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The hidden potential of digested sewage sludge: a recycling approach to combat desertification

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I. Summary

Wastewater management and desertification are two of the main problems of the contemporary world. The purpose of this thesis is to suggest and validate possible solutions to combat these problems. Although they may appear to have very little in common, they can in fact solve each other's issues. Indeed, one cause of desertification is soil chemical degradation which is produced by five phenomena, which include a lack of nutrients and the deficiency of organic matter. In order to purify water nowadays, the increase in wastewater in the world means that it is necessary to develop water treatments which are more and more efficient. The byproduct of these treatments is sewage sludge (SS). SS represents the central core of this thesis because its high presence of nutrients and organic matter means it (or compounds extracted from it), could be a possible solution for soil chemical degradation and, hence, for desertification. Anaerobic digestion is one of the most common SS stabilization strategies because it simultaneously allows SS stabilization, energy recovery, and biomass waste reduction. For these reasons, anaerobically digested sewage sludge (SSAD) was chosen to perform all the experiments for this thesis. Firstly, four SSADs, derived from the same wastewater treatment plant but differently treated (primary, secondary, centrifuged and dried SSADs), were chemically characterized. A significant amount was found of nitrogen, phosphorus, organic matter and many meso- and micro-nutrients which are essential for plant growth. On the other hand, contaminants such as heavy metals were also found. The first two experiments evaluated whether the direct application of these digestates could have beneficial effects on plants and soils and therefore be considered a possible solution to fight desertification. Hence, at the beginning of the experiment, a desert-like soil was selected: alkaline, low in nutrients and in organic matter. Then, this soil was treated in six different ways: with the four SSADs, a mineral fertilizer and a control sample with sand. Finally, evaluation was made on whether beneficial effects on plants and soil occurred, or, whether, due to the presence of contaminants, there were phytotoxicity effects. Both experiments were performed in a controlled environment: firstly, in a climatic chamber and, secondly, in a greenhouse. From the results analysis it emerged that beneficial effects were found in plants with the use of SSADs when compared to plants grown on non-treated soil. For example, the biomass produced with the application of dried SSAD was up to 37.5 folds higher than the control samples. Moreover, in some cases, there were even better results with SSADs than with the mineral fertilizer; this was caused by the higher quantity of phosphorus, organic matter and microelements in SSADs. Generally, the

dried and centrifuged SSADs gave better results than the liquid ones (primary and secondary) and the soil balance of nitrogen confirmed this result two months after the SSADs addition: in liquid SSADs, there was more loss of nitrogen, probably due to volatilization of $NH4^+$. Positive effects were also found in the soil treated with SSADs, with an increment of nutrients and organic matter after two months from the treatments. In order to complement this research, further investigation was made on phosphorus and organic matter: two specific compounds that gave SSADs better performances compared with mineral fertilizer.

Nowadays, phosphorus reserves are being depleted while demand for P fertilizer is increasing. Furthermore, the phosphorus used in fertilizers is mainly derived from non-renewable resources (such as phosphate rocks) with high environmental costs of extraction. However, SSADs can be considered as an alternative and renewable source of phosphorus. Using a modern phosphorus fractionation method (SMT method), it was found that the majority of P contained in centrifuged and dried SSADs was inorganic. Additional fractionation of inorganic phosphorus showed that a higher proportion of inorganic P was non-apatite inorganic phosphorus (NAIP) rather than apatite phosphorus (AP). According to many authors, high values of NAIP fraction correspond to high percentages of bioavailable-P. In fact, it is well known that the quantity of total phosphorus (P-Tot) has little or no relationship with the P availability for plant nutrition and only a small proportion of P-Tot is bioavailable. One of the most used methods for bioavailable-P quantification in soils is the Olsen method which consists of a spectrophotometrical quantification after the phosphorus extraction with $NaHCO_3$. Hence, an experiment was carried out over a three-month period in which P fractions and $NaHCO_3$ extractable-P in soils were measured at different times. Three treatments were applied to the sandy alkaline soil: control (no treated soil), centrifuged (soil + centrifuged SSAD) and dried (soil + dried SSAD). From the results it emerged that, after the SSADs addition, there was a significant increment of $NaHCO_3$ extractable-P. Furthermore, during the 90 days of the experiment, the P-Tot remained constant in all cases and, the bioavailable-P (P extracted by $NaHCO_3$) decreased in control and centrifuged-treated soils. This decrease could be due principally to the precipitation of phosphorus with calcium: in fact, with the soil alkaline pH and with a high quantity of Ca^{2+} ions in soil solution, the formation of sequential calcium phosphates, less soluble over time, could be the principal process. Furthermore, it was even possible that P adsorption had occurred in a lesser quantity. These explications are in line with other literature studies. To describe the behavior of bioavailable-P ($NaHCO_3$- extractable P) on soils, four kinetic models were tested. The Elovich model seems to best describe the adsorptive-precipitate process of bioavailable-P in control and centrifuged-treated soils. None of the models tested described the behavior of bioavailable-P in dried SSAD tested soil, probably because, differently from the other treatments, there was an increment in P-bioavailability on the 14th day from treatment addition. The same behavior was also found in other works and the possible reasons for this increment may include the anoxic environment, the microorganisms or the presence of organic matter.

In addition to the direct land application of SSADs, there might be another strategy for fighting desertification: the extraction of specific compounds that can help combat the desertification process. For this reason, promising compounds contained in organic matter were studied: the humic acids (HAs). These natural polymers are also defined as “the black gold of agriculture” due to their beneficial effects on soil quality and plant growth. Extraction, quantification, characterization and encapsulation of HAs recovered from SSAD were performed. The protocol adopted for the extraction made it possible not only to obtain a HA extract with a HA concentration (26.87%) double that of the starting material (SSAD: 12.53%) but also to get rid of heavy metals. Electron microscopy and infrared spectroscopy provided insights into the peculiarities of the HA extract, revealing features on isolated HA comparable to those reported in the literature for what concerns morphology and chemical moieties. After that, the extracted HAs were encapsulated in alginate beads in order to have a slow release of HA in a soil solution. In fact, alginate molecules can form a physical hydrogel, which creates an ionic cross-linking between monomers of the alginate and two adjacent polymer chains of HA. Afterwards, HA-alginate beads were tested in a greenhouse experiment with lettuce plants on poor sandy soil. An increment on the root apparatus of lettuce was revealed: +63% above the untreated control. In conclusion, the encouraging results of this last experiment suggest that HA extraction from SSAD is a promising strategy for the sustainable production of HAs. Indeed, a slow-release bio-stimulant containing HAs derived from waste was produced and positively tested, fulfilling the circular economy principles.

Future objectives could include the improvement of extraction yields of HAs, the implementation of the encapsulation technique and the investigation of other possible high-value compounds that can be extracted from SSADs. Finally, it is possible to affirm that both SSADs valorization strategies mentioned here are suitable for fighting desertification.

