soil pH was determined using a soil to water ratio of 1:2.5. Total carbon content was
178 determined in soil, digestates and manure by dry combustion (Nelson D.W. 1982). at 1050°C,
179 using a Leco Carbon Determinator EC12. Instrumental analysis was performed shortly after

180 collecting the samples. Concentration of dissolved organic carbon (DOC) in soil extracts, manure
181 and digestates was determined using a Shimadzu TOC analyser. Determination of NO_3^- and NH_4^+
182 in digestates and animal manure was carried out in fresh samples by flow injection analysis using
183 FIAstar 5000, FOSS, after extraction with 2M KCl.

184

185 *2.6 Extraction of POMs*

186 Prior to extraction, organic fertilizers, grain and soil samples were dried (24 h, 105°C).
187 Two separate methods were used for extraction and analysis: one for PFAAs and the second for
188 non-fluorinated micropollutants. For PFAAs, the extraction procedure was a modified version of
189 the Powely method (Powley et al. 2008). Briefly, 1 g of dry solid sample (i.e. soil, fertilizers, or
190 grain) was spiked with surrogate standards and extracted two times with methanol. After
191 centrifugation and supernatant collection, the extracts were evaporated to approximately 1mL. The
192 concentrated extract was cleaned-up with activated carbon, centrifuged and supernatant were
193 stored at -20 °C prior to analysis by liquid chromatography tandem mass spectrometry (LC-
194 MS/MS). Fertilizers, soil, and grain samples were extracted and analysed in triplicate.

195 Extraction of non-fluorinated POMs was carried out using 1 g of dry soil, organic fertilizer
196 and grain samples. Samples were spiked with surrogate standards, dispersed with 1.5 mL of
197 methanol, and then vortexed for 3 minutes and left in the fume hood for the methanol to evaporate
198 overnight. Thereafter, the samples were supplemented with 20 mL of acetonitrile (ACN) sonicated
199 for 15 minutes in an ultrasonication bath, and then centrifuged for 5 minutes at 2000 RPM. The
200 supernatant was subsequently transferred to a clean 15mL tube. The ACN extraction was repeated
201 and the extracts were combined. The resulting extract was placed in a Buchi evaporator and

202 reduced to a volume of <1 mL. The final extraction volume was diluted with 4 mL of water and
203 stored at -20 °C for 48 h prior to instrumental analysis.

204

205 *2.7 Instrumental analysis of POMs*

206 Instrumental analysis of PFASs was carried out by liquid chromatography tandem mass
207 spectrometry (LC-MS/MS) using a Waters Acquity UPLC coupled to a Xevo TQ-S triple
208 quadrupole mass spectrometer (Waters Corp., Milford, MA, U.S.). The instrument was operated
209 in negative electrospray ionization (ESI), selected reaction monitoring (SRM) mode (see SI for
210 details). Quantification was performed by isotope dilution using a 5-point calibration curve (linear,
211 1/x weighting), which was run before and after samples. For most targets, analogous isotopically
212 labelled internal standards were available.

213 Non-fluorinated micropollutants were analyzed by LC-MS/MS using a Thermo Scientific Ultimate
214 3000 UHPLC system coupled to a Thermo Scientific Quantiva triple-quadrupole mass
215 spectrometer (Thermo Scientific, Dreieich, Germany). The instrument was operated under polarity
216 switching, SRM mode using an ESI source. Details can be found elsewhere (Posselt et al. *In*
217 *preparation*). Calibration standards were measured before and after each batch of samples and
218 quantification was performed using a linear calibration curve for all targets.

219

220 *2.8 Quality control*

221 Accuracy and precision were evaluated using matrix-specific spike/recovery experiments.
222 Approximately 6 ng of individual PFAAs were used for fortification of BDs (n = 2) and ~14.5 ng
223 of individual PFAAs were used for fortification of soil (n=5) and grain (n=2). For fluorinated
224 POMs the LOQs was defined as the concentration producing a signal-to-noise ratio of 3,

225 extrapolated from the lowest calibration point. If there was a blank contamination present the LOQ
226 was set at blank + 3×STD. For non-fluorinated POMs fortification was in the range 387.28 – 500.5
227 ng for all three matrices. Blanks were included during analysis of both fluorinated (n=6) and non-
228 fluorinated (n=6) POMs to account for laboratory background contamination. Matrix-specific
229 LODs and LOQs for the non-fluorinated POMs were defined as the concentration producing a
230 signal-to-noise ratio (s/n) of 3:1 and 10:1, respectively. Exceptions were carbamazepine and
231 venlafaxine, in which calculated LODs were negative due to the calibration curves having larger,
232 positive y-intercepts. In these cases the LOD was set to the LOQ.

233

234 *2.9. Statistics*

235 Statistical analyses was carried out using Sigma plot version 14. One-way Analysis of
236 variance (ANOVA) with Tukey's posthoc test was carried out to determine the significant
237 differences among investigate targets for each of the digestate/manure treatments or significant
238 difference between the treatments for the crop uptake study.

