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“Integrated water cycle sustainability: NEW CHARACTERIZATION PERSPECTIVES FOR SLUDGE”

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Contents

Summary

The increasing interest towards recycling the sewage sludge as fertilizer and soil amendment through agricultural use, according to the current policy of the European Commission and national authorities, on one hand and increase in the concentration of some emerging contaminants in the waste water and as a consequence in sewage sludge due to the changes in the lifestyle of the humankind, on the other hand, highlighted the need for constant monitoring and characterizing of sewage sludge.

Therefore, this PhD research has a focus on the new characterization perspectives for sewage sludge within the framework of the concept of integrated water cycle sustainability to evaluate its suitability for agricultural use. Friuli-Venezia Giulia region (north-east of Italy) was selected as sampling area and the sewage sludge samples were taken from 10 waste water treatment plants located in this region that vary in their treatment capacity, process units and sludge treatment sequences.

The work started with characterizing of the sewage sludge samples considering their nutrients content and the concentration of toxic metals (TM), extractable organic halogen (EOX), linear alkylbenzene sulfonate (LAS), polychlorinated biphenyls (PCBs), Polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs) and Polycyclic aromatic hydrocarbons (PAHs) to evaluate their agricultural reutilization potential. The obtained results showed that the TM content and the concentration of studied organic contaminant in sewage sludge were lower than maximum permitted limits from Italian and European regulations for agricultural use in all tested samples, but a general increase in maximum concentration values with respect to a previous monitoring is

highlighted, due to some upgrade in wastewater treatment plants processes. We hypothesized that this might be linked to a better degree of stabilization which is reflected by production of humic substances.

Furthermore, humic substances (humic and fulvic acid) as the most important fraction of organic matter content of sewage sludge were extracted and analysed considering their chemical and spectral properties. In this part of the work we aimed to ascertain the integrity of HS use as markers of biological transformations at the storage stage of sewage sludge where biological transformation is generally considered to be minimal. For this purpose, we tried to create a relation between the organic contamination and humification degree of sewage sludge and also the use of UV-vis, FTIR and Fluorescence spectra to assess properties of humic substances as a valid and cost effective methods was evaluated. As a result, we could show that several qualitative and quantitative changes can occur in the humic fraction of sewage sludge during the storage stage, both under aerobic and anaerobic conditions and also some correlations between LAS and changes in organic matter during storage is confirmed.

Prior to evaluate the stabilization degree of stabilization of sewage sludge it became necessary to assess the integrity of extraction procedures for the isolation of HA and FA. In the third chapter of this work, we answered to the question whether humic substances are artifacts or not of the extraction procedure. In a recent work, Lehmann and Kleber (2015), rejected the humic substances concept and proposed the Soil Continuum Model, in which organic matter exists as a continuum of organic fragments and humic substances are considered to be artifacts of the alkali extraction procedure. The goal of our work was to test assumptions made by Lehmann and Kleber (2015) this allowed us to demonstrate that humic substances are real components of natural organic matter and sewage sludge.

List of Tables

List of Figures

Chapter I

Introduction

In this chapter, the key concepts for the present research are defined; briefly we will speak about sewage sludge and its composition, both considering the potential fertilizer properties due to its nutrient and organic components and the potential health risks associated with the toxic metals and pharmaceutical residues content. In addition, some common treatments that sludge usually undergo in wastewater treatment plants as well as its final disposal methods are introduced with particular focus on sewage sludge recycling through agricultural use. Moreover, the concept of humic matter as the most important fraction of sewage sludge organic matter is explained. And finally the aim of the present research, which was monitoring of sewage sludge proposed for agricultural use by focusing on its new characterization perspectives, will be stated.

1.1 What is sewage sludge

The process of treatment of waste water results in the production of a residue that must be managed properly. This residue which is commonly termed sewage sludge (SS) is a semisolid and odiferous material with a two phase system: a water phase with dissolved substances and a dispersed phase which is a solid insoluble in water. The quality and quantity of sewage sludge is largely dependent on type of sewage and the treatment technology (Gawdzik et al., 2015; Sanin et al., 2011). Fresh and untreated sludge may contain pathogens, a high ratio of water and high biochemical oxygen demand (BOD) due to its large organic matter (OM) content. Nevertheless, sludge also can be potentially used as fertilizer since it contains macro and micro-nutrients which are essential for plant growth.

The quantity of sludge will continue to increase as a result of rapidly increasing population, urbanization and industrialization. About 9.5 million tons dry matter of SS are produced in the Europe in 2015 (Eurostat, SS production and disposal, last update 19.11.2018). In fact, upgrading of wastewater treatment plants and implementation of new Urban Waste Water Treatment Directive necessitate the development of sludge management methods and choice of the right treatment, disposal and reuse approach.

Figure 1.1 shows the operation of a typical sewage treatment plant's process in which the various types of sludge are generated. Primary sludge as a result of primary treatment, comprises floating material and heavy solids separated from liquid waste which is simply produced through settling at the bottom of the primary clarifier. Since this sludge has a high concentration of oxygen-demanding materials and pathogenic microorganisms and has high percentage of water, sub sequent treatments (most commonly anaerobic digestion) is used to make it less objectionable.

In the secondary treatment, effluent from primary treatment undergoes further treatment to remove the residual organics and suspended solids. This treatment method consists of an aeration tank followed by a secondary clarifier. In the treatment process, oxidation of wastewater is done through mixing air and liquid in the presence of high concentrations of microorganisms allowing a proper time for biological activity to achieve a sufficient degree of transformation. Microorganisms metabolize dissolved OM in the wastewater and produce more microorganisms and inorganic end-products and, as a result, the oxygen demand of the liquid is reduced.

In the secondary clarifier, the microorganisms are separated from the mixture by sedimentation and the clarified liquid is removed from the surface of the clarifier and discharged as secondary effluent. To balance the amount of the microorganisms in the system, a part of the microorganisms is recycled to the aeration tank and the remainder is removed from the process that is called activated sludge.

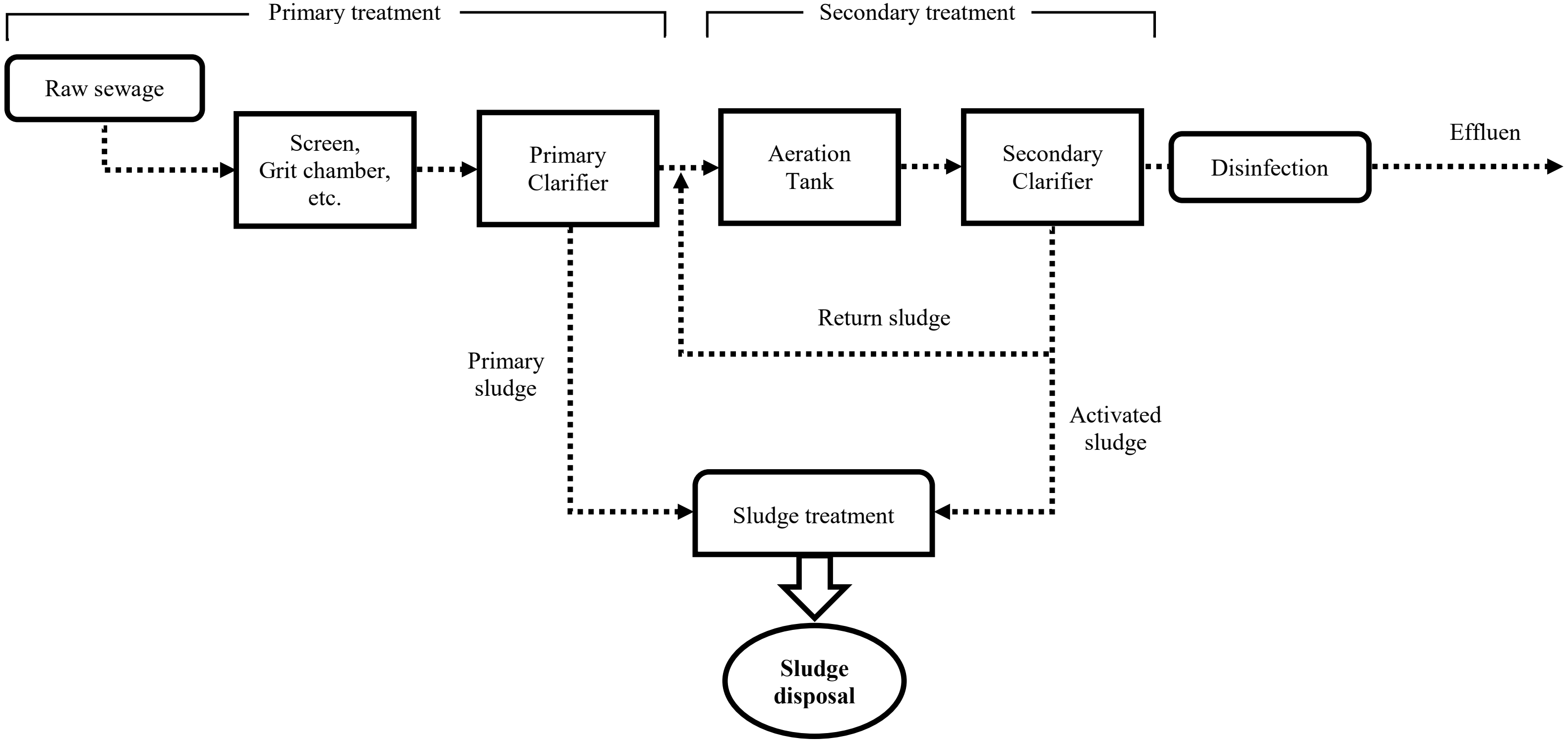

Figure 1. 1 Primary and secondary treatment of wastewater using the activated sludge process

1.2 Composition of sewage sludge

1.2.1 Nutrients

One of the low cost disposal option of SS is the utilization of this waste material as soil fertilizer in agriculture. The recycling of fundamental nutrients and metals required for plant growth from treated SS is going to be essential for future sustainable development. Treated SS contain useful concentration of nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg) and micro nutrients; however, nutrients content of the sewage sludge depends on both treatment efficiency and the sources of the sewage (Roig et al., 2012).

Nitrogen and phosphate are the principal nutrients in SS and along with Potassium are considered to be macronutrients in agriculture as crops need large amounts of these elements, but their concentration in sewage sludge is usually low. Ca, Mg and S are also essential for the plants growth as secondary nutrients and moreover they affect the availability of nutrients in the soil through regulating the pH (Maathuis, 2009; McCauley et al., 2009).

Some of the metals such as iron (Fe), boron (B), manganese (Mn), zinc (Zn), nickel (Ni) and copper (Cu) are classified as micronutrients, which are essential for the development of soil microorganisms and plants but in very small amounts (Lobo and Grassi Filho, 2009). For instance, zinc as a very important micronutrient, plays an important part in soil fertility and it is required in a large number of enzymes in plants (Milieu, 2013).

1.2.2 Organic matter

SS is a rich source of OM and its use as an amendment can improve significantly the physical, chemical and biological properties of soils and greatly contributes to its productive capacity. Incorporation of OM into the soil surface positively affect its structure (aggregation, porosity and bulk density), water holding capacity, Cation exchange capacity (CEC) and permeability (Clapp et al., 1986); and some chemical properties such as electrical conductivity, pH and redox potential are going to be changed as well.

Organic matter in SS promotes microbial activity in the soil and consequently increases its fertility. Important organic elements (C, N, P and S) are mineralized during OM decomposition and converted into their inorganic forms by soil microorganisms in order to be up taken by plants (García-Gil et al., 2000). Due to lack of stabilization of OM in SS, the soil bio-system can be altered through the addition of new energy sources for organisms, resulting in micro- and macro-biological population changes, which in turn affect synthesis and decomposition of microbially-produced humic substances in soil, interactions with soil inorganic components, nutrient availability, and other exchanges with soil physical and biochemical properties (Clapp et al., 1986). Therefore, it is important to determine maturity and stability degree of SS prior to its application to the agricultural soil (Hernanez-Apaolaza et al., 2000).

1.2.3 Pathogens

Municipal sewage is a complex mixture containing large numbers of pathogenic microorganisms excreted by humans or animals: bacteria such as salmonella, helminthes such as ascaris, viruses such as poliovirus, and protozoa such as cryptosporidium. Wastewater treatment process removes pathogenic organisms from wastewaters. The pathogens like cysts of protozoa and ova of parasitic worms concentrate into the sludge in primary treatment processes, and several bacterial pathogens are absorbed onto the settling particles and flocs. The density of pathogenic microorganisms will be reduced in secondary treatment to a certain extent, however the concentrations of pathogens in both raw primary sludge and waste activated sludge is much higher than in the incoming raw waste water (Yanko, 1988; 2004; Sanin et al., 2011).

Pathogens reduction in sludge largely depends on treatment methods applied to stabilize sludge in sewage treatment plants. Among these various sludge treating methods, composting is considered as one of the most efficient treatments in reducing pathogen concentrations and produces a valuable organic amendment for agricultural land (Dumontet et al., 1999; Sahlström et al., 2004).

Storage after anaerobic digestion for a certain period of time may produce suitable sludges for application to agricultural land for food crop production. Drying sludge higher than 80% DS was also shown to be very effective to completely destroy parasites and pathogens. Also, storage of air-dried sludge for 6 – 12 months further increases the level of hygienisation (Pike, 1986; Czerska and Smith, 2008).

1.2.4 Toxic metals

Although some of trace elements (e.g., Cu and Zn) are essential to plants and animals, they could be toxic depending on their own properties, availability (chemical speciation), and concentration levels. In fact, concentrations of toxic metals in sludge are among the deciding factors for its use in agriculture. Continuous use of SS with high concentration of toxic metals may result in their accumulation in the soil, which has phytotoxic potential on various cereals, vegetables, fruits, pastures, and fodder crops. Toxic elements also enters the food chain through consumption of these commodities by human beings and animals (Hue 1995; Marcovecchio et al., 2007; Usman et al., 2012).

The concentration of toxic metals such as zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), mercury (Hg), and chromium (Cr) in SS vary from less than 1 mg to more than 1000 mg/kg dry weight (Chen et al., 2008). Although the total concentration of metals indicates the overall level of contamination in SS, sequential extraction analysis is essential to determine their mobility, bioavailability and eco-toxicity to plants through identifying the groups of compounds the metal is bound to (Chen et al., 2008; Zhao et al., 2012; Ignatowicz 2017). To reduce the content of these elements in SS, some pre-treatment procedures needed to be applied before disposal.

1.2.5 Pharmaceuticals and Personal Care Products (PPCPs) residues

Among the so called ‘new emerging contaminants’, much attention has been given to PPCPs due to their pharmacological activity and consumption at rates of tons per year (Kasprzyk-Hordern et al., 2008, Daughton and Ternes, 1999) and also the impact of these contaminants’ on the environment or risks to human health is relatively unknown. In addition, due to improving in health care systems and higher life expectancies in industrial countries, it is expected that their worldwide production will increase.

Pharmaceutical residues are excreted through the human body into sewage either as the parent compound or as their metabolites. Many of these residues pass through conventional wastewater treatment processes (flocculation, sedimentation, and active sludge treatment) almost unaffected (majority of macrolides, sulfonamides, penicillin, and imidazole) and reach the environment (Lishman et al., 2006; Santos et al., 2007; Lin et al., 2009; Peng et al., 2011). Therefore, WWTPs are considered as the main source of PPCPs.

However, depending on the applied sewage treatment method, a greater or lesser portion of pharmaceuticals can be removed from sewage, bonded to suspended solids and deposited in SS. Aerobic and anaerobic biodegradation showed diverse influence on different types of PPCPs, for instance diclofenac concentration declined mainly through anaerobic biodegradation, while higher removal of anti-inflammatory drugs (naproxen, indomethacin, ibuprofen) and lipid regulators (clofibric acid, gemfibrozil, bezafibrate) occurred through aerobic biodegradation (Huang et al., 2011). Membrane bioreactor, revealed better performance for the biodegradable species of PPCPs, e.g. caffeine and bezafibrate compare to other two biological treatment processes, i.e. active sludge and biological nutrient removal (Sui et al., 2011). Li and Zhang (2011) also found that some kinds of antibiotics, such as ampicillin and cefalexin could be eliminated through disinfection process.

Implementation of advanced treatment technologies, in order to improve removal efficiency, lead to even further increase in pharmaceutical drug residues concentrations in SS. Therefore, concerns arise over application of such sludge as fertilizer to the ground (Daughton and Ternes, 1999; Gómez et al., 2007; Wiechmann et al., 2013).

1.3 Sludge processing

Sludge processing includes all treatments that improve its suitability for beneficial use and prepare SS for transport, storage or disposal. While the quantity of produced sludge in a wastewater treatment plant is approximately 1% of the quantity of treated wastewater, due to time-consuming process of sludge treatment and the necessity of complex equipment usage, sludge management costs 40 to 50% of the total wastewater treatment costs (Turovskiy and Mathai, 2006; Wiechmann et al., 2013). Sludge treatment methods include thickening, biological stabilization, dewatering, drying and incineration. The latter process will be discussed briefly in Disposal Method section of this chapter.

1.3.1 Sludge thickening

Sludge thickening is a process of reduction in sludge volume by removing as much water as possible and increasing the solids concentration. The purpose of this process is to increase the efficiency and decrease the costs of further treatments such as digestion and dewatering. The resulting material is still fluid with less than 15 % concentration of solids and can be pumped without difficulty with conventional means. Some commonly used thickening processes are gravity thickening, dissolved air floatation thickening, gravity belt thickening, and rotary drum thickening (Turovskiy and Mathai, 2006; Sanin et al., 2011).

Gravity thickening is the simplest and most commonly used method for sludge thickening in wastewater treatment plants and it works best with heavy sludges. Gravity thickening mechanisms are similar in design to primary clarifiers where sludge solids precipitate and compact as a result of gravity so it has minimum power consumption.

Some disadvantages of this method are large space requirement, odor potential and poor solids concentration (2 to 3%) for WAS (Waste Activated Sludge).

Dissolved air floatation thickening provides better solids concentration (3.5 to 5%) of WAS than that of gravity thickening, especially for light and fluffy materials such as activated sludge, and require less space than a gravity thickening as well. But the operational cost and power consumption is relatively high compare to the gravity thickening method (Turovskiy and Mathai, 2006; Sanin et al., 2011).

Gravity belt thickening, a solid-liquid separation process, has high solids capture efficiency (4 to 6%) of WAS solid concentrations and it is based on coagulation and flocculation of solids through polymer addition in a dilute slurry. While this method is relatively low power consumption one but it depends on polymers and odor potential exists.

Rotary drum thickening (RDT) can be used to thicken sludge with initial concentration of 0.5% with high solid separation efficiency (4 to 6% WAS solid concentrations). Low space requirement, relatively low capital cost and less power consumption are some other advantages of this method. Like gravity belt thickening it is polymer dependent and also sensitive to polymer type.

Sludge thickening technologies and equipment are being developed in Europe, Japan, and Russia. New materials such as water-absorbing porous materials, elastic capillary materials and nonwoven fibrous fabric materials, are being examined (Turovskiy and Mathai, 2006; Sanin et al., 2011).

1.3.2 Sludge dewatering

The objective of dewatering is to reduce the water content of sludge and biosolids as much as possible to achieve a volume reduction greater than that achieved by thickening so that subsequent handling of the resulting solid–semisolid material will be much easier. Additionally, it can also help to keep disposal costs, including transportation, to a minimum.

Dewatering is required: prior to composting in order to improve airflow and structure, before thermal drying or incineration to decrease consumption of the fuel that is going to be used for the evaporation of excess moisture, and also before disposal to limit leachate production at the landfill site after disposing of sludge in landfills. The conditioning of sludge by biological, chemical, and/or physical treatment is required to enhance dewatering efficiency.

Generally, in dewatering process the incoming stream is called *feed*, the dewatered solid is called *cake* and the outgoing liquid stream, which is most often recirculated into the WWTPs, is called *filtrate* or *centrate* based on the process used (filter or centrifugation, respectively). To determine the efficiency of dewatering there are two parameters to consider: the clarity of the *filtrate* or *centrate*; and the solid concentration of the *cake* (Sanin et al., 2011).

Dewatered sludge can be obtained through natural process such as lagoon-based air-drying or sand bed, which allow the sludge to dry through evaporation and drainage, or mechanical systems such as centrifuges, belt filter presses, and pressure filter presses. Historically, the first method for dewatering of sludge was the use of drying beds and drying lagoons which are simply based on evaporation and drainage. The sludge solid content can be increased to 45-90%. These methods require large areas, and land costs are often the limiting factor; but otherwise, they require little investments, low energy and low chemical consumption. On the other hand, release of foul smell and stabilized sludge requirement are the drawbacks in this sludge dewatering method (Turovskiy and Mathai, 2006; Sanin et al., 2011).

Sludge dewatering can be performed through centrifuges which consist of a central pipe set in rotating bowl. Forced by the centrifugal force, the heavier solids sink to the bottom while the lighter liquid remains pooled on top. This process results in sludge with 25-35 % solids content. Contrary to drying beds, centrifuges require relatively less space and allow better control on diffusion of bad odors. However, they require much larger initial investments and use more direct power per unit of product produced (US EPA, 1999; Czerska and Smith, 2008).

Belt filter press is another mechanical system of sludge dewatering in which sludge is continuously squeezed between two belts: a press belt and a filter belt. The resulting sludge cake has 20-32% solids content. This method has relatively low capital and management costs but it is very sensitive to feed sludge characteristics, polymer type and dosage rate.

In a pressure filter press sludge is into a frame set between plates that are covered with a filter cloth. Dewatering is achieved by forcing the water through the cloth under high pressure. This method is quite expensive but the cake produced by this method is drier than those produced by any other dewatering alternative (35-45 % solids content) (US EPA, 1999; Turovskiy and Mathai, 2006; Czerska and Smith, 2008; Sanin et al., 2011).

