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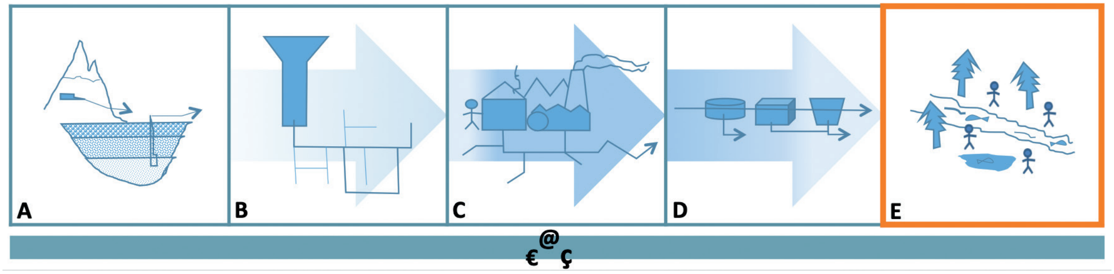
QUADERNI AUSIR E-2

new characterization perspectives for sludge

edited by
Ali Khakbaz



Printing
Impressum, Marina di Carrara (Ms)

Editrice Universitaria Udinese
FARE srl con unico socio
Società soggetta a direzione e coordinamento
dell'Università degli Studi di Udine
Via Palladio, 8 – 33100 Udine
Tel. 0432 26001 / Fax 0432 296756
www.forumeditrice.it

ISBN 978-88-3283-398-0

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FORUM

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FOREWORD

This 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. 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.

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 (TMs), 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 (HSs).

Furthermore, HSs (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 HSs 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, Fourier-transform infrared (FTIR) and fluorescence spectra to assess properties of HSs 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.

1. INTRODUCTION

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.

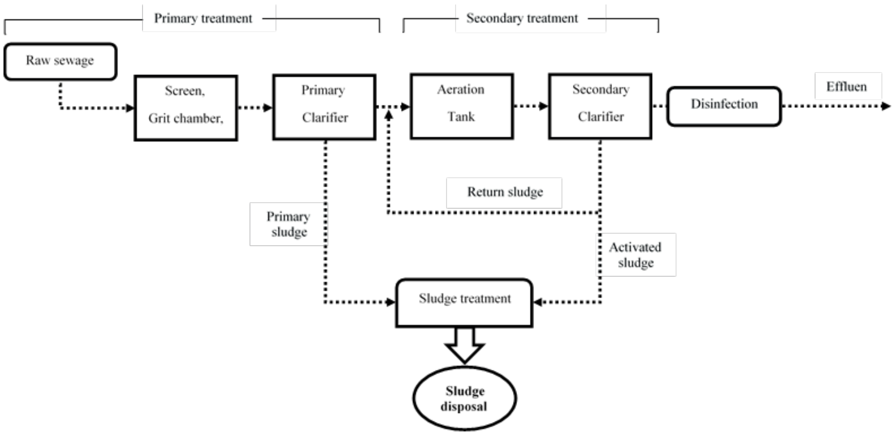
The quantity of sludge will continue to increase as a result of rapidly increasing population, urbanization and industrialization. About 9.5 million tons dry matter of SS are produced in the Europe in 2015 (Eurostat, SS production and disposal, last update 19.11.2018). In fact, upgrading of wastewater 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.

Fig. 1.1. *Primary and secondary treatment of wastewater using the activated sludge process.*



1.1. Composition of sewage sludge

Nutrients. One of the low cost disposal option of sewage sludge 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 sewage sludge is going to be essential for future sustainable development. Treated sewage sludge 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 sewage sludge 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).

Organic matter. Sewage sludge 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 sewage sludge 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 sewage sludge, 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 HSs 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 sewage sludge prior to its application to the agricultural soil (Hernanez-Apaolaza et al., 2000).

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% 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).

Toxic metals (TMs). 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 TMs in sludge are among the deciding factors for its use in agriculture. Continuous use of sewage sludge with high concentration of TMs 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 TMs such as zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), mercury (Hg), and chromium (Cr) in sewage sludge 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 sewage sludge, 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 sewage sludge, some pre-treatment procedures needed to be applied before disposal.

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, wastewater treatment plants (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 sewage sludge. 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 sewage sludge. 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).

1.2. 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.

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 TMs; 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).