239

240 **3. RESULTS AND DISCUSSION**

241 *3.1 Quality control*

242 With the exception of PFDS, PFTrDA, and PFTeDA, method accuracy and precision were good
243 for most PFAAs in all matrices, with mean percent recoveries ranging from 92-129, 89-108, and
244 from 93-120 in BDs, soil, and grain respectively. For PFDS, PFTrDA, and PFTeDA, lower
245 recoveries across all matrices were observed, likely due to an absence of exactly-matched,
246 isotopically-labelled internal standards (see SI for details). PFAA concentrations in blanks were
247 below detection limits for most targets (Table S5), with the exception of l-PFHxS and l-PFOA.

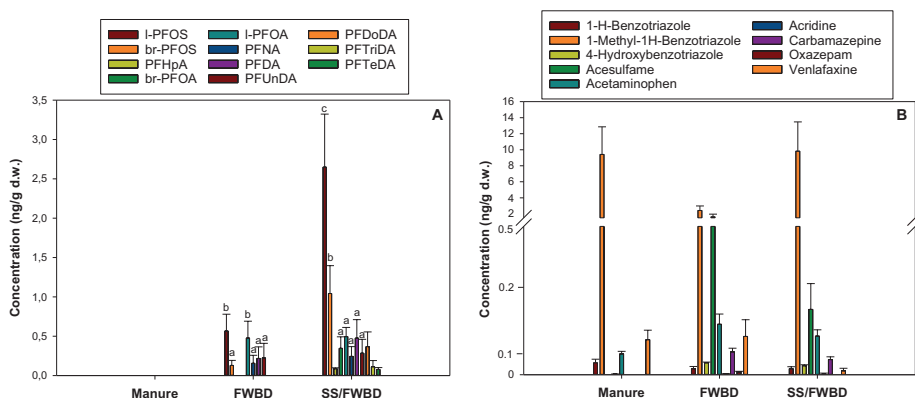
248 For non-fluorinated POMs, with the exception of acridine and 4-hydroxybenzotriazole, the mean
249 percent recoveries ranged from 118-147, 110-231, and 121-180% in BDs, soil, and grain
250 respectively. Acridine and 4-Hydroxybenzotriazole displayed lower recoveries, ranging from 40-
251 83 and 4-62 %, respectively. Anomalous apparent recoveries (Table S6) for some non-fluorinated
252 POMs are likely due to low fortified amounts relative to background concentrations for some
253 matrices and the absence exactly matched, isotopically labeled internal standards in other
254 instances. Reported concentrations for these targets should be considered semi-quantitative. In
255 general, concentrations of non-fluorinated POMs in blanks were below method detection limits
256 for most targets with the exception of 1H-benzotriazole, which was observed at a concentration of
257 0.002 ng/g, which is considerably lower than real samples.

258

259 *3.2 Occurrence of POMs in biogas digestates and animal manure.*

260 A total of 12 PFAAs and 9 non-fluorinated POMs were observed in at least 1 fertilizer (digestate
261 or manure) in the present work. Sum (Σ) PFAA concentrations (\pm standard deviation) were highest
262 in SS/FWBD (9.53 ± 0.20 ng/g) followed by FWBD (2.26 ± 0.10 ng/g), and non-detectable in
263 manure. In both digestates, the PFAA homologue profile was dominated by PFOS (Σ isomers; 0.6-
264 2.65 ng/g) followed by PFOA (approx. 0.50 ng/g). The main difference between PFAA
265 concentrations among the digestates was the significantly ($p < 0.05$) higher concentration of
266 Σ PFOS in SS/FWBD and the presence of numerous PFCA homologues. This is not surprising as
267 sewage sludge is a known source of PFAAs (Cervený et al. 2018; Eriksson et al. 2017; Paolini et
268 al. 2018; Yu et al. 2009). PFAS concentrations reported here are significantly lower than those
269 reported previously (3.4 to 35 $\mu\text{g kg}^{-1}$ d.w.) for a screening study on composts and digestates
270 (Brandli et al. 2007). For non-fluorinated POMs, 1-methyl-1H-benzotriazole (9.82-2.42 ng/g) and

271 acesulfame (0.2-1.62 ng/g) were the predominant substances detected in fertilizers. However,
 272 unlike PFAAs, concentrations did not consistently decrease in the order SS/FWBD > Manure >
 273 FWBD. For 1-methyl-1*H*-benzotriazole, concentrations were similar in both SS/FWBD and
 274 manure (9.2-10.3 ng/g), and at lower levels (0.5 ng/g) in FWBD. For acesulfame, concentrations
 275 were 1.62 ng/g in FWBD followed by SS/FWBD with 0.22 ng/g and non-detectable in manure. In
 276 contrast, acetaminophen was detected in both BD treatments at similar concentrations (0.13-0.17
 277 ng/g d.w.) and not detected in manure. Consequently, these data point to different sources of
 278 individual POMs and highlight the importance of considering a wide range of substance classes in
 279 risk assessments pertaining to fertilizers.