II. Acknowledgment

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VI. List of Abbreviations

ACA	Activated Carbon Adsorption
AD	Anaerobic Digestion
AOP	Advanced Oxidation Processes
AP	Apatite Phosphorus
A_N	Net Assimilation of CO_2
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
ATR	Attenuated Total Reflectance
BOD	Biological Oxygen Demand
C	Centrifuged anaerobic digestate from sewage sludge
C-85	Treatment with centrifuged anaerobic digestate from sewage sludge at 85 kg N/ha
C-170	Treatment with centrifuged anaerobic digestate from sewage sludge at 170 kg N/ha
C-255	Treatment with centrifuged anaerobic digestate from sewage sludge at 255 kg N/ha
CCI	Chlorophyll Content Index
CEC	Cation Exchange Capacity
COD	Chemical Oxygen Demand
C_i	Concentration of CO_2 in leaf substomatal cavity
CRF	Controlled Release Fertilizer
D	Dried anaerobic digestate from sewage sludge
D-85	Treatment with dried anaerobic digestate from sewage sludge at 85 kg N/ha
D-170	Treatment with dried anaerobic digestate from sewage sludge at 170 kg N/ha

D-255	Treatment with dried anaerobic digestate from sewage sludge at 255 kg N/ha
DNA	Deoxyribonucleic Acid
D.M.	Dry Matter
D.M.B.	Dry Matter Basis
DOC	Dissolved Organic Carbon compounds
EC	Electrical Conductivity
EDC	Endocrine Disrupting Compounds
EDX	Energy Dispersive X-ray
EmC	Emerging Contaminants
EPS	Extracellular Polymeric Substances
EU	European Union
EC50	Half maximal Effective Concentration
FA	Fulvic Acid
FAO	Food and Agriculture Organization of the United Nations
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transformed InfraRed
GGWSSI	The Great Green Wall of the Sahara and the Sahel Initiative
g_s	Stomatal Conductance
HA	Humic Acid
HM	Heavy Metal
HS	Humic Substances
HVC	High Value Compounds
IRGA	Infra-Red Gas Analyzer
M	Mineral fertilizer
M-85	Treatment with mineral fertilizer at 85 kg N/ha
M-170	Treatment with mineral fertilizer at 170 kg N/ha
M-255	Treatment with mineral fertilizer at 255 kg N/ha
MPN	Most Probable Number

NAIP	Non-Apatite Inorganic Phosphorus
OC	Organic Contaminants
OM	Organic Matter
P	Primary anaerobic digestate from sewage sludge
P-85	Treatment with primary anaerobic digestate from sewage sludge at 85 kg N/ha
P-170	Treatment with primary anaerobic digestate from sewage sludge at 170 kg N/ha
P-255	Treatment with primary anaerobic digestate from sewage sludge at 255 kg N/ha
P-inorg	Inorganic Phosphorus
P-org	Organic Phosphorus
PAH	Polycyclic Aromatic Hydrocarbons
PCA	Polychlorinated Alkane
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Dibenzodioxins
PCDF	Polychlorinated Dibenzofurans
PFC	Perfluorochemical
P-Tot	Total Phosphorus
QL	Quantification Limits
RDI	Root Development Index
RNA	Ribonucleic Acid
RO	Reverse Osmosis
S	Secondary anaerobic digestate from sewage sludge
S-85	Treatment with secondary anaerobic digestate from sewage sludge at 85 kg N/ha
S-170	Treatment with secondary anaerobic digestate from sewage sludge at 170 kg N/ha
S-255	Treatment with secondary anaerobic digestate from sewage sludge at 255 kg N/ha

SDG	Sustainable Development Goals
SE	Standard Error
SER	Society for Ecological Restoration
SMT	Standards Measurements and Testing (method of phosphorus quantification)
SOM	Soil Organic Matter
SS	Sewage Sludge
SSAD	Anaerobic digestate from sewage sludge
T	No treated, control thesis
Tukey HSD	Tukey's Honestly Significant Difference test - Post-hoc in ANOVA
U.M.	Unit of Measure
UN	United Nations
UNCCD	United Nations Convention to Combat Desertification
UNEP	United Nations Environment Programme
W.M.B.	Wet Matter Basis
XRD	X-Ray DiffractionWWTP Wastewater Treatment Plant

Chapter I

1. Introduction

1.1. Introduction to research themes

In this first part of the thesis, it will be firstly illustrated the phenomenon of desertification (what it is, why it happened, which are the consequences, etc.) and, secondly, it will be investigated the wastewater topic (what they are, why they are considered a problem, how they can be treated, etc.). In the following first paragraphs, these topics will appear far away from each other's, but in later sections, it will clarify how it will be possible to have a unique solution for both.

1.1.1. Desertification

The typical conception of the hot and sandy desert is just partially true. In fact, some deserts are hot such as Sahara, Arabian or Sonora but others are cold, as Gobi, Patagonian and Great Basin deserts. Moreover, just in the 20% of deserts there are sand dunes (Pye and Tsoar, 2008). Many definitions were assigned to the term desert based on mean values of precipitation, evaporation, temperature, geomorphic features and flora and fauna but, a universally accepted definition does not exist (Laity, 2009). A general explanation of desert defines it as a biome in which the potential for evapotranspiration (the combined process of evaporation and transpiration) is more than precipitations (Quinn, 2008). From a climatic point of view, deserts are lands with an extremely dried (hyper-arid) climate (**Figure 1**) (Middleton and Thomas, 1992). With this climatic approach and according to the United Nations Environment Programme (UNEP) classification, deserts occupy 16% of drylands (United Nations, 2010). Drylands are regions in which the production of crops, forage, wood and other ecosystem services are limited by water (Middleton and Thomas, 1992). Basing on the Aridity Index[1] values proposed by UNEP, drylands are subdivided in deserts, semideserts, grasslands and rangelands (Middleton and Thomas, 1992). All the drylands together occupied around 41.3% of Earth land areas where there lived a third of the global population (United Nations, 2010). Using this approach, the world desert area percentage results in about 6.6% of global emerged lands. Other authors increase estimated desert areas considering "true" deserts all the lands with a hyper-arid and

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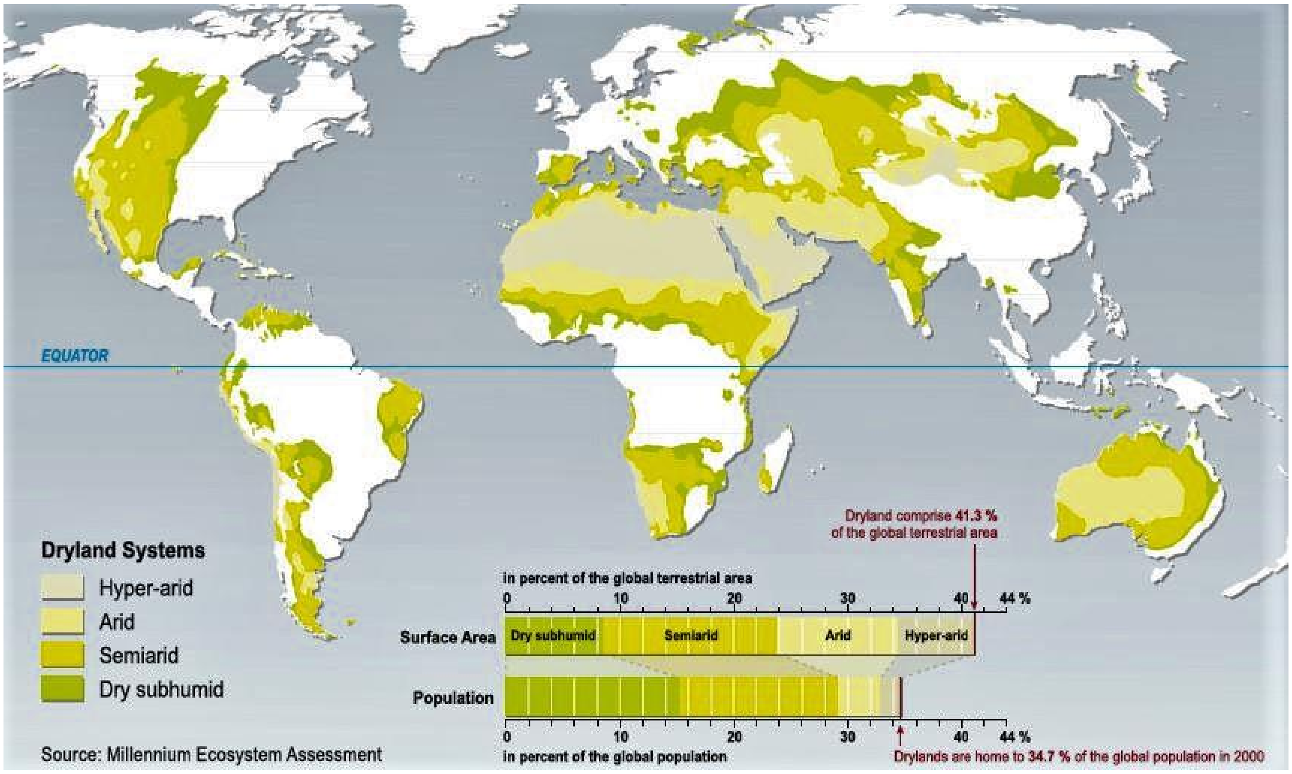
[1] Aridity Index is a numerical index that shows the grade of dryness of the climate of a definite area. Many AI exist (e.g. Thornthwaite and Budyko), but in this thesis it was chosen to refer to the Aridity Index proposed by UNEP (AIU). This index is defined as P/PET, where P are the mean annual precipitations of a specific area and PET is the potential evapotranspiration (ET) of the same area. ET corresponds to the sum of earth evaporation and plants transpiration.

