Speciation of heavy metals and nutrient elements in digestate

Tilstandsformer til tungmetaller og næringsstoffer i biorest

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Preface and acknowledgments

This master thesis presents a study of element speciation in digestate. The experiments of the thesis have been executed at the Isotope Laboratory, Department of Plant and Environmental Sciences (IPM) at University of Life Sciences (UMB) with funding from Bioforsk Soil and Environment – Ås.

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Contents

Abstract

Biogas and digestate constitutes the final products of anaerobic digestion of organic matter in biogas plants. Recycling of nutrients and organic material back to agricultural fields is desirable, and digestate might have great potential as organic fertilizer. However, insufficient knowledge about its properties limits its use. Heavy metals in digestate may pose an environmental risk if the digestate is to be spread on arable land, because the metals directly harm microorganisms and plants, and may accumulate in plants and lead to health effects, such as oxidative stress, in animals and humans. National regulations that may restrict the utilization of digestate apply only for total element concentrations. However, total element concentrations do not always reflect the real environmental threat. In fact, mobility, transfer within ecosystems, biological uptake and effect of trace elements are very much connected to the physico-chemical form of the specific element. This thesis aims at developing a size and charge fractionation procedure for element speciation of digestate, and examines the speciation of elements, with focus on Cr, Ni, Pb, Cd, Zn, Cu, K, Na, S, and P, in four samples of liquid digestate collected from the Mjøsanlegget AS biogas plant to assess the risk associated with utilization of digestate as fertilizer.

The total concentrations of Cr, Ni, and Pb were below limits set by Norwegian legislation in all digestates. The total concentrations of Cd, Zn, and Cu classified three of the digestates in quality classes I, II, and III.

The size fractionation techniques centrifugation and filtration and the charge fractionation technique ion exchange were performed to separate elements into four fractions: particulates > 0.45 μm, labile cation complexes < 0.45 μm, labile anion complexes < 0.45 μm, and non-labile complexes < 0.45 μm. Elements associated with particles were considered to be of low bioavailability, elements in labile cation and anion complexes of high bioavailability, and elements in non-labile complexes of low to variable bioavailability. The results showed that Cu, Zn, Cd, and Pb were to a large degree (> 95 %) present in the digestates as particulates > 0. 45 μm. In addition, Cu, Zn, Cd, and Pb species < 0.45 μm were mainly non-labile complexes. The proportion of Cr present as particulates > 0.45 μm was also relatively high (> 80 %). Between 42–58 % of Ni was present in the fraction < 0.45 μm, which consisted mostly of labile anion and non-labile complexes. For S, between 56–71 % was present as particulates > 0.45 μm, and labile anion and non-labile complexes accounted for the fraction < 0.45 μm. Phosphorus was mainly (> 95 %) present as particulates > 0.45 μm in two of the digestates, whereas both labile anion complexes < 0.45 μm (7–19 %) and non-labile complexes < 0.45 μm (16–17 %) were present in the other two digestates. Potassium and presumably Na were present as mainly labile cation complexes < 0.45 μm.

The results suggest that although total concentrations of heavy metals in some cases exceeded permissible levels, the actual risk associated with digestate fertilization is lower because a large degree of the heavy metals are actually present as species of low bioavailability.

Sammendrag

Biogass og biorest er de endelige produktene av anaerob nedbrytning av organisk materiale i biogassanlegg. Tilbakeføring av næringsstoffer og organisk material til jordbruksareal er ønskelig, og biorest kan ha stort potensial som organisk gjødsel. Manglende kunnskap omkring dens egenskaper begrenser imidlertid bruken. Tungmetaller i biorest kan utgjøre en miljørisiko dersom bioresten skal spres på dyrket mark, fordi metallene kan skade mikroorganismer og planter direkte, og kan akkumuleres i planter og føre til helseeffekter, som oksidativt stress, hos dyr og mennesker. Nasjonale forskrifter som kan begrense bruken av biorest gjelder bare for totale konsentrasjoner av grunnstoff. Totale konsentrasjoner reflekterer i midlertidig ikke alltid den virkelige miljøtrusselen. Faktisk er mobilitet, transport innen økosystemer, biologisk opptak og effekt av sporstoff i stor grad knyttet til den fysiokjemiske tilstanden til det spesifikke grunnstoffet. Denne avhandlingen sikter mot å utvikle en størrelses- og ladningsfraksjoneringsprosedyre for bestemmelse av grunnstoffers spesiering i biorest, og undersøker spesieringen av grunnstoff, med fokus på Cr, Ni, Pb, Cd, Zn, Cu, K, Na, S og P, i fire prøver av flytende biorest hentet fra Mjøsanlegget AS biogassanlegg for å vurdere risikoen forbundet med bruk av biorest som gjødsel.

Totalkonsentrasjonene av Cr, Ni og Pb lå under grensene gitt i norske forskrifter i alle biorestene. De totale konsentrasjonene av Cd, Zn og Cu klassifiserte tre av biorestene i kvalitetsklasse I, II og III.

Størrelsesfraksjoneringsteknikkene sentrifugering og filtrering og ladningsfraksjoneringsteknikk ved bruk av ionebytter ble utført for å separere grunnstoffer inn i fire fraksjoner: Partikulært > 0,45 µm, labile kationkomplekser < 0,45 µm, labile anionkomplekser < 0,45 µm og ikke-labile komplekser < 0,45 µm. Grunnstoff knyttet til partikler ble ansett for å ha lav biotilgjengelighet, grunnstoff i labile kation- og anionkomplekser for å ha høy biotilgjengelighet, og grunnstoff i ikke-labile komplekser for å ha lav til variabel grad av biotilgjengelighet. Resultatene viste at Cu, Zn, Cd og Pb var i stor grad (> 95 %) til stede i biorestene som partikulært > 0,45 µm. Spesier < 0,45 µm av Pb, Cd, Zn og Cu var i tillegg stort sett ikke-labile komplekser. Andelen av Cr til stede som partikulært > 0,45 µm var også relativt høy (> 80 %). Mellom 42–58 % av Ni var til stede i fraksjonen < 0,45 µm, som hovedsaklig besto av labile anionkomplekser og ikke-labile komplekser. For S var mellom 56–71 % partikulært > 0,45 µm, og labile anionkomplekser og ikke-labile komplekser utgjorde fraksjonen < 0,45 µm. Fosfor var hovedsaklig (> 95 %) til stede som partikulært > 0,45 µm i to av biorestene, mens både labile anionkomplekser < 0,45 µm (7–19 %) og ikke-labile komplekser < 0,45 µm (16–17 %) ble funnet i de to andre biorestene. Kalium og antageligvis Na var til stede hovedsakelig som labile kationkomplekser < 0,45 µm.

Resultatene tyder på at selv om totale konsentrasjoner av tungmetaller i noen tilfeller var høyere enn tillatte grenser, så er den reelle risikoen forbundet med biorestgjødsling lavere fordi en stor andel av tungmetallene er faktisk til stede som spesier med lav biotilgjengelighet.

Abbrevations

A	Labile anion complexes < 0.45 µm
AD	Anaerobic digestion
AE	Anion exchanged
As	Arsenic
BGP	Biogas plant
C	Labile cation complexes < 0.45 µm
C (element)	Carbon
Ca	Calcium
Cd	Cadmium
CE	Cation exchanged
CH_4	Methane
Co	Cobalt
CO_2	Carbon dioxide
Cr	Chromium
CRM	Certified reference material
Cu	Copper
DM	Dry matter
F	Filtered
Fe	Iron
HF	Hollow fibre
Hg	Mercury
HMM	High molecular mass
HNO_3	Nitric acid
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
K	Potassium
LOD	Limit of detection
LMM	Low molecular mass
LOQ	Limit of quantification
Mg	Magnesium
Mn	Manganese
N	Non-labile complexes < 0.45 µm
NaOH	Caustic soda
Ni	Nickel
OM	Organic matter
P (element)	Phosphorus
P	Particulates > 0.45 µm
Pb	Lead
T	Total digestate
TCF	Tangential cross flow
Zn	Zinc

1 Introduction

1.1 Biogas and digestate production

There is increasing national and international interest in biogas production by anaerobic digestion (AD) of organic matter (OM). Biogas is rich in methane (CH_4) (60–70 vol.%) (Appels et al. 2008) and constitutes an important renewable energy source that can be utilized in production of electricity, heating or as vehicle fuel. In addition, biogas production is important for the global climate, as it reduces the emissions of greenhouse gases such as CH_4 and nitrous oxide and substitutes the burning of fossil fuels. Furthermore, AD provides efficient management of manure and organic waste. In Europe, energy production from biogas totaled 6 million tons of oil equivalents in 2007, and the annual increase is believed to be 20 % (Weiland 2009). In addition to the valuable biogas, AD produces an organic digested substrate known as the digestate (Holm-Nielsen et al. 2009). Digestate may have great potential applied as fertilizer.

Good feedstock candidates for biogas production are easily degradable biomasses such as animal manure, sewage sludge, the organic fraction of municipal solid waste, energy crops, and agro-industrial residues (Tambone et al. 2010). The biomasses can either be used individually or in mixtures with other components.

1.1.1 Biochemical process of anaerobic digestion

The AD process is a strict anaerobic process which involves many different types of microorganisms. The AD process can be separated into four stages: hydrolysis, acidogenesis, acetogenesis/dehydrogenation and methanation (Weiland 2009). In the hydrolysis stage, high molecular mass (HMM) compounds such as carbohydrates, fats and proteins are degraded by a variety of hydrolyzing bacteria. During hydrolysis, components such as simple sugars, amino acids and fatty acids are produced. Through acidogenesis these compounds are converted into volatile fatty acids, and hydrogen-producing acetogenic bacteria degrade these into acetate, hydrogen and carbon dioxide (CO_2). Finally, methane-forming archaea (methanogens) produce CH_4 and CO_2 – the biogas – from acetate under the consumption of hydrogen. The remaining non-digestible fraction and the dead bacterial biomass constitute the digestate.

1.1.2 The fertilizing properties of digestate

Organic waste is a potential source of nutrients, and represents a good alternative to inorganic fertilizers. The digestate produced during AD of organic waste possesses many fertilizing advantages over other slurry types such as sludge and compost; AD significantly reduces odor (Powers et al. 1999), and digestate is generally more easily handled and has improved flowing

properties that allow for fast soil penetration and thereby reduced ammonia emissions (Weiland 2009). Depending on the biogas management strategy, weed seeds and pathogens such as bacteria, viruses, fungi and parasites present in the feedstock are also to a large degree inactivated (Sahlström 2003).

The short-term nitrogen (N) fertilization properties of digestate are beneficial because AD mineralizes organically bound nutrients, especially N, and the carbon (C)/N ratio is reduced during the formation of CH_4 (Pognani et al. 2009). Anaerobic digestion also increases the plant availability of nutrients such as phosphorus (P) and potassium (K) (Tambone et al. 2010). Due to the breakdown of easily degradable carbohydrates and conservation of lipids and recalcitrant lignin, Tambone et al. (2009) reported that the digestate is highly suitable as a fertilizer.

Despite all its advantages as a soil improvement agent, there are many properties of the digestate that has yet to be scientifically examined and may limit its use.