280
 281 **Figure 1.** Concentrations of PFAAs (A) and non-fluorinated POMs (B) in biogas digestates and
 282 animal manure used for the field experiment. Different letters indicate significant difference
 283 between specific targets ($p < 0.05$) for each of the treatments. Error bars represent standard
 284 deviation.

286 Overall, the PFOS and PFOA-dominant PFAA profiles reported here are consistent with prior
 287 measurements in organic wastes used in agriculture; however, concentrations are considerably
 288 lower than previously reported. For example, Suominen et al. (2014) investigated PFAAs in BDs
 289 produced from different mixtures of municipal sewage sludge, sorted municipal biowaste, food

290 industry by-products, and animal manure and reported Σ PFAA concentrations ranging from 1-170
291 ng/g dry weight with profiles dominated by PFOS (Suominen et al. 2014). Despite the low
292 concentrations in the present work, the occurrence of differing PFAA profiles between the two
293 BDs along with their absence in manure, indicates that the substrate used for the production of
294 biogas plays an important role in influencing the contaminant burden. While both BDs contained
295 low levels of PFAAs, overall, the FWBD appears to be more desirable in terms of lower PFAA
296 concentrations. This is perhaps unsurprising considering food waste in general contains lower
297 levels of PFAAs relative to sewage sludge (Allred et al. 2015; Sepulvado et al. 2011).

298 Among the non-fluorinated POMs, benzotirazoles have already been recognized as a potential
299 environmental problem in Norway because of their persistence in soils and sediments (Breedveld
300 et al. 2003). In our study, reported concentrations of benzotriazoles in BDs are comparable to those
301 found in earlier studies of biosolids application to agricultural soils (Hua-Jie et al. 2014). The
302 source of these corrosion and flame inhibitors is unclear, but may arise from the use of
303 anticorrosive covering in biogas facilities or storage tanks. The remaining non-fluorinated POMs
304 are regularly consumed by humans, and wastewater is identified as main pollution source for the
305 aqueous environment (Wang et al. 2016; Wu et al. 2015). Thus, there occurrence in the SS/FWBD
306 is not surprising. For acetaminophen and venlafaxine, concentrations were comparable to previous
307 measurements in digested organic material and fertilized soils, respectively (Martínez-Piernas et
308 al. 2018; Verlicchi & Zambello 2015) while carbamazepine levels (0.05-0.07 ng/g) were orders of
309 magnitude lower than concentrations in organic wastes or biosolids (140-260 ng/g) (Muñoz et al.
310 2009).

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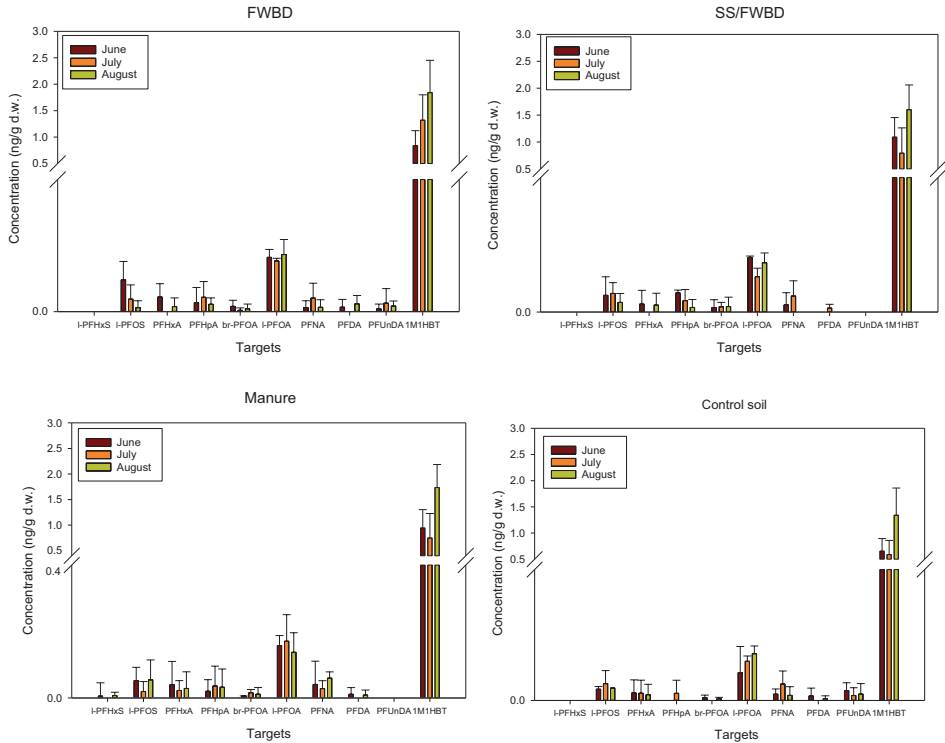
3.3 Occurrence of POMs in soils fertilized with biogas digestates and animal manure