arid climate (deserts and semi-deserts) while they classify the semiarid and drysub-humid areas (grassland and rangelands) as desert fringes (Laity, 2009). Studies revealed that comparing two 30-year periods, 1951-1980 and 1981-2010, drylands are globally increased by about 0.35% (Cherlet et al., 2018). Proportions in drylands subdivision are changing and, in fact, some deserts are expanding according to the process of desertization: “the seemingly irreversible extension of desert land forms and conditions to areas where they did not occur in a recent past” (Le Houérou, 2002). This phenomenon was confirmed by the demonstration of expansion of the Sahara desert (Thomas and Nigam, 2018) and Chinese deserts (Mitchell and Fullen, 1994). Many authors use as synonyms the terms desertization and desertification (Laity, 2009). If the first was previously clearly defined, the second has many definitions. The debate about the meaning of the term “desertification” is not only controversial between the scientific and academic community, but it can represent different approaches of governments and policies to combat the phenomenon (Batterbury et al., 2002). Le Houérou (Le Houérou, 2002) sustains that firsts appearances of the term were in 1927 by a French naturalist and forester called Lavaudance in relation to the destruction of the “Sahara forests” by nomads people and then, in 1949, by Aubreville, another French botanist and forester, who used it to describe the transformation of productive land into the desert as a result of human activity in Africa. From that time, many changes in the use of the term have occurred. Today, the most diffused and accepted definition is provide by United Nations Convention to Combat Desertification (UNCCD): “desertification is a land degradation that occurs in arid, semiarid and drysub-humid areas resulting from various factors including climatic variations and human activities” (Ma and Zhao, 1994). Hence, desertification is defined as land degradation which in turn is explains as the reduction or loss of the biological or economic productivity in drylands (Ma and Zhao, 1994).



Source: Millennium Ecosystem Assessment

Figure 1. Map of world drylands distribution. Deserts correspond to Hyper-arid regions. (Millennium Ecosystem Assessment, 2005)

Causes of desertification are many and it is possible to divide them in climatic and anthropic causes; to make a clear differentiation is difficult because many times they are very correlated to each other (Laity, 2009). For example, the Lake Chad basin has decreased by more than 90% over the last 40 years (Gao et al., 2011): in that area human activities amplified the water loss that was originally caused by drought. With the rainfall decreasing, cultivations needed more water and the level of the basin dramatically diminished causing desertification in all around areas.

The main general anthropic contributions are the increasing levels of population and the change in people's lifestyle that together generated a consequent increase in the use of resources and an unsustainable land use (Millennium Ecosystem Assessment, 2005). In fact, these induced a major request of lands provoking deforestation, overcultivation of the already cultivated areas, overgrazing by livestock, salinization of land by irrigation, urbanization, changes in hydrological systems, earth moving, etc., all causes of desertification (Laity, 2009).

As consequences of desertification, there are different reactions of the Earth depending on the area affected. Two evident phenomena are the reduction of cover vegetation and the increasing of aerosol and dust. These two consequences provoke modification in rainfall patterns, in the carbon cycle, in biodiversity and cloud formation. Furthermore, desertification induces an increase in wind and water erosion, in soil salinization besides causing a loss in topsoil. Moreover, all this environmental changings provoke a range of social problems: poverty, migrations and political instability are the three major effects on population (Millennium Ecosystem Assessment, 2005). In addition, considering that a third of the Mondial population lives in drylands, it is possible to affirm that desertification represents one of the bigger problems of our time. For these reasons, in 1994, it was created the UNCCD and, in 2015, all United Nations Member States adopted 17 Sustainable Development Goals (United Nations, 2020) with the objective of building a better world for people and our planet by 2030. The SDG number 15 is dedicated to reverse land degradation and to combat desertification. In particular, the objective of the target number 15.3 is: "by 2030, combat desertification, restore degraded land and soil, including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world". Furthermore, this agreement includes evaluation patterns to verify the compliance of the objective and inserts, as indicator of the phenomenon, the variation of the proportions of lands degraded over total land areas. Again, stand out the concept of land degradation, that from the UNCCD definition of 1994, has already change interpretation. Nowadays, in some cases, it does not only refer to drylands, but to all regions of the world. In fact, the last World Atlas of Desertification written by the European Commission sustain that: "land degradation leads to a long-term failure to balance demand for and supply of ecosystem goods and services" where goods and services include food, forage, fuel, building materials, potable water, control of agricultural pathologies, nutrient cycling, purification of air and water, the moderation of extreme weather, biodiversity and cultural and recreational benefits. (Cherlet et al., 2018). For this reason, it is possible to extent the concept of desertification also to all lands that are "simply" degraded, such as parts of northern Italy that are not drylands (Enne and Luise, 2006).

Furthermore, the 15.3 SDG introduces to the concept of restoration, one of the best practices for fighting desertification. The SER, Society for Ecological Restoration, defines restoration as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed". One of the main concepts of this discipline is that it is not necessary to rebuild the same ecosystem that was degraded, damaged, or destroyed, but it is "sufficient" to initiate or accelerate an ecosystem recovery that is capable of self-sustaining in future times. There are many ways to do restoration, but always it is previously required a combination of policies and technologies and the involvement of local communities: without these conditions a long-lasting restoration is not possible (Millennium Ecosystem Assessment, 2005). Restoration of soil functions, improving chemical, physical, pedological and microbiological characteristics of soils, is one of the best practices of restoration. Probably, the most evident practices of restoration are reforestation and afforestation. Reforestation is the establishment of a forest cover in a place where the forest has been destroyed in the recent past; differently afforestation means the establishment of a forest in a place where were not previously been or where forests have been missing for a long time. Many are the benefits of these two measures: less soil erosion by wind and precipitations, land stabilization (an examples is represented by the fixation of desert dunes), changing in microclimate (more humidity and lower temperature at ground level), more shadow on soil (other plants can take advantage from this situation to settle), increasing of the soil organic matter (from leaf falling and roots degradation), increasing of soil microorganisms, etc. Agroforestry is related to the forestry activity and it is another similar and already known possible solution to combat desertification (Miccolis et al., 2016). It is defined as "the sustainable management system for land that increases overall production, combines agricultural crops, tree crops, and forest plants and/or animals simultaneously or sequentially, and applies management practices that are compatible with the cultural patterns of the local population" (Bene et al., 1977). Other good practices for this purpose are the land terracing, the control of aliene species and, very important for this thesis, the chemical and organic nutrient replenishment in order to restock soil organic matter and organisms that promote higher plant establishment and growth (Millennium Ecosystem Assessment, 2005). Finally, it is important to underline that one of the best practices for combating desertification is its prevention. For this purpose, a good land and water management is required, avoiding overgrazing, overexploitation of crops and over-cementation of lands (Millennium Ecosystem Assessment, 2005).