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% dried sludge 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.

Tab. 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).

Land application. Due to population increase and development of 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 sewage sludge 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 sewage sludge 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).

1.3. 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, sewage sludge production and disposal, last update 06.08.2019).

Tab. 1.2. *Sewage sludge production and quantities recycled to agriculture in the EU (unit of measure: Thousand tonnes, Ths-t).*

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**

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 sewage sludge. 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 sewage sludge 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 sewage sludge 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 sewage sludge 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 sewage sludge 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).

Contaminants. The more diffuse contaminants of sewage sludge 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.

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 TMs (cadmium, copper, mercury, nickel, lead and zinc). The third draft of the *Working Document 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 TMs and organic compounds is presented.

Tab. 1.3. *Limit values for the concentration of TMs 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

Tab. 1.4. *Limit values for the concentrations of organic compounds in sewage sludge.*

Organic compound		**EU 2000 (third 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.

1.4. Humic substances

The chemical nature of HSs, 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. HSs present both hydrophobic and hydrophilic properties and can bind to mineral surfaces (MacCarthy, 2001).

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

Maintaining the physical and chemical properties of soil for healthy microbial activity and plant growth could be insured by HSs. 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 TMs 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 (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 (Boruvka and Drábek, 2004; Gusiatin et al., 2017).

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 sewage sludge 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 sewage sludge 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 sewage sludge compost maturity (Zucconi et al., 1981; El Fels et al., 2014).

2. MONITORING OF TMs, EOX AND LAS IN SEWAGE SLUDGE FOR AGRICULTURAL USE

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 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 TMs (Chen and Hu, 2019). A major cause of concern is the toxicity of these TMs at trace concentrations (order of magnitude of ng/L), in particular Cd, given its high bioavailability (Hu et al., 2017). TMs 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, TMs 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, TMs 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; 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 con-centrations 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, 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.

2.1. WWTPs and sample collection

Sewage sludge samples were collected from 10 different municipal WWTPs in Friuli Venezia Giulia region (Fig. 2.1); 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 (PE in the range of 850-9,000 P.E.) and treated mainly domestic wastewater, while two medium scale plants (identified by no. 1 and 2) were studied as well: plant no. 1 treated essentially municipal wastewater, while plant no. 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.

Fig. 2.1. *Sample collection sites in Friuli Venezia Giulia.*

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).

Tab. 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.

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

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.

2.3. 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 2.2); 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% dried sludge (DS) (0.27% dry weight (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).

2.4. 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 nitric acid HNO_3 (Merck solution IV) were added. The samples were digested in a microwave digester apparatus (CEM Mars Xpress, Matthews, NC, USA) at 180 °C for 10 min. After cooling, the resulting solution was filtered through a Polytetrafluoroethylene (PTFE) filter of 0.2 μm size, diluted to 20 mL in volumetric flasks by ultra-pure water and then stored at 4 °C until analyzed.

Tab. 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

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.

Tab. 2.3. *Some National and EU permissible limits of TMs 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

Table 2.3 shows the permissible limits for TMs, 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.

The concentration of TMs in the actual sludge samples, collected from various WWTPs is depicted in Table 2.4; and for all tested samples, TMs concentration were lower than maximum permitted limits (Tab. 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.

Generally, the concentration of TMs 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