1.1.3 Heavy metals in digestate

Heavy metals are naturally present in all organic materials, but organic waste and its digestate may also be contaminated by human activity. In Norway, regulation of heavy metals in fertilizers of organic origin is given by "Forskrift om organisk gjødsel" (FOR-2003-07-04-951). The heavy metals raising the highest concerns according to the Norwegian regulation are zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), chromium (Cr) and mercury (Hg). These heavy metals may be present in concentrations above natural background levels in environmental compartments such as soils, and can potentially harm man and environment, and affect crop quality, crop yield, and soil fertility. Heavy metal concentration increases during AD due to the microbial mineralization and loss of volatile solids (Ciavatta et al. 1993). Most national regulations prohibit the use of organic fertilizers, e.g. digestate, if the concentrations of one or more heavy metals are higher than the threshold concentrations. Organic fertilizers that are to be spread on arable land are divided into the 4 quality categories, 0, I, II and III, according to the concentration of heavy metals (Table 1).

There are evidences suggesting that AD increases the complexation of heavy metals with organic ligands and hence lower the mobility of heavy metals in the digestate (Lavado et al. 2005; Marcato et al. 2009).

Table 1: The heavy metal concentrations in the quality classes defined by Norwegian regulations for the utilization of organic fertilizers.

Quality classes	**0**	**I**	**II**	**III**	**Max. permitted level already present when applying class I, II or III**
Element			*Concentrations (mg kg^{-1})*		
Cadmium	0.4	0.8	2	5	1
Lead	40	60	80	200	50
Mercury	0.2	0.6	3	5	1
Nickel	20	30	50	80	30
Zinc	150	400	800	1500	150
Copper	50	150	650	1000	50
Chromium	50	60	100	150	100

(FOR-2003-07-04-951)

1.2 Consequences of heavy metal release to agricultural soil

The term "heavy metals" applies best as a collective term for all metals and metalloids that have relatively high densities, and may cause harm in humans or in the environment at low concentrations (Soghoian & Sinert 2009). The definition includes Cr, Fe, Co, Ni, Cu, Zn, arsenic (As), silver (Ag), Cd, Hg, and Pb. Heavy metals are ubiquitous and natural sources include minerals in soils and sediments as well as wind transport from enriched areas. Human activities, such as metal mining, chemical industry, burning of fossil fuels, discharge of products such as batteries, plastics, paints, inks, body care products, and medicines, and agriculture have led to the introduction and accumulation of hazardous high levels of heavy metals in the biosphere (Smith 2009). Some of the heavy metals are considered essential for humans and other organisms (e.g. Cr, Fe, Co, Cu, and Zn), while others are exclusively considered toxic (e.g. Cd, Hg, and Pb). The toxicity of heavy metals is frequently linked to their ability of causing oxidative stress in organisms at very low concentrations (Stohs & Bagchi 1995).

Increased concentrations of heavy metals by fertilization of agricultural soils may have far-reaching implications. Firstly, disturbance of the natural balance of elements in soil can adversely affect soil microorganism. The microbial biota of soils, represented by bacteria, fungi, algae, and other organisms, participate in many processes (e.g. oxygenation, N_2 fixation, nutrient recycling) which are important for the total health and fertility of the soil. Artificially high heavy metal concentration may disturb these processes, either by directly killing the microorganisms or biochemically disabling them. Regarding microorganisms, a commonly reported degree of toxicity is Cd > Cu > Zn > Pb (Bååth 1989).

Secondly, plants growing on contaminated soil may take up and accumulate heavy metals. Metals are taken up more efficiently by fast-growing plants such as lettuce, spinach, carrot and tobacco

than by slow-growing grasses (Bitton et al. 1997). On the one hand, accumulation of heavy metals by plants may be beneficial in the sense that toxic compounds are removed from the soil. One the other hand, plants cannot distinguish between essential and non-essential elements, and uptake may lead to damaging effects on the plant and to economic consequences for farmers. In addition, heavy metal accumulation in plants may lead to an unwanted transfer of toxic concentrations to higher compartments of the food chain, including animals and humans. The element considered to be the most prone to cause harm via this route is Cd (Verkleij et al. 2009). Dietary intake through consumption of plants may have long-term damaging effects on human health.

Thirdly, if animals are grazing directly on contaminated soil, they may accumulate toxic concentrations of elements by ingesting some of the soil (Nicholson & Chambers 2007).

Below are presented the biological effects, with main focus on human health, of Cu, Zn and Cd, as these are the heavy metals generally found in highest concentrations and thus most commonly limit the use of compost and in addition digestate, as fertilizer (Govasmark et al.).

1.2.1 Copper

1.2.1.1 General aspects

The transition metal Cu is positioned as the 29th element in the periodic table. Both the thermal and electric conductivity is very high, and Cu is being used in thermal and electrical conductors, building materials and as a constituent in metal alloys. Copper may be released to the environment during mining of Cu and other metals, from waste dumps and domestic sewage, by the burning of fossil fuels, waste incineration, wood production, production and utilization of phosphate fertilizers as well as from natural sources (e.g. dust, natural soils, volcanoes, forest fires and sea spray) (ATSDR 2004). The most common oxidation states of Cu encountered in nature is Cu(I) and Cu(II), the latter being the most important (ATSDR 2004). In soil, the concentration of Cu usually ranges between 2–250 mg Cu kg^{-1} soil (ATSDR 2004). The main intake route of Cu is through ingestion, and in adults the dietary intake ranges between 0.013–0.031 mg kg^{-1} body weight day^{-1} (WHO 1998). Foods such as oysters, liver, nuts, legumes, and whole grains are rich in Cu (Gaetke & Chow 2003). Copper also enters the human diet through drinking water (ATSDR 2004). Within the human body, the highest Cu concentrations are found in the liver (Gaetke & Chow 2003).

1.2.1.2 Essentiality

The Cu(II) ion is an essential trace nutrient involved in numerous biochemical functions in higher plants and humans. Excluding invertebrates and microorganism, Cu has, in comparison to other metals, relatively low toxicity. It contributes as a cofactor in many enzymes important for cellular respiration, defense against free radicals and Fe metabolism, such as oxidases and oxygenases (Stohs & Bagchi 1995). Copper may also be involved in gene transcription (Yonkovich et al. 2002). Copper deficiency is frequently correlated to diseases such as diarrhea in

children in developing countries, and may lead to effects like deformations of the skeleton, lung abnormalities, osteoporosis, vascular problems, and anemia (Muñoz-Olivas & Cámara 2001). In plants, Cu plays an important role in production of proteins and carbohydrates, N_2 fixation and desaturation and hydroxylation of fatty acids (Artiola 2005). Plant Cu deficiency symptoms are limited growth, malformation of fresh leaves, and necrosis of the shoot top (Artiola 2005).

1.2.1.3 Toxicity

Visible Cu toxicity symptoms in plants are chlorosis which can be observed in plants when the concentration in dry weight tissue typically exceeds 30 mg Cu kg^{-1} (Artiola 2005). As the fatal dose for acute orally intake of Cu in humans is relatively high (about 10 g), acute poisoning are usually related to accidents or suicide attempts (Nohr & Biesalski 2005). Characteristic effect of acute Cu poisoning is damage to the liver, since this is the first site where Cu is deposited after it enters the blood (Stohs & Bagchi 1995). Symptoms also include vomiting, diarrhea, and coma (Nohr & Biesalski 2005). Chronic effects of Cu overload include damage to the gastrointestinal tract, the liver, the kidneys, the nervous system, and immune system, and anemia (ATSDR 2004). Inhalation of Cu dust particles may lead to irritation in the respiratory tract (ATSDR 2004). No link between Cu exposure and cancer has been established.

On a molecular level, the toxicity of Cu lies in the special electron configuration of the element. Copper(II) ions have a high affinity for sulfur and nitrogen containing groups of biological molecules, and Cu can thus replace other essential elements in enzymes, or distort the function of macromolecules due to binding (Nohr & Biesalski 2005). Copper compounds may also participate in redox reactions and lead to oxidative stress. Oxidative stress disturbs metabolic pathways and may damage biological macromolecules as well as biological membranes. Copper compounds may oxidize thiol compounds and be oxidized by Fe which leads to the formation of free radicals (Stohs & Bagchi 1995). By the recombination of the free radicals, damaging reactive oxygen species are produced, which in turn may cause damage to DNA and to peroxidation of lipids of cell membranes (Chan et al. 1982).

Metallothioneins, which are small biomolecules rich in sulfur, decreases the Cu toxicity by binding to Cu and storing it in the liver where it can be excreted in bile or feces (ATSDR 2004). The counterpart to metallothioneins in plants is phytochelatins (Günther & Kastenholz 2005).

1.2.2 Zinc

1.2.2.1 General aspects

Zinc is the 30th element in the periodic table. The estimated average concentration on the earth's crust is 20–200 mg Zn kg^{-1} soil (ATSDR 2005). Zinc is being applied in protecting coatings of metals (e.g. iron and steel), various alloys (e.g. brass and bronze), paint coatings, and as a chemical catalyst (ATSDR 2005). Through Zn mining and smelting, steel production, coal and waste burning, Zn is released to the environment (ATSDR 2005). Zinc is relatively reactive and does not exist as free element in nature. The most common oxidation state is +2. Food of animal origin, especially sea food, generally contains more Zn than does vegetables and fruits. Ingestion

is the main route of Zn exposure, and the daily intake is estimated to be 0.07–0.23 $kg^{-1}day^{-1}$ (ATSDR 2005).

1.2.2.2 Essentiality

Zinc is an essential trace element which has been observed in the active center of about 300 enzymes, including superoxide dismutase, carbonic anhydrase, RNA polymerase, and alcohol dehydrogenase (Nagajyoti et al. 2010). Zinc is important for growth, development, healing of wounds, the immune system, skin metabolism, functionality of the nervous system, vitamin A metabolism, salvia secretion, senses of taste and smell, sperm production, prevention of carcinogenesis and aging, gonadal function and pregnancy, and glucose and lipid metabolism (Yanagisawa 2008). Zinc deficiency may lead to anorexia, reduced growth, skin symptoms (e.g. dermatitis), baldness, depression, loss of night vision, and dementia (Yanagisawa 2008). In plants, Zn deficiency leads to interveinal yellowing and reduced leaf size (Nagajyoti et al. 2010).

1.2.2.3 Toxicity

Acute toxicity effects following large orally doses of Zn (i.e. 2–8 mg kg^{-1} day^{-1}) include vomiting, abdominal cramps, and bloody diarrhea (ATSDR 2005). The effect most commonly reported following inhalation of Zn is "metal fume fever" (ATSDR 2005). This reversible condition manifests itself by symptoms such as chest pain, coughing, nausea, shortness of breath, and chills. Zinc may also reduce the human absorption of Cu, so a lot of effects of long-term, low doses Zn exposure may be associated with those of Cu deficiency, such as anemia (ATSDR 2005). Zinc overload can also deplete Fe stores. In plants, reduced growth, development and metabolism and induction of oxidative stress are all symptoms of Zn excess (Nagajyoti et al. 2010). Phytotoxicity caused by Zn is quite common as levels of Zn in contaminated soil are often above those recommended for nutrition (Nagajyoti et al. 2010).