Many are the concrete examples of fighting against desertification. A failing examples of this are some reforestation done in Sahel during the 1970s and 1980s in which many resources were spent: the failure was due mostly to the low involving of local people (Millennium Ecosystem Assessment, 2005). A totally different approach is the example of Yacouba Sawadogo, a burkinabé farmer (from Burkina Faso). He improved traditional farming techniques such as cordons pierreux and Zaï holes allowing a restoration of abandoned degraded desertic lands. In more than 20 years with, nearly no-money recourses and in opposition with local authorities, he created a forest of more than 20 hectares in those degraded drylands. In 2010, its interesting work was at the base of a documentary called "The man who stopped the desert". There are many other examples like this distributed all over the world. It does not exist an always valid "receipt" to combat desertification because every place has its own peculiar climate, soil, species, etc.

Taking into account all these considerations and experiences, we can move to a mastodonic multi-sectoral project against desertification began in 2007: "The Great Green Wall of the Sahara and the Sahel Initiative" (GGWSSI). In 1952 the English biologist and botanist Richard Barbe Baker had the idea of the creation of a "green barrier" made of plants in order to reduce the expansion of the Sahara Desert. From that "seed of idea", how previously shown, the concept of desertification and techniques and strategies to restore it changed many times, and, just in 2007 the African Union approved the GGWSSI. The purpose is to restore, by 2030, 100 million of hectares of currently degraded land in the Sahel region, one of the driest and poorest areas of the world. GGWSSI will create a transitional zone between the arid Sahara Desert and the humid savannas. 8000 km of plants across 12 countries, from Gambia to Djibouti passing from Senegal, Mali, Burkina Faso, Niger, Nigeria, Chad, Sudan, South Sudan, Eritrea and Ethiopia. In addition to the aforementioned countries, many other important partners are involved in the "construction of the wall" such as the UNCCD, the Food and Agriculture Organization of the United Nations (FAO), the European Union (EU), the World Bank and the World Agroforestry Centre (ICRAF). Thanks to the experience of all these partners, the mission is projected for applying an integrated approach between partners and local people using a wide knowledge in local climate, flora, soils and techniques. For example, in Burkina Faso 3 million hectares have already been restored using the previously mentioned technique of Zaï holes. Currently, the restoration is complete in 15% of the target area (Great Green Wall, 2020). Hence, one of the objectives of this thesis is to study the phenomena of desertification and land degradation and then, is to find some possible solutions. In particular, it will be proposed how to restore some degraded poor soils for the purpose of regenerate life where it has been lost.

1.1.2. Wastewater and sewage sludge

Although about 70.9% of Earth surface is covered by water, just 2.5% of total water is not saline and, hence, useful for drinking, industrial and agricultural purposes. Of this last 2.5%, 68.6% is trapped in ice caps and glaciers and 30.1% is groundwater: it means that only 1.3% of all freshwater (which was only 2.5% of all water) is the surface water (such as lakes and rivers) which serves most of life's needs (Shiklomanov, 1993). For its relative scarcity and the necessary human needing water is defined as "the blue gold" (Barlow and Clarke, 2002). Furthermore, considering the increasing of the human population, the expansion of industrial and agricultural activities and the aridity problems connected to climate change, the quantity and the quality of water are dramatically decreasing (Gleick et al., 2014). As a first consequence of these reductions, drinkable water is less available for human purposes. Nowadays, the World Health Organization estimated that 2.2 billion people are without safely managed drinking water services: it means that these people can't drink water from a water source that is located on-premises, or is not available when needed, or is not free from fecal or chemical contamination (World Healt Organization, 2019). Furthermore, by 2030 it has been estimated that 3.9 billion people will be water-scarce (Singh et al., 2020). For all these reasons, it is necessary to reduce the wastes of water resources, to rationalize the use of water and increase and improve water purification and wastewater treatment. It is precisely on this last theme that the research will focus in the following part.

Wastewater can be defined as ‘used water discharged from homes, businesses, industry, cities and agriculture’ (Asano et al., 2007). There are many types of wastewater depending on its previous water use. The three main categories are: urban wastewater, industrial wastewater and agricultural wastewater. If the wastewater is collected in a municipal piped system (sewerage) it is also called sewage (Bird and Adeel, 2015). In this thesis the attention will be focalized on urban (municipal) wastewater that usually includes the domestic blackwater (from toilets) and greywater (from kitchens and bathing), the water from commercial activities, institutions and hospitals, stormwater, the urban runoff and, finally, also industrial wastewater is occasionally included in this category (Mateo-Sagasta et al., 2015). Considering that an empirical study revealed that approximately 330 km^3 of municipal wastewater are produced worldwide yearly (Mateo-Sagasta et al., 2015) and due to the growth of population and industrialization, the wastewater produced all over the world is constantly increasing (Hong et al., 2009). Wastewater purification represents a “bulky” increasing problem.

Wastewaters can be reused for many purposes: agricultural irrigation, landscape irrigation (parks, golf course, cemeteries, greenbelts, etc.), industrial recycling and reuse (cooling water, boiler feed etc.), groundwater recharge and non-potable urban uses (fire protection, air conditioning, toilet flushing) (Intelligence, 2009). Its use can range from ultrapure recycled water for advanced industrial purposes to the untreated wastewater for agronomic purposes (Mateo-Sagasta et al., 2015). The direct reuse of wastewater (without treatments) is largely diffused although it is well known that wastewaters contain pathogenic microorganisms (bacteria, viruses, protozoa and helminths), nitrates and phosphates, hydrocarbons, heavy metals, endocrine disruptors, organic pollutants and pesticides (Akpor and OTohinoyi, 2014; Asano et al., 2007; Epstein, 2001). Technologies for disposal of urban wastewater received little attention until the negative effects of pollutants in aquifers, watercourses and seas were observed on human health and the environment, especially in western countries. By the 1970s, European countries and the United States started to prohibit the direct discharge of sewage into urban rivers and improved the sewer systems and wastewater treatment plants (WWTPs). As a result, water deterioration in many urban rivers decreased significantly while water quality gradually recovered (Xu et al., 2019). **Figure 2.** shows the level of wastewater treatment in the world. Very different from European landscape is the situation in developing countries: it is estimated that 90% of wastewater in developing countries is discharged untreated directly to water basins (UNEP, 2010). This last is one of the reasons why international attention has been focusing on water quantity and quality in the last years. In fact, 2005 – 2015 was the international decade for the United Nation action "Water for Life" and the SDG number 6 is dedicated to clean water and its sanitation. Target number 3 of SDG require that, globally, by 2030, will be improved water quality by reducing pollution, will be minimized the release of hazardous chemicals and materials in water, will be halved the proportion of untreated wastewater, will be increased the recycling and its safe reuse. Target 6a is also dedicated on this theme: it is expected by 2030 to expand international cooperation and capacity-building support to developing countries in water and sanitation-related activities and programmes, including water harvesting, desalination, water efficiency, wastewater treatment, recycling and reuse technologies. Therefore, the use of WWTPs for water purification appears as the best way for water reuse.