1.3.3 Sludge stabilization

Due to decomponibility of organic matter and hygienic requirements, stabilization of SS is necessary to reduce the potential for further anaerobic biodegradation, eliminate offensive odors and reduce pathogens, and thus generating a stable product for use or disposal.

There are some useful parameters which allow to evaluate the stability of SS such as:

- *Volatile solid (VS) reduction*: VS is generally used as an indicator of the amount of OM is sludge. Decomposition of organic matter results in release of carbon dioxide, so a reduction of the VS is linked to the effectiveness of the stabilization process (Evanylo, 2006).
- *Pathogen reduction*: Sludge stabilization treatments can significantly reduce pathogens and provide maximum options for sludge disposal so this parameter has been used as measure of sludge stability (Sanin et al., 2011).
- *Oxygen uptake rate*: Microorganisms use oxygen as they consume organic substances in aerobic systems. The rate at which the oxygen is consumed, is usually expressed as Specific Oxygen Uptake Rate (SOUR) represents the

biological activity in SS. A high oxygen uptake rate would indicate a very viable and active sludge. The respiration rate of an activated sludge and the amount of the biodegradable OM can be determined by this method. Reduction in oxygen uptake rate indicates that a sludge has been either seriously contaminated or that its easily degradable part has already been used up by microorganisms and therefore the biological activity decreased (Chandra et al., 1987; Sperling, 1994; Cokgor et al., 2007; Surerus et al., 2014).

- *Nitrification*: The transformation of organic N first to ammonia and then to nitrite and nitrate occurs during aerobic stabilization of sludge. Measuring the content of $NO3^{-}$ and the degree of nitrification can be used as indicator of stability under aerobic condition (Turovskiy and Mathai, 2006; Sanin et al., 2011).
- *Total Organic Carbon (TOC)*: If the original content of TOC is known, its decrease could be a reliable measure of the degradable fractions of OM in sludge. Higher microbial activity and hence greater OM mineralization lead to a higher loss of TOC (Hernández et al., 2006). The amount of biological or chemical oxygen demand (BOD or COD) are also important factors in the measurement of degradable OM and so stability of SS.

Common approaches used to assess sewage sludge stabilization include anaerobic digestion, aerobic digestion, lime/alkaline stabilization, composting, long term storage in lagoons or beds and thermal processes. The most frequently used stabilization methods are biological anaerobic and aerobic digestion (Goldfarb et al., 1999) which are discussed here briefly.

1.3.3.1 Aerobic digestion

Aerobic digestion is the most commonly used solid waste stabilization process in wastewater treatment plants. The aim is to produce stable biosolids that are suitable for land application, which have various beneficial features such as, reduced content of pathogens, and minimum release of foul odors. It is a biological process in which organic part of the sludge is oxidized and decomposed by microorganisms in the presence of oxygen. In activated sludge, bacteria consume organic matter with oxygen and convert it into carbon dioxide. When the soluble substrate is completely oxidized by the microbial population, the microorganisms must consume a portion of their cellular protoplasm in order to obtain energy for cell maintenance. This phenomenon that explains the destruction of biomass or volatile solids, is known as endogenous respiration. The basic reaction of the aerobic digestion process, can be illustrated by the following simplified equation.

$$\text{Organic matter/cell mass of microorganisms} + O_2 \xrightarrow{aerobic\ microorganisms} CO_2 + H_2O + NO_3^- + \text{heat}$$

However, all the cell tissue cannot be oxidized and the remaining material, that is about 20-30%, is composed of inert and organic compounds that are not biodegradable. The bio oxidation of biomass results in the reduction of the volume and the material that remains at the end of the process is biologically stable.

Aerobic digestion that is typically used in smaller treatment facilities, has no recovery of energy and the costs of supplying the oxygen required for continued aeration make the process to be expensive. However compared to anaerobic digestion, the capital costs are lower, the process is more flexible and safe in operation, and has a low odor potential (Bernard and Gray, 2000; Turovskiy and Mathai, 2006; Hani et al., 2012).

1.3.3.2 Anaerobic digestion

Stabilization of sludge through anaerobic digestion represents one of the oldest processes which is generally considered to be an economical and environmentally-friendly technology (Appels et al., 2011). In this process, a large fraction of the organic matter in sludge is biologically degraded into carbon dioxide (CO2) and methane (CH4), and a number of other gases in small amounts (ammonia, hydrogen sulfide, and mercaptans) (Nasir et al., 2012). Anaerobic microorganisms digest organic materials in the absence of oxygen, and the remainder product will be stabilized sludge with higher nitrogen content than that produced by aerobic digestion (Madur Electronics 2003; Monnet, 2003; Arthurson, 2008).

Anaerobic digestion involves series of stages of chemical and biochemical reactions. The process comprises three main degradation stages: hydrolysis, acidogenesis, and methanogenesis as illustrated in Figure 1.2.

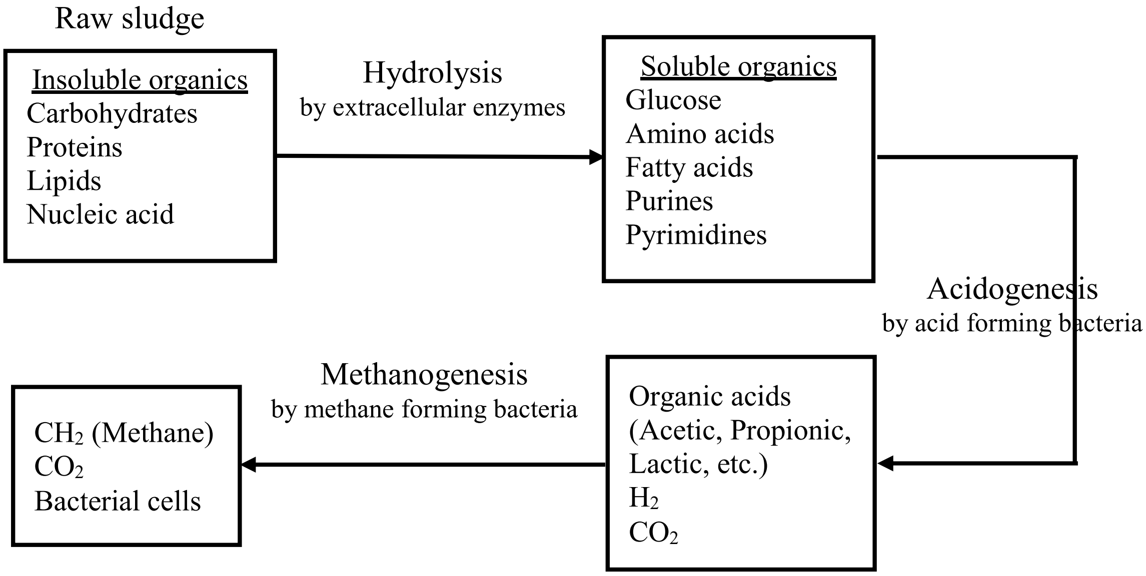

Figure 1. 2 Basics of anaerobic digestion process of organic waste

In the first stage, hydrolysis, complex insoluble polymeric matter such as carbohydrates, proteins, and lipids are hydrolyzed to their soluble monomers form such as sugar, amino acids and fatty acids by fermentative bacteria. In the second stage, acidogenesis, the products of the first stage are converted into simple organic acids, hydrogen and carbon dioxide by acid-forming bacteria. In the last stage, methanogenesis, methane is produced by a group of bacteria known as methane formers. This can happen in two ways: by conversion of acetic acid molecules to methane and carbon dioxide, or through reduction of carbon dioxide with hydrogen (Monnet, 2003; Turovskiy and Mathai, 2006; Sanin et al., 2011).

Even though the high dependency of this method on microorganisms which are sensitive to small changes in the environment (e. g., temperature, pH, toxic metals) necessitate continues monitoring of performance and close process control, anaerobic digestion provides several advantages over the other sludge stabilization methods, such as: methane production as an usable energy source and a high rate of pathogen distribution, especially through the thermophilic digestion process.

1.4 Disposal methods

Treated sludge can be disposed of or recycled through three main routes: landfilling, incineration or recycling to agriculture. Some developing combustion technologies such as pyrolysis, wet oxidation and gasification; and less developed disposal methods including silviculture and land reclamation are also exist. Each of these disposal routs and recycling methods has specific inputs, outputs and impacts.

1.4.1 Landfilling

Landfill, as the oldest form of wastewater sludge treatment, is a widely applied practice for sludge disposal in the European Union, and would be chosen when no other ways to dispose of the sludge exist (Bresters et al., 1997).

In this approach sludge could be deposited at municipal landfills (codisposal) or in dedicated areas alone (monodisposal) and finally covered up with a soil layer. In codisposal method sludge is deposited together with municipal solid waste in a landfill, while in monofill disposal, sewage sludge are buried alone in the trenches.

In sludge landfilling there are two important parameters that must be considered: biogas production and highly polluting leachate (ÓKelly, 2005). Landfill biogas, which mainly composed of methane, are generated as a consequence of slow anaerobic decomposition of biodegradable wastes in landfills (Hue, 1995, European Commission, 2004). This gas only partially captured and the further emissions contributes greatly to the greenhouse effect and climate change.

Sludge landfilling could also lead to the highly polluting leachate production. The excess moisture of sludge and rainwater generate sludge leachate which may contains considerable amounts of toxic organics and toxic metals; and could contaminate the surface water in downslope or ground water. Proper sludge dewatering prior to its landfill disposal could significantly reduce leachate production at the landfill site. Moreover, a clay-based or synthetic liner and a leachate collection system installed in the sludge landfill site would minimize potential environmental hazards of landfill (Luduvice and Fernandes, 2007).

1.4.2 Incineration

Incineration of wastewater sludge is a combustion reaction at high temperatures in an enclosed device called an incinerator (furnace). Sludge incineration techniques are classified into mono-incineration which refers to sludge incineration in dedicated plants, and co-incineration in which sludge is used with other municipal solid wastes as fuel in energy or material production (Guibelin, 2004; Rulkens, 2008). In this process the organic matter combusts and as well as energy production, flue gases, ashes, and wastewater are produced as outputs; therefore, to avoid environmental impact of emissions, Draft Directive on Incineration of Waste (94/08/20) laid down requirements for emissions released by sludge incineration plants (Bresters et al., 1997).

Mechanical dewatering and thermal drying, to obtain above 30% DS content, are essential to have an autothermic incineration (sludge burning without support fuel). In Table 1.1 the principal advantages and disadvantages of sludge incineration as a disposal method are shown.

Table 1. 1 Advantages and disadvantages of sludge incineration

Advantages	Disadvantages
• Sludge volume and weight reduction, thereby reduce in disposal requirements • Pathogen elimination • Low sensitivity to sludge composition • Energy recovery of sludge • Low odor potential due to high temperature and closed systems	• High operating and capital costs • Requirement of extensive treatment to avoid discharge of toxic or noxious emissions to the atmosphere • Dependence of its efficiency on the ratio of sludge mass to solid waste mass

However, according to several authors, incineration is not considered as a complete disposal practice but only a means of sludge minimization, since in this process dry solid residues (municipal solid waste incineration leaves around 30% of the initial waste mass) remain as an ash (European Commission, 2004; Turovskiy and Mathai, 2006; Tyagi and Lo, 2013).

1.4.3 Land application

Due to population increase and development of wastewater treatment plants (WWTPs), such as the construction of new treatment units or the improvement of existing facilities, sludge production has been on the rise. And land application is generally considered the most economical and beneficial way of biosolids disposal (Haynes et al., 2009). Land application of SS is using the biosolids as a soil conditioner for agricultural, horticultural or reclamation purposes based on its degree of stabilization provided. Land application of wastewater solids differs from surface disposal; that principally uses biosolids to enhance the productivity of the soil instead of using the land for final sludge disposal (Ontario, 2008).

Although many beneficial effects can be achieved through land application of SS such as: supplying nutrients to the crops, improving soil properties and increasing soil organic matter content, there are also concerns of the potential for nitrate or phosphate contamination of waters, possible toxic metal and pathogen transfer that must be addressed to insure applying sewage sludge to the soil as a safe and environmentally friendly approach (Singh and Agrawal, 2008).

1.5 Agricultural use of sewage sludge and its regulations

Recycling to farmlands and land restoration/reclamation as long-term sustainable solution to sludge disposal is the current policy of the European Commission and national authorities to reduce landfill as a common way of disposal; considering the quality of sludge that should be compatible with public health and environmental requirements (Schowanek et al., 2004). However, to have an acceptable sludge for agricultural use and society, well-regulated controlling and further reducing pollutant levels is necessary in order to improve the quality and public image of sludge (Andersen, 2001).

In the European Union the total sewage sludge production is around 10 million tons of dry solids per year, of which about 70% is produced in Germany, United Kingdom, France, Spain and Italy and about 40% is spread on land for agricultural use. However, the interest for sludge recycling to agriculture varies among EU countries; for instance, the development of stringent policies in Netherlands has actually lead to an effective ban on using sewage sludge for agriculture and some alternative options such as land reclamation, horticulture and landscaping, or energy recovery are used (Alabaster and Leblanc, 2008; Rovira et al., 2011). The sludge production of 27 European countries together with the percentage of agricultural disposal is presented in table 1.2. (Eurostat, SS production and disposal, last update 06.08.2019).

Table 1. 2 Sewage sludge production and quantities recycled to agriculture in the EU

Member State	**Year**	**Sludge production (Ths-t)**	**Agriculture**	
			(Ths-t)	**(%)**
Austria	2016	237.938	48.313	20.3
Belgium	2010	176.3	30.62	17.4
Denmark	2008	108	74	68.5
Finland	2015	146	-	-
France	2017	1174	229	19.5
Germany	2016	1794.443	423.497	23.6
Greece	2016	119.768	21.528	18.0
Ireland	2017	58.773	46.487	79.1
Italy	2010	1102.7	315.6	28.6
Luxembourg	2017	8.618	1.138	13.2
Netherlands	2016	347.6	0	0.0
Portugal	2016	237.938	13.885	5.8
Spain	2012	1082.69	754.74	69.7
Sweden	2016	204.3	69.5	34.0
United Kingdom	2012	1136.7	844.4	74.3
Sub-total EU 15		**7935.768**	**2872.708**	**36.2**
Bulgaria	2017	68.6	22.5	32.8
Cyprus	2016	7.408	1.613	21.8
Czech republic	2017	223.27	102.94	46.1
Estonia	2016	18.34	0.1	0.5
Hungary	2017	264.71	28.2	10.7
Latvia	2017	24.94	3.316	13.3
Lithuania	2017	42.488	20.817	49.0
Malta	2017	10.3	0	0.0
Poland	2017	584.454	108.52	18.6
Romania	2017	283.34	35	12.4
Slovakia	2017	54.52	0.52	1.0
Slovenia	2017	36.7	0.5	1.4
Sub-total EU 12		**1619.07**	**324.026**	**20.0**
Total		**9554.838**	**3196.734**	**33.5**

1.5.1 Nutrient recovery

The recycling of sewage sludge to agricultural land is the best possible environmental strategy for the recovery of their organic matter and inorganic elements content. Nitrogen and phosphate are the principal nutrients in SS. Since nitrogen is a constituent of all proteins and nucleic acids and therefore protoplasm, is an essential nutrient for plant growth. Phosphate is part of DNA and cells' energy pathways and it can never be substituted. The availability of nitrogen is more dependent on sludge treatment. Anaerobic digestion process increases the total nitrogen concentration of SS in the form of ammonia, which is readily available to plants (Usman et al., 2012; Van der Hoek et al., 2018).

Phosphate is part of DNA and cells' energy pathways and it can never be substituted. Based on the current extraction rates of phosphate, about 20 million tons (Mt) of P from phosphate rock, by the end of this century all today's mines will be exhausted. Therefore, the recovering of phosphorus in SS through reuse of sludge in agriculture could represent a renewable source of phosphorus for future demand (Driver et al., 1999; Cordell et al., 2009). According to European Commission (2001) around 25% of phosphorus in municipal wastewater in the European Union is recovered and reused mostly as sludge. It is reported that phosphorus concentration in sludge amended soils increase from the original 2-4 mg/kg of phosphorous in soil to 29-114 mg/kg of phosphorus in sludge amended soil. The availability of phosphorus content is independent of prior sludge treatment (Chen et al., 2012; Evans 2016).

Potassium content of SS is relatively low since most K components are soluble in water and remain in the sewage effluent or the aqueous fraction during sludge dewatering (Hue, 1995). Therefore, in crop production with SS applications, addition of potassium through an inorganic (such as KCl) or organic (such as wood ash or K-rich crop residues) sources may be critical and could significantly increase yields (Wen et al, 1997).

1.5.2 Contaminants

The more diffuse contaminants of SS can be divided in three main groups: (i) potentially toxic elements including cadmium, chromium, copper, mercury, nickel, lead and zinc; (ii) pathogenic microorganisms (bacteria, viruses, protozoa, and helminthes) and (iii) organic pollutants including PCBs, PAHs, NPE, DEHP, LAS, dioxins (PCDD) and furans (PCDF). The first two groups of pollutants were described in section 1.2 "Composition of sewage sludge" of the present document.

During waste water treatment a wide variety of hydrophobic organic contaminants are efficiently removed through binding to the sludge solids. The main categories of organic pollutants are:

> *Extractable Organic Halogens (EOX)* represent the total content of halogens (Cl, Br, I) in organochlorine compounds that is used for monitoring of their concentrations in sediments. One of its main sources has been the bleaching of paper pulp together with manufacture of polyvinyl chloride (PVC) and waste incineration (Pöykiö et al., 2008).
>
> *Polycyclic Aromatic Hydrocarbons (PAHs)* are produced through incomplete combustion and suspected to be carcinogens/mutagens. House fires, heat and energy power stations, waste incineration, vehicle traffic, and industrial plants are its main anthropogenic sources.
>
> *Linear alkylbenzene sulphonates (LAS)* is the predominant class of anionic surfactants which are the main agents in all detergents. Although LAS is readily biodegradable in aerobic condition, much of these compounds load into a treatment facility is associated with suspended solids and escapes from aerobic treatment into sludge management processes (Thornton et al., 2001).
>
> *Di-2-(ethyl-hexyl)-phthalate (DEHP)* is a phthalate ester and used as softeners in plastic (PVCs), as antifoaming agent in paper production and as an emulsifier for cosmetics. These compounds are degradable both aerobic and anaerobically but being absorbed by the particles, due to their lipophilic properties, decreases their degradation rate considerably. These compounds are toxic for soil organisms and some of them are suspected to have hormone mimic properties (Madsen et al., 1997; Erhardt and Pruess, 2001).

Polychlorinated biphenyls (PCBs) are organic chlorine compounds that produced by chlorination of biphenyl. Inputs of these persistent pollutants to the urban wastewater are basically from atmospheric deposition on the soil and runoff and industrial emissions. They absorbed by solids during wastewater treatments and accumulate in sewage sludge (Thornton et al., 2001).

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are polyhalogenated organic compounds with tricyclic structure that are not intentionally produced. They are by-products of organo-chlorine compounds production and of incomplete combustion of chlorine-containing substances. Household and laundry wastewater are the main sources of these persistent compounds in sewage sludge (Erhardt and Pruess, 2001).

As the land application of sewage sludge may result in an increase of the pollutants concentration in soil, to avoid the environmental risks associated with direct use of this waste in agriculture, some treatment is necessary to minimize and eliminate the undesirable effects.

1.5.3 Legal regulations

Although the use of sewage sludge in agriculture is the best strategy for its recycling, because of the high organic matter content and the presence of other essential elements such as nitrogen and phosphorous, providing regulations with the purpose of preventing noxious effects in plants, soils, animals and human beings as well as promoting its correct use is necessary.

In this regard, Directive 86/278/EEC was adopted for the use of sewage sludge in agriculture which prohibits the use of untreated sludge on agricultural land and required concentration limits for certain toxic metals (cadmium, copper, mercury, nickel, lead and zinc). The 3r draft of the “Working paper on sludge” suggested limit values for concentrations of organic compounds in sludge. In tables 1.3 and 1.4 all the limit values related to toxic metals and organic compounds is presented.

Table 1. 3 Limit values for the concentration of toxic metals in sewage sludge

Contaminants	**Directive 86/278/EEC (Current)**
Cd (mg/kg dm)	20 to 40
Cr (mg/kg dm)	-
Cu (mg/kg dm)	1000 to 1750
Hg (mg/kg dm)	16 to 25
Ni (mg/kg dm)	300 to 400
Pb (mg/kg dm)	750 to 1200
Zn (mg/kg dm)	2500 to 4000

Table 1. 4 Limit values for the concentrations of organic compounds in sewage sludge

Organic compound	**EU 2000 (3rd draft)**
AOX (mg/kg dm)	500
LAS (mg/kg dm)	2600
DEHP (mg/kg dm)	100
NPE (mg/kg dm)	50
PAH (mg/kg dm)	6
PCB (mg/kg dm)	0.8
PCDD/F (ng TEq/kg dm)	100

Although a health and environment impact due to sludge agricultural use has not evidenced, works are in progress on some aspects of the Directive to improve the long-term protection of Community soils.

1.6 Humic substances

1.6.1 Concept of humic matter

The chemical nature of humic substances, is still largely unknown, they were defined by MacCarthy (2001) as "an extraordinarily complex, amorphous mixture of highly heterogeneous, chemically reactive yet refractory molecules, produced during early diagenesis in the decay of organic matter, and formed ubiquitously in the environment via processes involving chemical reaction of species randomly chosen from a pool of diverse molecules and through random chemical alteration of precursor molecules".

These substances are generated from degradation of organic residues in soil, sediment or natural waters and can be operationally subdivided into three major fractions, that are Humin, Humic Acids (HA) and Fulvic Acids (FA), based on their solubility in water adjusted to different acid alkaline conditions. One of the more notable properties of HA and FA is their prominent chemical reactivity. They possess an abundance of carboxyl groups and also have weakly acidic phenolic groups which can dissociate and contribute to their buffering capacity and also contribute to the complexation and ion-exchange properties of humic materials. Humic substances present both hydrophobic and hydrophilic properties and can bind to mineral surfaces (MacCarthy, 2001).