1.2.3 Cadmium

1.2.3.1 General aspects

Cadmium has atomic number 48 and as Cd appears in group IIB in the periodic table along with Zn, Cd and Zn share many chemical properties. Cadmium is used in batteries, pigments, and electro-plating and -coatings. Cadmium enters the environment through quite similar routes as Cu and Zn, with the most important releases coming from non-ferrous metal mining and refining (ATSDR 2008). In nature, the only oxidation state of Cd is +2. The estimated average concentration of Cd on the earth's crust is about 0.1–0.5 mg Cd kg^{-1} soil (ATSDR 2008). It is recognized that this ubiquitous element accumulate in plants and animals (Verkleij et al. 2009), and hence the main route of Cd exposure in the non-smoking population is through ingestion of Cd-containing foodstuff (ATSDR 2008). The estimated daily intake of Cd is 0.3 μg $kg^{-1}day^{-1}$ (ATSDR 2008), of which 70 % is by vegetables and especially cereals (Günther & Kastenholz 2005). Depending on the Cd specie, about 3–7 % of Cd ingested is taken up in the gastrointestinal tract (Verougstraete 2005). The Cd uptake seems to be lowered if other trivalent or divalent cations such as calcium (Ca), Cr, Mn and Zn are present (Verougstraete 2005).

1.2.3.2 Toxicity

Cadmium has no known biological functions in higher organisms, and even minor amounts of ingested Cd can cause detrimental effects to the human body (Günther & Kastenholz 2005). The liver and the kidneys are the main target organ for Cd toxicity, but symptoms are also seen in the brain, lungs, heart and the central nervous system (WHO 1992). Main symptoms of acute Cd poisoning include nausea, vomiting, diarrhea, abdominal cramps, headache, and salivation (WHO 1992). The human skeleton is damaged when Cd doses are high and prolonged. Cadmium accumulate in the kidneys, making them the most vulnerable organ to chronic dietary doses of Cd. Cadmium has an estimated biological half-life of 10–40 years in the kidneys (CDC 2009). Renal effects include dysfunctional tubular reabsorption which may develop into End Stage Renal Disease (Järup & Akesson 2009). Chronic Cd exposure may also give mutagenic effects (Waalkes 2000). Cadmium is classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC) (IARC 1993) and has been linked to human lung cancer (Waalkes 2000). Although the evidence for other types of cancers is somewhat inconsistent, Cd is also believed to cause prostate cancer, renal cancer and breast cancer (Järup & Akesson 2009; Waalkes 2000). Visible symptoms of Cd phytotoxic effects include chlorosis, decrease in growth, root tips browning and death (Nagajyoti et al. 2010).

The mechanisms by which Cd exerts its toxicity are not yet fully understood. It is generally accepted that Cd causes lipid peroxidation and oxidative stress (Stohs & Bagchi 1995). In addition, depletion of the defense system, i.e. antioxidant glutathione and sulfhydryl groups of proteins may be important for the toxicity of Cd (Stohs & Bagchi 1995). Cadmium neurotoxicology is believed to be caused by the similarity between Cd and Ca (Foulkes 1990). When Cd competes with Ca at the synaptosome of neurons, Ca uptake is inhibited and thus the neuronal Ca channels are partially blocked. This severely disturbs normal neural function. In addition, Cd^{2+} posses the ability to mimic other divalent essential elements such as magnesium (Mg), Ca, Fe, Cu and Zn and may displace those elements in cationic centers of proteins (Foulkes 1990). Regarding cancer induction, Cd affects cell proliferation, cell differentiation, apoptosis, and other mechanism correlated to carcinogenesis (Mates et al. 2010). In plants, Cd disturbs the uptake and transportation of many essential micronutrients (e.g. Ca, Mg, P and K) and water (Nagajyoti et al. 2010). In addition, Cd inhibits nitrate reductase in the shoots and hence absorption and transport of nitrate is lowered (Nagajyoti et al. 2010).

The relationship between concentration of Ca in the body and Cd toxicity is believed to be of importance. It has been demonstrated that absorption of Cd increases when the body suffer from Ca depletion/deficiency (Foulkes 1990). This enhances the overall toxicity of Cd, and in particular the ability of Cd to act as a neurotoxicant. There also exists an important relationship between Zn and Cd. Due to the similarity between Zn and Cd, Zn is competing with Cd, thus reducing Cd plant uptake (Mengel & Kirkby 2001).

1.3 The concept of speciation

1.3.1 Definitions

Elements in the environment are present in a wide variety of different physico-chemical forms, often referred to as element species. An element's distribution amongst all these different forms is defined as the speciation of that element (Templeton et al. 2000). Trace element species are defined according to their physico-chemical properties; nominal molecular mass, charge properties and valence, oxidation state, structure and morphology, density, degree of complexation, etc. (Salbu 2000). In environmental chemistry, toxicology, and geochemistry, it has been widely accepted that these properties are essential for the elemental behavior, mobility, ecosystem transfer, and biological uptake and effect. Thus to avoid overestimation of the risk posed by heavy metals present in environmental compartments, the concentration of element species and not only total concentrations must be considered.

1.3.2 Environmental element species

Trace elements in soils are present in either the aqueous soil solution or in the solid phase, defined according to a 0.45 µm filter membrane. The association of elements with the solid phase can be reversible (physical and electrochemical sorption processes) where complexation with organic or inorganic ligands in soils is important, or irreversible (chemisorption) where elements are included in mineral lattices. Species in the soil solution include simple ions and complexes with organic ligands (e.g. amino-, carboxyl- and phenolic groups) and inorganic ligands (e.g. carbonate, chloride, hydroxide, nitrate and sulfate) as well as colloids and pseudocollidals (WHO 2006).

Molecular mass is an ecologically important property of chemical species. Figure 1 gives an overview of the size classification. In terms of size classification for species in water, particles are referred to as aggregates with diameters larger than 0.45 µm and due to gravity, these particles

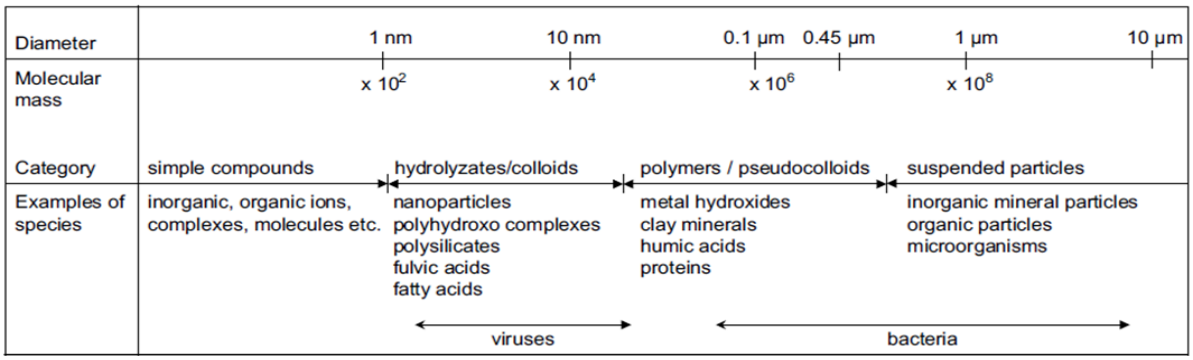

Figure 1: Schematic representation of size classification of species and representatives within the different domains found in the environment. In Salbu 2009.

will sediment in still water (Salbu 2009). Silt (0.063–0.002 mm) and sand (2–0.063 mm) are larger particles. Entities of larger diameters are defined as fragments. Colloids or pseudocolloids have diameters from 1 nm to 0.45 μm. Units smaller than 0.45 μm are not expected to sediment, due to repulsion and Brownian motion (Salbu 2009). Species with diameters less than 1 nm and mass in the range of 1–10 kDa are defined as low molecular mass (LMM) species. The smallest species are complexes, molecules and simple ions.

The elemental charge of the species is a relevant property for its environmental impact. The charge of species is governed by the valence and oxidation state of the element and their ability to form complexes with inorganic and organic ligands (Templeton et al. 2000). Since most soil components (e.g. clays) are slightly negatively charged, the ability to retain anionic species is rather poor and are often more susceptible to washouts than are positively charged species (Cooper et al. 1995).

1.3.3 Bioaccessibility and bioavailability

The presence of an element in the environment does not necessarily imply that biological uptake takes place and that the element affects living organisms. Firstly, the elements have to be accessible, i.e. have the potential of coming in contact with organisms (WHO 2006). For instance, elements included in insoluble particles will remain inaccessible for most organisms, although passive uptake of filtering organisms may occur. Substances located on particle surfaces may also be inaccessible, unless desorption takes place, if uptake of simple ions, molecules or complexes in organisms is required. In soils and sediments, the bioaccessibility of elements may be the factor controlling the biological uptake (WHO 2006). Secondly, the elements must be bioavailable in order to exert their nutritional or toxic effect. Bioavailable substances are those that can be transported from the environment through biological membranes and in to living cells where they can interact with certain target molecules within the cells (Ruby et al. 1999). Regarding humans, the term oral bioavailability is defined as the fraction of a given dose of an element that is actually taken up in the gastrointestinal tract and transferred to the central blood compartment (Ruby et al. 1999). Information on the physico-chemical form of an element is essential in order to judge if the element is bioavailable to living cells (WHO 2006). The amount of bioavailable substances is also dependent on the organism in question (Duffus 2005). Although non-bioavailable species can lead to physical stress and damage and may change the availability of other substances, species that are bioavailable are considered most relevant for nutrition and toxicity assessments. Thus when assessing the environmental risk posed by a specific element, the bioavailable species of that element should be the prime target in analysis (Duffus 2005). As ecosystems are dynamic, however, transformation processes influencing metal species, mobility and bioavailability should also be taken into account.

In general, when the size of the species decreases, the mobility and bioavailability are expected to increase as smaller entities have higher potential of crossing biological membranes (Ruby et al. 1999). In addition, small particles (i.e. colloids) have a larger surface area to volume ratio and are more rapidly dissolved. Simple ions in solution are generally considered to be both bioaccessible

and bioavailable (WHO 2006). Elements that are weakly associated with organic and inorganic soil substances or are complexed with dissolved ligands are believed to be more bioaccessible than are elements sorbed to soil particles or trapped in minerals (WHO 2006). The oxidation state of species is important for the bioavailability, as it to a large extent governs acid-base chemistry, charge, solubility and ligand reactivity, and hence absorption, transport over biological membranes, excretion, and toxicity (WHO 2006). Some elements (e.g. Hg, cobalt (Co), Pb, and As) have the potential of forming organometallic species. As organification changes solubility, lipophilicity, volatility and thus also bioavailability, the organometallic species are important to environmental risk assessments (WHO 2006).

1.3.4 Factors influencing speciation

In all risk assessments that include information on the element distribution between chemical species, it is important to pinpoint that the speciation of a particular element within an environmental system is not set. The elemental speciation may change over time due to physical and chemical weathering, biological processes, infiltration of water and anthropogenic activities (Ruby et al. 1999). Some mobilization processes that have the potential of increasing bioaccessibility are desorption, dissolution and dispersion, while growth mechanisms including hydrolysis, complexation, polymerization, formation of colloids and aggregation are expected to reduce the bioaccessibility (Salbu 2009). The physical and chemical properties of soils, such as pH, hardness, salinity, redox potential, the presence of inorganic substances, humidity, clay content, temperature, and level and form of OM present, greatly influence these processes (Duffus 2005; Vig et al. 2003). Among these, the soil pH is the factor considered to have the strongest impact on speciation. Generally, an increase in soil pH results in increased precipitation, coprecititation (if iron (Fe), manganese (Mn), and aluminum (Al) are present) and soil particle sorption. Conversely, a low pH favors species solubility and increases the fraction of bioaccessible species. The availability of OM is also an important factor to consider. For instance, Cd and Cu have high affinities for OM and may be immobilized if the soil has a high content of OM (ATSDR 2008). Soils with high clay content are generally associated with high retention of cationic elements (Vig et al. 2003).

