Chapter 6

Assessing fate and bioavailability of trace elements in soils after digestate application

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ABSTRACT

Anaerobic digestion (AD) is a biotechnological process in which organic matter is microbially converted into biogas and digestate. Many parameters affect the underlying microbial processes, including depolymerization of organic compounds, acidogenesis, acetogenesis and methanogenesis, as part of the AD cycle. Optimal concentrations of different nutrients and micronutrients are a prerequisite for optimum microbial growth and metabolism in AD processes. The effluent digestate can be used as a substitute for chemical fertilizers, recycling nutrients to create more sustainable agricultural production systems. Trace elements (TEs) can be transferred to soils during application of digestate as fertilizer, being subjected to environmental influences. To evaluate TEs bioavailability and uptake by plants (which can be transferred to the food chain), TEs leaching processes (which can prevent loss of soils nutrients and run off in ground waters), and TEs effects on soil organisms (which can affect soil fertility and productivity), it is relevant to assess the fate and availability of TEs after land application of digestate. This book chapter provides an overview of different type of biogas plants and digestate post-treatment processes.

Possible physicochemical interactions between digestate and soil components, which influence TEs speciation and availability for biological uptake, are also described. Finally, different TEs fractionation and speciation techniques are extensively discussed to give to the reader a good basis when investigating the fate of TEs in soils after digestate application.

KEYWORDS: anaerobic digestion, digestate, TE analysis, fractionation, speciation, trace element (TE)

6.1 INTRODUCTION

Anaerobic digestion (AD) is an established biotechnological process in which organic matter is broken down by microorganisms in controlled anaerobic conditions (biogas plant – BGP) leading to the production of two valuable products: biogas and digestate.

BGPs can be classified according to the treated organic substrates. The first type is BGP that handles livestock effluents, agricultural residues and agro-industrial by-products (agricultural BGPs). The second type handles organic compounds from sewage sludge and wastes of various origins (waste BGPs), and the third type is referred to landfills that handle solid wastes (Seadi et al., 2008). In most cases, the latter is not used as fertilizer due to the presence of many additional organic pollutants or excessive trace-metal burden. In 2016, 17,662 BGPs and 503 biomethane plants were operating in Europe, showing that the biogas industry is an essential part of European development (EBA, 2017). A general description of the main types of BGPs is summarized in Section 6.2.1.

Digestate is the effluent from BGPs and it is considered a natural fertilizer, mainly used for agricultural land, forest soil, re-cultivation of mining sites and brownfields according to national regulations. Digestate application increases organic matter content in the soil and improves land fertility because it is rich in mineral elements like nitrogen, phosphorus, potassium and other nutrients and micronutrients. Considering the carbon balance, about 65–80% of the carbon fed into an agricultural BGP is converted to biogas while the remaining carbon is largely unavailable for anaerobic microorganisms, consequently it is recovered in digestate and incorporated into the soil via digestate application (Dale *et al*., 2016), thus increasing the soil organic content.

Following AD, digestate usually undergoes solid–liquid separation. Such post-treatment allows concentrating the coarse solids and organic matter in the solid fraction, whereas most of the mineral nitrogen is kept in the liquid fraction (Tambone *et al*., 2015) (Section 6.2.2). In fact, both digestate fractions can be used as soil amendment, reducing the use of manufactured/chemical fertilisers and optimizing management costs which promote a circular economy (Delzeit & Kellner, 2013). The high nutrient and organic matter content depends on the composition of the input feedstock and on the efficiency of the biological process (Riva *et al*., 2016).

Optimal concentrations of different elements, such as Fe, Co, Ni, Zn, Mo, W or Se, hereafter referred as TEs, are a prerequisite for optimum microbial growth and metabolism in AD processes. These elements can already be present in the AD substrate, which will depend on the type of substrate, of digester and of employed digestion procedure (mono or co-digestion) (Ezebuiro & Körner, 2017). On the other hand, TEs could be externally added in digesters, either as single elements or as mixed "cocktails" (Garuti *et al*., 2018).

During application of digestate as a bio-fertilizer, TEs can be transferred to soils, and, thus, released into the environment. Seasonal changes (temperature, water content, rainfall), as well as soil porosity, carbon content, and mineral fraction, will affect the leaching extent of the deposited digestate. After land deposition, AD digestate is exposed to atmospheric air which affects the redox potential of the digestate matrix as well as the redox state of the TEs. TEs can also be adsorbed on to the charged surface areas of the soil's organic and inorganic particles. Additionally, TEs can be complexed by microbially produced molecules and can be taken up by soil microbial biomass as well as plants. In most cases, these processes take place simultaneously at various rates and in different layers of the soil.

The fate and availability of TEs after digestate land application must be assessed to evaluate TEs bioavailability and uptake by plants (which can be transferred to the food chain), TEs leaching processes (which can prevent loss of soils nutrients and run off in ground waters) and TEs effect for soil organisms (which can affect soil fertility and productivity). This book chapter provides an overview of different type of BGPs and post-treatment processes of digestate. The possible physicochemical interactions between digestate and soils, which influence TEs speciation and their availability for biological uptake, are also described. Finally, different TE fractionation and speciation techniques are discussed.

6.2 DIGESTATE – PRODUCTION AND APPLICATION ON SOIL

Digestate contains more inorganic nitrogen (N), which is more accessible to the plants, than untreated slurry. N-uptake efficiency increases considerably and nutrient losses, by leaching and evaporation, are minimized if digestate is used as fertilizer in line with good agricultural practices.