Tab. 2.4. *TMs in the analyzed sludge (mg/kg d.w.).*

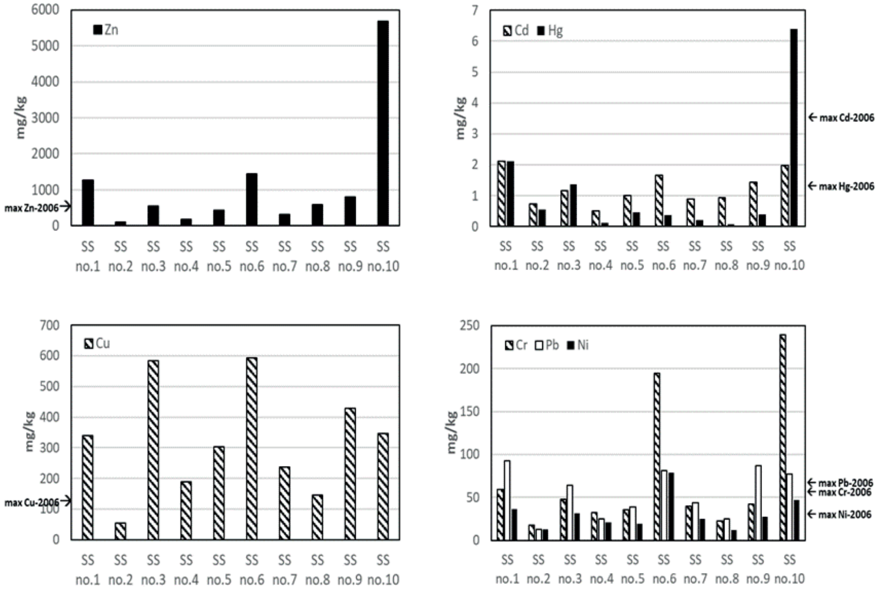
Sample	**Cd**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
SS no.1	2.12	60	340	2.12	37	92	1266
SS no.2	0.74	18	54	0.54	13	13	110
SS no.3	1.17	48	584	1.36	31	64	555
SS no.4	0.51	32	190	0.12	21	25	191
SS no.5	1.01	35	302	0.47	19	39	436
SS no.6	1.65	194	593	0.36	79	81	1446
SS no.7	0.88	39	238	0.20	25	43	309
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

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 (Fig. 2.2).

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.

Fig. 2.2. *Toxic metal concentration in sludge samples and maximum values monitored in 2006.*



2.5. 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.

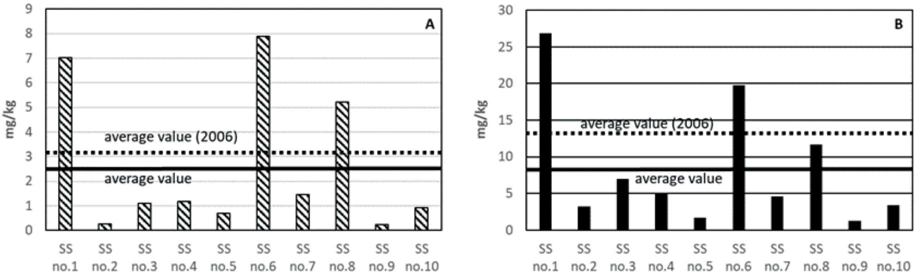
100 μL of residual extract were introduced into the instrument with an injection rate of 20 μL/min. At 950 °C and in an oxidizing atmosphere, combustion accompanied by pyrolysis of organochlorine compounds and

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.

EOX concentration in the analysed sewage sludge samples, obtained both by hexane and ethyl acetate extraction was presented in Figure 2.3 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.

Fig. 2.3. *EOX concentration measures (A: hexane extraction; B: ethyl acetate extraction) in sludge samples and average values comparison.*



Tab. 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

2.6. 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.

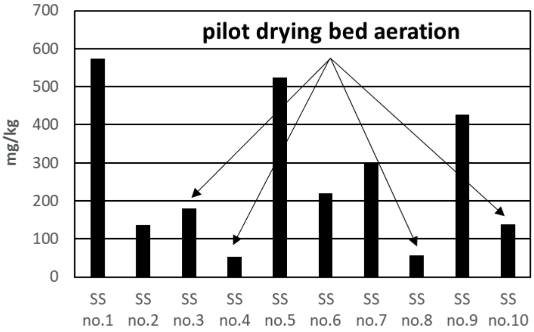
Microwave-assisted extraction (MAE) was performed on 0.5 g of dried sewage sludge samples. Methanol was used as a solvent because in literature it was the most common adopted solvent (Mortensen et al., 2001; García et al., 2005; Villar et al., 2007; Braguglia et al., 2014). 5 mL of solvent were added 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.

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 (Fig. 2.4). It is worth noting that LAS mean was significantly lower than the limit values proposed in the *Working Document on Sludge* (EU, 2000).

Fig. 2.4. *LAS concentration in sludge samples from analysed WWTPs.*



Indeed, quality of the investigated sludge (in terms of TMs, 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 TMs, 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.

Tab. 2.6. *Concentration of PCB, PCDD/F and PAH in studied sewage sludge samples and their limit values in sludge of Italy and as suggested in the third draft of the* Working Document 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 (third 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, benzo(a)pyrene, benzo(ghi)perylene, indeno(1,2,3- c,d)pyrene

[3] TEq: Toxicity equivalents

2.7. 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 third draft of the *Working Document 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 third draft of the *Working Document on Sludge* for EU.