1.3.5 Fractionation techniques

It has been well established during the last 30 years that properties of metal species may have profound impact on living organisms and that total concentrations alone poorly explain observed effects. This insight has encouraged the development of a great number of techniques and methods for determining speciation (Cornelis et al. 2003). Due to thermodynamic and instrumental restrictions it is most often difficult to distinguish accurately between the different, highly defined chemical species. In this case, fractionation techniques that separate and classify groups of species according to properties such as size, charge, solubility and reactivity may provide sufficient and useful information (Templeton et al. 2000). Fractionation techniques are

Table 2: Techniques for fractionation of trace elements. Modified from Salbu 2000.

Size fractionation	Charged fractionation	Combined techniques
Filtration	Exchange chromatography (cation, anion, adsorption)	Filtration/Ion exchange chromatography
Tangential flow/hollow fibre ultrafiltration	Electrochemical methods	Tangential flow ultrafiltration/Exchange chromatography
Continuous flow Centrifugation	Crown ether chromatography	Dialysis/Exchange chromatography
Ultracentrifugation	Ion selective electrodes	Electrodialysis
Density centrifugation		
Dialysis		
Gel chromatography		
Field-flow fractionation		
Other techniques		
Sequential extraction		
Liquid-liquid extraction		

techniques that physically separate fractions of species to allow for the elemental determination in each fraction. Examples of different fractionation techniques are contained in table 2.

Available techniques that separate species according to size (i.e. molecular mass) include filtration, centrifugation, tangential cross flow (TCF) or hollow fibre (HF) ultrafiltration, flow field-flow fractionation, and dialysis (Salbu 2007). As mentioned, bioaccessibility and bioavailability are dependent on species size, and size fractionation can hence provide useful information relevant for risk assessments.

Among techniques for charge fractionation are exchange chromatography, crown ether chromatography, and electrochemical methods (e.g. electrophoresis) (Salbu 2000). Combined size and charge fractionation techniques, which often greatly improve analysis (Salbu 2009), include filtration coupled with exchange chromatography. Sequential extraction is another fractionation technique that separate species in soil or sediment neither according to size nor to charge, but to their degree of binding to the soil or sediment. In the scheme of Tessier et al. (1979), elements are divided into five fractions: exchangeable, bound to carbonates, bound to oxides of Fe or Mn, bound to OM, and residual. Generally, water soluble and exchangeable forms are considered to be bioavailable; the fractions bound to oxides, carbonates and OM potentially bioavailable; and the mineral occluded portions not bioavailable to either plants or microorganisms.

1.3.5.1 Filtration

In the technique of filtration, a sample solution is passed through a filter (e.g. Millipore filters or Nucleopore membranes) with a specific pore diameter. Particles with sizes larger than the pore diameter are retained on the filter. By measuring element concentrations before and after filtration (e.g. by inductively coupled plasma mass spectrometry (ICP-MS)), the amount of particles with sizes equal to the chosen pore diameter is determined. Since 0.45 μm is commonly regarded as the cut off size for particles, filters of this pore diameter separate particular from dissolved matter. Colloids, pseudocolloids and LMM species can be separated by using membranes with pore sizes in the range 1–100 kDa in TCF or HF. Although filtration is quite popular due to its simplicity, a large source of error associated with the method is clogging of the filter (Salbu 2009). Clogging arises when particles accumulate on top of the filter, leading to a decrease in the effective pore diameter. Hence, the size of the species in the filtrate is no longer unambiguous.

1.3.5.2 Centrifugation

In a centrifuge, the effective gravity force is increased, repulsive forces and Brownian motions are overcome, and both particles and colloids will sediment. The species that have not sedimented can be collected from the supernatant by quick decantation or withdrawal with a Pasteur pipette. Centrifugation speed and time required to achieve a certain cut off size vary according to particle shape, particle charge, porosity, and viscosity (Bufflap & Allen 1995).

1.3.5.3 Exchange chromatography

This technique discriminates between species being positively charged, negatively charged or neutral (Ackley & Caruso 2003). Special resins containing ionic functional groups bonded to polymers such as polystyrene, divinylbenzene, or silica make up the stationary phase. The ionic group of the resin has the opposite charge of the species in the sample to be separated. Through electrostatic interactions, cationic species are sorbed onto cationic resins and anionic species are sorbed onto anionic resins. Activated C or XAD™ can be applied as stationary phase to retain neutral species (van Schaik 2008). Exchange resins retain only labile species, i.e. simple ions and easily ionizable species, whilst non-labile, i.e. stable complexes and colloids, will be kept in solution. Thus, exchange chromatography is highly applicable for distinguishing between the labile and most bioavailable species and the non-labile and less bioavailable species. The fraction of labile species can be calculated as the difference between total element concentration and element concentration in the eluate. However, particles and colloids may be held back by the filtering properties of the resins, leading to an overestimation in the amount of labile species. This source of error can be minimized by performing size fractionation techniques which remove particles and colloids in advance.

1.4 Objectives

The objectives for this thesis were to develop a size and charge fractionation method for speciation of trace elements, in particular the heavy metals Cr, Ni, Pb, Cd, Zn, and Cu, in digestate, and in addition apply this method for trace element speciation in digestate collected at four different times at the Mjøsanlegget AS biogas plant to estimate digestate's suitability as fertilizer. The techniques of centrifugation, filtration and ion exchange were chosen.

2 Materials and methods

General

All water used in analysis was purified in a MilliQ water purification system (> 18 MΩ cm^{-1}). The centrifuge tubes, filtering equipment and ion exchange columns were washed, soaked in 10 % nitric acid (HNO_3) for minimum 12 hours, and rinsed thoroughly with deionized water prior to use.

2.1 The Biogas Plant and sampling

The digestates were sampled at the Mjøsanlegget AS biogas plant (BGP) situated in the Lillehammer region, Norway. The BGP is certified to handle Category 2 material according to the EEC 1774/2002 legislation (European Commission 2002). Food waste from households and industry is the main substrate in the AD at the BGP. The BGP has a current capacity of 14 000 tons of food waste per year. Every fourteenth day, domestic organic waste are collected from households in the Lillehammer region and transported to the BGP where large objects and plastic are removed. Then the waste is grinded, heated to 137 ºC for 24 min at 2.4 bar and placed in a storage tank before it is pumped into the digestion tank where it undergoes a fermentation period of 14 days at 39 ºC. Through centrifugation, the digestate is separated into a fibre fraction and a liquid fraction. A part of the liquid fraction is recycled into the fermentation tank until the ammonia concentration is at maximum 3000 mg dm^{-3}. In order to stabilize the process, the BGP regularly uses an industrial foaming agent (NALCO® 71D5 PLUS, NALCO Norge AS). The pH is adjusted to 7.5 through the use of caustic soda (NaOH).

Four samples of liquid digestate of the anaerobic digested residue were collected from the BGP in (A) January 2009, (B) January 7th 2009, (C) January 13th 2009, and (D) December 15th 2008. The samples were collected from the pipeline and stored refrigerated (4 °C) on polypropylene bottles prior to analysis.

2.2 Analysis

2.2.1 Digestate characterization

The pH and the specific conductivity of each sample of digestate were measured by using InoLab pH 720 with electrode SenTix 21, both from Wissenschaftlich-Technische-Werkstätten (WTW).

Dry matter (DM) percentage was determined in three replicates by weighing the residue of about 15 ml of each digestate after drying in porcelain crucibles at 105 ºC for 24 hours.

The amount of carbon was determined as the ignition loss after heating of the dried samples at 550 °C for 8 hours.

2.2.2 Fractionation

A schematic presentation of the different steps in the fractionation procedure is presented in figure 2. For each of the four digestates A–D three replicates of total digestate (T), centrifuged, centrifuged and filtered (F) through 0.45 µm filters, cation exchanged (CE), and anion exchanged (AE) digestate were made. For the determination of total element concentration in the sample fractions, 5 ml was digested at 250 ºC for 20 minutes using an ultraclave (UltraCLAVE 3, Milestone) in 5 ml distilled ultrapure HNO_3 (Merck). After digestion, all samples were diluted to 50 ml prior to analysis of total concentrations of Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, and Zn by inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer, Optima 5300 DV) and total concentration of Cr, Mn, Co, Ni, Cu, Zn, Mo, Cd, Ba, and Pb by ICP-MS (Perkin Elmer, Sciex Elan 6000). The total digestate samples were diluted 1:5 times with MilliQ water prior to analysis.

A more detailed description of the development and execution of the fractionation procedure follow.

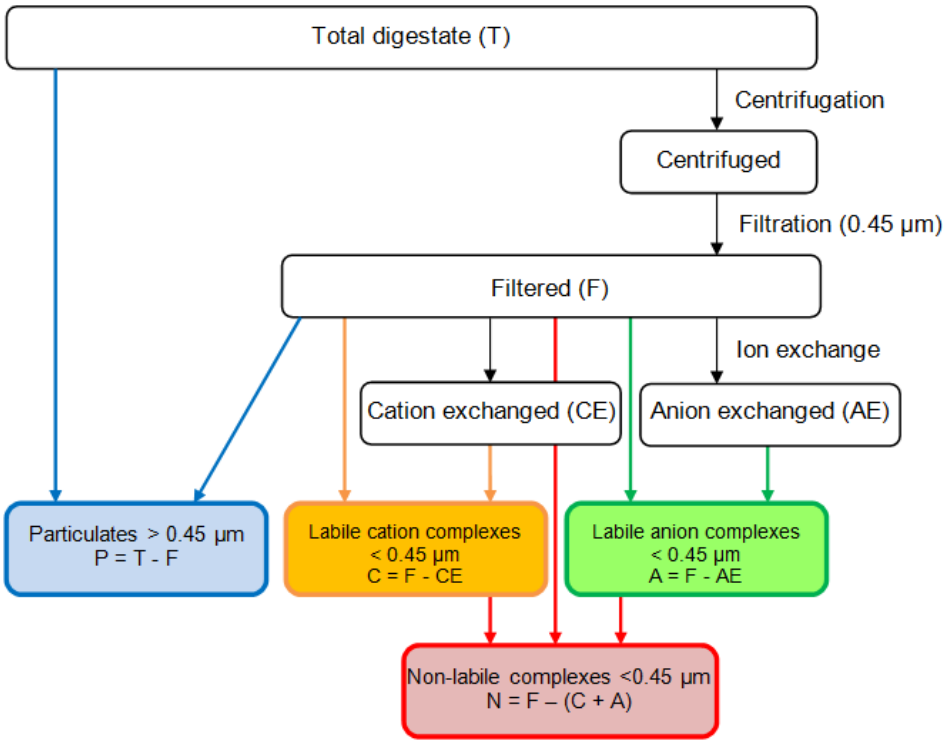

Figure 2: Chart of the steps involved in the digestate fractionation procedure. Analyzed fractions are total digestate (T), centrifuged, filtered (F), cation exchanged (CE), and anion exchanged (AE). Calculated fractions are particulates > 0.45 µm (P), labile cation complexes < 0.45 µm (C), labile anion complexes < 0.45 µm (A), and non-labile complexes < 0.45 µm (N).