During AD, organic nitrogen is partially mineralized to ammonium and this transformation is dependent upon feedstock used and upon efficiency of the biological process. For example, a longer hydraulic retention time (HRT) leads to lower organic matter content resulting from more efficient anaerobic digestion and methanogenesis due to decomposition of these organics and the breakdown of the resultant organic nitrogen compounds, too. For instance, in livestock slurries nitrogen is mainly ammonium (e.g., in pig slurry 65–70% of nitrogen is ammonium), whereas in energy crops the total nitrogen is mainly organic.

Subsequently, after AD, the ammonium quota could reach 70–85% in livestock slurries and 30–65% in energy crops (Rossi & Mantovi, 2012).

As mentioned previously, digestate is a product of BGP and inherits its characteristics from the substrates processed during anaerobic digestion. Consequently, depending on the BGP, different digestate, with different composition, are generated, which ultimately can affect TEs fate after soil application. In the next sub-sections a general overview of several BGPs and digestate post-treatment processes is described.

6.2.1 Biogas plants: a general overview

6.2.1.1 Agricultural biogas plants

Agricultural BGPs (Figure 6.1) represented about 69% of the total BGPs in the European Union in 2015 (EBA, 2017). AD substrates from agricultural BGPs derived from livestock effluent (e.g., pigs, cows, horses, poultry slurries and manure), energy crops (e.g., maize, sorghum, triticale, other cereals, grasses, sugar beet), agricultural residues (e.g., straw, stovers) and agro-industrial by-products obtained from manufacturing agro-food sector (e.g., molasses, straw, stovers, olive pomace, tomatoes peel, vegetable and fruits manufacturing residues).

Photo Credit: CRPA

Figure 6.1 Agricultural biogas plant.

An indisputable advantage of agricultural BGPs compared to other BGPs, such as waste BGPs, is that the digestate is usually a high quality organic fertilizer that does not contain excessive amounts of potentially hazardous compounds (Koszel & Lorencowicz, 2015).

6.2.1.2 Waste biogas plants

Waste BGPs (Figure 6.2) are involved in the processing of biological degradable wastes (BDW). BDW is a raw material from various origins and substrate compositions, including the organic fraction of municipal wastes (OFMW), waste from the food industry, retail trade (expired food), certain agricultural waste, sludge from wastewater treatment plants, etc. (Usták *et al*., 2004). The substrates used in these reactors exhibit non-homogeneous properties. Moreover, they can contain impurities, pathogens, trace metals, some organic pollutants and micro-organic pollutants which create waste management concerns. These concerns limit the potential uses of the digestate as a soil conditioner or fertilizer.

Figure 6.2 Waste biogas plant.

Therefore, it was recommended to incorporate a waste separating line before the AD process (Usták *et al*., 2005), to homogenize (i.e., softening substrate) and perform sufficient hygiene processes (i.e., inactivation of pathogen by pasteurization) of the raw input materials (Bernstad *et al*., 2013).

Digestate from waste BGPs can be used as fertilizer or soil conditioner eventually after aerobic post-treatment when element concentrations (Cd, Co, Cr, Cu, Ni, Pb and Zn) in composts, solid and liquid digestates are below permitted thresholds (Knoop *et al*., 2018; Tampio *et al*., 2016).

6.2.2 Separation of liquid and solid fraction of digestate

The high water content of the digestate requires transporting high volumes. Separation of digestate into liquid and solid fractions may provide a solution by

reducing the volume, which is why it is one of the most common first steps in digestate processing.

The most widely used definition of separation efficiency is the total mass recovery of certain components (total solids, volatile solids, total nitrogen, ammonium, total potassium, total phosphorous, etc.) in the solid fraction as a proportion of the total input (Svarovsky, 1985).

The separation efficiency of these mechanical separators can vary widely, due to differences in the efficiency of the separators and because the separation efficiency is affected by the variable physical and chemical composition of the slurry (i.e., the higher the dry matter content, the higher the proportion of solid phase after separation) (Bauer *et al.*, 2009).

6.2.2.1 Solid digestate

Solid digestate generated from agricultural BGPs represents about 3–15% of the wet weight of unseparated digestate. The total solids content is quite high, generally between 15–30%. Dry matter, volatile solids, carbon and phosphorus – in relation to the mass – are significantly accumulated in the solid phase, the amount depending on feedstock and separation technology used. Potassium, on the other hand, is distributed evenly between the digestate liquid and solid fraction (Rossi & Mantovi, 2012).

6.2.2.2 Liquid digestate

Liquid digestate consist ca. 85–90% of the wet weight of unseparated digestate. Total solids content is generally between 1.5–8% when screw press and belt press are used for separation (Rossi & Mantovi, 2012).

The more soluble forms of nutrients are normally contained in the liquid phase, which is characterized by a high phosphorus, nitrogen (35–90% of the nitrogen in this fraction is NH_4^+) and potassium content. The liquid fraction or its derivatives can function as either inorganic or organic fertilizers and/or soil conditioners, providing renewable substitutes for mineral fertilizers based on fossil resources (Vaneeckhaute *et al.*, 2013).

6.2.3 Technologies for application of digestate on soils

Digestates are used very frequently as fertilizers or soil conditioners, especially those from agricultural BGPs, because they contain plant nutrients such as N, P, K, Ca, Fe, Cu, Mn, Zn, Ni and diverse organic compounds. The stable organic matter in the digestate solid phase is excellent for improving soil properties. Increasing soil aeration can improve harvesting better than intensive fertilization of soils with unsuitable physical properties (i.e., poor aggregation, high compaction, inefficient drainage, shallow ploughing, low humus level, high clay content, etc.). Higher soil porosity ensures sufficient flow of nutrient and water. Before application on soil, the dosage of digestate has to be chosen according to

its total nitrogen and phosphorus content, crop nutrient requirements for the expected yield and available amount of nutrients in soils (Lukehurst *et al.*, 2010). The soil properties, mainly linked to the soil composition, pH, Ca, Mg and K content, are another factor that influences the dosage of digestate. Finally, the soil organic matter content and cultivation conditions, mainly referring to the previous crop, soil treatment and irrigation, are complementary factors for assessing the required digestate dosage.