2.8. 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 TMs and other persistent toxic compounds in sludge, restricting its reuse potential.

In this study TMs, 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. 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 (TMs, EOX, LAS, PCB, PCDD/F and PAH), with the risk of soil and groundwater contamination, as well as potential entrance into the food chain.

3. BIOLOGICAL TRANSFORMATION OF HSs AND CONTAMINANTS DURING THICKENING AND STORAGE OF TREATED SLUDGE

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 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 TMs 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 TMs 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 are nowadays a major reason of concern for the application of treated sewage sludge to agricultural soils.

LAS are the most used anionic surfactant, introduced in 1964 as the readily biodegradable replacement for 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 (Fig. 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; 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 HSs in order to evaluate the efficacy of the single stages of the treatment.

HSs, namely humic and fulvic 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 HSs as an indicator of the efficiency of transformation process in sewage sludge treatment and specifically of the storage period iii) application of UV-visible, Fourier-transform infrared (FTIR) and fluorescence spectroscopies as valid, simple and cost effective methods to pre-examine the quality of HS in sewage sludge.

3.1. Sewage sludge samples and treatments

Sewage sludge samples were obtained from four different small scale waste water treatment plants in Friuli Venezia Giulia, 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 no. 1 is first dewatered by a belt system and then stored under cover in a heap. The sludge from plant no. 2, after dewatering in a thickening bed, is stored in the open air on the ground. Samples from plants no. 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 Table 2.1) were used for following analyses as well.

3.2. HA and FA analysis

Extraction and purification of HSs. Sewage sludge samples of freshly treated (FT) sludge were dried at 45 °C 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 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.

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}) and the results are shown in Figure 3.1. 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.

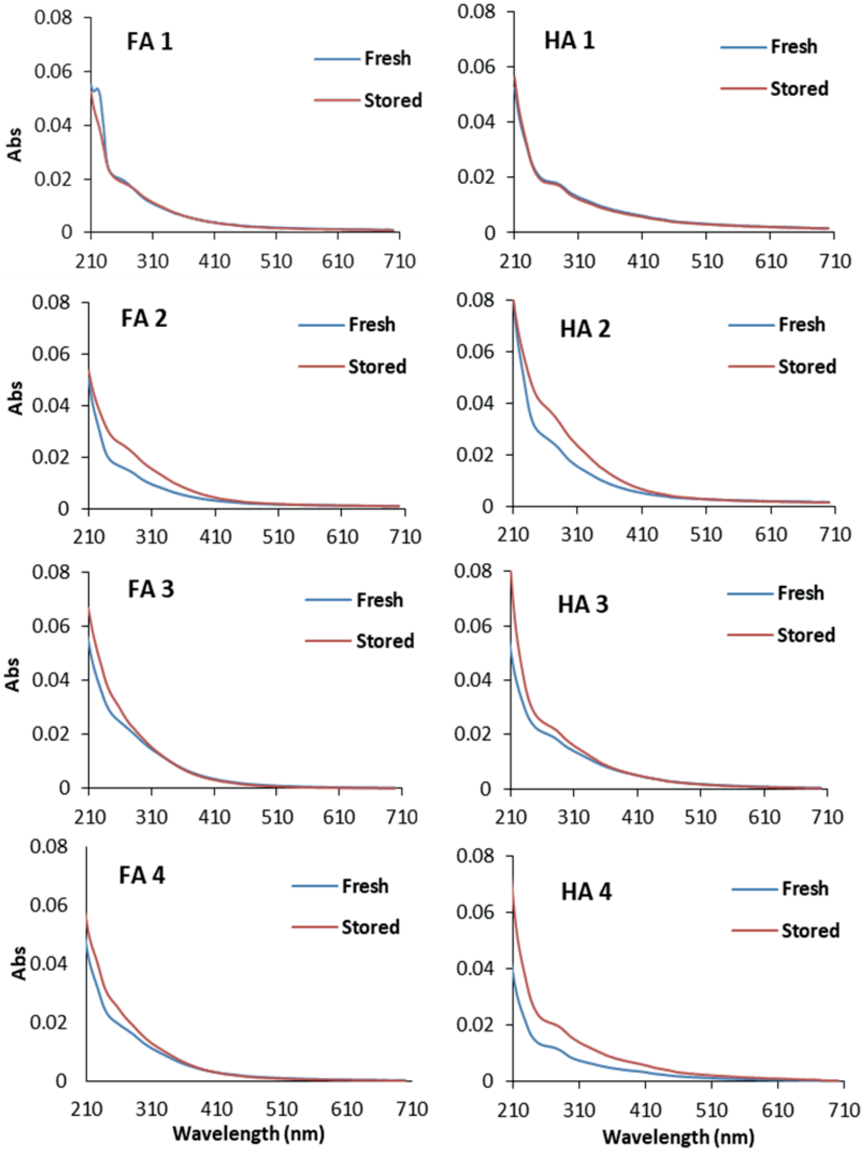
Presence of a shoulder between 240 and 290 nm is related to aromatic or unsaturated compounds (tryptophan, conjugation of quinone and ketones) (Chin et al., 1994). Higher absorption intensity in this region by HA and FA from stored samples compared to fresh ones indicates increasing aromaticity. Again aromaticity increased in both FA and HA in all cases, except for HS from plant 1, as also shown by both SUVA254 and calculated % aromaticity from slope absorbance data (Tab. 3.1). 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 HSs (Li et al., 2011; Awasthi et al., 2015).