2.2.2.1 Size fractionation

Due to the high concentration of particles, direct filtration of digestate through 0.45 μm filters would almost immediately clog the filters. Therefore, centrifugation was to be executed prior to filtration. The centrifugation speed and time to obtain a cut-off size as close to 0.45 μm as possible were found by centrifuging approximately 25 ml digestate (Nalgene, 50 ml, PPCO) in a Beckman, J2-MC centrifuge at three different combinations of speed and time. The digestate used for this part of the experiment was collected from the same BGP as digestates A–D. The combinations were set to 20 000 rpm for 30 minutes, 20 000 rpm for 1 hour, and 15 000 rpm for 30 minutes. Ten ml supernatant was collected carefully from each tube by an automatic pipette. The supernatant was filtrated through 0.45 μm nitrocellulose membrane filters from Millipore® by a vacuum pump (KNF Neuberger™, VDE 0530). Total element concentrations in three replicates of filtered and unfiltered sample for each of the centrifugations were determined as described above. By examining the results, the best combination of centrifuge speed and time to obtain a supernatant which did not clog the filters too much and at the same time did not give a cut-off size less than 0.45 μm was found to be 15 000 rpm and 30 minutes.

Size fractionation of the four samples of digestates A–D were then executed by centrifuging each sample at 15 000 rpm for 30 minutes and filtrating the supernatant through the 0.45 μm filters. The filters were replaced after filtration of 20 ml sample to prevent clogging.

2.2.2.2 Charge fractionation

Econo-Column® chromatography columns (ID: 1cm, length: 10 cm) packed with 7–8 g cation resin (Bio-Rad, Chelex® 100 Resin, 50–100 mesh, sodium form) and 5–6 g anion resin (Bio-Rad, AG® 1-X8 Resin, 50–100 mesh, chloride form) were used for respectively cation exchange- and anion exchange. In order to determine the appropriate time of sampling from the ion exchange columns and to check that the ion retaining capacity of the columns was not exceeded during the experiment, centrifuged and filtrated samples of digestate were run through the anion and cation exchange columns. The solutions were pressed by a peristaltic pump (ISMATEC, BVP *standard*) via hoses (ISMATEC, Tygon® ST R-3607, ID: 3.17 mm, wall: 0.86 mm) through the columns with a speed of about 7.5 ml min^{-1}. The filtrates were collected at the end of the columns after 1, 2, 3, 4, 5, 6, 7, and 8 minutes, acidified (2 % HNO_3) and stored dark at 4 °C prior to total element determination.

Three replicates of centrifuged and filtrated sample of each of the digestates A–D were then run through the anion and cation exchange columns at a speed of 7.5 ml min^{-1}. The ion exchange resin was replaced between replicates to avoid memory effects. Before each run, the hoses were washed with 50 ml 0.2 M HNO_3 and 50 ml MilliQ water, and the columns with 50 ml MilliQ water. Five ml of ion exchanged digestate were collected from the columns when about 15 ml of digestate had entered the system.

2.2.3 Calculations

The fraction of elements associated with particles > 0.45 µm is defined as particulates > 0.45 µm (P) and is the amount of elements removed during centrifugation and filtration. This fraction is considered to have low bioavailability. Elements in labile cation complexes < 0.45 µm (C) and labile anion complexes < 0.45 µm (A) are retained by respectively cation and anion resins in the ion exchange columns. The elements present in the fractions of C and A are considered to be the most bioavailable. The elements associated with non-labile complexes < 0.45 µm (N) are those remaining in the sample after ion exchange and this fraction may have low to variable bioavailability. The fractions were calculated as follows:

$$P = T - F$$
$$C = F - CE$$
$$A = F - AE$$
$$N = F - (C + A)$$

2.3 Quality assurance

Three replicates of blank samples to identify impurities and to calculate instrumental limits of detection (LODs) and limits of quantification (LOQs) were made for each step of the procedure: Total, before centrifugation, after centrifugation, after filtration and after cation and anion exchange. The blank samples were handled and analyzed in the same way as the rest of the samples.

To quality check the digestion procedure and analysis method, about 0.2 g of the certified reference materials (CRMs) whole wheat flour 1567a (National Institute of Standards and technology) and dogfish liver DOLT-4 (National Research Council Canada) were analyzed with the samples.

Solution was collected from the end of the ion exchange columns prior to pumping of digestate in order to detect any impurities from the columns. These samples were analyzed with the other samples.

Each sample was added 250 µl internal standard (IS) (20 µg l^{-1} Rh, In, T, and Tl) before digestion to correct for loss of sample during sample preparation, variances in dilution and instrumental drift.

2.4 Data treatment and presentation

For Cr, Ni, Cu, Zn, and Cd, the concentrations of two isotopes, namely Cr-52 and Cr-53, Ni-60 and Ni-62, Cu-63 and Cu-65, Zn-66 and Zn-68, and Cd-111 and Cd-114, were determined by ICP-MS. Certified concentrations were available for Cu, Zn, and Cd, and the isotopes whose

concentrations measured in the samples of CRM were closest to the certified concentrations were chosen for further data evaluation. For Ni and Cr, the isotopes of lowest concentrations were used because of less interference in the results of these isotopes.

Corrections for interference from Mo and Ba were executed for respectively Cd and Zn.

The blank samples were used to calculate LOD and LOQ for the samples. The LOD and LOQ were calculated as three and ten times the standard deviation of the blanks, respectively.

For the elements (Cr, Cu, Mn, Ni, and Zn) whose concentrations were analyzed by both ICP-MS and ICP-OES, linear regression analyses were executed in order to estimate degree of correlation. The results given by ICP-OES were plotted against the results given by ICP-MS in Microsoft Excel. Results given by ICP-MS were chosen for further presentation where concentrations were low (< 0.20 μg l^{-1}), and results given by ICP-OES otherwise.

3 Results and discussion

3.1 Data validation and interpretation

The isotopes chosen for data evaluation for those measured by ICP-MS were Cr-52, Ni-60, Cu-65, Zn-66, and Cd-114.

All results are expressed as mean ± standard deviation of the replicates. The standard deviations were generally < 5 % of the mean, with few exceptions. The standard deviations of samples with low concentration were somewhat larger, but generally < 10 %.

The total element concentrations in the two CRMs and their certified concentrations, LOD, and LOQ are presented in table 3. The measured CRM concentrations were within limits of the certified concentrations, with exception of Pb in DOLT-4 which was approximately two times larger than the certified concentration. The DOLT-4 was analyzed one more time together with a new sample of DOLT-4. In the new analysis, the Pb concentration of the old sample was still two times larger than the certified concentration, while the new sample fell within the certified concentration. This inconsistency was believed to be due to a contamination of the first DOLT-4, and the results were rejected.

In the linear regression analyses executed to compare the results given by ICP-MS and ICP-OES for Cr, Cu, Mn, Ni, and Zn, values of R^2 raging from 0.9863–1 were obtained. The correlation between the two analysis methods was thus high.

The relative standard deviations (RSD) of analysis by ICP-MS are presented in table 4.

Table 3: Element concentration measured in the CRM and its certified concentrations, LOD, and LOQ.

	Certified reference material					
	Whole wheat flour 1567a		**Dogfish liver DOLT-4**		**LOD**	**LOQ**
Element	*Measured (mg kg^{-1})*	*Certified (mg kg^{-1})*	*Measured (mg kg^{-1})*	*Certified (mg kg^{-1})*	*(µg l^{-1})*	*(µg l^{-1})*
Cr	0.063	-	1.6	-	2.7	9.0
Mn	8.3	9.4 ± 0.9	10	-	0.70	2.3
Co	0.0070	-	0.24	-	0.042	0.14
Ni	0.10	-	1.0	-	0.73	2.4
Cu	2.0	2.1 ± 0.2	32	31.2 ± 1.1	3.3	11
Zn	11	11.6 ± 0.4	126	116 ± 6	4.1	14
Mo	0.47	0.48 ± 0.03	1.1	-	1.2	4.1
Cd	0.025	0.026 ± 0.002	25	24.3 ± 0.8	0.052	0.17
Ba	1.2	-	0.75	-	1.8	5.9
Pb	0.011	-	0.31	0.16 ± 0.04	0.7	2.4

Table 4: RSD of analysis by ICP-MS.

	Digestate sample				
Element	*Total*	*Centrifuged*	*Filtered*	*Anion exchanged*	*Cation exchanged*
Cr	< 4.8 %	< 8.0 %	< 10 %	< 5.2 %	< 7.7 %
Mn	< 2.8 %	< 5.0 %	< 5.3 %	< 2.2 %	< 2.8 %
Co	< 4.0 %	< 3.3 %	< 6.1 %	< 2.7 %	< 3.7 %
Ni	< 3.7 %	< 3.5 %	< 4.4 %	< 2.7 %	< 4.0 %
Cu	< 3.0 %	< 2.4 %	< 3.6 %	< 3.2 %	< 5.0 %
Zn	< 2.5 %	< 2.2 %	< 4.3 %	< 1.9 %	< 2.5 %
Mo	< 5.5 %	< 4.0 %	< 3.5 %	< 6.3 %	< 3.0 %
Cd	< 7.8 %	< 91 %	< 70 %	< 120 %	< 62 %
Ba	< 2.3 %	< 2.1 %	< 2.9 %	< 2.2 %	< 2.2 %
Pb	< 2.1 %	< 2.5 %	< 3.7 %	< 2.4 %	< 4.4 %

3.2 Sample outliers and removal

The standard deviation for Cr concentration in the cation exchanged eluates of digestate C was about 50 % of the mean due to replicate concentrations of 36, 39 and 79 μg l^{-1}. As the standard deviations of the other elements in this sample were low, a contamination of Cr in the one replicate is plausible, and the result of 79 μ l^{-1} was rejected from the set of data.

Analysis of the water collected from the ion exchange columns right before the digestate samples were injected to the system generally gave element concentrations close to LOD. Therefore, contamination of elements from the columns can be considered to be insignificant compared to concentrations measured in the digestate eluates. The most pronounced exceptions are those of the high concentrations of Na in the samples collected from the cation exchange columns. However, as the resin applied for cation exchange was Na based, contamination of this element must be expected. Results of Na concentrations in the cation exchanged samples were thus rejected. Another exception is a high Zn concentration (197 μg l^{-1}) in one of the replicates of sample from the cation exchange column. However, as the Zn concentration in the corresponding digestate B eluate is not higher than in the other to replicates (45 μg l^{-1} vs. both 49 μg l^{-1}), the high Zn concentration is likely to be due to contamination during sample handling, rather than an actual Zn contamination from the column.

3.3 Digestate characterization

The pH, specific conductivity, DM and total carbon of the digestates A–D are compiled in table 5. The pH of the digestates ranged from 8.7–8.9, and the specific conductivity ranged from 17.5–23.9 mS cm^{-1}. The DM varied from 1.1–2.6 %, and the percentage of carbon from 0.51–0.88 %.

Table 5: Characteristics of the digestates expressed through pH, specific conductivity, dry matter and total carbon.