Despite all above mentioned chemical reactivity of humic substances, they are known to be refractory in terms of microbial degradation (Stott and Martin, 1990). According to Malcolm (1990) humic substances from all environments exhibit "a refractory nature to microbial decay".

1.6.2 Importance of humic substances

Maintaining the physical and chemical properties of soil for healthy microbial activity and plant growth could be insured by humic substances (HS). Since, as the most important fraction of organic matter, HS are directly involved in the slow release of nutrients, pH buffer capacity, high cation exchange capacity, and toxic metals and xenobiotic organic molecule retention (Anderson et al., 2001; Réveillé et al., 2003).

Due to the specific structural properties of HS (e.g. hydrophilic and hydrophobic structure, ability to reduce surface tension), they can be considered as washing agents for removal of contaminants from soil (Gusiatin et al., 2017). The toxic metal removal efficiency of HSs (e.g. Cu, Cd, Zn, and Pb) was demonstrated by several investigations (Soleimani et al., 2010; Borggaard et al., 2011; Hartley et al., 1014; Kulikowska et al., 2015) and it was shown that FA, with lower molecular weight and higher number of reactive groups, are able to form more soluble, mobile and bioavailable metal complexes than HA; therefore, they could have a higher metal binding capacity compare to HA (Donisa et al., 2003; Boruvka and Drábek, 2004; Gusiatin et al., 2017).

1.6.3 Humic matter in sewage sludge

In general, sewage sludge can be a rich source of HSs and considering the above-mentioned properties and importance of these compounds, the use of SS in agriculture as fertilizer and as conditioner in intensively-cropped and organic-depleted soils may have many beneficial effects; they enhance the metabolism of phosphorus and nitrogen in soil, help the absorption of nutrients by plants and microorganisms and improve the aggregation and cationic exchange capacity of soil particles (Réveillé et al., 2003; Li et al., 2013).

Composting is usually applied to reduce the degradable organic substances e.g., protein, polysaccharide and fat in SS and convert them to more oxidized and stable compounds. This process lead to increase the concentration of aromatic humic compounds with high molecular weight that indicates humification process takes place during composting treatments. Therefore, HSs could be used as an indicator of the degree of organic matter humification, and so the degree of SS compost maturity (Zucconi et al., 1981; El Fels et al., 2014).

1.7 The aim of this study

The aim of the research carried out during the PhD program and summarized in this thesis was to evaluate the suitability of sewage sludge, from wastewater treatment plants located in the Friuli-Venezia Giulia region (north-east of Italy), for agricultural use in the light of the new characterization perspectives for sewage sludge within the framework of the concept of integrated water cycle sustainability. To this purpose, the sewage sludge samples were taken from 10 representative waste water treatment plants that vary in their treatment capacity, process units and sludge treatment sequences. Sewage sludges were characterized considering the concentration of toxic elements and two different classes of organic emerging contaminants, Extractable Organic halogen (EOX) and Linear Alkylbenzene Sulfonate (LAS). Their degree of stabilization was evaluated by investigating the properties and content of humic and fulvic acids.

The objectives of this work are described in the three following chapters which will be separately submitted as manuscripts to appropriate peer reviewed journals:

Chapter II:

In this chapter, which is an updated version of a previous study, reported by Goi et al. (2006) in Friuli-Venezia Giulia region (north-east of Italy), is evaluated the evolution of selected contaminants in about 10 years due to upgrading of wastewater treatment processes. In addition, LAS concentration, as one of the emerging contaminants in sewage sludge, was studied as well. Since legislation for agricultural reuse of sewage sludge is becoming more and more stringent, this study represents a contribution to stakeholders to help the choice of which kind of sewage sludge is suitable to agricultural reuse and which should undergo additional treatments or different final destination.

Chapter III:

Before proceeding with the evaluation of the stabilization degree of stabilization of sewage sludge by examining the quality and quantity of humic substances, it became necessary to assess the integrity of extraction procedures for the isolation of HA and

FA. In this part, we will answer to the question whether HS are artifacts or not of the extraction procedure. In a recent work, Lehmann and Kleber (2015), rejected the humic substances concept and proposed the Soil Continuum Model, in which organic matter exists as a continuum of organic fragments and humic substances are considered to be artifacts of the alkali extraction procedure. The goal of our work was to test assumptions made by Lehmann and Kleber (2015) this allowed us to demonstrate that humic substances are real components of natural organic matter and sewage sludge.

Chapter IV:

This chapter describes modifications in the quality of sludge that occur during a stage, which is often neglected in the evaluation of the overall performance of a treatment plant: namely the period spent by treated sludge in thickening beds and in storage facilities. Our aim was also to ascertain the integrity of HSs use as markers of biological transformations at this stage of sewage processing where biological transformation is generally considered to be minimal.

To this purpose we will evaluate: i) the effect of sludge storage on degradation EOX, as representative of compounds best degraded under anaerobic conditions, and LAS as representatives of compounds best degraded under areobic conditions, ii) the use of humic substances as an indicator of the efficiency of transformation process in sewage sludge treatment and specifically of the storage period iii) application of UV-visible, FTIR and fluorescence spectroscopies as valid, simple and cost effective methods to examine the quality of HS in sewage sludge.

Preface

This work was an attempt to update information on the concentration of toxic metals and Extractable Organic Halogen (EOX) in sewage sludge from waste water treatment plants located in Friuli Venezia Giulia region reported by Goi et al. (2006). In addition, Linear Alkylbenzene Sulphonate (LAS) was added to the analyzed parameters as the most heavily used surfactant in domestic detergents since they can affect the fate and behavior of hydrophobic organic compounds in soil.

For this purpose, the samples were collected from 10 different waste water treatment plants that are vary in the treatment capacity (expressed as population equivalent, P.E.), process units and sludge treatment sequences.

Compared to the previous work of Goi et al. (2006), our present results showed that improvement in the wastewater treatment route can lead to the increase in the concentration of toxic metals in the sewage sludge.

Chapter II

Monitoring of toxic metals, EOX and LAS in sewage sludge for agricultural use: a case study

2.1 Introduction

According to the sustainable agriculture perspective, the reuse of sewage sludge is of great importance, due to the nutritional and organic matter content (Fijalkowski et al., 2017) and its low cost. However, the presence of some persistent organic contaminants and toxic elements in sewage sludge can cause environmental and health problems (Anjum et al., 2016). Given the continuous development of wastewater treatment plants (WWTPs), involving the construction of new treatment units or the upgrading of existing facilities, sludge management has become one of the most critical environmental issues in the sector: sludge treatment cost represents nowadays approximately 50% of total running costs in WWTPs (Quian et al., 2016). In fact, about

9.5 million tons dry matter of sewage sludge were produced in Europe in 2015 (Eurostat, 2018) that should be properly disposed.

There are several options for the final disposal of sewage sludge, including energy and resource recovery (Gherghel et al., 2019); treated sludge is a source of nutrients for the soil, when applied as fertiliser and soil conditioner (Yoshida et al., 2018; Ashekuzzaman et al., 2019), even if the risks of soil contamination and pathogen transmission have to be considered (Singh and Agrawal, 2008). Council Directive 91/271/EEC encouraged the land application of sewage sludge (European Commission, 1991) due to its fertilizing and soil conditioning properties for agricultural soil. However, this practice may also lead to environmental and health problems, due to accumulation of persistent organic contaminants and toxic elements contained in sewage sludge (Valentin et al., 2013). In order to utilize the sludge produced by urban WWTPs as a fertilizer in agriculture, precise specification of the properties and quality of sludge is required, to prevent the occurrence of health and environmental issues.

There are many aspects of interest when evaluating the quality of sewage sludge as fertilizer (USEPA, 1995; American Society of Civil Engineers and American Water Works Association, 1996), but most crucial and hazardous aspect that can prevent widespread agricultural use is the concentration of toxic metals (Chen and Hu, 2019). A major cause of concern is the toxicity of these toxic metals at trace concentrations (order of magnitude of ng/L), in particular Cd, given its high bioavailability (Hu et al., 2017). Toxic metals in sewage systems originate from household sewage, industrial wastewater or urban runoff (Sorme and Lagerkvist, 2002). As a result of heavy urbanization and the entry of untreated industrial wastewater into municipal wastewater sewerage, toxic metals such as lead, cadmium, mercury, nickel and chromium may be present in municipal wastewater (Pires and Mattiazzo, 2003; Singh et al., 2004; Hargreaves et al., 2018) and most of these accumulate in the sludge, since only a small amount is released with the final treated effluent (Sorme and Lagerkvist, 2002). Once sludge from WWTPs is applied to the land, by degradation of organic compounds in sewage sludge, toxic metals availability increases in soil and accumulation in plant biomass (which is one of the initial elements of the food chain) is observed (Gondek et al., 2014).

Apart from single harmful inorganic contaminants, recently particular attention has been given to organic compounds present in domestic and municipal wastewater which are similarly accumulated in sludge. The use of sum parameters, like Extractable Organic Halogen (EOX) and Linear Alkylbenzene Sulphonate (LAS), is particularly interesting, because it can be a good representation of a general wide-ranging organic pollution in the sludge by specific classes of organic compounds.

The EOX parameter represents the sum of organic chlorine (Cl), iodine (I) and bromine (Br) which can be extracted by organic solvents from environmental solids (Kannan et al., 1999). These compounds include not only synthetic polychlorinated biphenyls, organochlorine pesticides, polychlorinated dibenzo-p-dioxins or polychlorinated dibenzofurans and other substances, but also those naturally produced by microorganisms, flora and fauna (Niemirycz et al., 2005).

The importance and usefulness of this parameter in the evaluation of environmental quality have been demonstrated by some studies (Rodziewicz et al., 2004; Contreras Lopez, 2003; Goi et al., 2006; Rizzardini and Goi, 2014; Braguglia et al., 2014); however, there is still a limited number of investigations on EOX content in sludge.

LAS is one of the most used anionic surfactants, introduced in 1964 as a readily biodegradable replacement of branched alkylbenzene sulfonates (BAS). It is a mixture of homologues and isomers characterized by a hydrophilic sulfonate head-group and a hydrophobic alkylbenzene tail-group with 10-13 carbon units in its alkyl chain (Traverso-Soto et al., 2012). LAS are widely used as cleaners and household detergents, given their low cost, excellent detergent properties and general good biodegradability under aerobic conditions (Zhou et al., 2019).

The agricultural application of sewage sludge is the main source of LAS entrance to the agricultural soil (Jensen, 1999). Typical LAS concentrations in anaerobically digested sludge are in the range of 1,000-30,000 mg/kg, in aerobic sludges <1,000 mg/kg and in aerobically stabilized sludges <500 mg/kg of sludge (dry weight)

(Schowanek et al., 2007). During sludge transportation to the farmland, sludge storage and application on agricultural soil, aerobic conditions are restored and rapid degradation of LAS resumes (Jensen, 1999).

The third draft of future sludge directive “Working Document on Sludge” (EU, 2000) published by European Union in 2000, was the first comprehensive regulation in which an enhanced monitoring of sludge was proposed, but little advancing followed in the field in the following years. This work gives a contribution in the perspective of introducing EOX and LAS parameters in monitoring sludge from WWTP, as suggested by the “Working Document on Sludge”, only partially integrated in some national and regional legislation (Emilia-Romagna Region, 2005; Italian regulation, 2018).

In this paper, which is an updated version of a previous study, reported by Goi et al. (2006) in Friuli-Venezia Giulia region (north-east of Italy), the evolution of analysed parameters in about 10 years was evaluated, due to upgrading of wastewater treatment processes. In addition, LAS concentration, as one of the emerging contaminants in sewage sludge, was studied as well. Since legislation for agricultural reuse of sewage sludge is becoming more and more stringent, this study can be a contribution to suggest which kind of sewage sludge is suitable to agricultural reuse and which should undergo additional treatments or different final destination.

2.2 Material and Methods

2.2.1 WWTPs and sample collection

Sewage sludge samples were collected from 10 different municipal WWTPs in Friuli-Venezia Giulia region (North-east of Italy); the chosen plants are listed as WWTP 1-10 in Table 2.1, together with a brief description of the main characteristics of the plants, including treatment capacity (expressed as population equivalent, PE), process units and sludge treatment sequences. It can be noted that most of the analyzed plants were little scale plants (P.E. in the range of 850-9,000 P.E.) and treated mainly domestic wastewater, while two medium scale plants (identified by n. 1 and 2) were studied as well: plant n.1 treated essentially municipal wastewater, while plant n. 2 treated a mixture of municipal and industrial wastewater, with the main fraction coming from chlorine free pulp and paper industry.

In the same area samples coming, for most part from of these WWTPs, were studied during a similar monitoring in 2006 (Goi et al., 2006), during the time these plants were transformed and upgraded in size and processes to lead to the actual layout. Sludge samples were collected manually at the end of sludge treatment, by filling polyethylene bags with 5 kg of sludge from a wider selection about 50 kg.

2.2.2 Sample preparation

The samples were immediately transported to the laboratory and stored at 4 °C; for the subsequent analysis, the samples were freeze-dried and passed through a 1 mm sieve, to obtain well homogenized samples.

The sewage sludge samples were frozen at −20°C, then lyophilized by a Coolsafe 55-4 Touch lyophilizer with − 50 °C condenser temperature. The ultimate vacuum pressure was 0.4 mbar.

To test potential degradation of LAS throughout time, a fraction of sludge coming from the different size WWTPs No. 3, 4, 8, and 10 was put in a pilot-size aerobic drying bed, where aerobic condition was maintained by ideal surface venting for 6 months after sludge withdrawal (Table 2.1).

Table 2. 1 Main characteristics of the plants considered in the present study

WWTP	Plant size (P.E.)	Wastewater treatment sequence	Sludge treatment sequence
No. 1	>100000	Scr. - G.Tr. - O.Rm. - Pr.S.T. - A.S. (N-DN; SBR) - S.Cl. - Disnf.	Thk. - An.Dig. - B.Pr.
No. 2	>100000	Scr. - A.S. - S.Cl. - CoTr - Pr.S.T.	Thk. - Aer.Dig. - FP
No. 3 *	9000	Scr. - G.Tr. - A.S. (N-DN) - S.Cl. - Disnf.	Thk. - Aer.Dig. – P.D.Bd.
No. 4 *	7500	Scr. - G.Tr. - A.S. (N-DN) - S.Cl.	Thk. - P.D.Bd.
No. 5	6000	Scr. - G.Tr. - A.S. (N-DN; IFAS) - S.Cl.	Thk. - D.Bd.
No. 6	5000	Scr. - G.Tr. - A.S. (N-DN) - S.Cl. - Disnf.	Thk. - Aer.Dig. - D.Bd.
No. 7	4000	Scr. - G.Tr. - A.S. (N-DN; MBR)	Thk. - D.Bd.
No. 8 *	3500	Scr. - G.Tr. - A.S. (N-DN; MBBR) - S.Cl.	Thk. - P.D.Bd.
No. 9	1500	Scr. - G.Tr. - A.S. (N-DN) - S.Cl. - Disnf.	Thk. - D.Bd.
No. 10 *	850	Scr. - G.Tr. - A.S. (N-DN; SBR)	P.D.Bd.

Legend: P.E. = Population equivalent; Scr. = Screening; G.Tr. = Grit Trap; O.Rm. = Oil removal; Pr.S.T. = Primary settling tank; A.S. = Activated sludge; N-DN = Nitrification-Denitrification; SBR = Sequencing Batch Reactor; MBBR = Moving Bed Biofilm Reactor; IFAS = Integrated Fixed-film Activated Sludge; MBR = Membrane BioReactor; SBR = Sequencing Batch Reactor; S.Cl. = Secondary clarifier; CoTr = Coagulation-flocculation treatment; T.F. = Trickling Filter; Disnf. = Disinfection; Thk. = Thickener; B.Pr. = Belting press; FP = Filter Press; Aer.Dig. = Aerobic digestion; An. Dig. = Anaerobic digestion; D.Bd. = Drying bed; P.D.Bd. = Pilot Drying bed; (Dom) = Domestic wastewater; (URB-Dom) = Urban wastewater, mainly domestic; (URB-Ind) = Urban wastewater, mainly industrial

*** = Optimal aeration of the sludge was performed by a pilot drying bed (P.D.Bd.) for 6 months**

2.2.3 Toxic Metal analysis

Toxic metal content was determined basing on USEPA 3051 method. Briefly, 0.5 g of each sample, previously freeze-dried and grinded, were weighed into a Teflon microwave vessel and 10 mL of concentrated HNO3 (Merck solution IV) were added. The samples were digested in a microwave digester apparatus (CEM Mars Xpress, Matthews, NC, USA) at 180 ℃ for 10 min. After cooling, the resulting solution was filtered through a PTFE filter of 0.2 μm size, diluted to 20 mL in volumetric flasks by ultra-pure water and then stored at 4 ℃ until analyzed.

Toxic metal concentration in sewage sludge samples was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), Varian Vista Pro axial instrument, equipped with a cross-flow nebulizer; an auto-sampler was used. The calibration was performed using standard solutions (0.5, 1, 5, 10, 30, 50 ppm) prepared from an ICP-standard 23 elements solution in 5% HNO_3 (Merck solution IV) and yttrium (Y) was used as an internal standard. The method detection limit (MDL) was calculated as 3 s/m (where s is the standard deviation of 10 replicate blanks and m is the slope of the calibration curve) for each element.

2.2.4 EOX analysis

All the samples were freeze-dried, manually sieved through a 1 mm mesh sieve and grinded in a ball-grinder. Subsequently, 1.0 g of such pre-treated samples were extracted with 5 mL of solvent (ethyl acetate or n-hexane) by shaking for 24h. Most of the solvent was separated and then evaporated from the extracts under a nitrogen flow, until only 1 mL remained and the resulting sample was stored as refrigerated until analysis was executed.

Analysis were performed with Trace Elemental Instrument, Euroglas ECS 1000 upgraded with digital coulometer and control software (TEIS). This apparatus consisted of an injection system, a thermal extraction, a trapping section and a titration cell.

100 µL of residual extract were introduced into the instrument with an injection rate of 20 µL/min. At 950 °C and in an oxidizing atmosphere, combustion accompanied by pyrolysis of organochlorine compounds and hydrogen halides release took place. The reaction gases formed by the combustion process were carried by the gas stream to the titration cell after passing the absorber and were filled with concentrated sulfuric acid to remove the water from the gas flow. In the titration cell, constituted by acid solution and silver electrodes, the halogenated acid formed by the organic halogens combustion created a current which can be measured. From the integral of the current over time, by the halogenated ionic charge the global quantity of organic halogen compounds in the extract can be established.

2.2.5 LAS analysis

HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany), analytical grade sodium perchlorate (Aldrich). Sodium dodecyl-benzenesulfonate was used as standard (Sigma-Aldrich). Standard solutions of LAS were prepared in ultrapure water. Ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA).

All measurements were made with a Shimadzu high performance liquid chromatograph LC-20AT (Shimatzu Corporation Kyoto, Japan), fitted with an SIL-20AHT autosampler with a loop 20 µL, equipped with a diode array detector (DAD), a quaternary pump, a vacuum degasser and a thermostatic column compartment. The analytical cartridge column was a SUPELCOSIL LC-8 (SUPELCO, Bellefonte, PA, USA), 25.0 cm × 4.6 mm ID, 5 µm particle size. The microwave extraction system was a Microwave Mars 5 Digestion Oven apparatus (CEM, North Carolina, USA) with programmable power and irradiation time. The apparatus was equipped with a carousel, able to hold 36 extraction vessels.

Microwave-assisted extraction (MAE) was performed on 0.5 g of dried sewage sludge samples. Methanol was used as a solvent because in literature it was the most common adopted solvent (Mortensen et al., 2001; García et al., 2005; Villar et al., 2007;

Braguglia et al., 2014). 5 mL of solvent were added to the samples and then the extraction vessel was closed and introduced into the microwave cavity. Microwave oven power and irradiation time were 340 W and 10 min, respectively. After extraction, the vessels were allowed to cool at room temperature before they were opened. The extracts were filtered through glass wool and analyzed by HPLC.

A commercial mixture of linear alkylbenzene sulfonates with C10–C13 chain length was used. Standard solutions at different concentrations were prepared in ultrapure water. Different solvents gradient programs were tested, to obtain a good resolution of all LAS peaks. Good results were obtained by using as mobile phase acetonitrile–water containing 0.1M NaClO4 (55:45) and isocratic elution. Compounds were eluted isocratically over 6 min at runtime at a flow rate of 0.8 mL/min after a 20 µL injection. Since polar interferences were eluted between 0 and 2 min, they did not interfere in the analysis. The column was thermostated (35°C). DAD-UV (λ ex =225nm) detector were used for LAS determination. The instrumental response was preliminarily tested through the use of standard LAS solution, highlighting an excellent HPLC cleaning and separation process.

2.3 Results and Discussion

2.3.1 Nutrient content and agricultural reutilization potential

Sludge nutrient concentration is an important factor which should be considered, to obtain a successful land application. Since P requirement of most crops is four to ten times less than N requirement and in a major portion of sludge P is present in bioavailable forms (inorganic), sludge application rate would be much lower if P requirement of crops was considered instead of N (Hue, 1995). Generally, a wide variation was highlighted for plant macronutrient concentration in the analyzed sewage sludge (Table 2); the obtained results were coherent with other literature studies. For example, data collected from 10 WWTPs in the analyzed area (FVG's sewage sludge) showed total N ranging from 3.1 to 6.8% dry weight; Sommers (1977), Mumma et al. (1988) and Mtshali et al. (2014) reported ranges from 0.5 to 7.6, 1.19 to 4.93 and 0.5 to 4.5% dry weight, respectively.