The digestate liquid phase can be applied by means of devices used to spread raw sludge, and the digestate solid phase by means of the same devices used for solid manure application. Some devices are shown in Figure 6.3.

Figure 6.3 Digestate application methods: (A) trailing hose, (B) trailing shoe, (C) injection, and (D) splash plate.

Once applied on the soil surface, the digestate has to be incorporated into the soil during the first 24 h, or alternatively, some special machinery capable of soil injections needs to be used. In fact, for the incorporation of digestate to soil, the best application method is by machinery with soil injectors. If only the solid phase is applied, the digestate incorporation could occur in the course of 48 h.

As discussed above, digestate might contain diverse TEs that, despite being nutrients for plants and soil microorganisms, can be toxic at high concentrations. This will depend on digestate origin and treatment. In fact, origin and treatment

of digestate will control the digestate composition and the fate of TEs in it. TEs present in digestate can be transferred to soil during digestate application (Kupper *et al.*, 2014). Trace elements may leach to surface or ground waters and/or be taken up by plants and, consequently, transferred into the food chain. All these mechanisms depend on TE speciation and TE bioavailability. Moreover, once digestate is spread on soils, the fate of TE in the environment is influenced by soil properties (soil physical and chemical characteristics).

6.3 SOIL PHYSICO–CHEMICAL CHARACTERISTICS AND TRACE ELEMENT MOBILITY

Soil is a heterogeneous, complex living ecosystem that represents a unique balance between physical, chemical and biological properties. The colour, moisture content and permeability are major physical characteristics of soils.

In addition to the physical characteristics, a lot of other parameters can be considered for soils characterisation. Electrical conductivity (EC), pH, redox potential (Eh), cationic exchange capacity (CEC), exchangeable potassium (K_{exch}), total nitrogen (N_{tot}), nitrates (NO_3^-), sulfur (S), carbonates ($CaCO_3$, $MgCO_3$), ammonium (NH_4^+), organic carbon (OC), total phosphorous (P_{tot}), available phosphorous (P_{avail}), microbial biomass carbon (MBC), TE, and soil respiration are some of the major physicochemical parameters that are essential for soils characterization (Alef & Nannipieri, 1995; Anderson & Domsch, 1989; Bremner & Mulvaney, 1982; Di Bene *et al.*, 2013; Keeney & Nelson, 1982; McLean, 1982; Moscatelli *et al.*, 2005; Nelson & Sommers, 1982; Olsen & Sommers, 1982; Thomas, 1982).

Minerals are major constituents of soils, mainly originating from weathering of parent material. Quartz, feldspars, carbonates (calcite, dolomite), metal oxides/hydroxides and clay minerals (hydrous aluminosilicates) are some of the most important constituents because they play a vital role in the fertilization of soils through adsorption-desorption processes. However, different kinds of minerals show different adsorption-desorption ability and cation exchange capacity (Churchman & Lowe, 2012).

6.3.1 Total trace elements in soils

TEs occur in soils from the natural environment (the Earth's crust) reaching concentrations lower than 100 mg Kg^{-1} (Hooda, 2010). However, anthropogenic activities contribute to the total TE concentrations in soils. One of the major TE sources contributing to diffuse soil pollution is the agricultural practice of spreading mineral and organic fertilizers and pesticides on soil (Kabata-Pendias & Pendias, 2001; Lukehurst *et al.*, 2010).

Some of TEs can be considered as micronutrients because of their involvement in plant growth and animal nutrition. Such TEs are copper, iron, manganese,

molybdenum, sodium, zinc, cobalt, chromium, nickel as well as iodine, selenium, boron, chlorine and fluorine (Bennett, 1993; Johnston, 2005).

6.3.2 Mechanisms regulating trace elements distribution in soils

Many TEs in soil solutions exist as a single oxidation state, however, some of them like selenium, chromium and arsenic can occur in different oxidation states according to the existing redox conditions. Common TE speciation in soil solutions includes hydrated cations, oxyanions, organometallic compounds, inorganic complexes or neutral species (Kabata-Pendias, 2011). In the solid part of the soils, the most immobile fraction of TEs occur in the minerals' structure, whereas the most mobile fraction corresponds to TEs bound on the surface of minerals and organic matter of the soil.

Understanding the mobility of TE in soils and their chemical speciation is important to comprehend whether plants or soil microorganisms can easily uptake TE from soils. Moreover, this information will also allow classification of elements as essential or toxic.

The speciation of TE in soils is strongly affected by the physicochemical properties of soils and the toxicity or fertility of soils is influenced by the interactions between the TEs and the soil components (Kabata-Pendias & Pendias, 2001).

The major processes that determine the distribution of TEs in soils include dissolution-precipitation, ion exchange, adsorption-desorption and absorption-mineralization by the biomass (Figure 6.4).
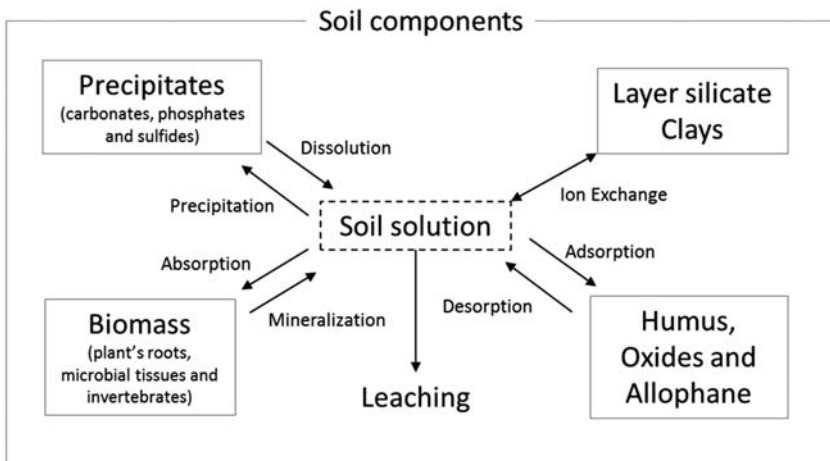

Figure 6.4 Interactive binding mechanisms affecting the distribution of TE between the liquid and solid phases of a common soil (modified from Adriano, 2001).