	Digestate sample			
	A	**B**	**C**	**D**
pH	8.9	8.9	8.9	8.7
Specific conductivity (mS cm^{-1})	23.9	19.6	17.5	19.8
Dry matter	1.80 % ± 0.010 %	2.07 % ± 0.002 %	1.10 % ± 0.001 %	2.60 % ± 0.010 %
Total carbon	0.74 % ± 0.001 %	0.72 % ± 0.004 %	0.51 % ± 0.009 %	0.88 % ± 0.003 %

Specific conductivity is a measure of a materials ability to conduct electricity. Its determination has been used along with DM as a cheap and rapid method to determine the amount of nutrients, such as NH_4^+, K, and P, in liquid fertilizers (Stevens et al. 1995). The specific conductivities of the digestates are comparable to those of liquid animal manure reported in the literature (Marino et al. 2008). The specific conductivity and DM of the digestate thus indicates that the fertilizing properties of digestate with regard to amount of nutrients are acceptable.

Due to adjustment with NaOH in the reactor of the BGP, the pH of the digestate is slightly higher than the pH of liquid animal manure reported in the literature (Marino et al 2008; Whalen et al. 2000; Paul & Beauchamp 1989). Fertilizers of high pH are favorable because they increase the pH of acid soils, hence improving crop production and reducing the need for liming agents. Increased pH favors species growth mechanisms, such as precipitation, coprecititation, and soil particle sorption, and in soils with high pH, element bioaccessibility and toxicity is thus expected to be low.

3.4 Total element concentrations

Total concentrations of elements, classified as anions (S and P) and metals (Cr, Co, Ni, Zn, Mo, Cd, Pb, Ba, Cu, Mn, Al, Ca, Fe, K, Mg, and Na), in the samples of digestates A–D analyzed by ICP-MS and ICP-OES are presented in table 6. The lowest concentrations of elements were found in digestate B, while digestate D generally contained highest element concentrations. The element concentrations in digestate A and C were quite similar. The major elements in the digestates are Ca, K, and Na.

The concentration of Cr, Ni, Pb, Cd, Cu, and Zn in DM basis and in which quality class the concentration classifies the digestate are presented in table 7. Concentrations of Cr, Ni, and Pb were within the concentrations for quality class 0 in all digestates. Due to the concentrations of Cd and Zn, digestate A is classified in quality class II. The presence of all heavy metals (omitting Hg) in digestate B was so low that it can be classified in quality class 0. The high Zn concentration in digestate C places this digestate in quality class III. The level of Cd, Zn, and Cu all classifies digestate D in quality class I. Thus, the concentration of Cd, Zn and Cu in digestate may limit its application as fertilizer.

Table 6: Total concentrations of elements in the digestates A–D.

	Digestate sample			
	A	**B**	**C**	**D**
Anions				
SO_4^{2-} -S (mg l^{-1})	130 ± 2	100 ± 0.5	150 ± 1	170 ± 2
PO_4^{3-} -P (mg l^{-1})	200 ± 4	200 ± 5	240 ± 3	330 ± 5
Metals				
Cr (µg l^{-1})	450 ± 12	120 ± 2	480 ± 7	500 ± 7
Co (µg l^{-1})	67 ± 0.4	16 ± 0.1	68 ± 0.4	74 ± 1.1
Ni (µg l^{-1})	210 ± 4	74 ± 1	180 ± 3	200 ± 2
Pb (µg l^{-1})	220 ± 1	70 ± 0.3	230 ± 6	240 ± 2
Mo (µg l^{-1})	110 ± 11	48 ± 1.6	72 ± 0.6	85 ± 0.5
Cd (µg l^{-1})	17 ± 0.3	7.1 ± 0.17	16 ± 0.1	19 ± 0.1
Zn (mg l^{-1})	9.1 ± 0.07	2.8 ± 0.02	9.4 ± 0.14	10 ± 0.1
Ba (mg l^{-1})	4.0 ± 0.03	0.35 ± 0.003	6.2 ± 0.05	6.2 ± 0.09
Cu (mg l^{-1})	1.8 ± 0.02	0.65 ± 0.001	1.6 ± 0.03	1.7 ± 0.01
Mn (mg l^{-1})	0.97 ± 0.231	0.14 ± 0.007	13 ± 0.2	13 ± 0.2
Al (mg l^{-1})	61 ± 0.3	15 ± 0.8	76 ± 1	100 ± 0.4
Ca (mg l^{-1})	510 ± 8	450 ± 2	590 ± 13	980 ± 87
Fe (mg l^{-1})	42 ± 0.5	55 ± 0.5	30 ± 0.4	39 ± 0.1
K (mg l^{-1})	1600 ± 7	910 ± 2	1300 ± 18	1500 ± 11
Mg (mg l^{-1})	99 ± 3.7	63 ± 4.3	170 ± 4	230 ± 4
Na (mg l^{-1})	1200 ± 8	870 ± 1	1000 ± 15	1200 ± 6

Table 7: Element concentration on dry matter basis and quality class of the digestates A – D.

	Digestate sample							
	A		**B**		**C**		**D**	
Element	*Concentration (mg kg^{-1} DM)*	*Quality class*	*Concentration (mg kg^{-1} DM)*	*Quality class*	*Concentration (mg kg^{-1} DM)*	*Quality class*	*Concentration (mg kg^{-1} DM)*	*Quality class*
Cr	25	0	5.6	0	14	0	6.5	0
Ni	10	0	3.6	0	17	0	7.8	0
Pb	12	0	3.4	0	21	0	9.1	0
Cd	0.92	II	0.34	0	1.4	II	0.72	I
Zn	510	II	140	0	860	III	390	I
Cu	100	I	32	0	140	I	67	I

Compared to concentration of Cr, Ni, Pb, Cd, Cu, and Zn in digestates of maize and horse manure measured by Selling et al. (2008), the concentrations presented in this experiment are comparable but generally higher. However, as the main substrate in the anaerobic digestion at the

Mjøsanlegget BGP is food waste from households and industry, which potentially also include non-organic waste such as plastics, cans and electrical waste, higher levels must be expected.

The soil content of P often governs the choice of fertilizer, whereas N is most probably the nutrient deciding the amount (kg daa^{-1}) of fertilizer applied. If we assume that the N requirement is 12 kg N daa^{-1}, which is common for wheat production, the fertilizers NPK 22-2-12, NPK 22-3-10, and NPK 19-4-12 will add approximately 1, 1.5, and 2 kg P daa^{-1}. Using the concentrations of digestate C (since heavy metal concentrations, except Cr in digestate A, are highest in this digestate), amount of heavy metals applied with digestate to meet the P demands can be calculated. Total amount of heavy metal added to the field is estimated and presented in table 8. For comparison, average annual amounts of heavy metal added if applying 4 tons sludge within quality class I per daa every 10^{th} year, which is the regulations for application of sludge quality 1 as fertilizer to agricultural land (FOR-2003-07-04-951), and the percentages of heavy metal added with digestate at P demand 2 kg in parenthesis are also presented in table 8.

Table 8 shows that due to the high concentration of P in digestate, lower amounts of heavy metal are delivered to soil by application of digestate than by application of sludge. Even for Zn, which concentration placed the digestate in quality class III, the amount added from sludge is 2 times higher than that added from digestate when the P demand is 2 kg.

Table 8: Amount heavy metal added to field from digestate at different P levels and average annual amount heavy metal added to field from 4 tons of sludge within quality class I delivered per daa every 10^{th} year.

	Fertilizer			
	Digestate C			Sludge
Element (mg daa^{-1})	*1 kg P*	*1.5 kg P*	*2 kg P*	quality class I
Cr	2 000	3 000	4 100	24 000 (580 %)
Ni	780	1 200	1 600	12 000 (750 %)
Pb	960	1 400	1 900	24 000 (1260 %)
Cd	67	100	130	320 (250 %)
Zn	40 000	60 000	80 000	160 000 (200 %)
Cu	6 700	10 000	13 000	60 000 (460 %)

3.5 Size fractionation

The concentration of elements remaining in solution (filtrate) after centrifugation at three combinations of centrifuge time and speed and subsequent 0.45 μm filtration are presented in table 9. The element concentration in the filtrate is expressed as percentage of element concentration in the unfiltered supernatant. Except for Cr, Mo, and Cd, lowest percentages are found in the filtrate of the digestate sample centrifuged at 15 000 rpm for 30 minutes. Hence, this centrifugation removed the lowest amount of particles > 0.45 μm. This was important to ensure that as few particles < 0.45 μm as possible were removed during centrifugation, which would lead to an overestimation of elements in the fraction of particulates > 0.45 μm.

The concentrations of Cr, Ni, Pb, Cd, Zn, Cu, K, Na, P, and S in digestates A–D after centrifugation and after 0.45 μm filtration are presented in table 10. In the parentheses, the concentrations are expressed as percentages of the total concentrations.

The element concentrations in the centrifuged samples are higher than in the filtrated samples, except for Cr in digestates B and C, and K in digestate C. Contaminations from the filters, memory effect from previous samples, and contamination during further sample handling may explain these deviations.

The concentrations of Pb, Cd, Zn and Cu in the filtrate of all digestates were less than 5 % of total element concentrations. Since such a large fraction was removed during centrifugation and filtration, these elements were mostly bound to particles > 0.45 μm. Lead, Cd, Zn and Cu are known to occur as divalent ions and to bind strongly to negatively charged groups of OM. It has been demonstrated that Cd in particular bind very strongly to OM (Vig et al. 2003). It appears credible that production of digestate increase the complexation of the metals.

Table 9: Element concentration in filtrate expressed as percentage of element concentration in unfiltered supernatant of centrifugation at different combination of time and speed.

Time (h)	0.5	1	0.5
Speed (rpm)	20 000	20 000	15 000
Element	*% of unfiltered sample concentrations*		
Cr	95	99	105
Co	92	99	89
Ni	94	98	94
Cu	89	185	75
Zn	77	84	50
Mo	87	70	91
Cd	98	100	124
Ba	90	97	85

Table 10: Element concentrations in the samples of digestates A–D after centrifugation and filtration. Percentage of total element concentration is given in parentheses.