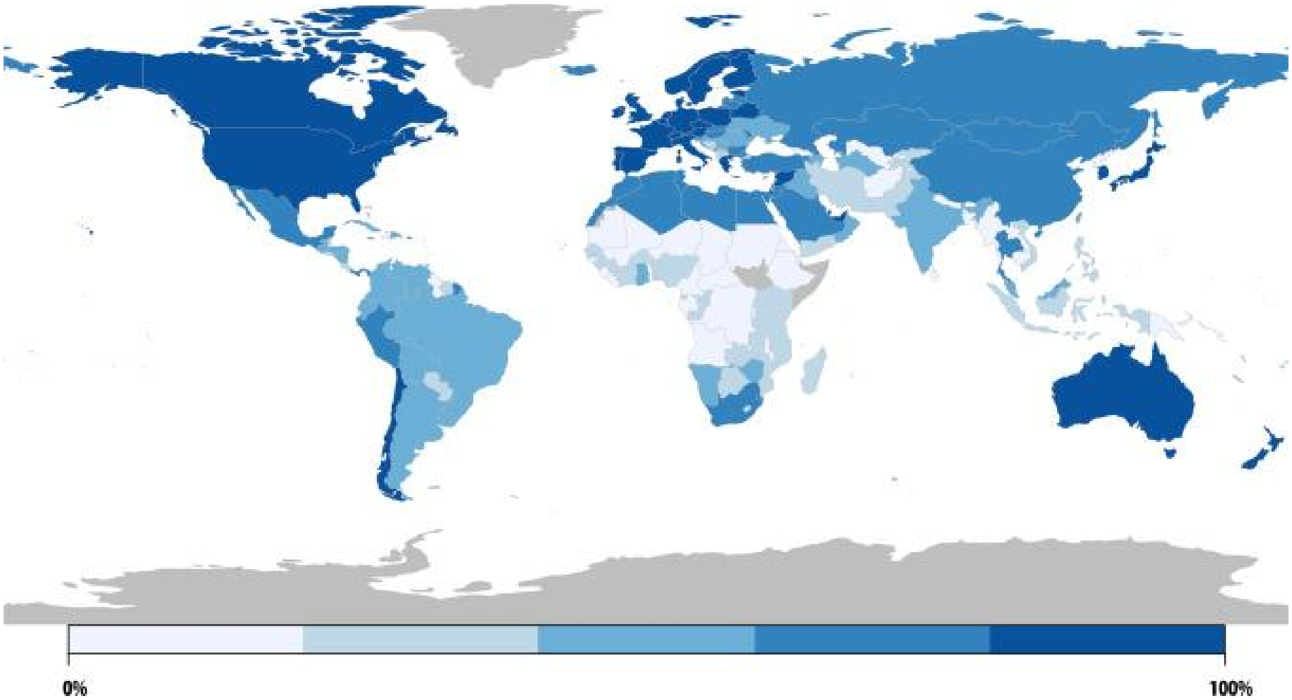


Figure 2. The world states level of wastewater treatment as the percentage of wastewater treated normalized by connection rate. Wastewater treatment was calculated as the amount of wastewater that is treated within a country relative to the amount of wastewater that is collected, generated, or produced. Connection rate refers to the population connected to municipal sewerage systems relative to the people living in that country. (Malik et al., 2015).

WWTPs can be subdivided in two main categories: centralized or decentralized. Centralized WWTPs are more common in Europe and receive wastewater from highly developed sewerage systems that may be far from the plant, and therefore common in densely populated areas. Usually, centralized WWTPs serve a relatively large portion of the population and exploit a synergic combination of techniques for water cleaning (such as physical and biological treatments). Otherwise, decentralized wastewater systems are more common in rural and remote areas with difficulties to connect to the sewerage network. Typically, they are smaller and less advanced under a technological point of view. WWTPs are engineered to remove bulky and suspended solid and to effectively removal contaminants in the effluent: after that treatments water is clean enough to be returned to natural water bodies. Specifically, the organic load reduced from the wastewater, is described by indicators such as chemical oxygen demand (COD) and biological oxygen demand (BOD). The intensity of the wastewater treatment and the design of the plant depends on the contaminant load of the wastewater and the desired quality of the effluent water (Masotti, 2012).

When urban wastewater reaches the WWTP, it first undergoes preliminary treatment. These operations remove solids, oil, soil and sand, to avoid damaging pipelines, pumps, the WWTP equipment or the process. Bulky solids are removed by racks, while smaller ones are retained with screens. Once solids are removed, larger particles can be grinded through different comminutors. Furthermore, grit chambers allow to remove sand and other inert solids by gravity, and oil and grease by flotation (Riffat, 2012a).

Primary treatment reduces the 50-70% of the organic solids suspended in the wastewater through sedimentation. Sedimentation tanks are used for flocculent sedimentation of solids and flotation of residual oils and greases. Solids are scraped off the bottom of the tanks and recovered as primary sewage sludge, which then goes to sludge treatment (Paragraph 1.3.3.). Sedimentation can be enhanced by adding chemicals that agglomerate particles, which also improves the flocculation performance. This primary treatment can reduce BOD from 25% to 40% (Riffat, 2012b).

The secondary treatment further reduces the organic solids (BOD) through biological processes. This rids a cocktail of organic molecules (e.g. carbohydrates, proteins, and fats), which are consumed by microorganisms. The microbial action results in an effective degradation of organic molecules. Technically, two types of treatment processes are used: suspended growth and attached growth. In suspended growth processes, the primary effluent is delivered to an aeration tank where organic matter is degraded by a biomass rich in microorganisms, so-called activated sludge. As the sludge volume increases, it is sent to a secondary clarifier where it is separated from the water by decantation. The sedimented sludge is then partially recirculated to the aeration tank to maintain the process (i.e. return activated sludge) and partially to treatment as secondary sludge (i.e. waste activated sludge) (Riffat, 2012c). For attached growth processes, trickling filters, biotowers, and rotating biological contactors are widely used. In these processes, microorganisms live in biofilms that are attached on apposite support to maximize the contact surface with the wastewater, e.g. rocks and gravel. In this case, sludge recirculation is not required, however less sludge is produced, and the BOD abatement may be less efficient. Whichever strategy is adopted, water can be further cleaned with advanced treatments before being returned to groundwater bodies (Riffat, 2012d).

Tertiary treatment (advanced treatment) is a process that remove residual polluting components, such as nutrients and remaining solids.

Nutrients, such as nitrogen (N) and phosphorous (P), are removed to prevent algae eutrophication of groundwater. Nitrogen is typically removed by a nitrification-denitrification process, a biological treatment that often is integrated with the secondary treatment. Nitrogen is mainly present in wastewater as ammonium (NH_4^+), which first is oxidized to nitrate (NO_3^-) by nitrifying bacteria, then converted by denitrifying bacteria into molecular nitrogen (N_2) and released as gas to the atmosphere.

Phosphorous can be removed through chemical and biological processes. In the chemical process, salts induce the phosphate precipitation as insoluble salts, for example from ferric chloride to ferric phosphate. Biological phosphorous removal is conducted in bioreactors with phosphorous accumulating organisms (PAO), which uptake and store P in their cellular structures. This bio-inspired approach allows a subsequent recovery of phosphorus, which can be cleverly recycled as fertilizer.

After, solids and non-degraded contaminants are removed. Solids are typically removed through filtration, which can be granular media filtration or membrane filtration, mainly microfiltration and ultrafiltration. Residual volatile organic compounds and specific ions are removed through carbon activated adsorption and ion exchange resins, respectively. Finally, depending on discharge limits, the effluent may be directly discharged to surface waters or, alternatively, water can be disinfected through UV or chlorine treatments (Riffat, 2012e).The main objective of all these treatments is water purification: if from one side the main result is clean water that can be re-used or discharged into water bodies, on the other side, abundant quantity of waste will be separated and collected. This type of waste, in WWTP of municipal wastewater, is called sewage sludge (SS). Considering the huge increasing quantity of municipal wastewater produced worldwide yearly (Hong et al., 2009; Mateo-Sagasta et al., 2015) and the UN intention of increasing wastewater treatments (SDG n.6), a high and growing volume of SS is globally produced and its quantity will increase. The SS production has started to rise thanks to policies dealing with the improvement of wastewater treatment and of standard quality of effluents, such as the E.U. directive 91/271/EEC (Council of the European Communities, 1991). For example, an increase of 50% of annual SS production has been estimated in EU-15 countries (old member states of European Union) between 1992 and 2005, passing from 6.5 to 9.8 million tons of dry solids (Kelessidis and Stasinakis, 2012). In Italy, SS production is estimated in about 1103 thousend tonnes/year (Eurostat, 2021). Treatment and disposal of SS represented the bottleneck of WWTP: it is estimated that they affect between 50 and 60% of WWTP operational costs (Guangyin and Youcai, 2016). For this reason, in the last decades efforts of researchers and engineers have been focused on strategies of SS reduction and reuse. Nowadays SS is considered a waste, but due to its composition it can represent a resource in order to combat desertification. Paragraph 1.3 will be exclusively and exhaustively dedicated on SS, from its origin to its disposal.