The distribution of TEs between the solid and liquid phase (i.e., soil solution) depends on soil parameters such as its pH, redox potential (Eh), cation exchange capacity and it is strongly affected by the presence of carbonates, oxides of manganese, iron and aluminium, clays, low-molecular weight organic substances and microorganisms (Kabata-Pendias & Pendias, 2001). The general observed trend is that cations such as cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) are dissolved at low pH and Eh, pH having a more important role in TE solubility than Eh (Chuan *et al.*, 1996). Therefore, soils with neutral and alkaline pH behave as a reservoir of TEs that slowly supplies them to plants. On the other hand, in acidic soils TEs are quickly dissolved, thus the TEs are relatively readily available for uptake by plants (Kabata-Pendias & Pendias, 2001).

Another phenomenon which includes the majority of the reactions between TE and the soil components is sorption (Adriano, 2001; Kabata-Pendias & Pendias, 2001), which includes all interaction mechanisms between TE and metals oxides, biomass, carbonates, phosphates, sulphides, basic salts and clays. Moreover, it includes intermolecular interactions (e.g., van der Waals' forces), mechanisms based on valence forces and interface forces such as ion exchange.

A particular sorption process is adsorption, where TEs dissolved in the soil solution interact with the surface of solid particles of soils (Kabata-Pendias & Pendias, 2001). Two types of TE adsorption can be distinguished: a physical adsorption characterized by the weak electrostatic forces where TE are bound through cation exchange, and a surface complexation adsorption, also called chemisorption, which involves stronger chemical bonds between TE and solid particles (Adriano, 2001).

Another binding mechanism is absorption, which involves the assimilation of TE by microorganisms and plants' roots (Adriano, 2001). Indeed, the process of plant transpiration induces the absorption of TE from the soil solution by the roots of plants. In addition, the microbial population surrounding the plant roots will immobilize the TE ions in the microbial biomass. In addition, microbial uptake of TEs is severely affected by positively charged ions supplied along with digestate due to their interference with the direct availability of TEs to microbial and plant siderophores and soil polyaromatic compounds, such as humic acids and lignin residues.

All of the aforementioned binding mechanisms will strongly depend on the composition of the soil and will help to determine the speciation of TEs and their mobility and bioavailability into the environment (Adriano, 2001).

6.3.3 Trace elements mobility in soils and availability for plants

In general, the mobility of selected TE in soils decreases in the following order:

cadmium $>>>>$ nickel $>>>$ zinc $>>$ copper $>$ lead (Tlustoš *et al.*, 2007).

The most important factors regulating TE solubility and availability for plants are the redox potential and pH values. Trace element availability is mostly lower in

heavy soils with neutral or alkaline pH values and higher in light soils (Wenzel *et al.*, 1999). Generally, TE content in plant tissues decreases with increasing pH values in plant tissues (Tyler & Olsson, 2002).

Redox potential is mainly important in relation to iron, manganese and elements adsorbed onto Fe and Mn oxy-hydroxides, such as arsenic, copper, mercury and lead. Under reducing conditions, Fe and Mn oxyhydroxides dissolve releasing adsorbed elements into soil solution (Chuan *et al.*, 1996). Elements with higher oxidation numbers are usually less soluble.

Organic compounds participate in the processes that reduce element concentration in soil solutions either by nonspecific or specific sorption (soil ability to bond ions or molecules of different compounds from soil solution into soil solid phases) or by complexation that protect elements against adsorption or precipitation (Alloway, 1990; Shaheen *et al.*, 2013). The solubility of soil organic matter increases with an increase in pH, which influences complex stability consisting of humus and trace elements. Humic acids are insoluble at neutral and acidic pH values and they can cause the retention of harmful TEs in soils, and the decrease of TE concentration in soil solution. Conversely, fulvic acids and their complexes are soluble even at low pH values and they may increase TE mobility, to which they are bonded (Shaheen *et al.*, 2013; Tlustoš *et al.*, 2007).

The cation adsorption of TE depends on the amount of negative electric charge on the soil surface, which is also influenced by pH values. However, some cations can have a higher displacement force than others and they can selectively bond to sorption sites. In addition, competition for sorption sites with more abundant ions, such as calcium and magnesium, will also influence sorption capacity. Cation exchange capacity influences the uptake of TE both directly, by the amount and force of bonded sites for elements in soils, and indirectly, by the influence of other soil properties (Shaheen *et al.*, 2013; Tlustoš *et al.*, 2007).

To summarize, TE mobility and transport in soils and availability to plants will depend on soil physico-chemical characteristic as well as on the TE itself.

6.3.4 Effects of digestates on soil properties and TE content

The digestate application leads to an improvement of soil properties by reducing the bulk density, increasing saturated hydraulic conductivity (water ability to move through pores), raising soil capacity of moisture retention and improving aggregate stability compared with soils without digestate addition. The favourable influence on soil aggregates is caused also because compounds such as lignin, cellulose and hemicellulose complexed with lignin and humic compounds are difficult to degrade during AD, but they are highly reactive with soil surface (Nkoa, 2014). On the other hand, digestates with high sodium content contribute to the dispersion of soil particles.