	Digestate sample							
Element	**A**		**B**		**C**		**D**	
Centrifuged								
Cr (µg l^{-1})	66 ± 2.4	(15 %)	19 ± 0.3	(16 %)	37 ± 0.3	(7.7 %)	44 ± 1.1	(8.8 %)
Ni (µg l^{-1})	110 ± 4.4	(52 %)	43 ± 0.4	(58 %)	77 ± 1.2	(43 %)	100 ± 2	(50 %)
Pb (µg l^{-1})	9.2 ± 2.42	(4.2 %)	< 2.4		5.5 ± 0.12	(2.4 %)	6.2 ± 0.242	(2.6 %)
Cd (µg l^{-1})	0.77 ± 0.070	(4.5 %)	0.079 ± 0.025	(1.1 %)	0.26 ± 0.124	(1.6 %)	0.18 ± 0.022	(0.95 %)
Zn (µg l^{-1})	480 ± 20	(5.3 %)	70 ± 0.4	(2.5 %)	220 ± 12	(2.3 %)	210 ± 6	(2.1 %)
Cu (µg l^{-1})	50 ± 4.0	(2.8 %)	19 ± 0.4	(2.9 %)	61 ± 1.8	(3.8 %)	58 ± 1.8	(3.4 %)
K (mg l^{-1})	1600 ± 18.0	(100 %)	920 ± 12	(101 %)	1300 ± 2	(100 %)	1500 ± 10	(100 %)
Na (mg l^{-1})	1200 ± 13	(100 %)	880 ± 11	(101 %)	1000 ± 3	(100 %)	1200 ± 11	(100 %)
S (mg l^{-1})	59 ± 0.1	(45 %)	43 ± 0.5	(43 %)	52 ± 1.2	(35 %)	57 ± 2.4	(34 %)
P (mg l^{-1})	50 ± 0.2	(25 %)	69 ± 0.2	(35 %)	15 ± 0.2	(6.3 %)	20 ± 0.1	(6.1 %)
Filtered								
Cr (µg l^{-1})	63 ± 5.4	(14 %)	22 ± 0.7	(18 %)	42 ± 1.2	(8.8 %)	41 ± 0.4	(8.2 %)
Ni (µg l^{-1})	110 ± 2	(52 %)	43 ± 0.6	(58 %)	76 ± 1.4	(42 %)	97 ± 0.6	(49 %)
Pb (µg l^{-1})	8.2 ± 1.89	(3.7 %)	<2.4		3.2 ± 0.12	(1.4 %)	3.8 ± 0.39	(1.6 %)
Cd (µg l^{-1})	0.66 ± 0.036	(3.9 %)	<0.1		0.12 ± 0.012	(0.75 %)	<0.1	
Zn (µg l^{-1})	400 ± 12	(4.4 %)	61 ± 1.8	(2.2 %)	160 ± 5	(1.7 %)	140 ± 6	(1.4 %)
Cu (µg l^{-1})	40 ± 2.5	(2.2 %)	15 ± 0.6	(2.3 %)	32 ± 3.0	(2.0 %)	30 ± 3.3	(1.8 %)
K (mg l^{-1})	1600 ± 7	(100 %)	920 ± 3	(101 %)	1400 ± 2	(108 %)	1500 ± 9	(100 %)
Na (mg l^{-1})	1200 ± 8	(100 %)	870 ± 2	(100 %)	1000 ± 2	(100 %)	1200 ± 8	(100 %)
S (mg l^{-1})	57 ± 0.3	(44 %)	42 ± 0.7	(42 %)	47 ± 2.7	(31 %)	50 ± 1.1	(29 %)
P (mg l^{-1})	48 ± 0.4	(24 %)	69 ± 0.5	(35 %)	12 ± 0.3	(5.0 %)	17 ± 0.2	(5.2 %)

The concentration of Cr was less than 20 % of total concentration in all digestates, suggesting that a relatively large portion of this heavy metal was bound to particles > 0.45 µm.

The concentrations of Ni in the filtrate lay between 42–58 % of total concentrations, pointing to a medium degree of particle binding for this metal. This is consistent with results in the literature stating that Pb, Zn, and Cd are more strongly bound to OM than is Ni (Smith 2008).

The percentages of S were between 29–44 %, indicating a somewhat high degree of particle binding for S. Within the samples of digestate, it can be assumed that S exists in the form of the anion sulfate. At high pH, such as the one found in the digestate, sulfate is known to sorb more weakly to surfaces than are cations (Gustafsson et al. 2000), explaining the lower percentage of S present in the fraction of particulates > 0.45 µm.

For P, there seems to be a distinction between digestates A and B and C and D. While only approximately 5 % of P was present as species < 0.45 µm in digestates C and D, between 24–35

% was present as species < 0.45 µm in digestates A and B. Thus, a large amount of P was associated with particles, although to a less degree in digestates A and B than in digestates C and D. In soils, P has low solubility and is mainly associated with soil particles (Busman et al. 2002). The results imply that this also applies for P in digestate.

The percentages of Na and K were 100 % for all digestates. These results appear to be unrealistically high, as some Na and K were probably not mineralized. However, from these results, this cannot be determined. Nevertheless, the results indicate that Na and K do not associate with particles to a large degree. Being alkali metals, Na and K are usually present as monovalent cations and do generally not bind to organic groups, and the results are thus expected.

Removal of particles < 0.45 µm during centrifugation and adsorption of species < 0.45 µm on the filter, both effect leading to an overestimation in the amount of element present in the fraction of particulates > 0.45 µm, must be taken into consideration when evaluating the results. It is possible that the elements are less associated with particles than stated above.

3.6 Charge fractionation

The concentrations of Cr, Cu, Ni, and Zn in the collected eluates after elution time from the cation- and anion exchange columns are presented in figure 3. Except for Ni in the cation exchange eluate, the ion exchange capacity of the columns was not exceeded in the duration of the experiment. The element concentrations, except Ni, in the eluates seemed to level out after approximately 2 minutes, i.e. when about 15 ml digestate had entered the system.

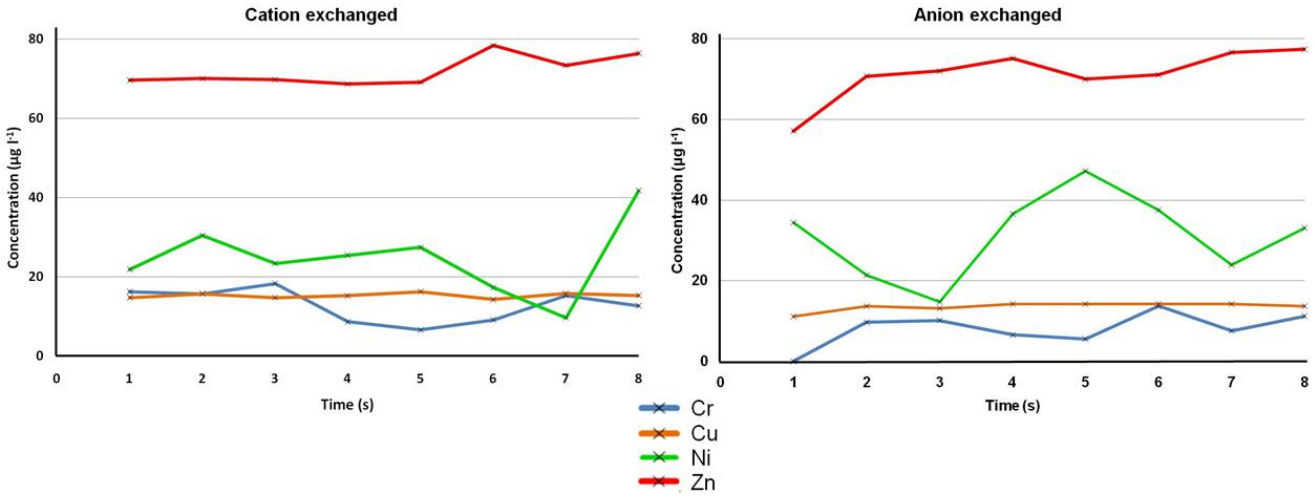

Figure 3: Element concentration in residue from cation (left) and anion (right) exchange columns as a function of time after digestate ingestion.

Element concentrations in the eluates of digestates A–D after the cation and anion exchange are presented in table 11. The concentrations in percentages of concentrations in the filtrates are presented in parentheses.

The element concentrations in some ion exchanged eluates were more than 100 % of the filtrate concentrations as presented in table 10. Most of these discrepancies lay within the analytical uncertainty. However, in the anion exchange eluates, the Cu concentration of digestate B and the Pb concentrations in digestates A and C were 127, 134, and 135 %, respectively, and cannot be explained by a variation between replicates and instrumental uncertainty. In these cases, it appears to have been a release of elements from the anion exchange resin. As mentioned, however, the element concentrations in the samples taken from the ion exchanged columns prior to injection of digestate were found to be very low. Nevertheless, it is possible that the high concentration of ions in the digestates released elements present in the ion exchange material that distilled water could not. Other explanations for the higher concentrations in the eluates than in the filtrates include contamination during further sample preparation, and variations between the filtrate samples digested directly and those used for ion exchange.

Table 11: Element concentration in samples of digestates A–D after centrifugation, filtration and cation and anion exchange. Percentage of element concentrations in filtrates is given in parentheses.

	Digestate sample							
Element	**A**		**B**		**C**		**D**	
Cation exchaged								
Cr (µg l^{-1})	63 ± 1.8	(100 %)	20 ± 0.5	(91 %)	38 ± 1.9	(89 %)	39 ± 0.4	(95 %)
Ni (µg l^{-1})	110 ± 1	(100 %)	38 ± 1.4	(88 %)	66 ± 2.2	(87 %)	85 ± 0.9	(88 %)
Pb (µg l^{-1})	6.7 ± 1.48	(82 %)	<2.4		2.8 ± 0.14	(87 %)	3.3 ± 0.28	(86%)
Cd (µg l^{-1})	0.66 ± 0.047	(100 %)	<0.17		<0.17		<0.17	
Zn (µg l^{-1})	310 ± 6	(78 %)	48 ± 2.3	(79 %)	120 ± 3	(75 %)	110 ± 5	(79 %)
Cu (µg l^{-1})	38 ± 2.0	(95 %)	13 ± 0.8	(87 %)	28 ± 0.6	(88 %)	25 ± 1.5	(83 %)
K (mg l^{-1})	810 ± 98	(51 %)	160 ± 51	(17 %)	55 ± 25.2	(3.9 %)	62 ± 11.9	(4.1 %)
S (mg l^{-1})	53 ± 1.1	(93 %)	39 ± 3.2	(93 %)	43 ± 0.5	(91 %)	39 ± 0.5	(80 %)
P (mg l^{-1})	48 ± 1.0	(100 %)	69 ± 7.5	(100 %)	11 ± 0.3	(92 %)	14 ± 0.4	(82 %)
Anion exchanged								
Cr (µg l^{-1})	57 ± 3.8	(90 %)	19 ± 0.5	(86 %)	28 ± 1.6	(67 %)	26 ± 1.4	(63 %)
Ni (µg l^{-1})	83 ± 5.0	(75 %)	27 ± 3.1	(63 %)	47 ± 3.4	(62 %)	60 ± 5.5	(62 %)
Pb (µg l^{-1})	11 ± 0.8	(134 %)	3.1 ± 0.14	-	4.4 ± 0.30	(135 %)	3.7 ± 0.11	(96 %)
Cd (µg l^{-1})	0.69 ± 0.167	(105 %)	<0.17		<0.17		<0.17	
Zn (µg l^{-1})	400 ± 23	(100 %)	66 ± 1.3	(108 %)	140 ± 7	(88 %)	100 ± 1	(71 %)
Cu (µg l^{-1})	36 ± 2.3	(90 %)	19 ± 0.6	(127 %)	23 ± 1.3	(72 %)	18 ± 0.3	(60 %)
K (mg l^{-1})	1500 ± 69	(94 %)	900 ± 20	(98 %)	1200 ± 22	(86 %)	1200 ± 52	(80 %)
Na (mg l^{-1})	1100 ± 51	(92 %)	840 ± 23	(97 %)	880 ± 16	(88 %)	940 ± 38	(78 %)
S (mg l^{-1})	28 ± 1.5	(49 %)	15 ± 0.6	(36 %)	19 ± 0.6	(40 %)	18 ± 1.3	(36 %)
P (mg l^{-1})	34 ± 2.0	(71 %)	31 ± 5.5	(45 %)	9.7 ± 0.31	(80 %)	12 ± 0.3	(71 %)

In all digestates, the concentrations of Cr, Ni, Cu, S and P in the cation exchanged eluate were more than or equal to 80 % of concentrations in the filtrates. The percentage for Zn was found to be 70–80 % in all digestates. A relatively small fraction of these elements was retained by the resin of the cation exchange column and are thus only present to a small degree in the filtrate as labile cation complexes < 0.45 μm.