While in the presented results the average P concentration of sludge samples was about five times less than the average N content, Sommers (1977), Mumma et al. (1988) and Mtshali et al. (2014) found relatively higher P/N ratio (0.56, 0.28 and 0.68, respectively).

As it is shown in Table 2.2, sewage sludge can be considered as an imbalanced fertilizer due to loss of soluble nutrients from sludge during wastewater treatment; such as K, with typical range of 0.1 – 0.4 % DS (0.27 % d.w. for the actual samples), so supplements such as KCl, wood ash and K-rich crop residues could compensate this shortage and make it more suitable for agricultural use (Hue, 1995; Czerska and Smith, 2008; Pakhnenkoa et al., 2009).

Table 2. 2 Total concentration of selected plant nutrients in sewage sludge

Variable	**Total Nutrient, % d.w.**				
	N	**P**	**K**	**Ca**	**Mg**
FVG's sewage sludge (10 samples)					
Range	3.1-6.8	0.37-1.7	0.17-0.38	1.88-17.13	0.22-4.78
Mean	4.21	0.93	0.27	6.88	1.95
Median	4.15	0.85	0.29	5.47	1.63
Sommers, 1977 (250 samples)					
Range	0.5-7.6	1.1-5.5	0.08-1.1	0.6-13.5	0.03-1.1
Mean	4.9	2.9	0.52	3.3	0.52
Median	4.8	2.7	0.3	3	0.41
Mumma et al., 1988 (15 samples)					
Range	1.19-4.93	0.22-3.13	0.03-0.46	0.32-15.9	0.04-0.81
Mean	2.9	1.2	0.19	3.92	0.35
Median	2.78	0.78	0.15	2.17	0.34
Mtshali et al., 2014 (7 samples)					
Range	0.5-4.5	0.7-2.5	0.04-0.49	0.12-1.59	0.04-0.43
Mean	2.47	1.69	0.15	0.92	0.22

2.3.2 Toxic metals

Toxic metals concentration in sludge is the main concern among the determining factors for sludge reutilization on land. In this monitoring, following suggestions of "Working Document on Sludge and Biowaste" (European Union, 2004) "toxic metals are meant to be cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) in metallic form as well as their salts and oxides". Toxic metal presence is essential for plants and animals, but an excessive concentration of these elements can damage crops and threaten human health by entering the food chain (Usman et al., 2012). Therefore, before land application toxic metals concentration should meet the limits defined by legislations on sewage sludge management.

Table 2.3 shows the permissible limits for toxic metals, suggested by some national legislations and European Council Directive 86/278/EEC (European Commission, 2009) on the protection of environment, in particular of soil, when sewage sludge is available for agricultural applications.

Table 2. 3 Some National and EU permissible limits of toxic metals in sludge for agricultural use (mg/kg d.w.) (EC 2009; Stylianou et al., 2008; Italian regulation, 2018)

Element	Limit 86/278/EEC	Limit Italy	Limit Netherlands	Limit France
Cd	20-40	20	1.25	20
Cr	-	200	75	1000
Cu	1000-1750	1000	75	1000
Hg	16-25	10	0.75	10
Ni	300-400	300	30	200
Pb	750-1200	750	100	800
Zn	2500-4000	2500	300	3000

The concentration of toxic metals in the actual sludge samples, collected from various WWTPs in this study, is depicted in Table 2.4. As shown in Table 4, toxic metals concentration in all the tested samples were lower than maximum permitted limits (Table 2.3), boosting for agricultural reuse of the analyzed sludge, except for Cr and Zn concentration in sample no.10, which were 239.63 and 5,676.4 mg/kg respectively; this could be due to the advance wastewater treatment of plant no. 10, that is a small WWTP actually applying nitrification-denitrification in a sequential batch reactor (SBR), a technique in which sludge remain a lot of time in the tank leading to an important metal adsorption potential.

Table 2. 4 Toxic metals in the analyzed sludge (mg/kg d.w.)

Sample	Cd	Cr	Cu	Hg	Ni	Pb	Zn
SS no.1	2.12	60	340	2.12	37	92	1266
SS no.2	0.74	18	54	0.54	13	13	110
SS no.3	1.17	48	584	1.36	31	64	555
SS no.4	0.51	32	190	0.12	21	25	191
SS no.5	1.01	35	302	0.47	19	39	436
SS no.6	1.65	194	593	0.36	79	81	1446
SS no.7	0.88	39	238	0.20	25	43	309
SS no.8	0.94	23	146	0.07	12	25	587
SS no.9	1.44	42	428	0.39	27	87	796
SS no.10	1.97	240	347	6.40	47	77	5676
Range	0.51-2.12	18-240	54-593	0.07-6.4	12-79	13-92	110-5676
Mean	1.243	73.1	322.2	1.203	31.1	54.6	1137.2
Median	1.09	40.5	321	0.43	26	53.5	571

Generally, the concentration of toxic metals in sludge is affected by the plant potentiality and the type of influent wastewater, i.e. whether it is domestic or industrial (Spanos et al., 2016; Chanaka Udayanga et al., 2018), but some process upgrading in the wastewater treatment route can lead to the increase of the metal content on the sludge.

In practice, a general growth in maximum Toxic metal concentration was observed, comparing the actual data with the previous work by Goi et al. (2006) on the same area. The maximum concentrations of Cd, Hg, Cr, Pb, Ni, Cu, and Zn reported by Goi et al. (2006) were 3.6, 1.4, 51.5, 58.7, 35.9, 105.8, and 410.1 mg/kg, respectively, while the highest concentrations of these elements obtained in the present study were 2.12, 2.12, 239.63, 92.19, 78.81, 593.48 and 5676.4 mg/kg, respectively (Figure 2.1).

This interesting outcome could be explained because of last ten years' process improvements in wastewater treatment lines of the WWTPs, which maximized the removal efficiency of contaminants from wastewater, allowing to a larger transport of metals to the residual solid fraction.

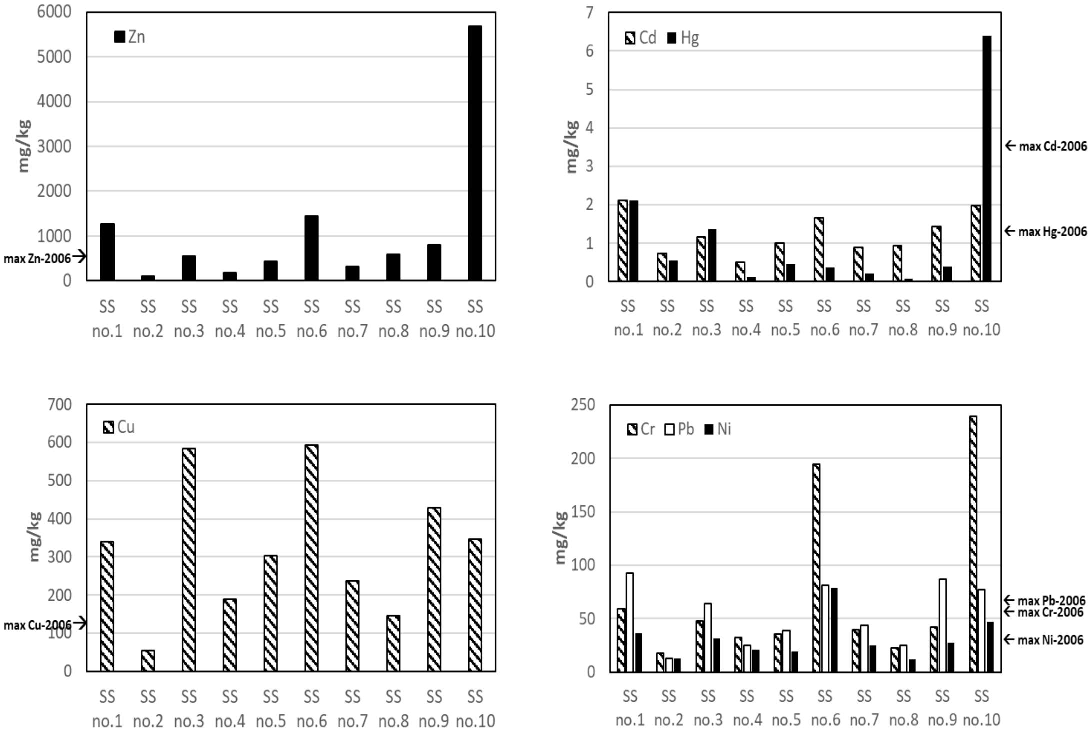

Figure 2. 1 Toxic metal concentration in sludge samples and maximum values monitored in 2006

2.3.3 EOX

In last two decades a great interest in data about occurrence, behaviour and fate of organohalogen compounds in water, sludge and environment was arisen. The presence of organic halogen compounds in the sludge was a key point in the EU new proposed monitoring (European Union, 2004). These compounds, in fact, are very persistent and do not degrade over the passage of time; moreover, they are not absorbable by the soil (especially the polar halogenated organics) and directly reach the groundwater, contaminating it. Therefore, a sludge which has higher concentration of these harmful compounds is generally deemed as unsuitable for agricultural reuse (Rizzardini and Goi, 2014). As well as monitoring of single compounds with particular toxicity, a very interesting perspective in monitoring organic halogen content in soils and terrains by the agricultural utilization of sewage sludge, is the utilization of EOX as sum parameters for a global information. Moreover, EOX parameter can be important in quality control prior to the sludge management choice as already observed in previous case study (Goi et al. 2006).

EOX concentration in the analysed sewage sludge samples, obtained both by hexane and ethyl acetate extraction was presented in Figure 2.2 and Table 2.5. The maximum concentration of EOX found in the present work was 26.86 mg/kg (related to sample no. 1), extracted by ethyl acetate. This EOX content was associated to the largest WWTP, with combined municipal and urban sewage sources; EOX concentrations in small municipal WWTPs have usually low values in this monitoring, except for sample 6 and 8. Differently from the toxic metal assay, the maximum EOX values measured in the samples are comparable to those of previous monitoring.

The EOX content in ethyl acetate extractions was 2-6 times higher, if compared with hexane extractions and it conformed to the primary results reported by Reemtsma and Jekel (1996). It indicated that the polar halogenated organics were in larger quantity than non-polar compounds, and these compounds could only be extracted by ethyl acetate.

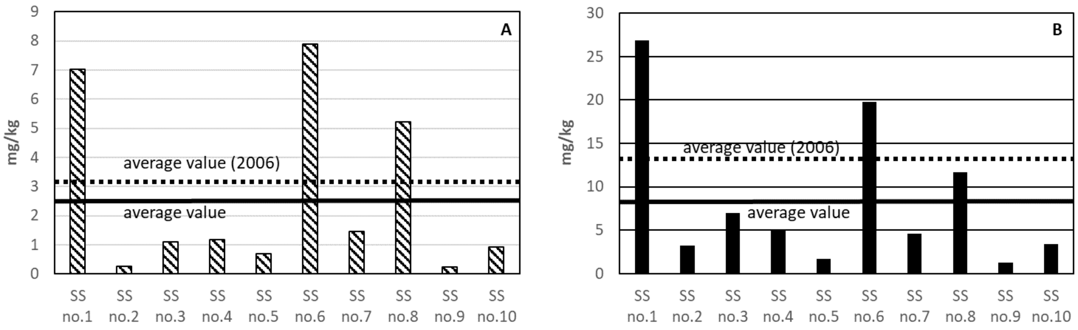

Figure 2. 2 EOX concentration measures (A- Hexane extraction; B- ethyl acetate extraction) in sludge samples and average values comparison

Table 2. 5 EOX and LAS concentration in sludge samples from analysed WWTPs (mg/kg d.w.)

Sample	**LAS**	CV%	**EOX**			
			By hexane	CV%	By ethyl acetate	CV%
SS no.1	574	9.6	7.03	5.3	26.86	1.3
SS no.2	136	14.8	0.25	45.0	3.26	38.8
SS no.3	181	14.1	1.11	20.6	6.95	4.3
SS no.4	54	26.3	1.17	28.6	4.98	5.9
SS no.5	523	23.6	0.69	55.2	1.74	11.9
SS no.6	220	22.7	7.89	16.0	19.81	7.7
SS no.7	302	12.4	1.46	15.1	4.56	5.1
SS no.8	56	17.3	5.22	7.8	11.7	14.4
SS no.9	428	9.2	0.24	31.7	1.24	24.2
SS no.10	138	16.3	0.93	11.8	3.38	3.8

2.3.4 LAS

Since surfactants are extensively used for domestic and industrial applications and most of these surfactants are LAS, their presence in sludge samples is assured (Granatto et al., 2019). Moreover, these compounds can only be partly degraded by WWTPs and the extent of degradation largely depends on LAS content in raw sewage, sludge age after storage and process nature, i.e. whether the process is aerobic or anaerobic. Therefore, a measurable portion of LAS always accumulates in solids and that is why it is important to monitor LAS in sludge (Villar et al, 2007).

In Europe LAS concentration in sewage sludge lies between <1 g/kg d.w. and 30 g/kg d.w. (Gawlik and Bidoglio, 2006). Table 2.5 shows the measured concentration of LAS in the analysed sludge samples. The lowest concentrations were found in samples 4 and 8 (55.88 and 53.75 mg/kg, respectively), while the highest amount was highlighted in sample no. 1 (574.41 mg/kg). Stock et al. (2002) analysed more than 150 sludge samples from different WWTPs in a comprehensive study in Westphalia. They found a correlation between WWTPs size and LAS concentration; it should be reminded that extended aerobic sludge treatment is common in smaller treatment plants, reducing LAS concentration.

In accordance with the above explanation, the LAS concentration in sample 2 was three times lower than sample 1, considering the same size of WWTPs and the sludge sample 2 was treated using aerobic digestion, while sample 1 by anaerobic one. Considering LAS amount in the investigated sludge, it could be observed that this parameter was lower (between 53, 75 and 180, 95 mg /kg d.w.) in those plants in which the sludge was treated in the pilot drying beds, with optimal aeration, or by aerobic digestion. It is worth noting that LAS mean was significantly lower than the limit values proposed in the "Working Document on Sludge" (EU, 2000).

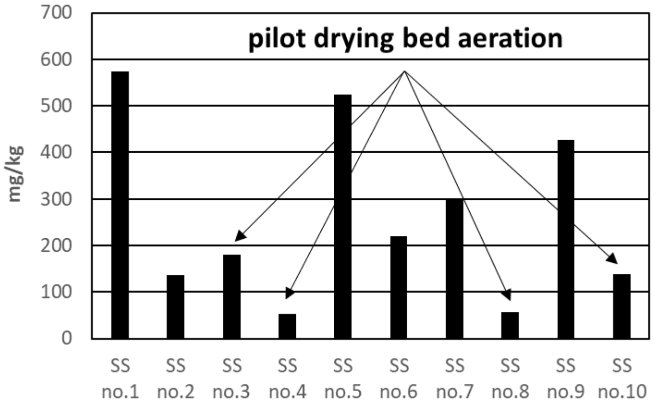

Figure 2. 3 LAS concentration in sludge samples from analysed WWTPs

Indeed, quality of the investigated sludge (in terms of HM, EOX and LAS), depended on multiple factors, including plant size (PE), type of treated water (Dom., Ind., or Urb.), wastewater treatment process and sludge treatment sequence. It was not possible to directly correlate each parameter to the pollutant quantity in the sludge, because all these variables were strictly interconnected and strongly matrix-dependent. Despite the differences and the variability of the analysed parameters, all the investigated sludge respected the Italian limits suggested by the regulations for agricultural reuse.

The comparison with a previous study (Goi et al., 2006) highlighted a crucial factor: the progressive wastewater treatment improvement seems to produce a higher concentration of some harmful pollutants in the sludge. When we monitor together HM, EOX and LAS, we introduce a first basic way to manage how much a more efficient treatment in water line increases inorganic/organic substances transferred into the sludge. This means to accept a critical evaluation of further process development in wastewater treatment, considering future perspectives in sludge agricultural use.

2.3.5 Other organic contaminants

polychlorinated biphenyls (PCBs), Polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs) and Polycyclic aromatic hydrocarbons (PAH) are the main organic pollutants of concern; they are very persistent due to the complexity and stability of their molecular structures and they show affinity for sediment and bind to the sludge during treatments in WWTPs because of being strongly hydrophobic.

Table 2.6 presents the concentration of these contaminants in sewage sludge samples followed by their limit value concentrations in sludge of Italy and as suggested in the 3rd draft of the “Working paper on sludge” for EU. The maximum concentration of PCDD/F and PAH found in the present work was 4.39 and 4.79 mg/kg (related to sample no. 1) which was associated to the largest WWTP, with combined municipal and urban sewage sources. Considering PCBs, the highest concentration found was 0.070 mg/kg obtained from sample no. 10.

Except PAH in sample no. 1 that was almost close to the suggested limitations, the concentration of contaminants in all samples were much lower than both Italian legislation and the one suggested in the 3rd draft of the “Working paper on sludge” for EU.

Table 2. 6 Concentration of PCB, PCDD/F and PAH in studied SS samples and their limit values in sludge of Italy and as suggested in the 3rd draft of the "Working paper on sludge" for EU

Sample	**PCB** (mg/kg)	**PAH** (mg/kg)	**PCDD/F** (ng TEq[3]/kg dm)
SS no.1	0.037	4.39	4.79
SS no.2	0.003	0.04	0.04
SS no.3	0.017	0 .40	2.08
SS no.4	0.007	0.10	1.28
SS no.5	0.015	0.40	2.24
SS no.6	0.014	0.44	2.7
SS no.7	0.021	0.96	3.25
SS no.8	0.023	0.45	2.70
SS no.9	0.008	0.37	4.68
SS no.10	0.070	0.41	4.12
Range	0.007-0.037	0.04-4.39	0.04-4.79
Mean	0.0215	0.84	2.788
Median	0.016	0.41	2.7
EU 2000 (3rd draft)	0.8[1]	6[2]	100
Italy (D.Lgs. 152/2006)	0.8	6	50

[1] Sum of 6 congeners PCB 28, 52, 101, 138, 153, 180

[2] Sum of acenapthene, phenanthrene, fluorine, fluoranthene, pyrene, benzo(b+j+k) fluoranthne, [3] benzo(a)pyrene, benzo(ghi)perylene, indeno(1,2,3- c,d)pyrene

TEq: Toxicity equivalents

2.4 Conclusion

With increasing wastewater treatment coverage, especially in high- and middle-income countries, the produced sewage sludge should be treated and valorised in an ecological and economic way, contributing to the circular economy perspective. While wastewater treatment plants efficiently remove pollution from water, they accumulate toxic metals and other persistent toxic compounds in sludge, restricting its reuse potential. In this study HM, EOX and LAS, as well as PCB, PCDD/F and PAH, as the main limiting factors for sewage sludge land application, were monitored in sewage sludge samples from 10 different low-middle potentiality wastewater treatment plants in Friuli-Venezia Giulia region (north-east of Italy). The results showed that the concentration of these compounds was much lower than permissible limits suggested by Council Directive 86/278/EEC for sewage sludge agricultural reuse, in particular for sludge coming from small municipal wastewater treatment plants. Sewage sludge from the studied wastewater treatment plants could be used for sustainable agriculture, to exploit its macro and micronutrients content, without being a threat for the environment and human health. On the other side, the present study pointed out a future scenario in which the constant progress in wastewater treatment, by decreasing pollutant levels released to the receptor body, therefore improving water quality, transfers higher quantities of potentially harmful compounds in sewage sludge, making it unsafe for agricultural use. This study, considering also a similar monitoring performed in 2006, questioned the future possibility sewage sludge agricultural use and raised the need for further constant and regular sludge checking, to prevent the agricultural use of sludge contaminated with excessive quantities of organic and inorganic substances (HM, EOX, LAS, PCB, PCDD/F and PAH), with the risk of soil and groundwater contamination, as well as potential entrance into the food chain.

Preface

This chapter is an attempt to answer the question that are humic substances artifacts of the extraction procedure or not? In a recent work, Lehmann and Kleber, rejected the humic substances concept and proposed the Soil continuum model, in which organic matter exists as a continuum of organic fragments and humic substances are considered to be artifacts of the alkail extraction procedure.

Since in chapter IV of the present document, humic substances are suggested as tracers for biological transformation of some organic contaminants and to understand the efficiency of treatments in WWTPs, in this chapter the integrity of extraction procedures for the isolation of HA and FA was assessed. In fact, the main goal of this work was to refuse the Soil continuum model and demonstrate that humic substances are real components of natural organic matter.

Chapter III

Integrity of extractions of humic substances by alkaline extractants

3.1 Introduction

Humic substances (HS) are defined as “naturally occurring materials found in or extracted from soils, sediments, and natural waters. They result from the decomposition of plant and animal residues (McCarthy, 2001).The reliability of the extraction of humic substances (HS) by alkaline extractants had been frequently questioned in the past decades and has been once again challenged recently. Lehman and Kleber (2015) and Kleber and Lehman (2019) rejected the classical humification model proposing the soil continuum model, in which soil organic matter exists as a continuum that spans from intact plant material to highly oxidized carbon, excluding any secondary synthesis during the decomposition process. The main argument put forward against what the authors call the humic paradigm (i.e. the existence of HS) is the alkaline extraction used to isolate these compounds. The alkaline extraction is considered "incomplete, selective and prone to create artefacts" (Lehman and Kleber, 2015).

It is reported that "alkaline extraction cannot distinguish between humic substances and non-humic substances" and that "alkaline extraction cannot discriminate for products of secondary synthesis" (Kleber and Lehman, 2019). In other words, according to these authors, HS are not real compounds that form in natural environments (soil, compost, water, etc.) from decomposition of plant and animal residues, but merely artifacts from the alkaline extraction procedure. However, the fact that HS can be extracted by neutral solvents (Hayes, 2006) was never considered in their criticism.

As shown by Olk et al. (2019), studies on HS have helped to understand biochemical processes underlying several important environmental and agronomical issues and have been used for the characterization of NOM in water systems. It is therefore important to re-examine the possibility of artifacts formation during the alkaline extraction of HS.