Analysis of soil samples collected from the field experiment revealed low and consistent concentrations (i.e. < a few ppb dry weight) of up to 8 PFAAs and a single non-fluorinated POM (1M-1HBT) in both treated and control plots. The small differences in profiles and concentrations among plots, and the limited differences in time suggested that the BDs had only minor impact on the contaminant loadings. This observation was confirmed by estimating the quantity of POMs added to each plot in the fertilizer. The calculation assumed a mixing depth of 15 cm, plot size of 3 × 7 m, soil density of 1.22 g cm⁻³. As shown in Table 2, the quantity of POMs added via fertilizer is negligible relative to the concentration in the soil, consistent with field observations. This is indeed a promising result for the use of BDs as a safe fertilizer in agriculture. Calculations done for the non-PFAS POMs are located in the SI and the same observation has been seen as for the PFAS in regards to their soil concentrations.

Table 2. Comparison of calculated theoretical Σ PFAS concentrations applied per each of the field plots (0.0022 ha) and the ones measured in soils samples from plots treated with biogas digestates and control plots.

Treatment	% dry matter	Added amounts / L	Dry fertilizer amount applied per plot / g	Σ PFAS in treatments / ng g ⁻¹	Total quantity of PFAS applied per plot / ng	Theoretical* concentrations in soil after treatment addition / ng g ⁻¹	Measured Σ PFAS in soil (June) / ng g ⁻¹	Measured Σ PFAS in control soil (June) / ng g ⁻¹
FWBD	3	183	5673	2.26	12 821	0.0025	0.52	0.31
SS/FWBD	2	232	3712	9.53	35 375	0.0069	0.48	

*Based on the mass of soil of 5124 kg for one plot (calculated based on the depth of digestate incorporation to soil and soil density).



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Figure 2. Total concentration of PFAAs and POMs in soils from plots fertilized with FWBD and SS/FWBD and animal manure (including control plots). The 1M1HBT corresponds to 1-Methyl-1H-benzotriazole. There was no significant difference ($p < 0.05$) found for any of the targets between June and August sampling. Error bars represent standard deviation.

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Several studies have reported on the occurrence of POMs in soil following application of organic fertilizer. For example, an earlier study on the field application of municipal biosolids (dewatered sewage sludge) has showed that venlafaxine was not measured 2 months after application to the soil (Gottschall et al. 2012), consistent with the absence of this substance in our field study. The highest concentration of 1-Methyl-1H-Benzotriazole in soil was approx. 2 ng g⁻¹ dw. These concentrations are comparable to study that included a soil type with similar characteristics (clay content) as ours treated with biosolids (Hua-Jie et al. 2014). Nevertheless, in the same study

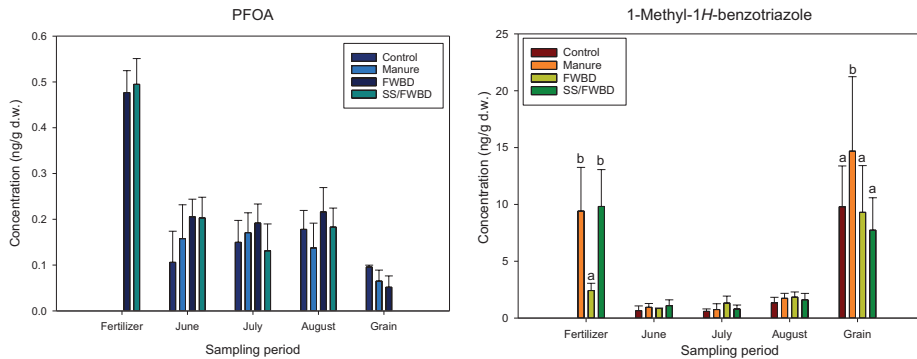
345 application rate was 60 t ha⁻¹ of the agricultural land and sampling was done independent of the
346 growing season (12 months) with the same depth of organic waste soil incorporation (20 cm).
347 Since most of the benzotriazoles are hydrophobic, it was expected that they would sorb onto soil
348 particles, which would influence the soil/plant distribution and make them more persistent in the
349 soil (Lai et al. 2014).

350

351 *3.4 POM Crop uptake*

352 Among all POMs, PFOA and 1-methyl-1H-benzotriazole were detectable in grain (0.05-0.13 ng/g
353 and 8-10 ng/g), but the concentrations were not significantly different among fertilized plots for
354 either substance. For PFOA, these concentrations are low when compared to prior measurements
355 in grain reported for field and greenhouse studies involving dewatered sewage sludge (Blaine et
356 al. 2013; Navarro et al. 2017) and oat grain (12 ng/g) (Stahl et al. 2009). Concerning 1-methyl-
357 1H-benzotriazole, to the best of our knowledge this is the first detection of this substance in cereal
358 grains (oats). Previous studies on uptake of benzotriazoles in other type of plants (lettuce and
359 strawberries) have also indicated uptake of these compounds in the fully-grown edible plant parts
360 that ranged between 15-35 ng/g d.w. (LeFevre et al. 2017). The same study has included the use
361 of a treated wastewater for growing plants, which means that even applied methods of wastewater
362 treatment cannot guarantee the removal of benzotriazoles. This could additionally explain why
363 benzotriazoles were detected in the SS/FWBD were treatment of wastewater (sewage sludge
364 production) is a part of the biogas production process.