E465/665 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 E465/665 ratios), whereas anaerobic conditions during storage (plants 3 and 4) result in an apparent increase in molecular sizes in both FA and HA.

Tab. 3.1. *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

Fig. 3.1. *UV-vis spectra of HA and FA extracted from four samples before and after storage.*



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). But 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).

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:

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

where I is the fluorescence intensity at each wavelength.

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 3.2 and 3.3.

As shown in Figure 3.2, 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 (Fig. 3.3), 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.

Fig. 3.2. *EEM contour plots for FA extracted from fresh and stored sewage sludge samples collected from 4 different waste water treatment plants.*

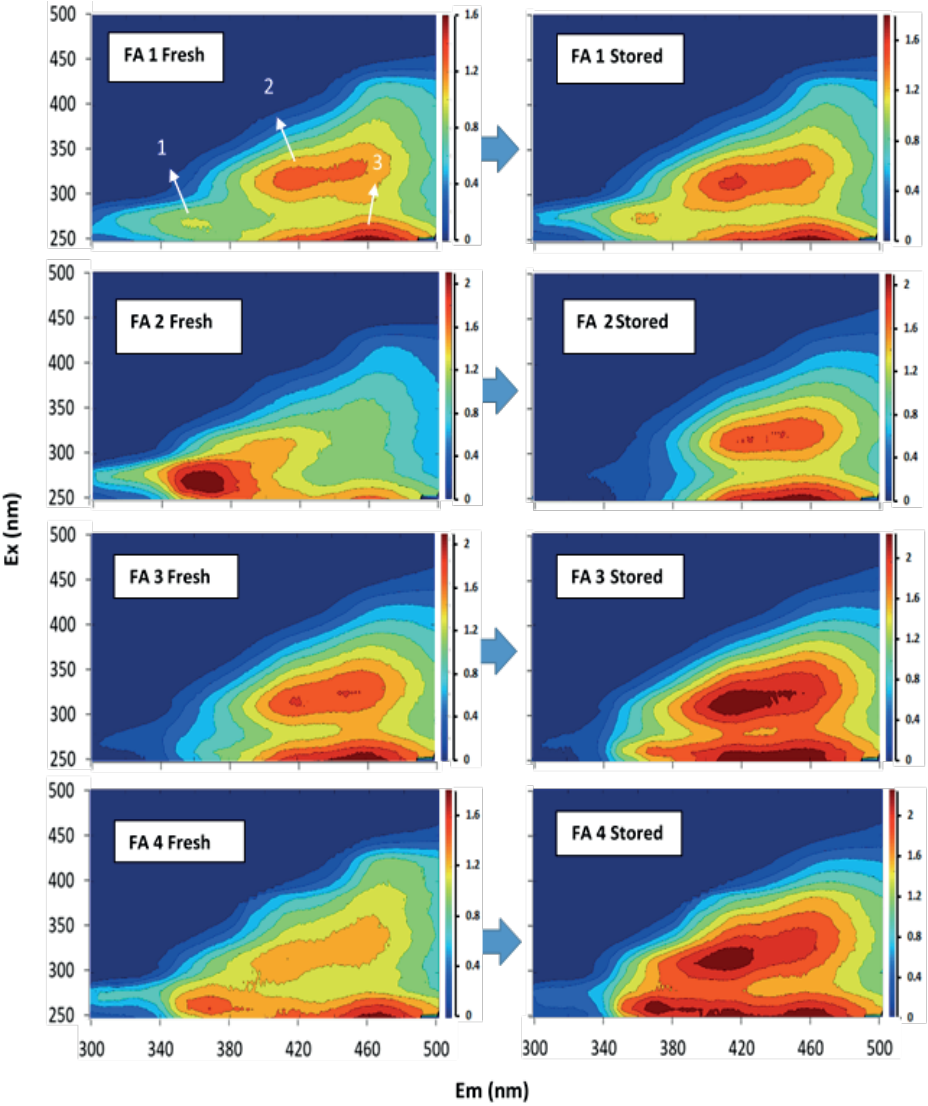
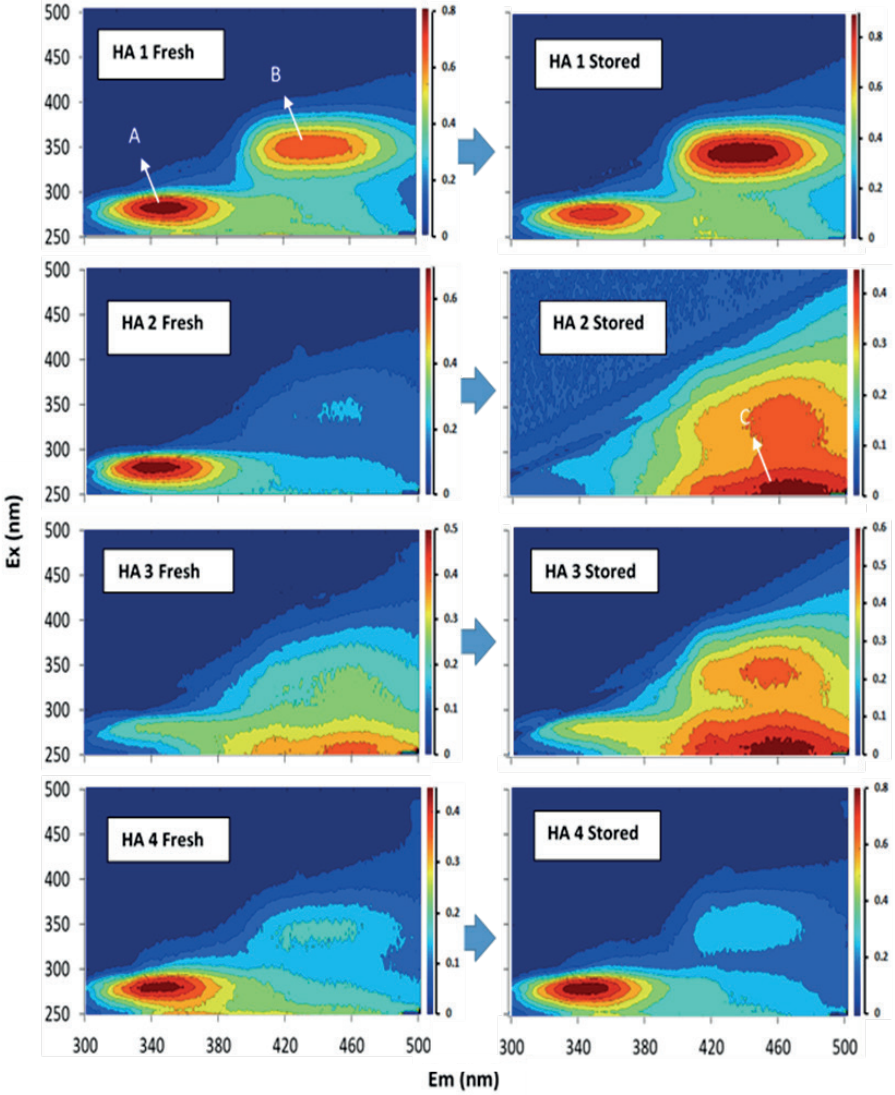


Fig. 3.3. *EEM contour plots for HA extracted from fresh and stored sewage sludge samples collected from 4 different waste water treatment plants.*



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 3.2 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 by-products.