In the anion exchanged eluates, the percentages for Cr were more than 80 % in digestates A and B and between 60–70 % in digestates C and D. The percentages in the anion exchanged samples were for Ni between 60–80 % in all digestates. For Zn, the percentages in the anion exchanged eluate were more than 80 % in digestates A–C and 71 % in digestate D. More than 90 % of Cu in digestates A and B and between 60–80 % of Cu in digestates C and D was present in the anion exchange eluate. Thus, Cr, Ni, Zn and Cu were mostly present in the filtrate as non-labile complexes < 0.45 μm.

For S, between 30–50 % was present in the anion exchange eluates. In the filtrate, S is thus mostly present as labile anion complexes < 0.45 μm. As S assumedly appears as the anion sulfate in the digestate, the relatively high retention of S in the anion exchange column seems credible.

The percentages of P in the anion exchanged samples were between 70–80 % in digestates A, C, and D and 45 % in digestate B. Hence, P is mostly present as non-labile complexes < 0.45 μm in digestates A, C, and D, and as labile anion complexes < 0.45 μm in digestate B.

As 100 % of Cd in the filtrate was present in the cation and anion exchanged eluates of digestate A, Cd was not present as neither labile cation complexes < 0.45 μm nor labile anion complexes < 0.45 μm. The Cd concentrations fell below LOQ for digestates B–D. Assuming that the results of digestate A are representative for all the digestates, the results indicate that Cd is mostly present as non-labile complexes < 0.45 μm in the filtrate.

In digestates A, C, and D, 18, 13, and 86 %, respectively, of Pb was held back by the cation exchange resin, while no Pb was retained by the anion exchange resin. Lead is thus present in the filtrates as mainly non-labile complexes < 0.45 μm and some labile cation complexes < 0.45 μm. The charge fractionation results of digestate B cannot be used to estimate Pb speciation as the concentration in the filtrate fell below LOQ.

The percentages of K in the cation exchanged eluates were below 20 % in digestates B–D, and 51 % in digestate A. In the anion exchanged eluates of all digestates, concentrations of K were more than 80 % of those in the filtrates. Thus, in the filtrates, K was mainly present as labile cation complexes < 0.45 μm. Although the results of Na concentrations in the cation exchange eluates was rejected, it can be assumed that Na will follow K as they are positioned in the same group in the periodic table. As mentioned, monovalent cations of Na and K bind only weakly to OM, explaining their high retention within the cation exchange column. As free Na and K cations are readily taken up by cells, Na and K present in the digestates have a high degree of bioavailability (Mäser et al. 2002).

The cation exchange resin applied in the experiment has higher selectivity for divalent ions, such as heavy metals, than for monovalent ions, such as K and Na (Chelex instruction manual). In a solution of high ionic strength and basic pH, the selectivity of the resin is Ni > Cd > Cu > Zn. Therefore, should the capacity of the resin be exceeded, K and Na will be washed out from the column before the heavy metals. Zinc and Cu will be washed out before Cd and Ni. As 51 % and 17 % of K in the filtrate was present in the cation exchanged eluate of digestates A and B, respectively, it is possible that the capacity of the resin was exceeded. The amount of Zn and Cu present in labile cation complexes < 0.45 μm can hence have been underestimated.

3.7 Element speciation and possible implications for the environment

Concentrations in the calculated fractions defined as particulates > 0.45 μm, labile cation complexes < 0.45 μm, labile anion complexes < 0.45 μm and non-labile complexes < 0.45 μm expressed as percentages of total element concentrations in the digestates A–D are shown for Cr, Ni, Pb, Cd, Zn, Cu, S, and P in figure 4.

In all digestates, Cu, Zn, Cd, and Pb were mainly present as particulates > 0.45 μm, with the fractions < 0.45 μm accounting for less than 5 %. In addition, the species < 0.45 μm were in addition almost completely composed of non-labile complexes. Generally, elements associated with particles are believed to be less accessible to organisms than are LMM species, and non-labile complexes are considered less susceptible for uptake than are labile complexes. Plants may thus not be able to directly take up the heavy metals supplied by digestate, and subsequent accumulation and transfer to higher strata of the food chain are minimized. So although the results showed that total concentrations of Cu, Zn, and Cd exceed limits given by Norwegian regulations in three of the four digestates, heavy metals present in digestate may in fact not pose a large environmental threat due to the fact that the metals are mainly present bound to particles > 0.45 μm and else as non-labile complexes. The plant availability of heavy metals after further degradation of the OM after soil application is not possible to predict from the presented results.

Less than 20 % of Cr in the digestates were present in the < 0.45 μm fractions, and within these fractions Cr were mainly present as non-labile complexes. This suggests that a relatively large portion of this heavy metal too, was present as species that has low availability to plants and thus exerts low toxicity and potential for food chain transfer.

For Ni, 42–58 % was present in the > 0.45 μm fraction, and anion and non-labile complexes constituted most of the < 0.45 μm fractions. Among the heavy metals in the digestate, Ni can thus be considered to be the most available for plant uptake. However, total concentrations of Ni in the digestates were below quality class 0, so Ni does not limit the use of digestate as fertilizer.

Between 29–44 % of S was found in the > 0.45 μm fractions, where the anion complexed fraction accounted for the highest amount of S. As the most form most available to plants is the anion sulfate (Rennenberg 1984), S appears to be among the most bioavailable elements in

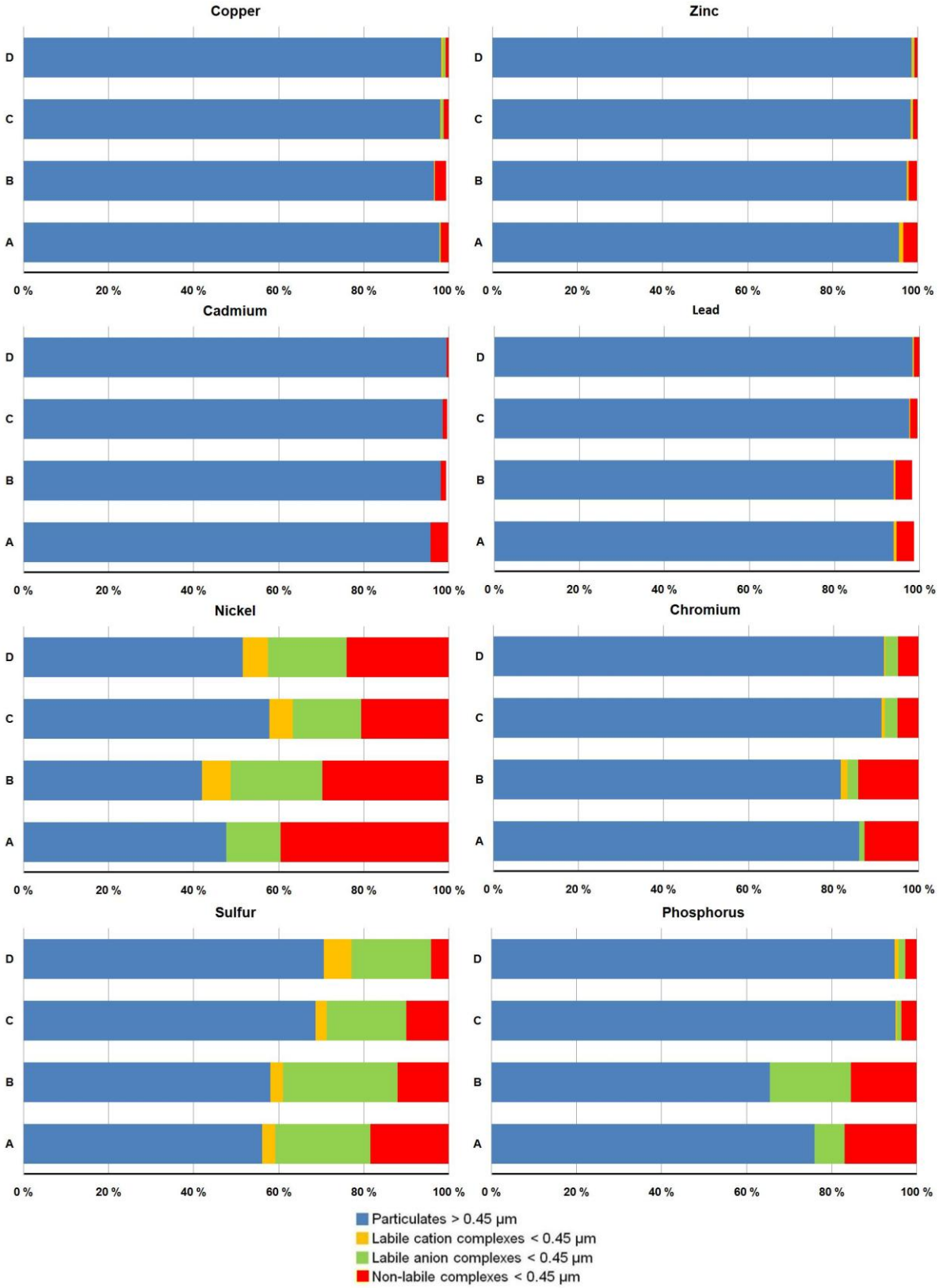

Figure 4: Distribution of Cr, Ni, Pb, Cd, Zn, Cu, S and P amongst fractions of particulates > 0.45 µm, labile cation complexes < 0.45 µm, labile anion complexes < 0.45 µm, and non-labile complexes < 0.45 µm in digestates A–D. Results are shown as percentage of total concentrations.

digestate. In contrast to heavy metals, however, S is a macronutrient required in relatively high amounts (Ceccotti 1996), and an addition may be beneficial to plant growth.

In digestates C and D, more than 95 % of P was found in the particulate fraction, and most of the < 0.45 μm fraction in these two digestates consisted of non-labile complexes. In the other two digestates, P was bound to particles to a less extent (between 65–75 %), and a higher amount of anion complexes made up the < 0.45 μm fraction. Thus, P seems to be more bioavailable in digestates A and B than in digestates C and D. Although plants require P for growth, a P excess may lead undesirable eutrofication (Schindler & Vallentyne 2008). The high degree of P associated with particles as seen in digestate C and D, contributes to immobilization and less leaching of P. Digestate may thus be more favorable to the environment than other fertilizers.

The author has not been able to obtain other literature describing size and charge fractionation of elements in digestate. This work is thus possible the only currently available research regarding this subject.

4 Conclusion

In this experiment, the speciation of elements in liquid digestate collected from the Mjøsanlegget AS biogas plant was investigated by performing size fractionation (centrifugation and filtration) and charge fractionation (ion exchange). The obtained results suggested that, with respect to heavy metal toxicity, digestate is a good fertilizer candidate. Although total concentrations of Cu, Zn, and Cd were found to be greater than minimum limits set by Norwegian regulations, the experiment demonstrated that a large amount of Cr, Cu, Zn, Cd, and Pb present in digestate was mostly unavailable for direct uptake by plants since the elements were mainly associated with particles. Nickel in digestate was more bioavailable as about half of Ni present was found in the form of labile anion and non-labile complexes < 0.45 μm. However, total concentration of Ni in digestate was low. The results could not predict plant availability of heavy metals after degradation of organic matter after application of digestate to soil.

The distribution of elements amongst the different fractions was found to be relatively similar in all four samples, indicating that the results may be representative for more digestates than those examined in this study.

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