365



366

367 **Figure 3.** Concentration of PFOA and 1-Methyl-1H-benzotriazole in biogas digestates (fertilizer),
 368 soil samples and grains from the field experiment. The letter *a* presents no significant difference,
 369 while letter *b* shows significant difference between the treatments for a specific target ($p < 0.05$).
 370 Error bars represent standard deviation.

371
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373 Still, detection of 1-Methyl-1H-benzotriazole in control plots and in FWBD indicates a possibility
 374 of local sources of these compounds. This may be attributed to the storage of digestates in the
 375 tanks protected with anticorrosive coating containing benzotriazoles, especially in the case of
 376 FWBD. The presence of the bezotriazoles in control plots can originate from the field preparation
 377 for the growing season, since the field experiment has been conducted for two years with the same
 378 treatments before the sampling for the current study was done.

379

380 4. CONCLUSIONS

381

382 Our study focused on the presence and disposition of 21 POMs (12 PFAAs, 5
 383 pharmaceuticals, 3 benzotriazoles and an artificial sweetener) in two different biogas digestates
 384 both before and after field application with oats as a growing crop. For PFAAs, concentrations
 385 generally decreased in the order SSFWBD>FWBD>manure. In contrast, non-fluorinated POMs

386 generally displayed similar concentrations in SSFWBD and FWBD and lower levels in manure.
387 Overall, levels of all POMs were low relative to other studies where waste water or dewatered
388 sewage sludge (biosolids) are used as fertilizer. We conclude that these particular digestates do not
389 contribute significantly to the background POM concentrations in soil. Still, potential
390 transformation products, other contaminants that were not included in the target list or even other
391 type of feedstock and applied biogas production technology require further research of biogas
392 digestates use in agriculture. Of the POMs investigated, only PFOA and 1-Methy-*H*-benzotriazole
393 where detectable in grains, but this appeared to be unrelated to fertilizer treatment. Overall, these
394 results point to the benefits of using BDs as agricultural fertilizer due to relatively low
395 concentrations of POMs.

396

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399 organic pollutants from recycling of organic waste as risk factors for human exposure - *NovelPol*).

400

401 **5. LITERATURE**

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SUPPORTING INFORMATION

**OCCURRENCE OF POLAR ORGANIC MICROPOLLUTANTS IN SOIL,
GRAIN AND LEACHATE FOLLOWING FIELD APPLICATION OF
BIOGAS DIGESTATES**

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542 **Table S1.** Summary of selected method parameters for PFAS POMs: retention time, quantitative
 543 and qualitative (Qual.) product ions (m/z) and the internal standards, IS ion and data quality used
 544 for PFASs POMs.

Target	Retention Time (min)	Quant. Ion (m/z)	Qual Ion (m/z)	Standard	Internal Standard	IS Ion	Data quality
L-PFHxA	1.78	313/269	313/119	L-PFHxA	13C-PFHxA	315/270	Quantitative
L-PFHpA	2.18	363/319	363/169	L-PFHpA	13C-PFHpA	367/322	Quantitative
L-PFOA	2.49	413/369	413/169	L-PFOA	13C-PFOA	417/372	Quantitative
L-PFNA	2.76	463/419	463/219	L-PFNA	13C-PFNA	468/423	Quantitative
L-PFDA	3.02	513/469	513/269	L-PFDA	13C-PFDA	515/470	Quantitative
L-PFUnDA	3.27	563/519	563/269	L-PFUnDA	13C-PFUnDA	565/520	Quantitative
L-PFDoDA	3.52	613/569	613/169	L-PFDoDA	13C-PFDoA	615/570	Quantitative
L-PFTrDA	3.74	663/619	663/169	L-PFTrDA	13C-PFDoA	615/570	Quantitative
L-PFTeDA	3.97	713/669	713/169	L-PFTeDA	13C-PFDoA	615/570	Quantitative
L-PFHxS	2.92	399/80	399/99 399/119	L-PFHxS	18O-PFHxS	403/84	Quantitative
Br-PFHxS	2.50	399/80	399/99 399/119	L-PFHxS	18O-PFHxS	403/84	Semi-quantitative
L-PFOS	3.07	498.9/80	498.9/99	L-PFOS	13C-PFOS	503/80	Quantitative
Br-PFOS	~2.95	498.9/80	498.9/99	L-PFOS	13C-PFOS	503/80	Semi-quantitative
L-PFDS	3.57	598.9/80	599/99	L-PFDS	13C-PFOS	503/80	Quantitative
Recovery standards							
Target	Retention Time (min)	Quant. Ion (m/z)					
M8-PFOA	2.49	421/376					
M8-PFOS	3.07	506.9/80					

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549 **Table S2.** Compound detection (LOD) and quantification (LOQ) limits for PFAS and non-PFAS
550 POMs both in ng g⁻¹.