Soil biota after digestate application is enhanced, especially soil microbial activity (sometimes even macro-organism such as worms are stimulated), because

the digestate promotes the activity of some enzymes as urease (decomposes urea to ammonia), phosphatase (important in the phosphorus cycle), β-glucosidonase and dehydrogenase. In addition, the comparisons of parameters describing soil microbial activity, such as basal respiration (the respiration without substrate addition), induced respiration by substrate, specific growth rate, metabolic quotient or nitrogen mineralization capacity, suggest that the effect of digestate application on the promotion of soil microbial activity is lower than the effect of undigested feedstock application in the short-term. Besides, the effects of digestate on different microorganisms can vary.

Digestate compositions combined with soil compositions will affect TE mobility and bioavailability when considering all the mechanisms mentioned in Section 6.3.2 and the organic matter that is being introduced. In fact, once the digestate is applied on soil, TEs will redistribute between the solid and liquid phase of the soil and their mobilization and speciation will change according to the physico-chemical characteristic of the soils and digestates (Guala *et al.*, 2010). Although TE can be already present in the soil, the addition of digestate can lead to the introduction of excess TE. Digestate is produced in anaerobic conditions. However, a fast oxidation of the digestate will occur when it is spread or injected into the topsoil. But, as the digestate moves down through the soil depth, oxic conditions can change to sub-oxic and even anoxic in the deeper soil layers (Borch *et al.*, 2010). This is related to the rate of oxygen penetration and to the soil organic carbon content. Therefore, the solid fraction of digestate can be destabilized and dissolution of TEs can occur together with desorption and/or leaching of metals, a subject that demands further investigation.

So, to fully determine possible TE toxicity during digestate amendment in soil, one must ensure TE analysis not only on the digestate itself but also on soil and on soil amended with digestate, analysing not only total TE content but specially TE speciation and fractionation to establish TE bioavailability. For that one must start with proper sample collection, ensuring sample preservation before analysis.

6.4 COLLECTION, PRESERVATION AND PREPARATION OF SAMPLES

Adequate sampling, sample preservation and preparation are important prerequisites in obtaining representative, reproducible and comparable results of soil analyses (Theocharopoulos *et al.*, 2004) as each of these steps may introduce errors greater than the analytical error.

Collecting, storing and processing samples should keep them as unaltered and free of contamination as possible (Mester & Sturgeon, 2003). van Hullebusch *et al.* (2016) recently reviewed the main methodologies suitable for collecting and preparing anoxic samples. Sampling includes planning of locations, size, number of samples, which should ensure their required quantity and representativeness.

Adequate sampling tools should also be used to obtain undisturbed and contamination-free samples.

To evaluate TE bioavailability and toxicity during digestate application on soil, samples of soil, digestate and soil blended with digestate should be collected and properly preserved and treated. Digestate should be collected before soil application, either liquid or solid fraction depending on what is being applied on soil.

As mentioned previously, digestate, which is in anaerobic conditions in BGPs, is oxidized once outside the reactor and particularly after liquid-solid separation. Therefore, digestate samples can be collected as regular oxic liquid or solid samples according to standard protocols. In contrast, for speciation and fractionation analysis, sampling methods should be carefully evaluated to minimise the conversion of the original chemical species. Furthermore, one should be aware that samples collected should ideally be representative of the whole system. Hence, precautions should be taken to ensure homogeneity of digestate material. With respect to the size of each sample, one needs to consider spatial, that is, the 3D heterogeneity of each soil under analysis. As there are numerous valid approaches to assess the characteristics of soil, one of the most commonly adopted strategies is to distribute the study area into quadrants, sampling a number of cores ($n > 10$) within each of the quadrants and assessing the TE content with depth and location of cores.

The procedure selected for sample preservation depends on the form of the samples stored for analysis (wet or dry) and the parameters to be analysed. Wet samples should be stored deep frozen ($-20°C$ to $-80°C$) and analysed after a short period of time. More often soil samples are dried, either at a room temperature (air dried) in the dark or by freeze drying (lyophilisation) for samples in which volatile compounds can be lost during this step (Mester & Sturgeon, 2003). Dry samples can be stored for several years, at room temperature at a dry place for the total metal analysis or frozen for speciation analysis which includes determination of organometallic compounds, which can be slowly degraded even in dry samples (Dubiella-Jackowska *et al*., 2007). Sample preparation methods should also be carefully evaluated to minimise the influence on original TE speciation and fractionation.

In general, the soil fraction selected for analysis (total sample of fine fraction) depends on the purpose, but the coarse fraction (>2 mm) is removed from soil samples by sieving after drying the samples up to constant weight. A further step comprises crushing samples (either manually or mechanically) to obtain a homogenous sample with fine particles of uniform size which are suitable for extraction (by organic solvents or leaching reagents) or total dissolution (by mineral acids for total TE analysis) (Mester & Sturgeon, 2003). Although this is valid for total TE content assessment, fractionation and speciation of TE may require that the sample remains at $-20°C$. For instance, the results of fractionation analysis may be affected by drying (humidity level, formation of

metal oxides) and grinding (high surface-to-volume ratio, therefore enhanced metal-oxide formation) prior to extraction of different TE fractions (Baeyens *et al*., 2003). Solid digestate can be collected as a soil sample, with the same concerns being applied.

As for liquid digestate, the total TE content can be determined on the whole sample or for different size fractions, the first involves sample digestion with concentrate acids or simple acid addition, whereas the second can include sequential filtration (van Hullebusch *et al*., 2016). For TE speciation, different techniques can be applied (see Section 6.5) all including specific sample preparation protocols. In general, the sample should be kept at −20°C.