In the years from 1940 to 1980, extraction of HS from soil had been often carried out with different extractants, including solutions of neutral salts (Choudri and Stevenson, 1957) diluted acids (Evans, 1959) and chelating substances (Posner, 1966). By employing extractants different from sodium hydroxide, HS are extracted from soil with different yields and ash content and sometimes with different chemical characteristics. Anyway, the suspect that these substances might be of a different nature compared to those extracted under harsh alkaline conditions was never raised (Yuant, 1964; Bremner, 1946). All these works agreed on that structural differences among HA extracted from different soils are indeed more pronounced than differences observed in isolates obtained from the same soil by milder extraction procedures (Martin and Reeve, 1957; Azuma and Shiro, 1956; Hayes et al., 1975; Zaccone et al., 2007).

In this work, sphagnum moss and two sphagnum peat samples at different stages of decomposition were extracted using extraction conditions spanning from acid to strongly alkaline. Our aim was to verify whether harsh alkaline conditions are responsible for the formation of artifacts or if HS are indeed pre-existing entities which are progressively solubilized by increasing the extractant pH because of their polyphenolic, polycarboxylic nature.

3.2 Materials and Methods

3.2.1 Part I: Extractions and characterization of extracts

Sphagnum moss and two sphagnum peat samples at two different stages of decomposition (Table 3.1) were air dried, sieved at 2 mm and extracted with four different extractants: a) 0.5 M NaOH, pH 13.7; b) 0.1 M $Na_4P_2O_7$, pH 10.0 (A-NaPP); c) 0.1 M $Na_4P_2O_7$, pH 7.0 (N-NaPP); d) Milli-Q water, pH 5.8. All extractants had been degassed and saturated with N2 before use to remove dissolved O_2. Extractions (40:1 extractant/sample ratio) were carried out for 4 hours in a reciprocating shaker after closing containers under bubbling nitrogen.

Extracts were centrifuged (14000 rpm, 1 h), filtered under vacuum at 0.45 μm and the final extraction pH measured in the supernatants immediately at the end of the extraction. To precipitate humic acids (HA), extracts were acidified to pH 1 with 6 M HCl. After overnight precipitation, HA were separated by centrifugation (5000 rpm, 45 min). Supernatants (fulvic acid fractions) were passed through a DAX-8 resin column (previously washed and equilibrated with 0.1 M HCl). The eluate, which represents the non-humic fraction (NHU, hydrophilic acids and hydrophilic neutrals), was collected and stored. The retained fulvic acids (FA) were eluted with 0.1 M NaOH and immediately neutralized to pH 7 with 6 M HCl before storage at 4°C.

Organic carbon (OC), carbon stable isotope composition ($\delta^{13}C$) and total nitrogen (TN) of peat samples before and after extractions were determined by an Elemental Analyzer (Costech Instruments Elemental Combustion System).

The OC of whole extracts and of their fractions was determined, after appropriate dilution and pH adjustment to neutral values, by high temperature catalytic oxidation and subsequent non-dispersive infrared spectroscopy and chemo luminescence detection (TOC-VCPN, Shimadzu).

All UV-vis spectra of extracts were recorded at pH 7 on a Cary Varian Spectrophometer using 1 cm quartz cuvettes over an interval from 220 to 700 nm (scan rate 60 nm min^{-1}). Specific absorbance (SA) was calculated by normalizing absorbance by the optical path length (cm) and C concentration (mg L^{-1}).

FTIR spectra of HA were recorded with a FTIR spectrum (100 PerkinElmer Spectrometer) equipped with an ATR device, over an interval from 4000 to 800 cm^{-1}, with a 4 cm^{-1} resolution. A linear baseline correction was applied to compare spectra; intensity ratios were calculated for specific pairs of bands (Inbar et al., 1989).

Fluorescence EEM measurements of extracts were conducted at pH 7 using a Cary Eclipse Fluorescence Spectrophotometer (Agilent). Excitation and emission wavelength ranges were set to 240 – 400 nm (10 nm intervals) and 280 - 550 nm (2 nm intervals), respectively. Fluorescence intensities were normalized by the C concentration in the cuvette.

The ^1H NMR spectra of HA were recorded on a Bruker spectrometer. Spectra were divided into the following diagnostic regions: 0–1.7 ppm (methyl and methylene groups of methylene chains, methylene of alicyclic groups and CH_2 and CH groups at least two carbons away from aromatic rings or polar functional groups); 1.7–3.0 ppm (protons of methyl and methylene groups α to aromatic or carboxylic acid groups); 3.0–5.0 ppm (protons α to carbon attached to oxygen groups in polysaccharides or carbohydrates); 5.0–6.5 ppm, (olefins); and 6.5–9.0 ppm, (aromatic protons). Areas of the chemical shift regions were integrated and expressed as percentages of total area (relative intensity).

3.2.2 Part II: Strong alkaline conditions- effect of time

Samples of well-humified peat were extracted by 0.5 M NaOH, 0.1 M A-NaPP and 0.1 M N-NaPP following the procedure described above, reducing the extraction time to 5 min. After filtration, the extract was divided in two equal parts: one was immediately

(T0) processed and the other was stored in the dark for 24 h (T1) under N2 atmosphere. Quantification and characterization of extracted fractions were performed at T0 and T1. Moreover, an aliquot sample of the whole extract was taken at t =T0 and put in a quartz cuvette, purged with N_2 and closed (to avoid contact with air). The absorbance at 465 nm was monitored for 4 h and the Vis spectrum (450 and 700 nm) was recorded at beginning and at the end of this period.

3.2.3 Statistical analysis

All measurements were analytically replicated three times, and reported in tables and figures as mean ± standard error of the mean. Kruskal-Wallis one-way analyses of variance and Mann-Whitney test were applied to compare the parameters of extracts obtained from the different extractants. Difference between treatments was considered significant at $p < 0.05$. Regression analysis and test of significance were carried out by R software (Miller and Miller 2010; Development Core Team 2018).

3.3 Results

3.3.1 Quantification and composition of extracts

The amount of C extracted per unit of organic C (total extractable carbon, TEC) increased linearly with pH up to 10 (Figure 3.1a). A sharp increase of both TEC and NHU-C (Figure 3.1b) was conversely observed when the pH was raised from 10 to 13. However, this trend was not mirrored by a corresponding increase of HA-C/TEC ratios (Figure 3.1c). If strong alkaline extraction had induced the artifactual production of HA or FA, then HU-C/TEC ratios should have followed and even magnified the sharp increase displayed by TEC at the highest pH. On the contrary, the proportion of HU-C is even lower than would be expected from a linearized model (Figure 3.1d). This result is coherent with an enhanced extraction of NHU-C (due to hydrolysis of hemicellulose and proteins), but not with the artefactual formation of HS.

As expected, the HU fraction in the two peats extracts was significantly higher than in the sphagnum moss extracts for all the extractants used.

Strong alkaline extracts (NaOH and A-NaPP) of sphagnum moss contain substances that, similarly to HA, precipitate at low pH. However, these substances could not be directly re-solubilized in phosphate buffer, like all other HA, but only by addition of 0.1 M NaOH. So, these substances are likely artifacts of the procedure, which differ from HA in their solubility behaviour. No visible trace of such irreversibly precipitated components was observed when HA from the two peats were dissolved in phosphate buffer.

Table 3.1 reports the OC, TN and ^{13}C content of raw materials and of the extracted HA. The elemental and carbon isotopic composition of artefactual HA extracted from sphagnum moss was significantly different from that extracted the two peats. Moreover, C/N ratios were much different and closer to the C/N ratios of proteins than to those of HA.

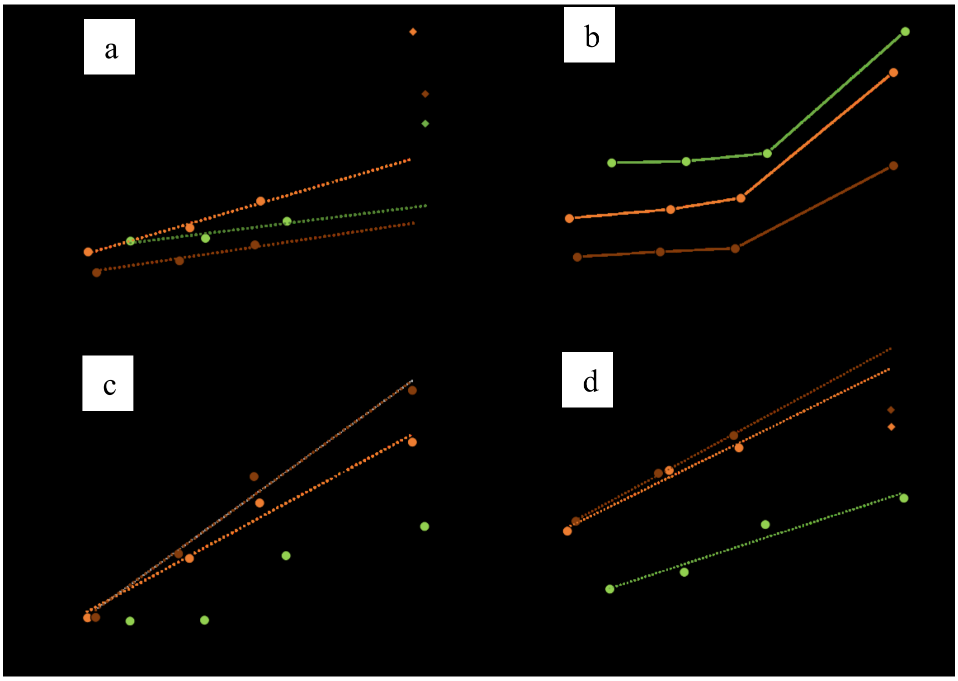

Figure 3. 1 Trends of a) extraction yields (total extractable carbon -TEC- per unit of organic carbon), b) not-humic carbon (NHU), c) ratio of humic acid carbon (HA) to TEC, d) ratio of humic carbon (HU) to TEC as a function of the final pH at the end of the extraction.

Table 3. 1 Elemental composition and 13C content of raw materials and of the extracted HA. Numbers in parenthesis represent standard deviations from the mean

	Extractant	**C (%)**	**N (%)**	**C/N**	**$\delta^{13}C$ (‰ vs VPDB)**
Sphagnum	raw	42.75 (0.20)	1.03 (0.01)	41.64	-27.39 (0.10)
	NaOH	50.77 (0.80)	5.86 (0.02)	8.67	-29.54 (0.02)
	A-NaPP	45.20 (0.71)	6.06 (0.01)	7.46	-29.11 (0.03)
	N-NaPP	42.07 (0.67)	5.87 (0.01)	7.17	-28.77 (0.02)
	Water	n.d.	n.d.	n.d.	n.d.
Poorly-humified peat	raw	45.24 (0.33)	0.81 (0.01)	56.09	-25.58 (0.17)
	NaOH	48.94 (0.79)	2.15 (0.01)	22.82	-26.60 (0.17)
	A-NaPP	48.78 (0.08)	2.48 (0.01)	19.71	-26.35 (0.09)
	N-NaPP	47.02 (0.81)	2.51 (0.04)	18.93	-25.65 (0.07)
	Water	46.73 (0.56)	2.96 (0.03)	15.79	-25.85 (0.10)
Well-humified peat	raw	45.86 (1.00)	1.10 (0.06)	41.69	-26.78 (0.12)
	NaOH	48.80 (0.51)	2.22 (0.01)	21.98	-27.08 (0.01)
	A-NaPP	49.01 (0.59)	2.00 (0.04)	24.50	-27.27 (0.03)
	N-NaPP	48.35 (0.42)	2.58 (0.02)	18.74	-27.07 (0.05)
	Water	48.93 (0.35)	2.81 (0.03)	17.41	-27.21 (0.04)

3.3.2 UV-Vis

Although NaOH extracts are visibly darker than extracts obtained under milder pH conditions (Figure 3.2), the color of HS is not an artifact produced by alkalinity, but the combined effect of the increased concentration of organic C (particularly HU-C) and the bathochromic shift caused by dissociation of weak acid groups at increasing pH. Once spectra are normalized with respect to concentration of organic C (specific absorbance, SA) and recorded at the same pH (Figure 3.3), differences among spectra of extracts obtained from the same material, virtually disappear in the visible region. On the contrary differences related to the degree of humification of the extracted material remain.

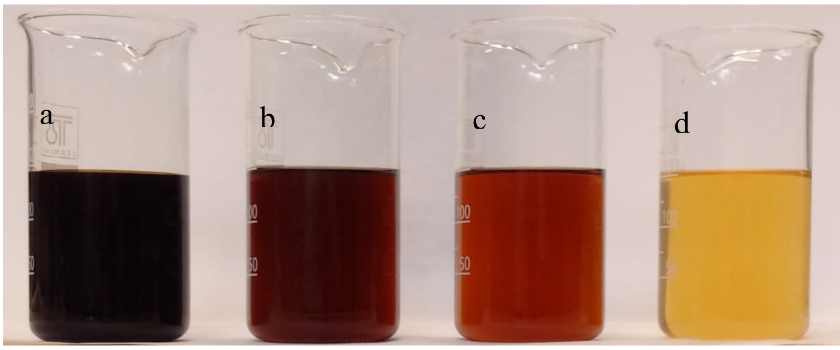

Figure 3. 2 Total extracts from well-humified peat using a) 0.5 M NaOH, b) 0.1 M A-NaPP, c) 0.1 M N-NaPP and d) water

In the UV region from 220 to 240 nm, the SA of sphagnum HA extracted by NaOH is about 25% higher than that of HA extracted by milder solutions. The SA of all HA strongly increase at all wavelengths with the degree of humification of peat, whereas the shoulder at about 270-280 nm, more pronounced in the spectra of sphagnum HA (generally attributed to tryptophan residues), becomes less visible.

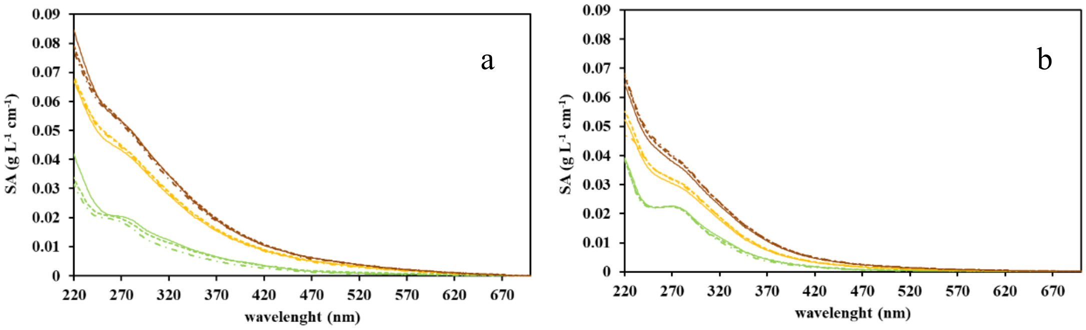

Figure 3. 3 Organic C normalized UV-Vis spectra from 220 to 700 nm of HA (a) and FA (b) from Sphagnum (green), partly-humified (yellow) and well-humified (brown) peat. HA and FA were extracted with 0.5 NaOH (continuous line), A-NaPP (dashed line) and N-NaPP (dash dot line). All solutions were adjusted to pH 7 before recording spectra

Specific absorbance at 254 nm ($SUVA_{254}$) makes possible to statistically evaluate compositional differences from UV spectra (Figure 3.4). This evaluation confirmed that no significant difference existed among materials extracted from the same material by extractants of increasing alkalinity, but aromaticity of HA and FA extracted from different substrates were indeed significantly different. At the same time there was no significant difference in aromaticity among hydrophilic fractions (NHU) isolated from all extracts: this means that the NHU fraction, contrary to the HU one, is conserved not only among extracts of different pH but also among different materials. This again is coherent with the conservation of chemical nature and absence of artefactual modifications even under strongly alkaline conditions.

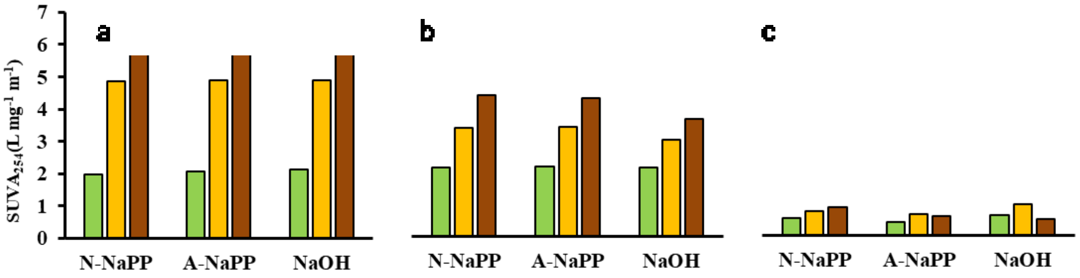

Figure 3. 4 $SUVA_{254}$ values for a) HA, b) FA (hydrophobic acids) c) hydrophilic (not-humic) fraction extracted with neutral pyrophosphate (N-PP), alkaline pyrophosphate (A-PP) and sodium hydroxide (NaOH) from sphagnum (Green), poorly humified peat (Yellow) and well humified peat (Brown)

3.3.3 FT-IR

FT-IR spectra of the extracted HA are reported in Figure 3.5. All spectra are characterized by HA typical absorption bands: a broad band at about 3280 (O-H stretching vibrations); twin peaks at 2920 and 2850 cm^{-1} (asymmetric and symmetric C-H stretching of CH_2 and CH_3 groups; a shoulder at 1710 cm^{-1} (C-O stretching of carboxyl and ketonic carbonyl) merged with the more intense band at 1610 cm^{-1} (conjugated carbonyl C=O and aromatic C=C); a discrete peak at about 1515 cm^{-1} (uncondensed aromatic compounds bound to N and O atoms); two small peaks at 1450 and 1420 cm^{-1} (C-H bending of CH_2 and CH_3 groups); a band at 1215 cm^{-1} (stretching

C-O and bending O-H vibrations) and stretching of carbohydrate or alcoholic C–O at 1040 cm^{-1}.

Regardless the used extractants, several spectral differences are evident between the three materials, from sphagnum to well-humified peat: a) the band at 3280 cm^{-1} became broader; b) the twin peaks at 2920 and 2850 cm^{-1} are less intense and resolute; c) the peak absorbance at 1710 cm^{-1} increased, while the ones at 1215 and 1040 cm^{-1} decreased. Considering the same material, the spectra of HA extracted by NaOH, A-NaPP and N-NaPP do not present clear differences among them. However, the HA extracted by water present the lowest 1710/1040 and 1215/2920 intensity ratios.

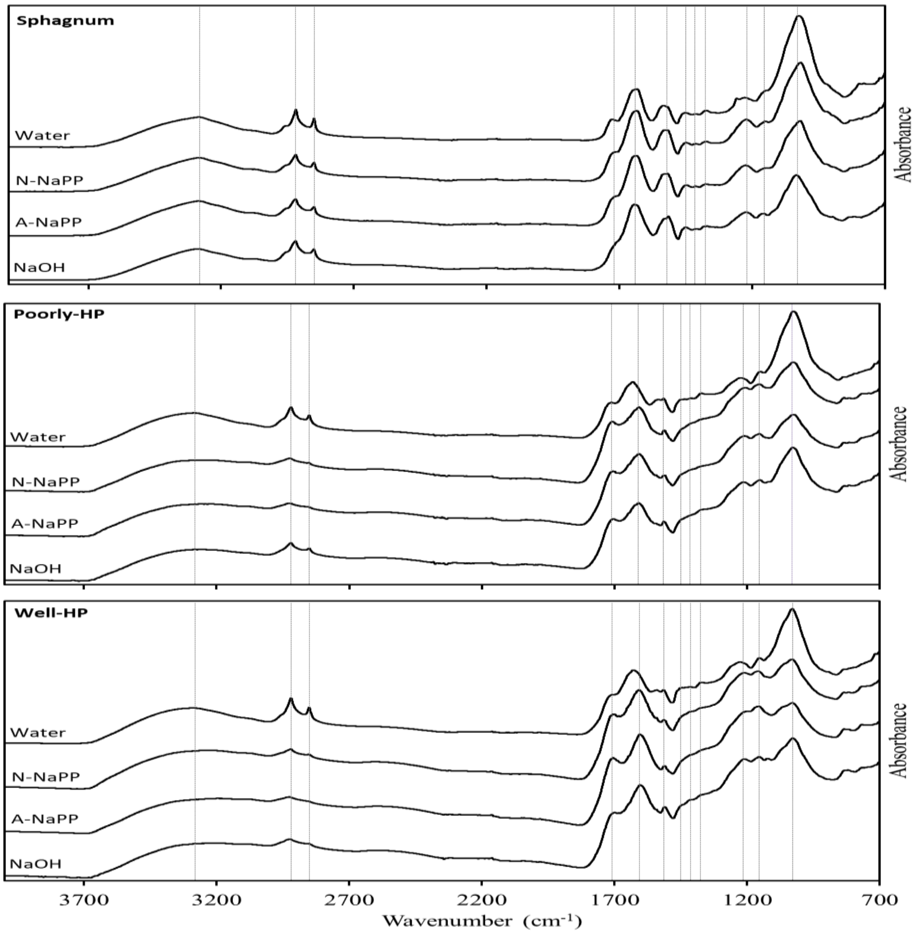

Figure 3. 5 FT-IR spectra of HA extracted from sphagnum, partly-humified and well-humified peat using different extractants

3.3.4 ^{1}H NMR

^{1}H NMR spectra of HA are reported in Figure 3.6 and their proton distribution is summarized in Table 3.2. These results confirmed evidence derived from FT-IR: in fact, well-humified peat presented the highest aromatic and lowest alkyl percentage. Moreover, the differences using different extractants are within the instrumental deviation (± 5%).