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Compound	LOQ
	ng g ⁻¹
L-PFHxA	0.08
L-PFHpA	0.05
PFOA (L- and Br-)	1.01
L-PFNA	0.04
L-PFDA	0.05
L-PFUnDA	0.43
L-PFDoDA	0.04
L-PFTriDA	0.04
L-PFTeDA	0.04
L-PFHxS	0.04
PFOS (L- and Br-)	0.05
L-PFDS	0.05
Venlafaxine	1.10E-01
1H-Benzotriazole	1.10E-01
1-Methyl-1H-Benzotriazole	1.10E-01
4-Hydroxy-1H-benzotriazole	4.82E-01
Acesulfame	9.20E-02
Acetaminophen	5.14E-01
Acridone	1.14E-01
Carbamazepine	4.00E-02
Oxazepam	8.40E-02
Valsartan	7.80E-02

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553 **Table S3a: MS/MS parameters for the PFAS POMs.**

Source Temp	150°C
Desolvation Temp	350°C
Cone / Desolvation Gas Rate	650 L/hr / 150 L/hr
Capillary voltage	1.0 kV

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555 **Table S3b: MS/MS parameters for the non-PFAS POMs.**

Positive spray voltage	3.7 kV
Negative spray voltage	3.5 kV
Sheath gas	38 arbitrary units
Auxiliary gas	10 arbitrary units
Ion transfer tube temperature	305 °C
Vaporizer temperature	350 °C

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558 **Table S4:** Summary of selected method parameters for non-PFAS POMs: electrospray
 559 ionization mode (ESI), precursor (Pre.), quantitative (Quan.) and qualitative (Qual.) product ions
 560 (m/z) and the internal standards used for quantification.

Chemical	Retention Time (min)	ESI	Pre.	Quant. Ion (m/z)	Qual Ion (m/z)	Internal standard
1H-Benzotriazole	3.29	+	120.1	65.2	39.3	1H-benzotriazole-d4
1-Methyl-1H-benzotriazole	3.59	+	134.1	77.1	51.2	1H-benzotriazole-d4
4-Hydroxy-1H-benzotriazole	2.7	+	136	53.2	80.1	1H-benzotriazole-d4
Acesulfame	1.9	-	162.1	82.1	78	Acesulfame-d4
Acetaminophen	2.35	+	152	65.2	65.2	Acetaminophen-d4
Acridone	4.64	+	196.1	167.1	178.1	Carbamazepine-d8
Carbamazepine	4.87	+	237.1	194.1	179.1	Carbamazepine-d8
Oxazepam	5.1	+	287.1	241	104.1	Oxazepam-d5
Venlafaxine	3.68	+	278.2	58.2	121.1	Venlafaxine-d6

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Table S5. Recoveries calculated from a spike recovery test for PFAS POMs in digestates, soil and grain samples.

Name	I-PFHxS	I-PFOS	PFDS	PFHxA	PFHpA	I-PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrIDA	PFTeDA
Spiking amount (ng)	6.1225	6.1875	6.252	6.48	6.48	6.48	6.48	6.48	6.48	6.48	6.48	6.48
Mean (n=2) digestates recovery (%)	118	127	33	104	94	92	113	129	133	128	82	566 ¹
STDEV	7	38	17	10	10	9	12	17	14	15	24	567 ³¹
Name	I-PFHxS	I-PFOS	PFDS	PFHxA	PFHpA	I-PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrIDA	PFTeDA
Spiking amount (ng)	14.17	14.32	14.47	14.995	14.995	14.995	14.995	14.995	14.995	14.995	14.995	14.995
Mean (n=6) Soil recovery (%)	96	108	38	93	89	89	95	104	104	104	67	570
STDEV	13	13	4	12	11	10	12	10	10	9	7	572
Name	I-PFHxS	I-PFOS	PFDS	PFHxA	PFHpA	I-PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrIDA	PFTeDA
Spiking amount (ng)	14.17	14.32	14.47	14.995	14.995	14.995	14.995	14.995	14.995	14.995	14.995	14.995
Mean (n=2) Grain recovery (%)	113	125	44	101	98	93	101	108	120	119	59	574
STDEV	16	2	4	1	4	5	1	12	13	1	5	575

Table S6. Recoveries calculated from a spike recovery test for non-PFAS POMs in digestates, soil and grain samples.