Regarding soil amendment with digestate, samples can be collected as regular soil samples with the same principles regarding sample collection, preservation and preparation being followed, and taking into consideration further sample analysis. Nevertheless, as mentioned above, as the digestate moves down through soil depth, oxic conditions can change to sub-oxic and even anoxic in the deeper soil layers (Borch *et al*., 2010). This can change TE mobility and speciation and therefore, soil amended with digestate should be collected over time and at different depths. To ensure the anticipated levels of TE mobility, the oxic/anoxic conditions of the samples need to be maintained comparable to in-situ conditions. In van Hullebusch *et al*. (2016) a review is made on methodologies appropriate for collecting and preparing anoxic samples.

Therefore, standard protocols must be established to set unified procedures for sampling, storing and preparing samples before further analyses, taking in consideration the oxic/anoxic conditions of the samples.

6.5 ANALYSIS OF SAMPLES

In assessing the fate and bioavailability of trace elements in soils after digestate application, one must analyse soil amended with the digestate. Analysis of the digestate (either liquid or solid fraction) and the soil prior to digestate application can also be considered as this will give an indication of the expected total TE concentrations as well as a picture of the changes in TE availability due to digestate application thus allowing for a more proper evaluation of possible TE toxicity and limitations that could be imposed upon digestate application into soil.

As the fate of TEs can be influenced by various parameters such as climate, soil and digestate characteristics as well as the digestate incorporation method (Mantovi *et al.*, 2005) it is crucial to conduct laboratory and field experiments under different but defined conditions. The fate of TEs following short and long-term application of biosolids is relatively well reported in the literature, but this issue remains deeply controversial within the scientific community. Some published data have shown that long-term biosolid spreading would result in the release of metals into the soil due to organic matter mineralization; this is the "time bomb hypothesis" (Bergkvist *et al*., 2005; McBride, 1995; Stietiya & Wang, 2011). Other authors

have suggested that the long-term application of biosolids would present no environmental risk, due to the high adsorption capacity of mineral phases within biosolids; this is the "protection hypothesis" (Hettiarachchi *et al.*, 2006; Li *et al.*, 2001).

Consequently, to better predict the environmental fate and mobility of contaminants, in addition to analysis of the total TE content, determination of TE speciation and fractionation is a prerequisite that ultimately will allow assessment of the possible health risks posed. As indicated by Templeton *et al.* (2000): "TE speciation analysis is the analytical activity of identifying and/or measuring the quantities of individual chemical species" whereas "fractionation analysis refers to separation procedures with insufficient separation power to differentiate between individual chemical species, classifying a group of analytes according to their physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties".

The methodologies commonly used to determine total TEs and TE fractionation and speciation in digestate samples have been recently reviewed by van Hullebusch *et al.* (2016). In the following sub-sections a review of analytical methodologies that can be applied to soil amended with digestate is presented.

6.5.1 Common parameters and total TE concentrations in digestate-amended soils

The general set of sample analyses include measurement of common parameters like pH, EC (electrical conductivity), CEC (cationic exchange capacity), CNS (carbonate, nitrogen and sulphur), organic matter, TOC (total organic carbon), total N, –NH_4, total P, total K, temperature, and water content. These analyses are similar to those used for soil prior to digestate amendment and several standard protocols are available.

Total concentration of major (e.g., S, Ca, Mg, Na, Cl, Fe, Mn, Cu, Zn, B) and trace (e.g., As, Co, Cd, Cr, Ni, Pb) elements is often measured and reported (e.g., Alburquerque *et al.*, 2012; Kathijotes *et al.*, 2015; Ramezanian *et al.*, 2015; Szilágyi & Szentmihályi 2009). Atomic absorption (AAS) and atomic fluorescence spectrometry (AFS), as well as inductively coupled plasma spectroscopy (ICP-AES) and spectrometry (ICP-MS), are the main techniques used for total TE concentration determination in soils (Hanlon, 1998; Isaac & Johnson, 1998; Karathanasis & Hajek, 1996).

Electrochemical techniques can also be used for the determination of trace amounts of a wide range of TEs. There are a numerous electrochemical techniques, but they can be subdivided into two groups: potentiometry and voltammetry.

In TE analysis, anodic stripping voltammetry (ASV) has been a popular voltammetric technique because of its speed, good sensitivity and selectivity. In this method, TE is pre-concentrated at the mercury working electrode by the reduction of metal to the metallic form. However, some metals do not form

amalgams with mercury. In these cases, adsorptive stripping voltammetry (AdSV) is an adequate technique for their determination in a variety of matrices. In AdSV, high sensitivity can be achieved by adsorptive deposition of a metal complex exploiting the appropriate complexing agent. For instance, for cobalt and nickel dimethylglioxime and nioxime (Ferancova *et al*., 2016; Korolczuk *et al*., 2005) can be used, whereas for zinc alizarin is the most suitable choice (Deswati *et al*., 2016). In the case of selenium, the most useful electrochemical technique is cathodic stripping voltammetry, where high sensitivity is obtained through the formation of an insoluble salt on the mercury electrode followed by its reduction (Grabarczyk & Korolczuk, 2010).

Potentiometry with ion-selective electrodes (ISEs) allows determination of free ion concentrations as well as total metal content in various aqueous samples. The most attractive features of this technique, besides low cost and quick analysis time, are the wide measuring range, the portability of the device, the non-destruction of the sample and the requirement of minimum sample pretreatment. TEs important in AD bioprocesses, for example, cobalt, nickel, zinc and copper, can be determined by ISE (Wardak 2008, 2014; Wardak & Lenik, 2013).