1.2. Degradation of soils

1.2.1. Soils characteristics

Desertification, defined as a kind of land degradation, was the main subject of paragraph 1.1.1. The focus of this paragraph is dedicated to one of the factors that cause desertification: soil degradation. In this section of the thesis, the objective is to know the phenomena with particular attention to one of the main causes of soil degradation: soil chemical degradation.

Firstly, it is necessary to have a definition of soil, but it changes depending on the field of study of the author. European Commission defined it as the top layer of the earth's crust (European Commission, 2020). A more complex definition could be that soil is the result of the interactions between the atmosphere (as regulated by climate), the biosphere (local vegetation and animal activities, including those of humans) and the geosphere (the rocks and sediments that form the upper few meters of the Earth's solid crust) (Orgiazzi et al., 2016). These geological, chemical and biological interactions which have acted upon the parent material over the lifetime of the soil determined the formation of different horizons, overlapping levels distinguishable from each other with the naked eye according to their physical and chemical characteristics. The soil pedological profile is the succession of these horizons. Proceeding from the surface to the bottom, the horizons are marked by the letters O, A, E, B, C and R. Not all horizons are present in every type of soils. For example, young soils, such as those on the river sediments, sand dunes, volcanic ash or soils degraded by wind or water erosion, may have indistinct or even no horizon formation.

O is the most superficial layer, characterized by its limited thickness and the presence of non-decomposed or only partially decomposed organic matter usually in form of humus materials. A is the richest layer of organic matter, in which the decomposition activity is particularly strong. It is within the horizon where most of biological and chemical activities occur (e.g. biomass growth, dead litter and root decay and release of nutrients, formation of organic acids and their reactions with minerals, etc.). E is the eluvial horizon, since in it is intense the removal of soluble, inorganic and organic components by the action of water that infiltrates the soil and transports them into the deepest layers. B is also called illuvial horizon, since it is the one in which the materials removed from the water from the upper horizon are concentrated. C is made mostly of the rock altered. R: is the layer of hard bedrock under the soil.

The average composition in volume of a soil sample is made of minerals (45%), water (25%), air (25%) and organic matter (5%) (Orgiazzi et al., 2016). Soils, therefore, must be viewed in a holistic way: they represent the interconnection between lithosphere, hydrosphere, atmosphere and biosphere (Wali et al., 2010). Different percentages in mineral particles size (sand, silt and clay) give to the soil its texture.

The process of soil formation (called pedogenesis) is regulated by the effects of interaction between place, environment, and history. This process causes specifics soil texture, structure, color and chemistry. These features occur in patterns of soil type distribution, forming in response to differences in soil forming factors. In 1941 the American soil scientist Hans Jenny proposed the following mathematical relationship that states that the observed properties of soil are the result of the interaction of variables, which allows the soil formation:

$S = f(cl, ø, r, p, t, \dots)$

The soil formation (S) is influenced by: climate (c), organisms (ø), topography – relief (r), parent material (p) and time (t). Furthermore, dot factors were added in order to better describe soil formation with others occasional state variables such as fires sweep the ecosystem and dust storms (Jenny, 1980). This equation is still used for understanding and describing soil pedogenesis.

1.2.2. Importance of soil

An old Chinese proverb affirms: “Man, despite his artistic pretensions, his sophistication, and his many accomplishments, owes his existence to a six-inch layer of topsoil and the fact that it rains” (Wali et al., 2010). Soil importance is ignored by most people despite it makes up the outermost layer of the planet and carries out a multitude of tasks that are essential to human wellbeing. Firstly, soil is the medium that provides the development of most of the human and animal food sources, in which the majority of Earth life lives. It represents a very high source of biodiversity with the possibility of containing more than 10000 species/m^2. According to these estimations, millions of individual cells and thousands of species of bacteria may live in just 1 g of soil (Orgiazzi et al., 2016). Furthermore, up to 5 tons of animal life can live in one hectare of soil (European Commision, 2005). Secondly, soil represents a natural filter where potential pollutants are neutralized and broken down by chemical, physical and biological processes. Thirdly, soil can absorb the excesses of water and it works as a repository of water for dry periods: a fully functioning soil can store as much as 3750 tons of water per hectare (European Commision, 2005). Then, soils represent an important stock for C: soils contain about 1500 Gt of organic carbon (Scharlemann et al., 2014), about 1.8 more than the atmosphere and between 2.3 and 3.3 folds more than C stocked in vegetation (IPCC, 2013). Lastly, soils protect archeological heritage from damages and depletion and represent the foundation upon which artificial structures are built (Wali et al., 2010).

1.2.3. Soil degradation

The soil formation rate varied from 100 years for 2 - 5 cm on volcanic ash in warm humid climates to 1 cm in 5000 years on hard rocks in cool temperate climates (Orgiazzi et al., 2016). Due to the very low regeneration rate, soil is considered a non-renewable resource. Most ancient civilizations, such as the Harappan in western India and Mesopotamic in western Asia, flourished on fertile soils but after the soil degradation they have disappeared (Olson, 1981). A study of the United Nations Environment Program (UNEP) estimates that from time of agricultural arrival to the end of the 80’s, 2 billion hectares of soils were degraded (Oldeman, 1994). Soil degradation is a processes in which there is a decline in soil quality which leads to the loss of the functions previously mentioned (Lal, 1990). Causes are complex and, although non-uniformly distributed, their scale can be continental or local and they are frequently inter-linked. When many causes occur at the mean time, their effects are added and, hence, the problem increases (Orgiazzi et al., 2016). In the beginning of the 90’s the UNEP's project 'Global Assessment of Soil Degradation (GLASOD), subdivided soil degradation in 3 main categories: erosion, physical degradation and chemical degradation (Oldeman, 1994). A recent map of world soil degradation is provided in **Figure 3**.

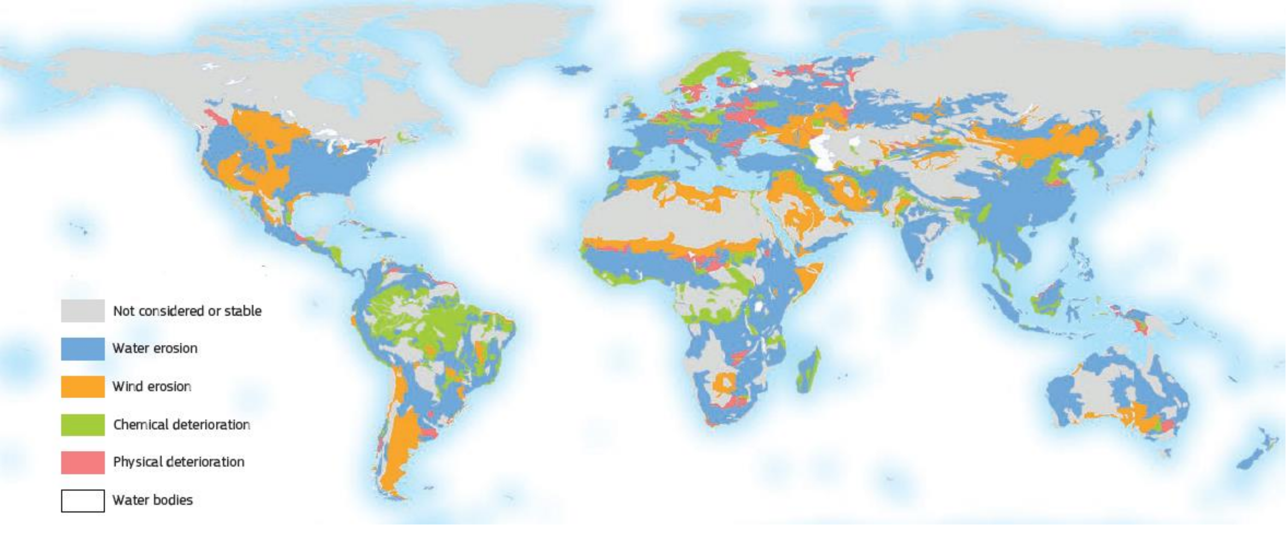


Figure 3. World map of soil degradation. Type, extent and main causes of soil degradation were assessed and mapped within loosely defined physiographic areas. (Orgiazzi et al., 2016, modified).