Tab. 3.2. *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

3.3. EOX analysis

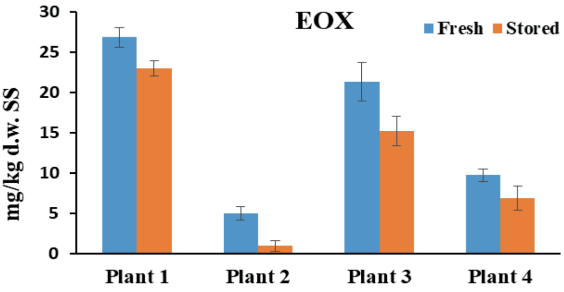
Extraction procedure. All sludge samples were freeze-dried, sieved manually through a 1 mm mesh sieve and ground in a ball-grinder. Subsequently, 1 g of freeze-dried samples were extracted with 5 ml of ethyl acetate or n-hexane solvent by shaking for 24 h. Most of the solvent was then stripped from the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

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.

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 3.4. 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.

Fig. 3.4. *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.

3.4. LAS analysis

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).

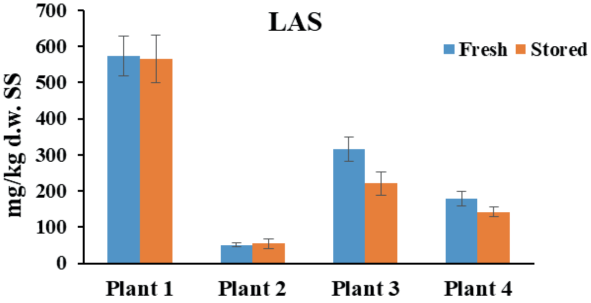
Microwave-Assisted Extraction method. Microwave-Assisted Extraction (MAE) was performed on 0.5 g of dried sewage sludge samples. Methanol was used as a solvent because in the literature it is the most common solvent used. 5 ml of solvent was added to the samples and the extraction vessel closed and introduced 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.

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 °C). DAD-UV (λ_{ex}=225nm) detector were used for the determination of LAS.

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 (Fig. 3.5) even if storage occurred under anaerobic conditions.

Fig. 3.5. *The concentration of LAS (mg/kg d.w.) in sewage sludge samples (fresh and stored) from four different wastewater treatment plant.*



3.5. 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 3.3. The four sludges displayed different degrees of loss of organic C (ΔC loss, Tab. 3.3) 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 no. 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.

Tab. 3.3. *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 (Tab. 3.3) 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. At the same time, the percent content of HA-C with respect to C_{org} increased significantly during the months that followed the end of the treatment in all stored sludges, except in sample from plant 1 (Fig. 3.6). 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 (Fig. 3.7).

Fig. 3.6. *Concentration of FA and HA as percentage of organic C in fresh and stored sewage sludge samples.*

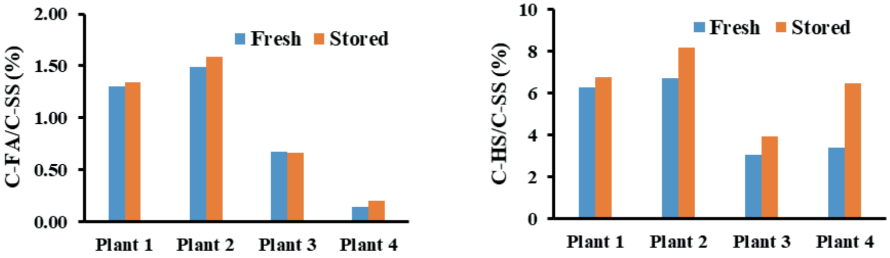
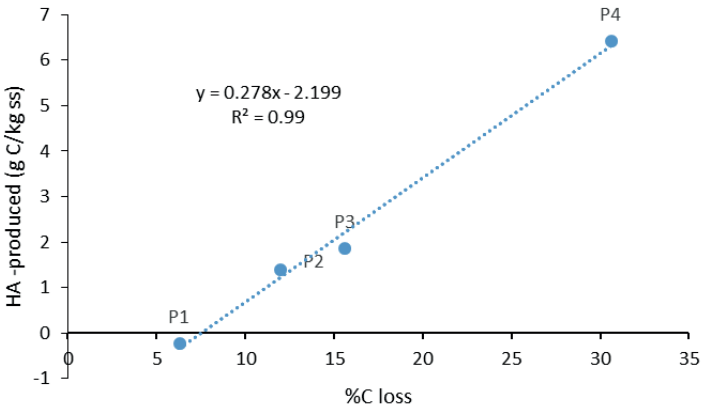