As mentioned above, soil layers with different redox conditions can be encountered and, as such, the oxidation state of the sample should be taken into account upon analysis. Nevertheless, for total TE concentration assessment redox condition is of minor importance.

6.5.2 Fractionation techniques for trace-element partitioning in soils amended with digestate

To investigate the partitioning of TEs in soils, fractionation techniques such as sequential extraction procedures can be adopted. Fractionation can be obtained by wet chemical extraction methods where a series of specific reagents are applied to extract operationally defined species which illustrate elements bound in various soil phases (Tack & Verloo, 1995).

Although the accuracy of sequential extraction methods can be queried due to the numerous extraction steps, operating conditions and reagents involved in extracting TEs, the outcomes of the procedures can provide information on the chemical form of TEs and their mobility in soils (Filgueiras *et al*., 2002). However, one should be aware that the sequential extraction fractions are operationally defined, that is, the extracted TEs are directly related to the procedure of extraction used (Quevauviller *et al*., 1997).

In general, sequential extraction procedures consist of consecutive additions of different reagents (extractants) on an aliquot of solid sample to extract diverse fractions of TEs. The extracted fractions have different degrees of mobility into the environment (Filgueiras *et al*., 2002). Usually, the first extracted fraction corresponds to TEs weakly bound to the solid phase, which have a higher mobility potential compared with later fractions.

Among sequential extraction procedures, the methods most utilized are the ones proposed by Tessier and by the Community Bureau of Reference (BCR). In the Tessier method the fractions are exchangeable, bound to carbonates, bound to iron and manganese oxides, bound to organic matter and the residual fraction (Tessier *et al*., 1979). Later, the method was modified and adapted for trace-metal extraction in sludge samples in the work of van Hullebusch *et al*. (2005). This sequential extraction method may be performed in anoxic conditions, which can be important because after digestate amendment different soil layers may have different redox conditions. van der Veen *et al*. (2007) observed that manganese, nickel and zinc extracted in carbonates and organic matter/sulphide fractions were significantly affected by oxidation, while other metals were not.

Regarding the BCR method, the original method was successively modified (Mossop & Davidson 2003), the fractions being the exchangeable fraction, bound to iron and manganese oxides, bound to organic matter and sulphides and finally the residual fraction (Mossop & Davidson, 2003; van Hullebusch *et al*., 2005). The performance of the "revised BCR" protocol is, however, more time-consuming compared with the "modified Tessier" protocol.

Despite some drawbacks, such as the uncertainties in the selectivity of the various extractants and the possibility of re-adsorption and partial oxidation of oxygen-sensitive elements (which can be important when dealing with anoxic soil layers), sequential extraction procedures are well established, thus allowing for study of metal partitioning among the various solid phases of soils (Filgueiras *et al*., 2002).

6.5.3 Speciation of TEs in digestate amended soils

The study of TEs speciation in digestate-amended soils can be achieved by traditional chemical methods such as adsorption/desorption experiments as a function of various parameters for example, pH, contact time, concentration, ionic strength or column experiments in lab or field scale. Such "macroscopic" experiments allow researchers to obtain thermodynamic and kinetic parameters of the sorption processes.

To obtain an in-depth understanding of the processes involved, these experiments can be combined with spectroscopic techniques, such as X-ray absorption spectroscopy (XAS) and X-ray fluorescence microscopy (Donner *et al*., 2011, 2012). These synchrotron radiation-based techniques are the best available for examination of metal speciation and associations in complex environmental media due to their high resolution and their selectivity for the chosen element. This combination of approaches in term of scale gives useful information to predict metal mobility and bioavailability in biosolid-amended soils. Indeed, the results gained in batch-scale experiments can be compared with those obtained in soil column studies and are then validated with the analytical results attained from the field measurements. If molecular scale studies can also

be performed, this enables a profound and general understanding of the fate and behaviour of TEs entering the soil *via* agricultural application of biosolids or being "naturally" present in soils.

As already mentioned above, solid-phase speciation in soil (D'Amore *et al.*, 2005) can be achieved by direct determination of species *in situ* through physical instrumental methods (X-ray methods like XRD, XPD, XAS; magnetic spectroscopies like NMR, EPR; electron techniques like SEM-EDX, TEM-STEM; vibrational spectroscopies like IR, Raman or mass spectrometry like LA-ICPMS). In addition, various hyphenated techniques such as GC-ICP-MS or LC-ICP-MS are used to determine specific compounds of elements like Hg, As, Sb or Cr, which appear in different oxidation states or form stable organometallic compounds in the environment (Bakirdere, 2013; Szpunar, 2000).

Another approach to estimate TE speciation in soil and digestate-amended soil could be through mathematical modelling, using for instance, geochemical equilibrium models. In fact, analytical techniques used to determine TE speciation often are not able to provide information on overall TE speciation. Therefore, mathematical modeling of TE speciation can be used as a theoretical approach to either compile or verify the analytical results.

Whichever methodologies are used, one must be aware that total TE concentrations are not directly linked to TE effects and toxicity and that TE bioavailability is the key factor, a factor that is strongly influenced by TE speciation.

6.5.4 Methods for estimation of TE mobility and bioavailability in soil

6.5.4.1 Diffusive gradient in thin films

In situ determination of bioavailable TE fraction can be estimated by applying passive sampling. The diffusive gradients in thin film (DGT) passive sampling is based on the diffusion of the targeted TE through a diffusive layer (porous gel) followed by an irreversible sorption onto a layer of binding phase. This technique was originally developed to determine labile TE in water (Davison & Zhang, 1994), but its applications have been extended to sediments and soils. During exposure to the device, a concentration gradient of the analyte is established between the exposition media and the binding phase, which results in a flux of analyte correlated with its concentration in the exposition media. After a given time, the accumulated TE is eluted from the binding phase and quantified, usually using AAS or ICP-MS. The original concentration in the exposition media is then determined according to the amount of sorbed TE, the exposition duration and diffusion parameters (TE diffusion coefficient through the diffusive layer, layer thickness) (Davison & Zhang, 1994).