Within the category of erosion, two different types are identified: water and wind erosion. Erosion can provoke the loss of topsoil (the more fertile part of soil), terrain deformations (such as rills and gullies in water erosion and hollows and dunes in wind erosion) and overblowing (it is the coverage of the land surface by wind-carried particles).

Physical degradation is due by many phenomena. Firstly, very diffused in urban areas, there is the soil sealing that is the loss of soil resources due to the covering of land for housing, roads or other construction work (European Commision, 2005). Secondly, the phenomena of soil compaction and crusting caused the densification of soil by the elimination of pores and consequently caused an acceleration of runoff and erosion. Finally, waterlogging can be caused by inundations by river water and submersions by rainwater.

Chemical degradation, one of the central cores of the thesis, does not refer to the progressive changes in chemical composition due to the soil pedogenesis neither to the cyclic fluctuations of soil chemical conditions of agricultural systems. Soil chemical degradation can be caused and distinguished by five main processes: salinization, acidification, pollution, loss of nutrients and loss of organic matter.

Salinization is the modification of the salinity of the soil. It can be caused by improper management of irrigation schemes or when excessive use of groundwater in coastal areas caused an input of seawater.

Acidification can appear in coastal regions after the drainage of soil containing pyrite. Alternatively, it can be caused by the overapplication of acidifying fertilizer.

Soil pollution is caused by the cumulation of organic and inorganic contaminants. It can be caused by industrial or waste accumulation, the use of herbicides or pesticides, chemicals and oils spills, excessive manuring or fertilizing, etc. It is the dominant type of human-induced soil degradation in Europe and industrial and bio-industrial activities are the main agents for pollution of the soils (Oldeman, 1994)

Loss of nutrients and loss of organic matter normally occur because of poor management practices that do not replenish the nutrients taken out the soil by the crops. These last processes will be further explicated in the next paragraph.

Many times, the different kinds of soil degradation are interlinked together: for this cause, it is difficult to clearly distinguish one from the other. For the same reason, it is possible to improve one condition for ameliorating all the others. A clear example of this strategy is the formation of natural barriers of trees for contrasting soil erosion. If this solution works, not only wind erosion will be contrasted, but also water erosion (with major water uptake by roots and minor drop violence to the soil due to the interception by canopy trees) and loss of organic matter (because of a reduced loss of topsoil that is rich in organic matter).

1.2.3.1. Chemical degradation by nutrients and organic matter losses

The thesis will focus on the contrast against chemical degradation and in particular to soil nutrients loss and organic carbon loss. Nutrients are helpful chemical elements for plant growth. Usually, nutrients are subdivided into macronutrients and micronutrients. Macronutrients or macro-elements are the essential elements for plant nutrition and they are in percent concentration in plant tissues (Jones Jr., 2012). They are: C, H, O, N, P, K, Ca, Mg and S. C, H, and O are the structural compounds in plants. The assimilation of these last three elements does not depend on chemicals dissolved in the soil solution, but they derive from soil water (for H and O) and air (for C). For this reason, they are not considered for fertilization practices and neither in this thesis for the nutrient loss. N, P and K are called “fertilizer elements” because they are the principal elements in commercial chemical fertilizers. The nutrient loss in chemical degradation is usually referred to the loss of this last category. Ca, Mg, and S were called “secondary elements” but this term is no more used preferring to it the term meso-nutrients. In comparison to the macronutrients, micronutrients, or minor-elements, are found and required at lower concentrations (mg/kg). Usually are identified as B, Cl, Cu, Fe, Mn, Mo, and Zn. Some researchers added to the micronutrient category other beneficial elements such as Co, Si and Ni (Jones Jr., 2012).

Normally, in a natural system such as a forest, there is a balance in the nutrient cycles. In agricultural systems or in case of deforestation the nutrient cycles are imbalanced because of the removing of biomasses. In fact, if there would be not an external addition of nutrients (such as fertilization), the removed nutrients would be replaced by soil reserves. Nutrients would be found by the desorption of strongly adsorbed chemical molecules, the dissolution of mineral precipitates and by mineralization of organic matter. These phenomena caused soil degradation by loss of nutrients because the total nutrient reserves would be inadequate for future biomass production or, more in general, the rate at which nutrients are mobilized is less than biomass demand (Lal, 1990). This kind of soil degradation is mainly caused by agricultural mismanagement (56%) and deforestation (28%) (Oldeman, 1994). In agricultural systems the nutrient loss can be restored by the addition of nutrients into the soil. Differently, deforestation must be avoided, opting instead for sustainable forest management.

Soil organic matter (SOM) is any organic carbon assembly in soil space, independently of the source, the dimension, if it is dead or alive (but excluding roots of living plants) or the stage of decomposition (Jones Jr., 2012). From 10 to 40% of SOM is composed by microorganisms and the remaining part is composed by residues of plants and animals. In these residues, a part is composed by unaltered material (potential food for microorganisms) and transformed material. The transformed material can still be subdivided into humic substances (HS) and non-humic substances (Bot and Benites, 2005). Further details on HS and non-HS will be provided in Chapter V.

Thanks to SOM physical characteristics, SOM presence in soil improves soil structure by stabilizing soil aggregates. In fact, while it reduces soil compaction and erosion, surface crusting, waterlogging, pesticide and herbicide use at the same time it increases water infiltration and aeration, water-holding capacity, fertilizer efficiency, yields and, finally, biodiversity (Lal et al., 2005; Murphy, 2015). Hence, the increasing of SOM will improves soil functionality and soil health and inversely, its reduction causes a deterioration of soil quality (Koch et al., 2013; McBratney et al., 2014; Stockmann et al., 2013). It is a well-known problem that globally SOM quantity is decreasing (Henry et al., 2018; Lal et al., 2007): it is estimated that agricultural soils reduce SOM levels by 20-70% with their conversion from natural ecosystem (Crews and Rumsey, 2017). Environmental factors that influences SOM variations are temperature (high temperatures increases SOM degradation), soil moisture (generally, more soil moisture allow higher biomass production which provides more residues in soil), water saturation (periods of water saturation allow less soil aeration which leads a reduction of the mineralization rate and consequently the accumulation of SOM, such as in peat substrates), soil texture (SOM increases when the clay content rises), salinity, toxicity and extremely alkaline or acid soils (these soils allow a low biomass production and consequently low addition of OM to soil), topography (accumulation of OM is favored in lowlands) and the type of vegetation (there are plants species that increases SOM and vice-versa). The causes of the global reduction of SOM are many and mostly due to incorrect agricultural practices: from burning or removing crop residues to the overgrazing, from tillage practices to lands drainage, from the replacement of mixed vegetation with monoculture of crops and pastures to the replacement of perennial vegetation, from the replacement of indigenous varieties of species with high-yielding varieties to the use of fertilizer and pesticides (Lal et al., 2005).

In order to enhance SOM many are possibilities, but it is essential to use different specifics approaches for each kind of soil and climate. Nevertheless, in all cases, the objective will be to increase the biomass production for the purpose of creating habitat and resources for beneficial soil organisms which in turn, can improve soil quality, water holding capacity and make available nutrients for plants. Some of the practices that can enhance SOM are: agroforestry, zero or reduced tillage, the use of cover crops, the use of balanced fertilization, reforestation, afforestation and also the previously cited technique of “zai”. Furthermore, in line with the objective of this thesis, the addition of manure or other of carbon-rich wastes (such as sewage sludge) are further recognized methods that can enhance SOM (Lal et al., 2005).