Fig. 3.7. *Amount of HA-C produced during storage as a function of % organic C loss during storage.*



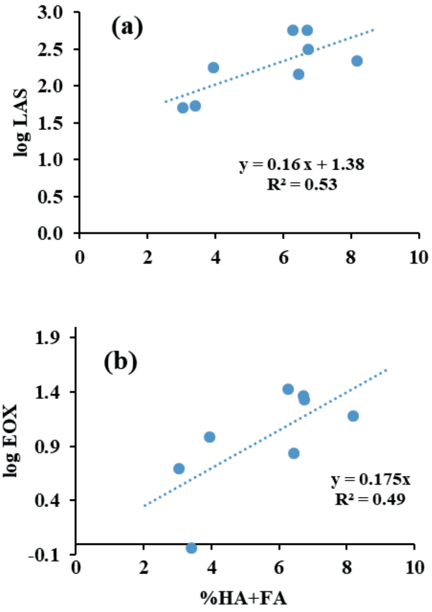
3.6. Relationship between HSs in sludge and contamination by LAS

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 HSs.

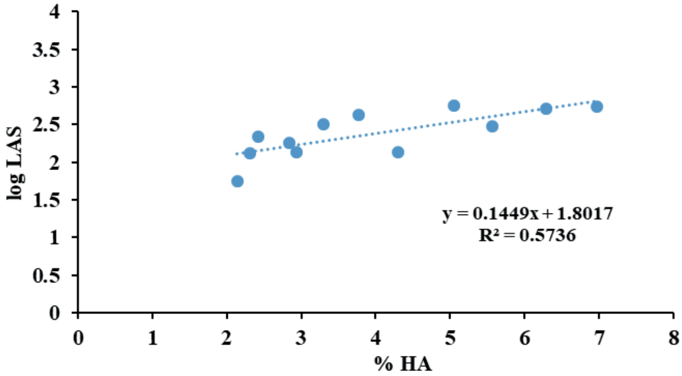
In the four samples examined a significative correlation was found between the percent content of HS in the dry matter of fresh and stored sludge and its contamination by LAS (Fig. 3.8a). A similar, but less significative relationship of this kind was observed for EOX (Fig. 3.8b).

Fig. 3.8. *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 HSs content. The trend observed for LAS was confirmed by a even more significant correlation with % of HA, whereas that for EOX was not confirmed (Fig. 3.9).

Fig. 3.9. *Logarithm of the concentration of LAS in sewage as a function of the percentage of HA in the sludge.*



3.7. 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 HSs.

The objectives of this work were also to measure, quantify and eventually correlate these changes with the presence and behaviour, 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.

4. 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 research the suitability of sewage sludge, from WWTPs in Friuli Venezia Giulia, was evaluated based on permissible limits suggested by European regulations for its agricultural use. 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 this work was to introduce a new characterization perspectives for sewage sludge which I reported in section 4. In fact, in accordance with the need for constant monitoring of sewage sludge, 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 HSs extracted from sewage sludge in different stages of decomposition to assess their properties and behaviour during storage. Also the correlation of qualitative and quantitative changes of HSs 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.

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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Quaderni AUSIR notebooks, a convergence idea by Massimo Canali, Marcello Del Ben, Daniele Goi and Lorenzo Tosolini, represent a form of dissemination of the work carried out in collaboration between the University of Udine and the Authority for Water and Waste Services of the Region of Friuli Venezia Giulia (Italy), on the areas of water and waste. They report innovation and research activities on: collection (A), treatment-distribution (B), use-discharge (C), treatment (D), recovery-return to the environment (E) of water resource sector. It also includes other topics such as: communication (ç), economic-legal (€) and management (@) focuses related to the complex world of water, waste and more. The manuscripts contain various levels of commitment: parts of the thesis by students from various degree programs, descriptive summaries of results obtained in research activities, research reports, studies in PhD programs, description of the outcomes achieved in researches published in national and international journals.

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ISBN 978-88-3283-398-0
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