During deployment in a soil, the accumulation of the TEs in the DGT device induces a decrease in the TEs concentration in the soil solution in the vicinity of the device. To compensate this decrease, some TEs can be released from the soil

solid phases. As a consequence, the TEs accumulated by the DGT will reflect the initial pore water concentration and its potential resupply from the solid phase (Hooda & Zhang, 2008). Such behavior can be considered as mimicking the uptake of TEs by plants, and thus TE concentration determination by DGT could be more effective in estimating TE bioavailability compared with total analysis or chemical extractions (Sun *et al.*, 2014). Several studies concluded that DGT measurements were actually correlated to the bioavailability of TEs, such as Zn or Cu (Agbenin & Welp, 2012; Almas *et al.*, 2006; Cornu & Denaix, 2006; Quasim *et al.*, 2016; Sun *et al.*, 2014; Tandy *et al.*, 2011; Tian *et al.*, 2008; Wang *et al.*, 2016), but there is no consensus yet. Indeed, poor or no correlation was observed for high TE concentrations (Almas *et al.*, 2006) or during experiments conducted on field cultivation (Agbenin & Welp, 2012). This could be due, for example, to the influence of soil humidity on DGT sampling (Hooda *et al.*, 1999), to competition between major elements of the soil and TEs for sorption onto the binding phase of the DGT device (Mundus *et al.*, 2011), or to the fact that high concentrations of TEs can modify uptake by plants (inhibition of uptake, activation of regulation mechanism, and so on) but not by DGT devices. More studies that include a large variety of TEs, soils and plant species are thus required to evaluate the effectiveness of DGT as a phytoavailability assessment tool.

By taking into account the effective concentration based on pore water and solid-phase releasable amounts, DGT can reveal different rates of release between TEs present in the soil and freshly amended TEs (Zhang *et al.*, 2004). Furthermore, its accumulation capability allows the detection of very low concentrations of labile TE. Finally, DGT can be also used to obtain a depth profile or TE repartition in soils, with an even higher resolution than piezometric sampling (Leermakers *et al.*, 2016; Luo *et al.*, 2013). Such profiles may be helpful to understand the mechanisms of TE release and allow the detection of TE microniches, corresponding to localized highly concentrated mobile TEs, which is not possible with conventional techniques.

6.5.4.2 Other methodologies
Biological activities can also influence TE fractionation and speciation through a range of possible mechanisms including precipitation, dissolution, sorption, chelation, and redox transformation. In soils, both plants and microorganisms can have an active role in this. So, TE fractionation and speciation can also be indirectly assessed by specific protocols involving plants and/or microorganisms.

For instance, potential availability of TEs for plant uptake (bioavailable fraction) is usually determined by a single extraction, for which $CaCl_2$, NH_4NO_3 or some complexing agents like EDTA may be used (Chojancka *et al.*, 2005; Pueyo *et al.*, 2004). Other extractants can also be used. For instance, Almeida *et al.* (2005) used low molecular weight organic acids, commonly exuded by plants, to assess metal bioavailability in sediments.

On the other hand, simple toxicity tests can be carried out to evaluate TE effects on soil microorganisms. For instance, commonly used tests are Microtox™ and ToxScreen. ToxSreen is a bioassay that uses a highly sensitive variant of the luminescent bacterium *Photobacterium leiognathi* (Ulitzur *et al.*, 2002). Although these tests are for aqueous samples, they can be also used to test leaching solutions from soils amended with digestate or a slurry of it.

Results obtained through these methodologies should be interpreted jointly with TE fractionation and speciation results to fully assess TE bioavailability and toxicity in sols amended with digestates.

6.6 CONCLUSIONS AND RESEARCH NEEDS

Many countries adopted in their national energy strategies the production of energy from renewable sources, like biogas/biomethane obtained from the anaerobic decomposition of organic substrates, including agricultural residues, biowaste and sludge from sewage treatment.

Digestate is the main out stream from anaerobic digestion process that contains mineral nutrients (nitrogen, phosphorus, potassium) and TEs. The digestate, particularly when it is produced from agricultural substrates, can be used as fertilizer or soil conditioner according to legal requirements. In terms of absorption of nutrients by plants, digestate can resemble mineral fertilizers improving agriculture sustainability.

The analysis of nutrients and TEs in the digestate (e.g., potassium, calcium, iron, copper, manganese, zinc, nickel and chromium), when one considers the digestate as a fertilizer, is required to improve the existing and future digestate application technologies (i.e., precision farming). This knowledge is also necessary to evaluate the cumulative effect of TE content in soil, as well as the soil salinization effect due to excessive content of microelements from the digestate. After application in soil, TE bioavailability will depend on soil characteristics and on digestate composition.

To assess TE bioavailability, TE fractionation and speciation studies should be performed after applying appropriate sampling and sample preservation methods. Several fractionation and speciation methods commonly applied to soil samples can be used for soil amended with digestate. However, the diversity of digestate chemical characteristics and soil chemical properties poses difficulties in setting up an accurate method to investigate the fate of TE in soils amended with digestate.

Long-term field studies should be performed to investigate the fate of TE in soils amended with a large set of digestates. Further research is also needed to assess how much TEs present in digestates may be transferred to plants. It is important to assess how digestate application may affect soil microbial diversity. The outcome of these research works could contribute to improving the knowledge on nutrients and TEs present in anaerobic digestion effluent, and to the establishment of new regulations

in the field of organic fertilizers to incorporate digestate, at national and European level.

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