Table 3. 2 Proton distribution percentage calculated from ^{1}H NMR spectra

		Alkyl-H 0-1.7 ppm	**Alkyl-H 1.7-3.0 ppm**	**Carbohydrate-H 3.0 -5.0 ppm**	**Olefins 5.0-6.5 ppm**	**Aromatic-H 6.5-9.0 ppm**
Sphagnum	NaOH	41.1	20.5	24.9	6.4	7.0
	A-NaPP	45.1	20.3	23.6	4.1	6.9
Poorly-HP	NaOH	34.0	19.2	28.8	6.3	11.6
	A-NaPP	33.0	21.6	26.4	6.5	12.5
	N-NaPP	30.1	21.8	28.1	6.7	13.4
Well-HP	NaOH	25.8	21.6	26.9	8.9	16.8
	A-NaPP	27.3	24.2	23.7	8.2	16.6
	N-NaPP	29.3	23.9	25.7	7.9	13.2

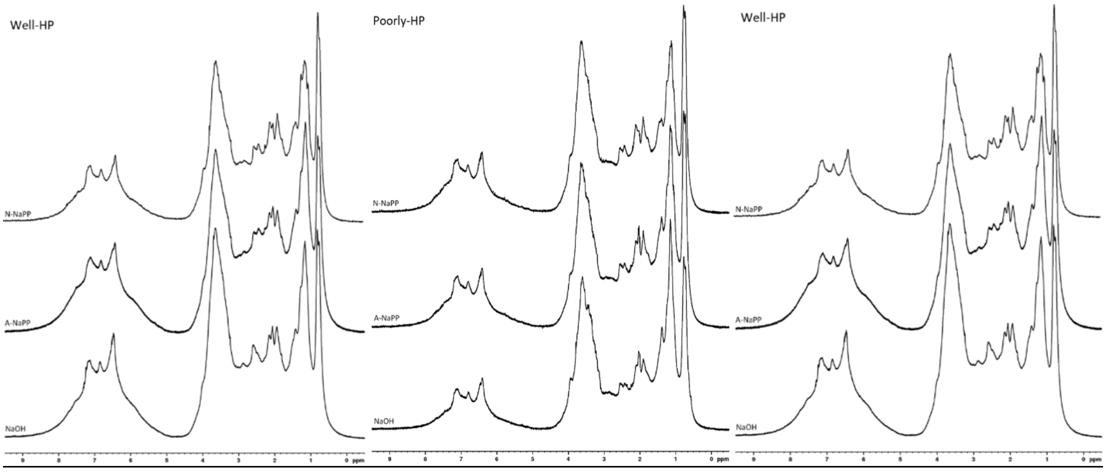

Figure 3. 6 ^{1}H NMR spectra of HA extracted from sphagnum, partly-humified and well-humified peat using different extractants

3.3.5. Fluorescence spectroscopy

EEM spectra of total extracts (Figure 3.7), fulvic acids (Figure 3.8) and the not-humic fraction (Figure 3.9) reflect much more the effects of different humification degree of the original material than the pH of extractant solutions.

These results support the hypothesis that the alkalinity of extractants is not responsible of artefacts as claimed by Lehman and Kleber.

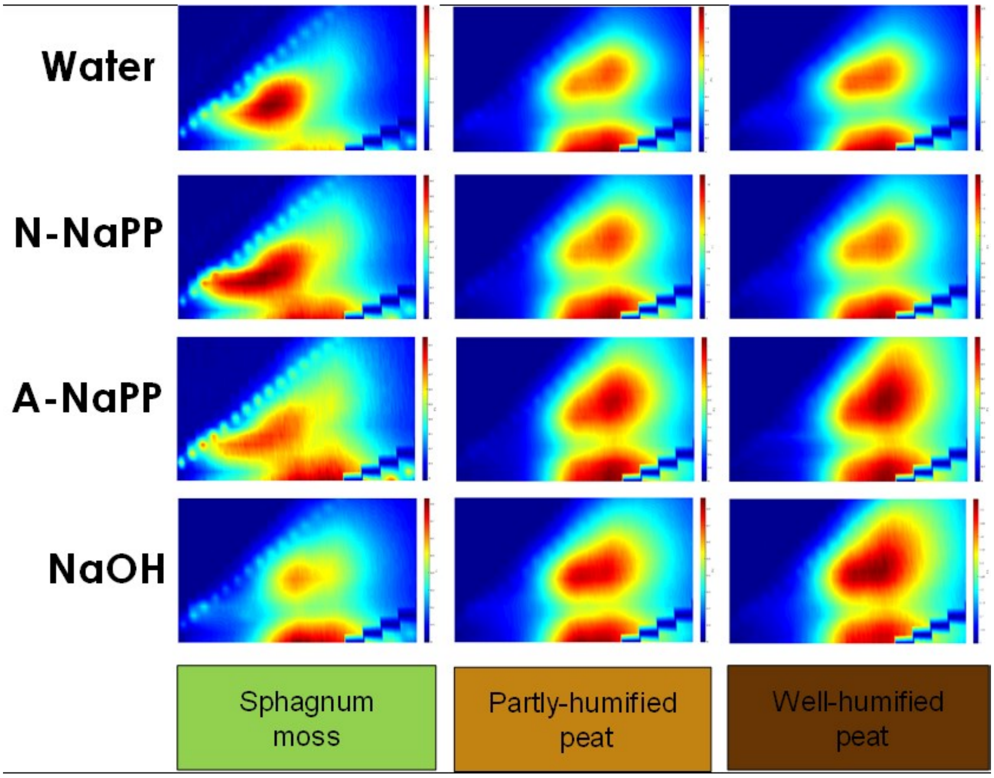

Figure 3. 7 Fluorescence excitation-emission matrix spectra of total extracts.

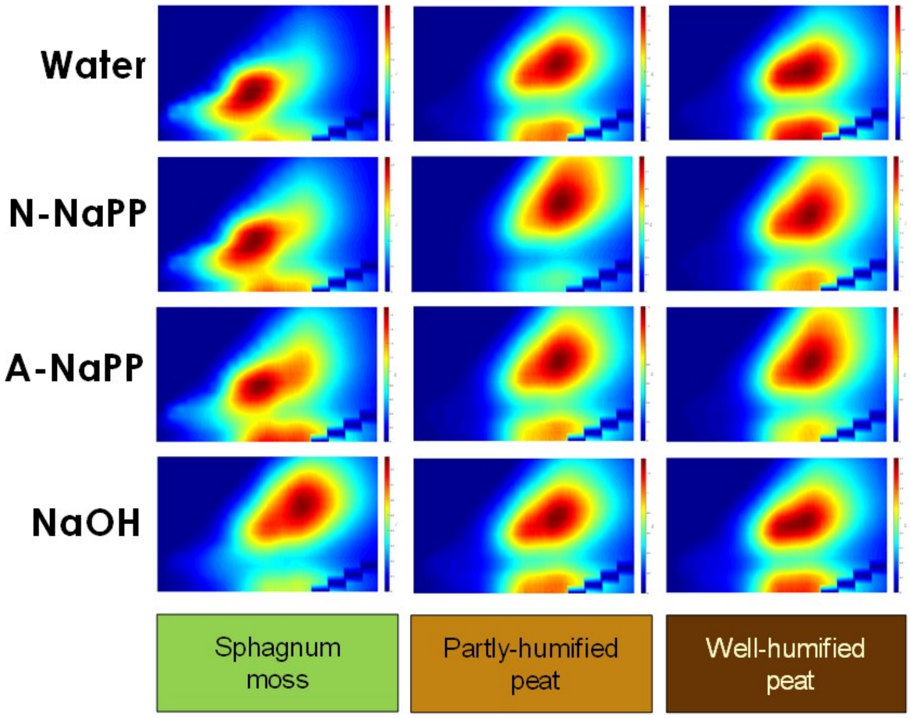

Figure 3. 8 Fluorescence excitation-emission matrix spectra of fulvic acids.

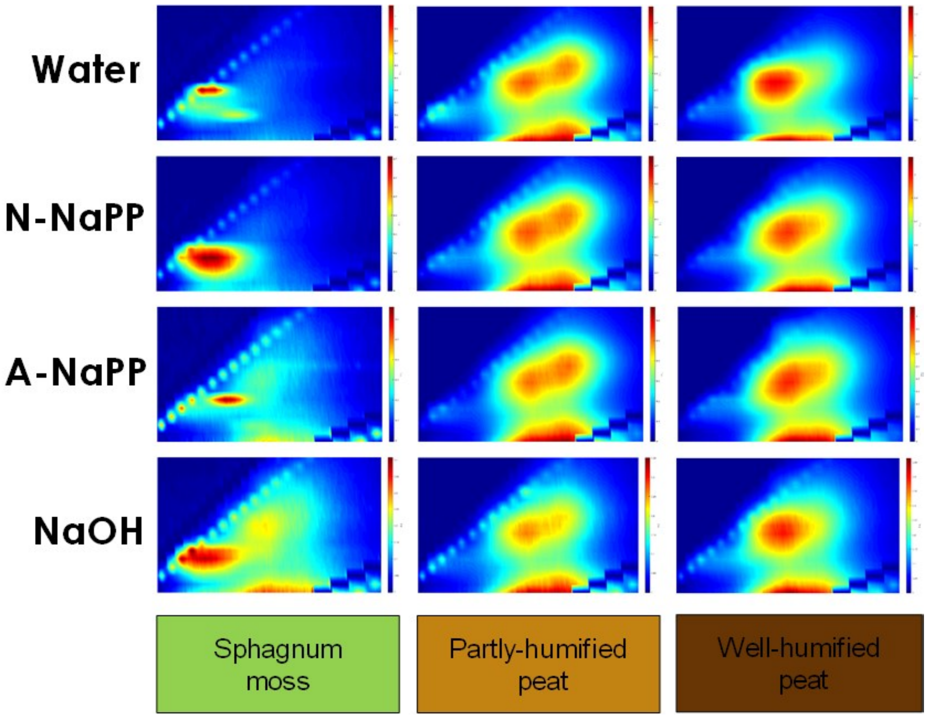

Figure 3. 9 Fluorescence excitation-emission matrix spectra of the not-humic fraction.

3.3.6 Strong alkaline conditions- effect of time

Results are summarized in Table 3.3. TEC extraction yields after 5 min of extraction were significantly lower than after 4 h of extraction (39, 26 and 22 % respectively for NaOH, alkaline and neutral $Na_4P_2O_7$). After 24 h of storage the TEC did not vary, excluding any spontaneous precipitation. The pH of the extracts did not change, confirming that no decarboxylation (as also confirmed by the elemental and isotopic composition of HA) nor H^+ consuming hydrolysis reactions occurred between T0 and T1. Moreover, also the amount of the HU fraction did not change, indicating that even at very alkaline conditions there is not creation of "artifactual" HS. In fact, if HS were merely created by alkaline conditions, keeping extracts at high pH for longer times should cause an increase of the HU fraction with respect to NH/HU. Regarding the optical properties, the UV-Vis spectra of HA and FA did not change between T0 and T1. This indicates that the color of HS is not created by the alkaline conditions and even storing the solutions at pH > 12 for 1 day did not result in any modification in the optical properties of extracts. EEM fluorescent data confirms UV-Vis results (no changes in peaks position and intensities). FT-IR spectra of HA overlapped between T0 and T1 and consequently the ratio 1605/1030 did not change.

Considering the NaOH extract, after 4 h the Abs (465 nm) decreased of 2.4%. This led an increase of the E4/E6 ratio from 6.80 to 7.34 (Figure 3.10). On the other side, the changes in the E4/E6 ratio for the alkaline and neutral pyrophosphate are lower and not significative (from 6.65 to 6.69 and from 4.45 to 4.42, respectively). The change of the E4/E6 ratio for the NaOH could be explained with a not completely remove of O_2 from the cuvettes. In fact, letting the extract in contact with the atmospheric air, the decrease in Abs (465 nm) is 8.45 %, and consequently the E4/E6 ratio increase to 8.53. In fact, in alkaline solutions the presence of O_2 could lead to decarboxylation. For these reasons it is important to de-aerate extractants before extraction.

Table 3. 3 Variation in the considered parameters of HA between T0 and T1, for the 3 different extractants

	N-NaPP		**A-NaPP**		**NaOH**	
	T0	**T1**	**T0**	**T1**	**T0**	**T1**
pH	6.70	6.72	9.04	9.02	12.81	12.83
TEC (mg-C/g-C)	8.03	7.94	14.69	14.85	97.36	99.21
HU (mg-C/g-C)	7.36	7.48	14.12	13.68	89.31	89.34
NHU/HU	0.11	0.10	0.08	0.09	0.12	0.12
%C	48.30	47.55	47.64	47.13	48.84	48.92
%N	2.14	2.08	1.69	1.67	1.96	1.93
C/N	22.57	22.86	28.19	28.22	24.98	25.34
$\delta^{13}C$	-27.02	-26.95	-27.00	-27.32	-26.88	-26.87
$SUVA_{254}$	5.15	5.27	5.42	5.33	5.38	5.30
% Arom					34.01	34.60
1605/1030 cm^{-1}	1.40	1.39	1.35	1.35	1.36	1.36

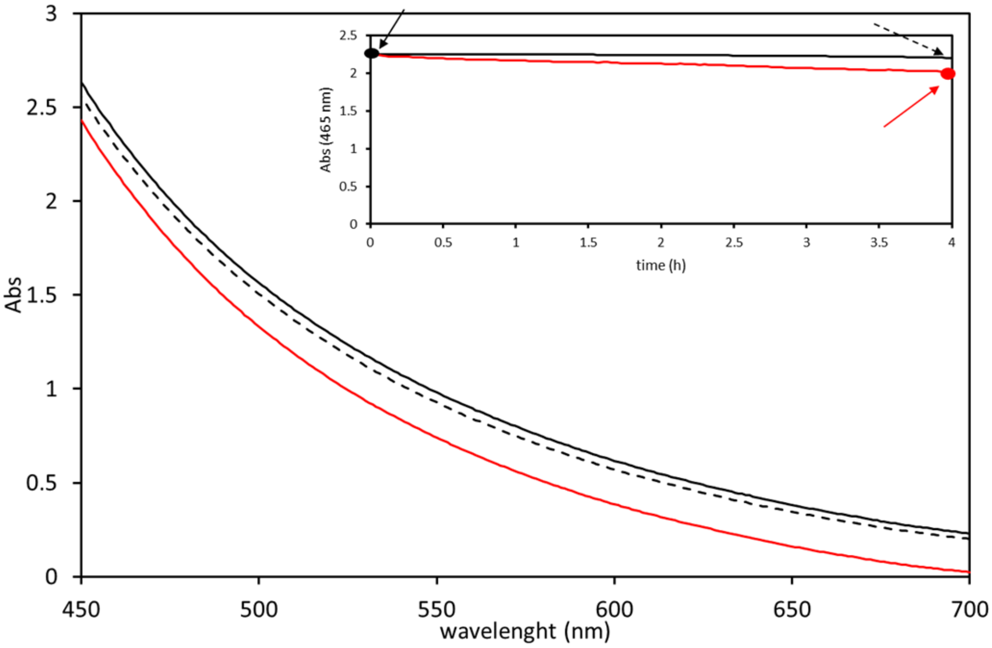

Figure 3. 10 Vis spectrum of NaOH extract at t=0 (continuous line) and at t=4h (dotted line). The insert represents the variation of the absorbance at 465 nm for 4 h. The red curve represents the UV spectrum of the extract stored 4 h in contact with the atmospheric air

3.4 Conclusion

All the obtained results are coherent with the classical humification theory, demonstrating that humic substances are not artifacts of the extraction process. In fact, humification consists in demolition and loss of labile substrates and in structural changes of the hydrophobic acid fraction. Moreover, not only spectroscopic properties are conserved when different extractant are used, but also extraction of substrates at different stages of humification allows to observe structural differences among corresponding fractions.

Preface

This work answered to the need for some valid and cost effective methods to evaluate the suitability of sludge for agricultural use through the application of humic substances as tracers for degradation of organic contaminants during storage. Humic substances are the most important fraction of sludge organic matter, and as demonstrated in the previous chapter are resulted from humification that could occur during storage.

The need was felt more especially when the use of sewage sludge in agriculture was taken into consideration, and expanded as an alternative method for landfill. Moreover, by the increase in the concentration of emerging contaminants in waste waters as well as constant progress in wastewater treatment in WWTPs, it is expected that the concentration of contaminants in sewage sludge is increased and limits their agricultural use. Previous studies (Thornton et al., 2001) highlighted the possibility that contaminants, such as LAS could not only become sorbed on organic materials during flocculation, but their content in sludge might increase as a consequence of improved stabilization treatments that result in formation of humic substances. Therefore, monitoring of sewage sludge before land application is essential; and since the regular evaluation methods for the content of organic pollutants are expensive and time consuming, this part will be a try to create a relation between the organic contamination and humification degree of sewage sludge and also the use of UV-vis, FTIR and Fluorescence spectra to assess properties of humic substances as a valid and cost effective methods.

Chapter IV

Biological transformation of humic substances and contaminants during thickening and storage of treated sludge

4.1 Introduction

Increasing sludge production, high cost of disposal and enforcement of more stringent environmental quality standards have led to the current pressing need for a cost effective and environmentally safe alternative disposal method.

In Europe, the Council Directive 91/271/EEC encouraged land application of sewage sludge (European Commission, 1991) as a suitable recycling strategy, considering its large content of organic matter (OM) and plant nutrients, which permit its potential use in agriculture as soil conditioner and fertilizer. However, this practice has sometimes lead to soil contamination (European Commission, 2016) and health problems (Anjum et al., 2016) due to the accumulation of persistent organic contaminants and toxic metals and presence of pathogenic microorganisms (Singh and Agrawal, 2008). Therefore, a sustainable use of sludge derived from urban wastewater treatment plants as a fertilizer in agriculture, requires precise specification of its properties and quality and if necessary, sludge need to be processed properly.

Suitable treatments and regulation of industrial waste disposal have greatly reduced risks from toxic metals and pathogens, but other types of contaminants are now becoming increasingly diffuse in sewage and are not satisfactorily eliminated by treatments. Emerging contaminants, among which plasticizers, antibiotics, chlorinated compounds, Linear Alkylbenzene Sulfonates (LAS) are nowadays a major reason of concern for the application of treated sewage sludge to agricultural soils.

Linear Alkylbenzene Sulphonates (LAS) are the most used anionic surfactant, introduced in 1964 as the readily biodegradable replacement for Branched Alkylbenzene Sulfonates (BAS). They are a mixture of homologues and isomers which is characterized by having a hydrophilic negatively charged sulfonate head-group attached to a benzene ring and a long hydrophobic alkyl tail of 10-13 (Figure 3.1).

In the year 2000, the third draft of the future sludge directive “Working Document on Sludge” (EU 2000) where more restricted concentration limit values of LAS is included for the first time, was published by European Union and was adopted among others, by some Italian regional regulations (Emilia-Romagna Region, 2005; Italian regulation, 2018).

Apart from LAS, recently particular attention has been given to presence of organic halogen compounds in domestic and municipal waste waters. Extractable Organic Halogens (EOX) represent the sum of organic Chlorine (Cl), Iodine (I) and Bromine (Br) which can be extracted by organic solvents from environmental solids. These compounds include not only synthetic polychlorinated biphenyls, organochlorine pesticides, polychlorinated dibenzo-p-dioxins or polychlorinated dibenzofurans and other substances, but also those produced naturally by microorganisms, flora and fauna (Asplund and Grimvall, 1991). In municipal waste water treatment plants organic halogens adsorbed and concentrated on biomass (Bornhardt et al., 1997; Goi et al., 2006) therefore monitoring and control of sewage sludge is essential prior to application onto agricultural soil due to the toxicity and durability of these compounds. The importance and usefulness of the EOX parameter in the evaluation of environmental quality have been demonstrated by some investigations (Rodziewicz et al., 2004; Contreras Lopez, 2003; Goi et al., 2006), however, there are still limited number of investigations on sludge EOX content.

The concentration of these substances (LAS and EOX), which can undergo decomposition under anoxic conditions, may decrease during sludge storage in thickening beds.

Monitoring of these contaminants is time consuming and expensive. A more convenient approach could be to monitor changes in quantity and or quality of humic substances in order to evaluate the efficacy of the single stages of the treatment.

Humic Substances (HS), namely Humic (HA) and Fulvic (FA) Acids, are refractory natural compounds produced during decomposition of organic matter in soil and natural waters. Their quantification has been widely used in the evaluation of the quality of sewage sludge for their use in agriculture. Changes in their easily measured spectral properties (UV and fluorescence) could represent a convenient way to assess the degree of biological transformation that occurs in sewage sludge. In this work we considered a stage, which is often neglected in the evaluation of the overall performance of a treatment plant: namely the period spent by treated sludge in thickening beds and in storage facilities. Our aim was to ascertain the integrity of HS use as markers of biological transformations at this stage of sewage processing where biological transformation is generally considered to be minimal.

To this purpose we evaluated: i) the effect of sludge storage on degradation EOX, as representative of compounds best degraded under anaerobic conditions, and LAS as representatives of compounds best degraded under aerobic conditions, ii) the use of humic substances as an indicator of the efficiency of transformation process in sewage sludge treatment and specifically of the storage period iii) application of UV-visible, FTIR and fluorescence spectroscopies as valid, simple and cost effective methods to pre-examine the quality of HS in sewage sludge.

4.2 Materials and Methods

4.2.1 Sewage sludge samples and treatments

Sewage sludge samples were obtained from four different small scale waste water treatment plants in Friuli Venezia Giulia (FVG), Italy. Freshly treated sludge samples were collected after primary sludges underwent a complete aerobic treatment. After this stage, before land application or disposal, the sludge from plant n.1 is first dewatered by a belt system and then stored under cover in a heap. The sludge from plant n.2, after dewatering in a thickening bed, is stored in the open air on the ground. Samples from plants 3 and 4 were sampled right after treatment and stored anaerobically under controlled conditions at about 25 °C. All stored sludges had been stored for approximately three months.