Name	1H-Benzotriazole	1-Methyl-1H-Benzotriazole	4-Hydroxybenzotriazole	Acetulfame	Acetaminophen	Acridine	Carbamazepine	Venlafaxine
Spiking amount (ng)	457	457	453.1	484.0	387.3	466.0	455.6	500.5
Mean (n=2) digestates, manure recovery (%)	118.45	130.23	62.2	108.8	147.05	40.0	131.4	139.7
STDEV	0.4	5.0	67.1	31.1	13.3	17.7	16.7	13.1
Name	1H-Benzotriazole	1-Methyl-1H-Benzotriazole	4-Hydroxybenzotriazole	Acetulfame	Acetaminophen	Acridine	Carbamazepine	Venlafaxine
Spiking amount (ng)	457	457	453.1	484.0	387.3	466.0	455.6	500.5
Mean (n=5) Soil recovery (%)	116.9	231.40	4.36	110.4	166.1	63.4	122.44	127.12
STDEV	3.6	72.8	1.36	15.5	8.72	15.1	6.0	8.4
Name	1H-Benzotriazole	1-Methyl-1H-Benzotriazole	4-Hydroxybenzotriazole	Acetulfame	Acetaminophen	Acridine	Carbamazepine	Venlafaxine
Spiking amount (ng)	457	457	453.1	484.0	387.28	466.0	455.63	500.5
Mean (n=2) Grain recovery (%)	130.7	180.8	40.4	121.4	166.8	83.84	139.9	132.5
STDEV	11.8	15.4	32.7	1.0	2.9	7.3	12.5	2.8


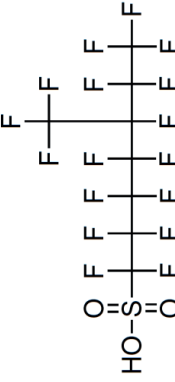
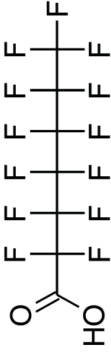
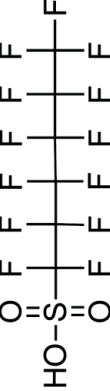
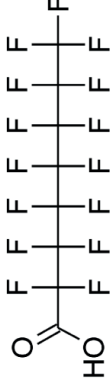
Table S7. IS recoveries for PFAS in digestates, soil and grain samples.

	MPFHxA*	MPFHpA*	MPFOA*	MPFNA*	MPFDA*	MPFUnDA*	MPFDoDA*	MPFHxS**	MPFoS**
Mean (n=3) digestates recovery (%)	73	69	70	62	46	34	25	77	116
STDEV	11	9	10	9	4	5	7	12	12
Name	MPFHxA	MPFHpA	MPFOA	MPFNA	MPFDA	MPFUnDA	MPFDoDA	MPFHxS	MPFoS
Mean (n=6) Soil recovery (%)	64	63	64	58	51	45	37	64	99
STDEV	7	6	6	7	6	6	6	9	12
Name	MPFHxA	MPFHpA	MPFOA	MPFNA	MPFDA	MPFUnDA	MPFDoDA	MPFHxS	MPFoS
Mean (n=2) Grain recovery (%)	80	57	73	70	60	43	29	75	118
STDEV	12	7	9	10	8	7	7	12	15

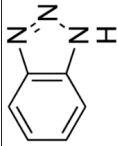
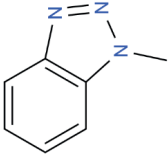
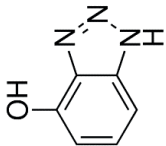
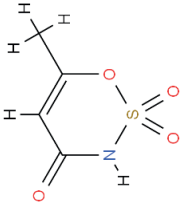
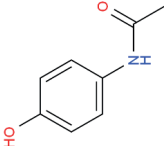
* - based on the M8PFOA

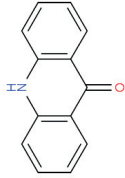
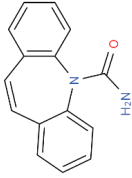
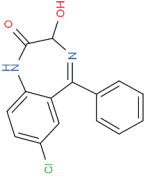
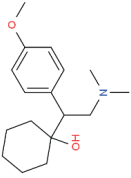
** - based on the M8PFOS

Table S8. Names, abbreviations, CAS numbers and structures of PFAS and non-PFAS POM targets used in the study.

Name	Abbreviation	CAS number	Structure
Perfluorooctane sulfonic acid, linear isomer	I-PFOS	1763-23-1	
Perfluorooctane sulfonic acid, branched isomer*	br-PFOS		
Perfluoroheptanoic acid	PFHpA	375-85-9	
Perfluorohexane Sulfonic Acid	PFHxS	355-46-4	
Perfluorooctanoic acid, linear isomer	I-PFOA	335-67-1	

Perfluorooctanoic acid, branched isomer*	br-PFOA		
Perfluorononanoic acid	PFNA	375-95-1	
Perfluorodecanoic acid	PFDA	335-76-2	
Perfluorodecanoic sulfonic acid	PFDS	678-39-7	
Perfluoroundecanoic acid	PFUnDA	2058-94-8	
Perfluorododecanoic acid	PFDoDA	307-55-1	
Perfluorotridecanoic acid	PFTriDA	72629-94-8	
Perfluorotetradecanoic acid	PFTeDA	376-06-7	