Other 10 fresh sludge samples collected from different WWTPs in FVG (detailed in chapter II) were used for following analyses as well.

4.2.2 HA and FA analysis

4.2.2.1 Extraction and purification of Humic Substances

Sewage sludge samples of freshly treated (FT) sludge were dried at 45 ℃ and ground to pass through a 2.0-mm sieve. Isolation of HS from all samples was performed according to the procedure recommended by the IHSS (Swift, 1996).

Briefly, 40g of dried sample was extracted by adding 400 mL fully deaerated 0.1M NaOH and shaking for 4 h under N_2. The alkaline suspension was left to settle overnight and after filtration, the solution was acidified with 6M HCl to about pH 1 and then allowed to stand for 16 h. Centrifugation for 20 min at 5,000 rpm allowed the separation of humic acids (HA) in the precipitate and of the fulvic acid fraction (supernatant).

The HA were redissolved by adding a minimum volume of 0.1 M KOH under nitrogen atmosphere with constant stirring. Solid KCl was added to attain a concentration of 0.3 M [K^+] and then centrifuged at high speed to remove the suspended solids. The HA was reprecipitated by adding 6 M HCl with constant stirring to pH = 1.0 and the suspension was allowed to stand for 16 h. The separation was carried out by centrifugation and the supernatant was discarded. For purification, the HA was suspended in 0.1 M HCl/0.3 M HF solution in a plastic container and shacked overnight at room temperature. After centrifugation, the precipitate was transferred to a Visking dialysis tube after being suspended in Milli-Q water and dialyzed against distilled water until the dialysis water gives a negative Cl^- test with silver nitrate ($AgNO_3$). At the end the purified humic acid was freeze dried.

In order to purify FA, the FA fraction extract was loaded on a XAD-8 resin column. The effluent was discarded and the XAD-8 column, containing the retained fulvic acids, was rinsed with 0.65 column volumes of distilled H_2O. The adsorbed FA were desorbed from the resin with one column volume of 0.1 M NaOH, followed by two column volumes of Milli-Q water. The solution was immediately acidified with 6 M HCl to pH equal to 1 and concentrated HF was added to a final concentration of 0.3 M HF. The acidified solution was passed again through XAD-8 resin and the FA recovered by the same process described above. The eluate was passed through H^+-saturated cation-exchange resin and finally freeze-dried.

4.2.2.2 UV-vis

UV-vis spectra were recorded at pH 7 on a Cary spectrophotometer (Varian) in 1 cm quartz cuvettes and scanned from 200 to 600 nm. Specific Absorbance (SA) was calculated through normalizing absorbance by the optical path length (cm) and the C concentration (mg L^{-1}).

4.2.2.3 FT-IR

Attenuated reflectance Fourier-transform infrared (ATR-FTIR) spectra were recorded with a FTIR spectrum (100 PerkinElmer Spectrometer) equipped with an ATR device, over an interval from 4000 to 500 cm^{-1}, with a 4 cm^{-1} resolution. A linear baseline correction was applied to compare spectra; the attribution of the main absorption bands was done according to Giovanela et al. (2004) and Filip et al. (1988). Intensity ratios were calculated for specific pairs of bands (Inbar et al. 1989).

4.2.2.4 EEM Fluorescence and Humification index (HIX)

The EEM fluorescence spectra of the FA and HA were recorded in 0.1 M phosphate buffer solution at pH=7 (50 mgl^{-1} of HS) with an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer. Solutions were irradiated in a 1 cm quartz cells (Agilent Technologies), thermostated at 20 °C. Scanning and recording of emission spectra (300– 600 nm) was carried out at sequential 5 nm increments of excitation wavelength (λ_{ex}) between 220 and 550 nm. Bandwidths for both excitation and emission were 4 nm, with emission wavelength (λ_{em}) increments of 1 nm and integration time of 0.1 s. The spectra were obtained by subtracting phosphate buffer blank spectra, recorded under the same conditions, to eliminate the phosphate solution Raman scatter peaks. The scans were used to generate three-dimensional contour plots of fluorescence intensity as a function of excitation and emission wavelengths. All fluorescence intensities were normalized to Raman units (R.U.), and divided by the sample's dissolved organic carbon concentration.

In this work the calculation of HIX values proposed by Zsolnay et al. (1999) was selected. HIX is calculated dividing the area of the emission at 435–480 nm by that at 300–345 nm at an excitation wavelength of 254 nm, as shown in the following equation:

$$\mathrm{HIX} = (\sum I_{435\rightarrow 480})/(\sum I_{300\rightarrow 345})$$

Where I is the fluorescence intensity at each wavelength

4.2.3 EOX analysis

4.2.3.1 Extraction procedure

All sludge samples were freeze-dried, sieved manually through a 1 mm mesh sieve and ground in a ball-grinder. Subsequently, 1 g of freeze-dried samples were extracted with 5 ml of ethyl acetate or n-hexane solvent by shaking for 24 h. Most of the solvent was then stripped from the extracts under nitrogen flow till the volume was reduced to 1 ml and was stored refrigerated until analysis.

4.2.3.2 Coulometric measuring procedure

Analyses were made with Trace Elemental Instrument, Euroglas ECS 1000 upgraded with digital coulometer and control software (TEIS). This apparatus consists of: injection part, thermal extraction, trapping section and titration cell.

A hundred microliters of residual extract were introduced into the instrument with injection rate of 20 µl/min. At 950 °C and in an oxygen atmosphere combustion accompanied by the pyrolysis of organochlorine compounds and the release of hydrogen halides takes place. The reaction gases formed are carried by the gas stream to the titration cell after passing the absorber. The absorber is filled with concentrated sulfuric acid and its function is to remove the water from the gas flow. In the titration cell there is a solution (acetic acid 70%) in which the silver ion concentration is maintained constant (approximately 10-7 M) by measuring the silver ion concentration continuously. As soon as the acid formed from the organic halogens reaches the cell, the halogen reacts with the silver ions present and precipitate as silver chloride (AgCl), silver bromide (AgBr) or silver iodide (AgI). From the integral of the current over the time, the quantity of silver generated and thus the quantity of halogen introduced can be calculated as chloride or as halogen molecules.

4.2.4 LAS analysis

4.2.4.1 Chemicals and reagents

HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany), analytical grade sodium perchlorate (Aldrich). Sodium dodecyl-benzenesulfonate was used as standard (Sigma-Aldrich). Standard solutions of LAS were prepared in ultrapure water. Ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA).

4.2.4.3 Microwave-Assisted Extraction method

Microwave-Assisted Extraction (MAE) was performed on 0.5 g of dried sewage sludge samples. Methanol was used as a solvent because in the literature it is the most common solvent used. 5 ml of solvent was added to the samples and the extraction vessel closed and introduced into the microwave cavity. The level of microwave oven power and the irradiation time were 340 W and 10 min, respectively. After extraction, the vessels were allowed to cool at room temperature before they were opened. The extracts were filtered through glass wool. The extract was analyzed by HPLC.

4.2.4.4 HPLC analysis

All measurements were made with a Shimadzu high performance liquid chromatograph LC-20AT (Shimatzu Corporation Kyoto, Japan), fitted with an SIL-20AHT autosampler with a loop 20 µL, equipped with a diode array detector (DAD), a quaternary pump, a vacuum degasser and a thermostated column compartment. The analytical cartridge column was a SUPELCOSIL LC-8 (SUPELCO, Bellefonte, PA, USA), 25.0 cm × 4.6 mm ID, 5 µm particle size. The microwave extraction system was a Microwave Mars 5 Digestion Oven apparatus (CEM, North Carolina, USA) with a programmable power and irradiation time. The apparatus is equipped with a carousel that is able to hold 36 extraction vessels.

A commercial mixture of linear alkylbenzene sulfonates with C10–C13 chain length was used. Standard solutions at different concentrations were prepared in ultrapure water. Different solvents gradient programs were tried to obtain a good resolution of all LAS peaks. Good results were obtained by using as mobile phase acetonitrile–water containing 0.1 M $NaClO_4$ (55:45) and isocratic elution. Compounds were eluted isocratically over 6 min run time at a flow rate of 0.8 mL min^{-1} after injection of 20 μL. Since polar interferences are eluted between 0 and 2min, they do not interfere in the analysis. The column was thermostated (35℃). DAD-UV (λ_{ex} =225nm) detector were used for the determination of LAS.

4.3 Results and Discussion

4.3.1 Characterization of organic fraction

Chemical characterization of sludge samples, including organic C (C_{org}), total N (N) and content of HA-C and FA-C before and after sludge thickening and storage are reported in Table 4.1. The four sludges displayed different degrees of loss of organic C (ΔC loss, Table 4.1) at the end of storage. The sludge sampled from plant 1 does not appear to have undergone much biological transformation after three months; on the contrary, sludge from plant 4 lost about 30% of its original organic C content and must have therefore undergone strong mineralization. Samples from plants 2 and 3 represented intermediate situations, so the chosen plants and treatments provide a well sorted representation of storage situations which, for different reasons, have different impacts on post treatment modifications of sludge quality.

Very slight variations in total nitrogen contents were found in all sludge samples before and after storage. This indicates that little ammonia volatilization occurred during this period in the examined samples.

The C/N ratio therefore simply reflects C losses through mineralization (evolution of carbon dioxide). This loss was very small for the dewatered sewage sludge from plant n.1 after 90 days of storage, while larger changes were recorded in samples from other plants. However, changes in C/N ratios appear to be small: the highest recorded decrement (from 8.9 to 7) was related to sample no. 4. So this parameter, which decreases during decomposition as a result of C loss as CO_2 and which is often used to characterize the degree of stabilization of sludges and composts is not sufficiently sensitive to monitor changes that may occur during storage of treated sewage.

Table 4. 1 Elemental composition and content of HA and FA in sewage sludge samples and percent changes in organic C and HA following storage

Samples		**N** (g/kg d.w.)	**C_{org}** (g/kg d.w.)	**C/N**	**ΔC_{org} %**	**FA-C** (g/kg d.w.)	**HA-C** (g/kg d.w.)	**HA** (g/kg d.w.)
P1	**Fresh**	32 a	224 a	8.2	-6.3	2.92 a	11.18 a	24.49 a
	Stored	31 a	210 a	7.9		2.82 a	11.40 a	24.25 a
P2	**Fresh**	35 a	223 a	7.5	-12.0	3.34 b	11.68 a	26.33 a
	Stored	34 a	197 ab	6.8		3.12 b	12.99 b	27.42 a
P3	**Fresh**	33 a	259 c	9.2	-15.6	1.75 c	6.13 c	13.36 c
	Stored	33 a	219 ab	7.7		1.45 c	7.19 c	15.21 c
P4	**Fresh**	42 b	319 d	8.9	-30.6	0.48 d	10.44 d	22.36 a
	Stored	37 b	221 b	7.0		0.46 d	13.81 e	28.78 b

Data were statistically analyzed by ANOVA. Numbers marked with different letters are statistically different ($P<0.05$).

A small decrease of FA-C (Table 4.1) was registered in all stored samples. The decrement, however, is only apparent as an increase was actually observed, after storage, in all samples if we consider the percentage of C_{FA} with respect to the total organic C content of the sludge (Figure 4.1). At the same time, the percent content of HA-C with respect to C_{org} (Figure 4.1) increased significantly during the months that followed the end of the treatment in all stored sludges, except in sample from plant 1. This was caused by biological transformations and the amount of HA produced during storage was strongly related to the loss of organic C occurred during this period (Figure 4.2).

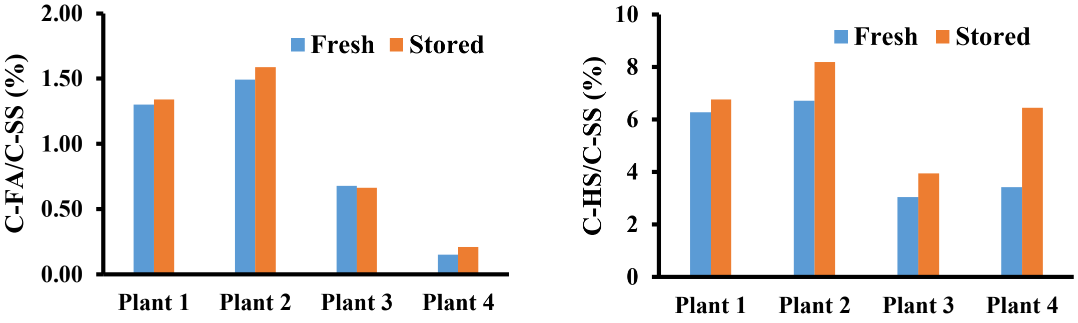

Figure 4. 1 Concentration of FA and HA as percentage of organic C in fresh and stored sewage sludge samples

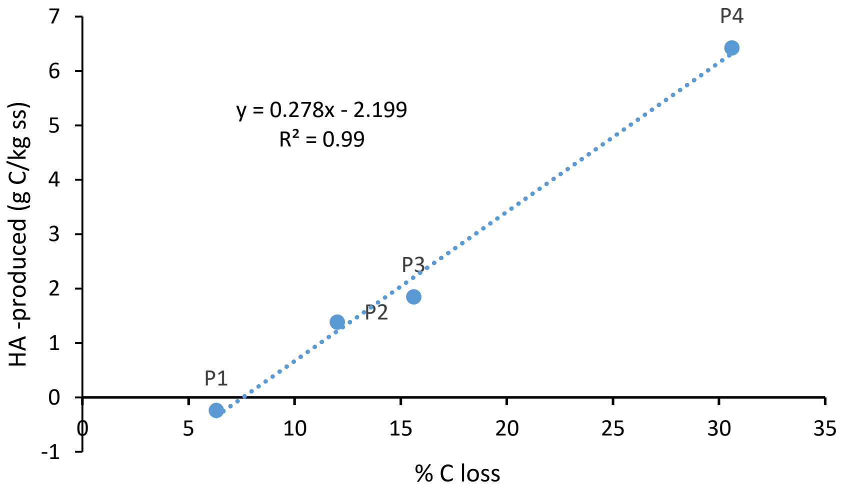

Figure 4. 2 Amount of HA-C produced during storage as a function of % organic C loss during storage

4.3.2 UV-vis parameters of HA and FA

UV-vis spectra of HA and FA extracted from the sludge samples before and after storage are shown in figure 4.3. In all spectra, except for fresh sludge FA from plant 1, which display a peak at about 210 nm, absorbance decrease monotonically with increasing wavelength.

Presence of a shoulder between 240 and 290 nm is related to aromatic or unsaturated compounds (tryptophan, conjugation of quinone and ketones) (Chin et al., 1994). Higher absorption intensity in this region by HA and FA from stored samples compared to fresh ones indicates increasing aromaticity. Again aromaticity increased in both FA and HA in all cases, except for HS from plant 1, as also shown by both $SUVA_{254}$ and calculated % aromaticity from slope absorbance data (Table 4.2). These changes that occur during storage could be due to the degradation of aliphatic structures like carbohydrates, polysaccharides or fatty acids which causes a relative increment of aromatic and more stable compounds in humic substances (Li et al., 2011; Awasthi et al., 2015).

$E_{465/665}$ ratios of HA and FA varied after storage and thickening in all samples except plant 1: apparently the more oxic (Plant 2) conditions observed during the thickening stage cause break down of humic molecules (corresponding to an increase in $E_{465/665}$ ratios), whereas anaerobic conditions during storage (Plants 3 and 4) result in an apparent increase in molecular sizes in both FA and HA.

Table 4. 2 E4/E6 ratios, $SUVA_{254}$ and % aromaticity of HA and FA extracted from fresh and stored sludge samples

Samples		**E4/E6**		**$SUVA_{254}$**		**Arom %**	
		HA	**FA**	**HA**	**FA**	**HA**	**FA**
P1	**Fresh**	7.20	5.26	1.94	2.05	13.0	13.6
	Stored	7.06	7.33	1.87	1.96	12.6	13.1
P2	**Fresh**	6.57	4.43	2.81	1.72	17.6	11.8
	Stored	7.48	7.09	4.03	2.64	24.0	16.7
P3	**Fresh**	6.62	9.81	1.98	2.58	13.3	16.4
	Stored	5.35	6.26	2.61	3.16	16.5	19.4
P4	**Fresh**	5.11	6.85	1.31	2.03	9.7	13.5
	Stored	4.51	5.51	2.23	2.63	14.5	16.7

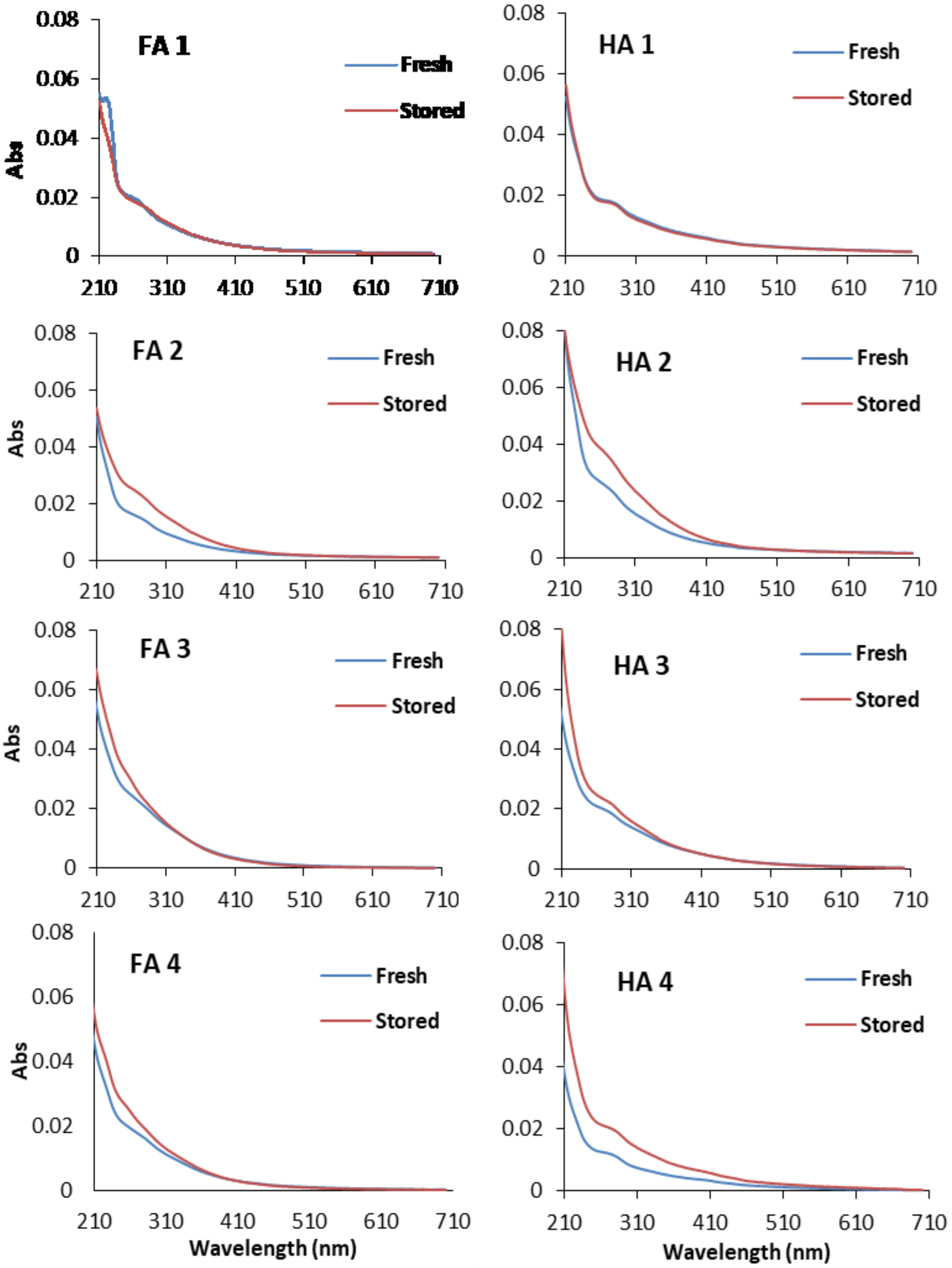

Figure 4. 3 UV-vis spectra of HA and FA extracted from four samples before and after storage

4.3.3 Fourier-transform Infrared Spectroscopy

ATR FTIR spectra of HA and FA extracted from sewage sludge samples before and after storage did not display any meaningful change and therefore are not reported.

Coherently with results from other techniques and parameters, small changes were observed in HA from all plants and were mostly limited to a decrease in intensity of absorbance in regions related to stretching and bending vibrations of carbohydrates and to a slight relative increase of carboxyls (1720 cm^{-1}).

4.3.4 Fluorescence spectra of HS

Fluorescence and even UV spectra appear to be much more sensitive ways to detect modifications of humic substance during thickening or storage.

Fluorescence is considered as a very sensitive tool to detect variations in the composition of dissolved organic substances in sewage sludge, however the displayed trends are complex and not always coherent with other data, particularly if we compare spectra from different sources. EEM spectra, normalized to the concentration of organic C, of FA and HA extracted from fresh and stored sewage sludge samples are displayed in Figure 4.4 and 4.5.

As shown in Figure 4.4, three peaks were detected in the normalized EEM spectra of FA extracted from freshly treated sludge from plant 1 before storage. Peak 1 is characterized by Ex/Em wavelength range of 260-280/330-350 that might be associated with soluble microbial byproducts (Chen et al., 2003) was already very little expressed in this sample after treatment compared to other fresh samples. As reported by Zbytniewski and Buszewski (2005), the level of non-humic substances is relatively high in sludge at the beginning of the treatment, but fluorescence emitted by these components is expected to progressively decrease with the stabilization degree achieved by the organic materials in the sludge. This indeed occurs in FA from plant 2 and 4, but is not even detectable in sample from plant 3, which already displays peaks typical of well humified FA in the fresh sample.

Peaks 2 and 3 are characterized by an Ex/Em wavelength range of 300-340/400-430 and 250-260/450-470, respectively, which are related to typical FA components. Comparing the EEM spectra of FA before and after storage, in all samples, storage caused increments in the intensity of peaks 2 and 3, with the only exception of fresh sludge of plant 1 where no significant changes were observed.

In the EEM spectra of HA (Figure 4.5), peak A was observe which is characterized, the same as peak 1 in EEM spectra of FA, by Ex/Em wavelength range of 260-280/330-350 that might associate with soluble microbial byproduct-like materials (Chen et al., 2003). As reported by Zbytniewski and Buszewski (2005), the level of non-humic substances is relatively high at the beginning of the process. Peak B was centered at Ex/Em wavelength range of 330-360/420-460 and could be defined as HA component. Considering HA 1 Stored, the F.I. of peak A and B presented very slight decreasing and increasing trends, respectively.

Much stronger changes were observed in the other three samples. In sample 2, the EEM spectrum of HA fresh differentiated significantly from that of stored. Before storage, the EEM spectrum of HA from plant 2 displayed a clear peak, peak A, which almost disappeared after storage and peak B with low intensity in HA 2 Fresh that became more intense in HA 2 Stored. Moreover, peak C as a new fluorescence peak appeared in HA 2 Stored, with an Ex/Em wavelength range of 250-275/450-480, which could be referred to FA substances (chen et al., 2003). These changes indicate that a good humification occurred during storage in this sludge sample. In HA 3 while no changes observed in peak A, the intensity of peak B and C increased almost twice.

Table 4. 3 Zsolnay humification index of HA and FA extracted from fresh and stored samples

	Plant 1		**Plant 2**		**Plant 3**		**Plant 4**	
	Fresh	**Stored**	**Fresh**	**Stored**	**Fresh**	**Stored**	**Fresh**	**Stored**
HA	1.77	1.46	1.09	10.98	3.54	4.43	1.07	1.35
FA	2.72	4.25	2.46	15.82	8.04	9.54	3.82	4.74

EEM features can be summarized and evaluated by calculating the Zsolnay humification index. HIX values increase as a consequence of shifting of the emission spectra toward longer wavelengths (red-shifting of fluorescence emission) that occurs with humification and aromaticity degree (Cox et al., 2000; Ohno 2002; Ohno and Bro, 2006). This trend is visible in FA, but may be masked in HA.

Changes in HIX values are reported in Table 4.3 and clearly show that HA and FA from plant 1 and plant 4 did not undergo strong modifications during storage, whereas these occurred in HA and FA from plant 2.

In HA from plant 4, Peak B which was barely detectable before storage doubled its intensity after anaerobic storage, still most of the EEM fluorescence spectra of stored HA from plant 4 display a very strong intensity of emission for peak A typical of poorly humified materials. Probably the increase of this peak is linked to the strong biological activity that occurred in this sludge during the 3 months of anaerobic storage, which is testified by the strong decrease in organic C registered and which may have caused a massive release of microbial byproducts.

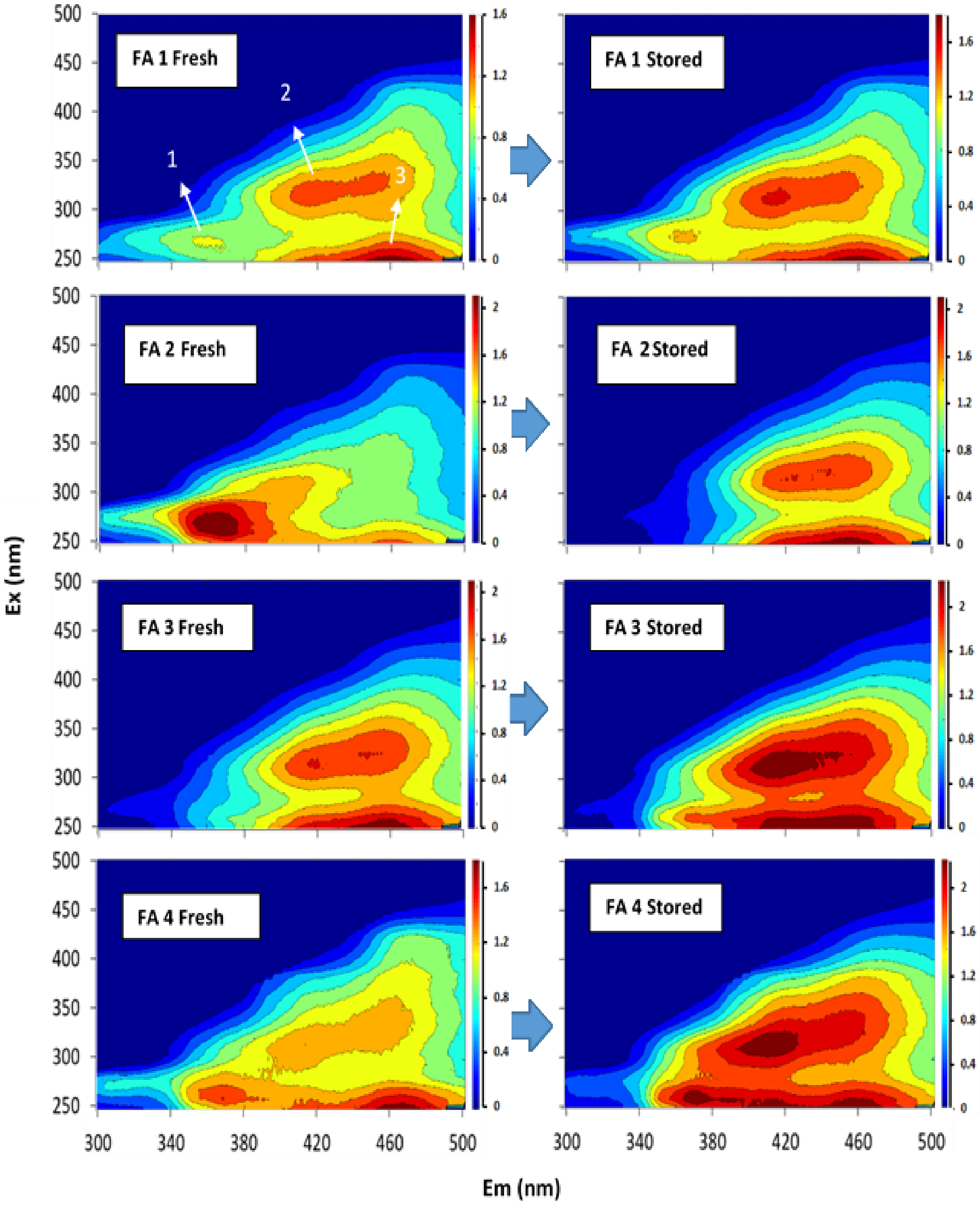

Figure 4. 4 EEM contour plots for FA extracted from fresh and stored sewage sludge samples collected from 4 different waste water treatment plants

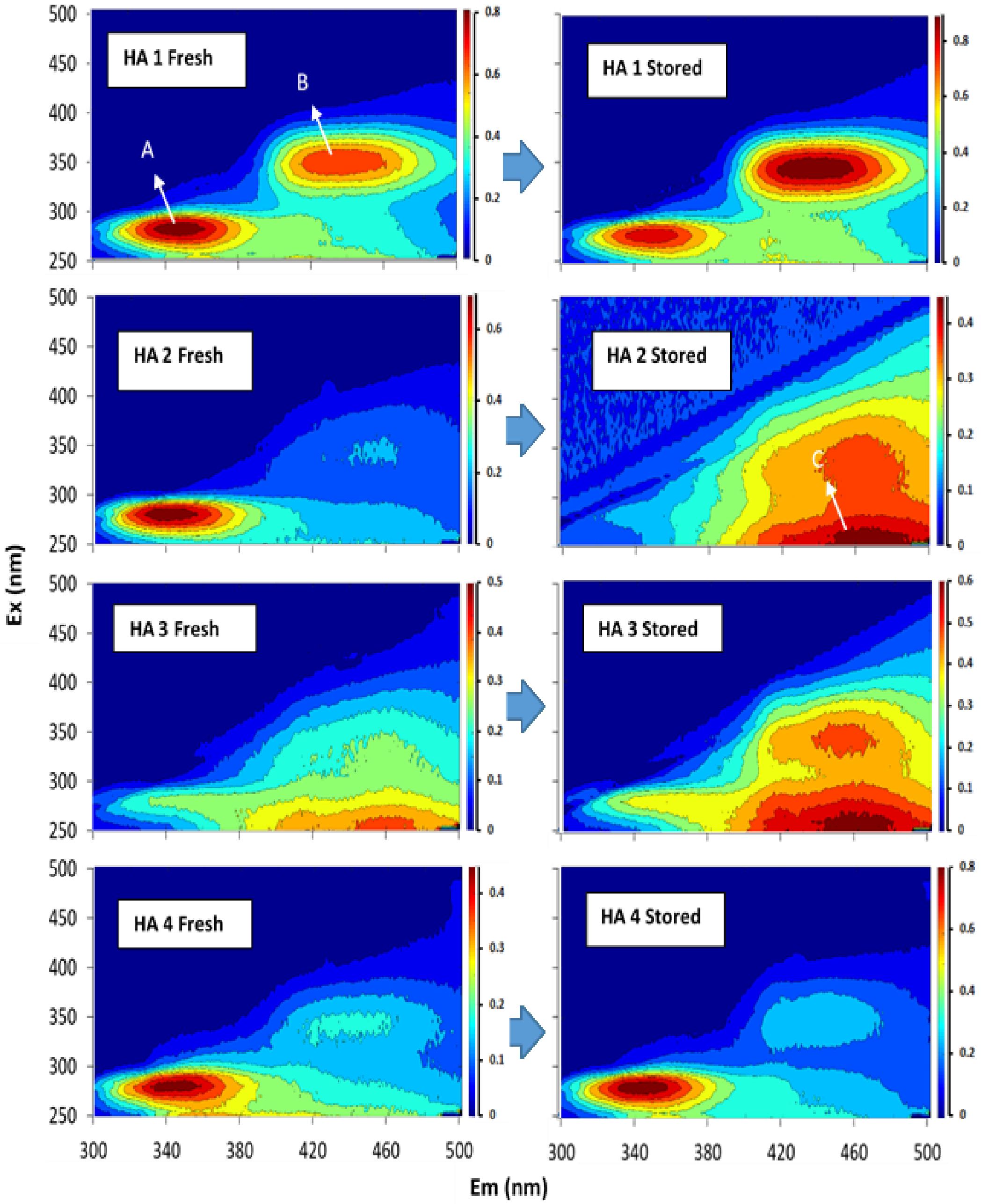

Figure 4. 5 EEM contour plots for HA extracted from fresh and stored sewage sludge samples collected from 4 different waste water treatment plants

4.3.5 EOX and LAS

The concentrations of EOX (mg/kg d.w.) in sewage sludge samples from the four different wastewater treatment plants before and after storage is shown in Figure 4.6. Results indicate that storage, in general, caused a decrease of the EOX concentration in sludge samples from all plants, showing that reductive biological activity went on during storage. The reduction of EOX concentration was 14, 81, 29 and 30% in plant 1, 2, 3 and 4, respectively.

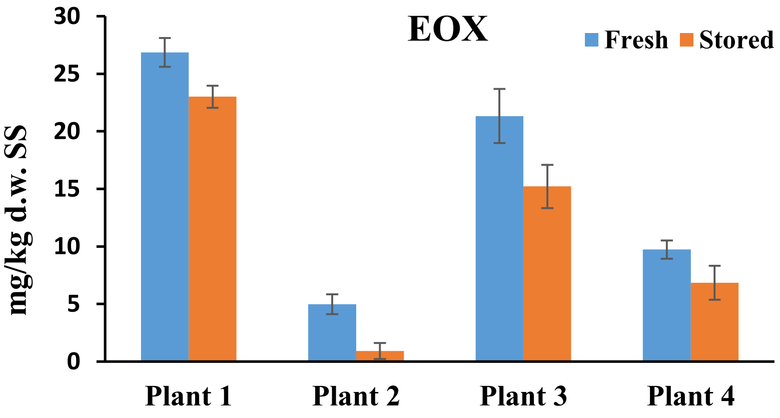

Figure 4. 6 The concentration of EOX (mg/kg d.w.) in sewage sludge samples (Fresh and Stored) from four different wastewater treatment plants

During storage, oxygen availability within the sludge is limited. Some oxygen may be taken in because of the progressive emptying of pores during drying. These conditions favor reductive dechlorination of chlorinated compounds which can be used as electron acceptors by anaerobic microorganisms.

For LAS, which are preferentially decomposed under aerobic conditions no significant changes were observed in plants 1 and 2, while, surprisingly LAS concentration decreased 30 and 20% in sludge samples from Plants 3 and 4, respectively (Figure 4.7) even if storage occurred under anaerobic conditions.

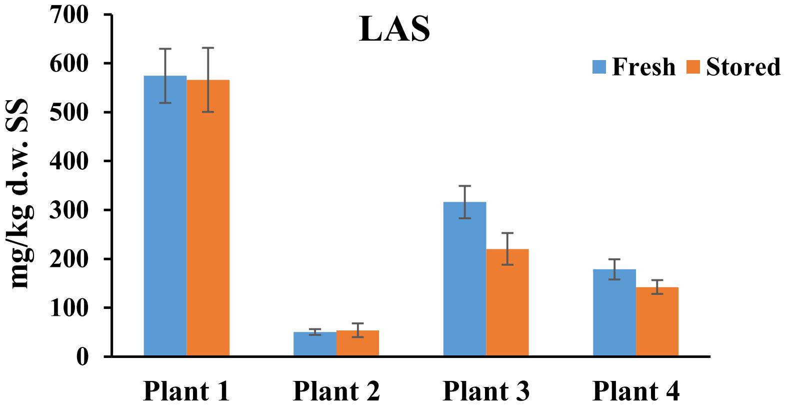

Figure 4. 7 The concentration of LAS (mg/kg d.w.) in sewage sludge samples (Fresh and Stored) from four different wastewater treatment plant

4.3.6 Relationship between humic substances in sludge and contamination by LAS

Both EOX and LAS are hydrophobic contaminants: they therefore have a tendency to become sorbed on to hydrophobic surfaces (Erhardt and Pruess, 2001; Villar et al, 2007). While EOX are sparingly soluble, LAS are on the contrary highly soluble because of the hydrophilic sulphonate groups in their structure (Erhardt and Pruess, 2001).

Contamination by EOX and LAS of sewage sludge may be therefore driven in one case by low solubility and sorption on hydrophobic parts in the structure of humic and fulvic molecules which constitute the hydrophobic acids fraction of dissolved and non-dissolved organic matter and in the other by trapping of these contaminants during flocculation by humic substances.

In the four samples examined a significative correlation was found between the percent content of HS in the dry matter of fresh and stored sludge and its contamination by LAS (Figure 4.8a). A similar, but less significative relationship of this kind was observed for EOX (Figure 4.8b).

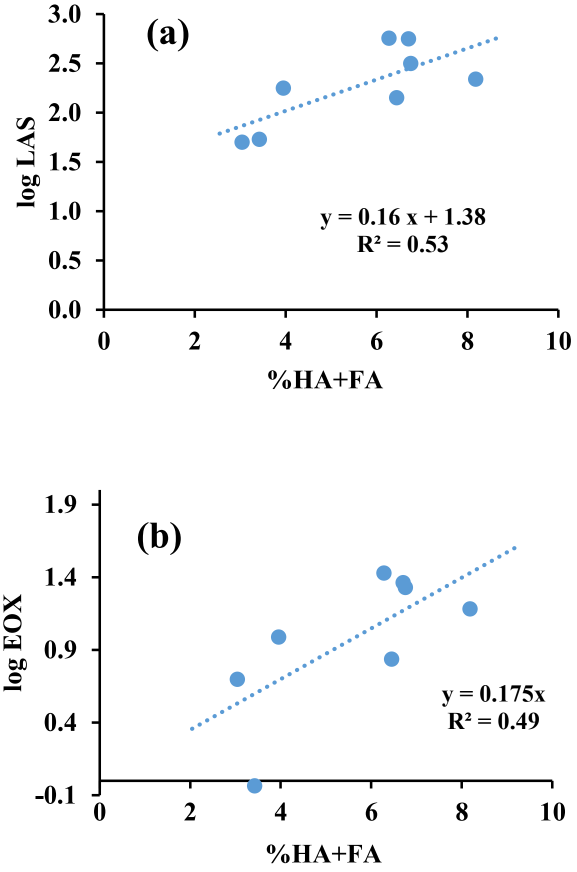

Figure 4. 8 Relationship between contamination of sludge by LAS (a) and EOX (b) and % content of HA+FA

To verify if this could be a generalizable behaviour, several sludges from other similar depuration plants were sampled and analyzed for their LAS, EOX and humic substances content (The comprehensive information about these samples: source of the waste water and treatment processes reported as Table 2.1 and the concentration of LAS and EOX is reported as Table 2.5 in chapter II of the present document). The trend observed for LAS was confirmed by a even more significant correlation with % of HA, whereas that for EOX was not confirmed.

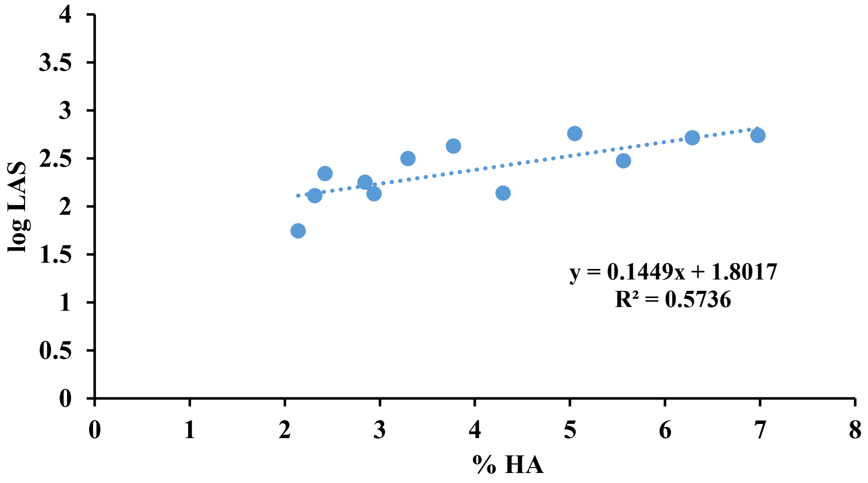

Figure 4. 9 Logarithm of the concentration of LAS in sewage as a function of the percentage of HA in the sludge

4.4 Conclusions

This work shows that several qualitative and quantitative changes can occur in the humic fraction of sewage sludge during the storage stage, both under aerobic and anaerobic conditions. In the absence of the possibility to monitor biological activity, the consequences of adopting different management practices, during thickening and storage of sludge can be inferred by examining modifications in the amount and structure of humic substances.

The objectives of this work were also to measure, quantify and eventually correlate these changes with the presence and behavior, during storage, of common organic contaminants such as EOX and LAS. This possibility was confirmed for LAS, whereas for EOX which, as a class may include compounds of much widely different nature, the trend was not confirmed.

This result has a practical bearing in that it suggests that amelioration of the efficiency of aerobic digestion treatment, might, by increasing the fraction of organic C transformed into humic acids, enhance sequestration of larger amounts of LAS in the sludge fraction.

General conclusion

Within the framework of the new concept of integrated water cycle, that encourages to implement a system with less pollutant loads to the environment, management of sewage sludge plays an important role. Due to sewage sludge fertilizing and soil conditioning properties, its agricultural use could be considered as an economically viable and environmentally sustainable disposal method compared to other disposal strategies.

In this PhD research the suitability of sewage sludge, from WWTPs in FVG, was evaluated based on permissible limits suggested by European regulations for its agricultural use (chapter II). Obtained results show that although the toxic metal content and the concentration of studied organic contaminants in sewage sludge were lower than maximum permitted limits in all tested samples, a general increase in maximum toxic metal concentration was observed, comparing the present data with the previous work on the same area, due to improvements in wastewater treatment lines of the WWTPs, which maximized the removal efficiency of contaminants from wastewater. Therefore we questioned the future possibility to use sewage sludge in agriculture and raised the need for further constant and regular sludge monitoring.

The novelty of my thesis was to introduce a new characterization perspectives for sewage sludge which I reported in chapter IV. In fact, in accordance with the need for constant monitoring of sewage sludge that was suggested in the second chapter, there should be some valid and cost effective methods to evaluate the suitability of sludge for agricultural use.

For this purpose, UV-vis, FTIR and Fluorescence spectra were used to characterize the humic substances extracted from sewage sludge in different stages of decomposition to assess their properties and behavior during storage. Also the correlation of qualitative and quantitative changes of humic substances with the degradation of common organic contaminants such as EOX and LAS during storage was studied. And this possibility was confirmed for LAS, whereas for EOX which, as a class of contaminants may include compounds of much widely different nature, the trend was not fully confirmed.

Since humic substances that used in chapter IV as tracers for transformation of organic matter in sewage sludge recently has been challenged by Lehmann and Kleber (2015), in chapter III, we tried to answer the question that "are humic substances artifacts of the extraction procedure or not". The obtained results were coherent with the classical humification theory.

In this work three samples at different stage of decomposition were extracted at different pH (from alkaline to neutral). Then fractions and whole extracts were quantified and characterized by UV-Vis, FT-IR, EEM fluorescence spectroscopy and 1H NMR. It was observed that even though the extraction yield increased by using alkaline extractants but spectroscopic properties were conserved for different extractants, demonstrating that humic substances are not artefacts of the extraction process.

To sum up, due to EU perspective of sewage sludge disposal and developing alternative strategies to reduce the application of conventional methods for environmental sustainability, this work was a contribution towards improving the safety of sewage sludge agricultural use through facilitating its monitoring and characterization.

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