1H-Benzotriazole	/	95-14-7	
1-Methyl-1H-benzotriazole		13351-73-0	
4-Hydroxy-1H-benzotriazole	/	26725-51-9	
Acesulfame	/	55589-62-3	
Acetaminophen	/	103-90-2	

Acridone	/	578-95-0	
Carbamazepine	/	298-46-4	
Oxazepam	/	604-75-1	
Venlafaxine	/	93413-69-5	

*-one of the possible isomers

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Table S8. Comparison of calculated theoretical non-PFAS POMs concentrations applied per each of the field plots (0.0022 ha) and the ones measured in soils samples/or limits of detection from plots treated with FWBD (added amounts 183 L, d. m. 3 %, dry amount of fertilizer 5673 g)

Compound	POM concentration in treatment / ng g ⁻¹	Total quantity amount of POM applied per plot / ng	Theoretical* concentration of the POM in soil after treatment addition / ng g ⁻¹	Measured POM concentration in soil soil (June) / ng g ⁻¹	Measured POM concentration in control soil (June) / ng g ⁻¹
1H-Benzotriazole	0.02	113.46	2.21×10^{-5}	<LOD	<LOD
1-Methyl-1H-benzotriazole	2.42	15 735	3.1×10^{-3}	0.9	0.8
4-Hydroxy-1H-benzotriazole	0.04	226.9	4.42×10^{-5}	<LOD	<LOD
Acesulfame	2.0	11 346	2.0×10^{-3}	<LOD	<LOD
Acetaminophen	0.17	980.9	1.74×10^{-4}	<LOD	<LOD
Acridine	0.003	17.2	3.35×10^{-6}	<LOD	<LOD
Carbamazepine	0.08	453.8	8.85×10^{-5}	<LOD	<LOD
Oxazepam	0.009	51.0	9.9×10^{-6}	<LOD	<LOD
Venlafaxine	0.13	737.5	1.44×10^{-4}	<LOD	<LOD

*Based on the mass of soil of 5124 kg for one plot (calculated based on the depth of digestate incorporation to soil and soil density).

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594 **Table S9.** Comparison of calculated theoretical non-PFAS POMs concentrations applied per each of the field plots (0.0022 ha) and the
 595 ones measured in soils samples/or limits of detection from plots treated with SS/FWBD (added amounts 232 L, d. m. 2 %, dry amount
 596 of fertilizer 3712 g)

Compound	POM concentration in treatment / ng g ⁻¹	Total quantity amount of POM applied per plot / ng	Theoretical* concentration of POM in soil after treatment addition / ng g ⁻¹	Measured POM concentration in soil (June) / ng g ⁻¹	Measured POM concentration in control soil (June) / ng g ⁻¹
1H-Benzotriazole	0.015	55.7	1.1×10^{-5}	<LOD	<LOD
1-Methyl-1H-benzotriazole	9.8	36.378	7.1×10^{-3}	1.3	0.8
4-Hydroxy-1H-benzotriazole	0.03	148.5	2.9×10^{-5}	<LOD	<LOD
Acetulfame	0.22	668.2	1.3×10^{-4}	<LOD	<LOD
Acetaminophen	0.13	482.6	9.4×10^{-5}	<LOD	<LOD
Acridone	0.002	7.4	1.44×10^{-6}	<LOD	<LOD
Carbamazepine	0.05	185.6	3.62×10^{-5}	<LOD	<LOD
Venlafaxine	0.01	37.1	7.24×10^{-6}	<LOD	<LOD

597 *Based on the mass of soil of 5124 kg for one plot (calculated based on the depth of digestate incorporation to soil and soil density.

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599 **Table S10.** Comparison of calculated theoretical non-PFAS POMs concentrations applied per each of the field plots (0.0022 ha) and
 600 the ones measured in soils samples/or limits of detection from plots treated with AM (added amounts 150 L, d. m. 6.6 %, dry amount of
 601 fertilizer 9900 g)

Compound	POM concentration in treatment / ng g ⁻¹	Total quantity amount of POM applied per plot / ng	Theoretical* concentration of the POM in soil after treatment addition / ng g ⁻¹	Measured POM concentration in soil (June) / ng g ⁻¹	Measured POM concentration in control soil (June) / ng g ⁻¹
1H-Benzotriazole	0.04	792.0	1.54×10^{-4}	<LOD	<LOD
1-Methyl-1H-benzotriazole	9.4	93 060	0.02	1.1	0.8
Acesulfame	0.002	19.8	3.86×10^{-6}	<LOD	<LOD
Acetaminophen	0.07	1 089	2.12×10^{-4}	<LOD	<LOD
Venlafaxine	0.13	1 287	2.51×10^{-4}	<LOD	<LOD

602 *Based on the mass of soil of 5124 kg for one plot (calculated based on the depth of digestate incorporation to soil and soil density)

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