Into the following parts of the thesis it will be theoretically demonstrate how the reuse of SS can fight against nutrient and organic matter losses in soils, hence how it can mitigate soil degradation and, consequently, combat desertification.

1.3. Sewage sludge

1.3.1. Origin and main characteristics

Sludge is a byproduct of many processes: water treatment plants, sewage treatment plants, coal and sand washeries, industrial manufacturing, agriculture and dredging of rivers and ports (Bowen et al., 1992). The process of wastewater origin is just one of the possible sludge categorizations. In fact, it can be characterized on the basis of many other parameters such as its chemical composition, the source of wastewater and on the basis of the stage or type of wastewater treatment in which sludge was created. With regard to chemical composition sludge can be mineral (if suspended mineral solids are > 50%) and organic (if suspended organic solids are > 50%). As far as wastewater source is concerned, there exist domestic, municipal and industrial sludges. After that, the stage or type of wastewater treatment can be another way to categorize: primary, secondary and tertiary sludges derive respectively from primary, secondary and tertiary treatments (paragraph 1.1.2.). Moreover, sludges can be categorized by the type of stabilization process in which they are involved (paragraph 1.3.3.): two of the most common techniques are the aerobic and the anaerobic digestion that allow classifying sludges in aerobically digested sludge and anaerobically digested sludge respectively (Gurjar and Tyagi, 2017). The focus of this thesis is centered on municipal sewage sludge (specifically to the anaerobically digested) that is the principal by-product WWTPs. In fact, an easy definition of SS is provided by Smith and co-workers as "the residue generated from the treatment of wastewater" (Smith et al., 2009).

The composition of SS varies depending on the composition and origin of wastewater. Usually, SS is made of microbial aggregates, filamentous bacterial strains, organic and inorganic particles, extracellular polymeric substances (EPSs), and a large amount of water. EPS derives from the adsorption of organic matter (mainly composed of proteins, polysaccharides, nucleic acids, humic substances, lipids..) and/or the microbial activity (secretion and cell lysis) (Guangyin and Youcai, 2016). Sewage sludge is a brownish and smelly slurry, with liquid to semisolid texture. Due to its richness in organic substances, it is a putrescible waste that can make SS even more malodorous as the degradation proceeds. Hence, also for this reason, the further handling of the SS it is critical.

In this paragraph there will be described the principal techniques of SS dewatering and stabilization in WWTPs, the main destinations of SS and its chemical profile, focusing on the components relevant to plant nutrition and growth. Finally, it will be evaluated the pros and cons of SS addition to soil.

1.3.2. Sewage sludge thickening and dewatering

Due to the high costs of SS disposal, the water removal from SS is a necessary process in order to reduce the final volume of SS (Guangyin and Youcai, 2016). Considering the volume of treated wastewater, SS normally represents a percentage between 1 and 2% (Guangyin and Youcai, 2016). Water can be removed from SS before or after stabilization. Specifically, it can be conducted as sludge thickening or sludge dewatering. Sludge thickening is used to increase the solid content in SS to make it suitable for further treatment (i.e., stabilization and/or dewatering) and can be done through gravitation, flotation or centrifugation (Gurjar and Tyagi, 2017). Dewatering of SS ensures that humidity is reduced to the required levels for SS disposal or reuse. SS dewatering makes mobilization easier and cheaper, allows SS thermal treatment and stops the decaying process (Campbell and Crescuolo, 1982). Technically, SS dewatering can be done through decantation or mechanical processes. Decantation processes are usually slower and suitable for medium-sized WWTPs. Sedimentation takes place in lagoons, drying beds or constructed wetlands with sludge drying reed beds (Uggetti et al., 2009). Mechanical dewatering is often done with devices such as vacuum filters, pressure filter presses and centrifuges. Prior to mechanical dewatering, two stages are required: thickening, as described above; and conditioning with polyelectrolyte, an ionic organic polymer with agglomerating properties. Mechanical dewatering is normally used in larger WWTPs, which handle larger amounts of SS. Regarding of the decantation or mechanical process, the resulting SS is a shovellable solid with a dry matter content ranging between 15 and 30% (Gurjar and Tyagi, 2017). Further water removal can be obtained by thermal drying, to reach dry matter content up to 95%. This also functions as sterilization and eliminates almost all viruses and pathogens. Unfortunately, drying SS is an energy-consuming process, and is therefore not the first-choice dewatering strategy. Instead direct or indirect dryers or combined mode drying systems are more common. Indirect dryers, such as rotary tray dryers, operate at lower temperatures and produce less vapor and therefore, they are easier to manage. However, they might generate lower dry matter content than direct dryers. Depending on the drying process, dried sewage sludge can take different forms and shapes, such as granular, pelletized, powdery, and beads (Chen et al., 2002; Lowe, 1995).

1.3.3. Stabilization

To remove smell and pathogens from SS in one single step, chemical or biological stabilization processes are required (Peirce et al., 2007). Particular attention will be given to anaerobic digestion due to its relevance in the experimental part of the thesis.

- Chemical stabilization: in chemical stabilization, chemical compounds are mixed with SS. Lime stabilization is the most common technique, where hydrated lime ($Ca(OH)_2$), quicklime (CaO) or fly ashes are mixed in appropriate amounts (20-30) to SS to reach a pH of 12 or more. The basic environment is favorable for accomplishing the stabilization purposes smoothly. Moreover, the process is exothermic, and higher temperatures helps to eliminate pathogens. However, the stabilization effect is only temporary and lasts until the pH drops back to neutral pH (Gurjar and Tyagi, 2017; Schanke Eikum, 1983; Valderrama et al., 2013).

- Vermistabilization: in vermistabilization, earthworms such as *Eisenia fetida*, digest organic matter and have a remediation effect on sewage sludge, reducing the levels of organic and inorganic pollutants, such as polycyclic aromatic hydrocarbons and heavy metals, respectively. The resulting product, called vermicompost, has fertilizing properties, such as improved carbon-to-nitrogen ratio and phosphorous plant availability (Rorat et al., 2017; Suthar, 2010).

- Biological aerobic processes for SS stabilization require oxygen and occur through aerobic digestion or composting.

Aerobic digestion is done in digesters, which are fed with SS and air or pure oxygen. During the first phase, biodegradable matter oxidizes as microbial consortia, mainly bacteria, feed on organic matter and generate water and carbon dioxide as by-products. Once the “feed” is consumed, the second phase (called endogenous respiration) starts. Here, microorganisms eat the cell structures of other microorganisms, generating water, carbon dioxide and nitrogen. When the digestion is completed, cell tissues have degraded by 80%, where the remainder is composed of inert compounds and recalcitrant cell components, such as cellulose, which require longer degradation times. The process efficiently reduces the SS volume and pathogens (Demirbas et al., 2017; Gurjar and Tyagi, 2017; Zhang et al., 2016).

Composting is a process to stabilize sewage sludge as well as waste from agriculture, food, or gardens. Microbial communities of bacteria, actinomycetes and fungi can reduce up to 30% of the volatile solids. Before composting, SS is dewatered to around 50% dry matter and mixed with a bulking agent, e.g. sawdust or fly ash, to improve aeration. The content is stocked in piles or containers and mixed to ensure proper ventilation. The composting process undergoes three phases: mesophilic (40°C) and thermophilic (70°C), where the organic matter is degraded, and the final curing phase, where degradation slow down until it stops. The degradation of organic matter properly occurs during the first two phases, while the third is the conclusion of the process. The resulting product is odorless, rich in humic acids and has good soil conditioning properties (Stentiford and de Bertoldi, 2010).

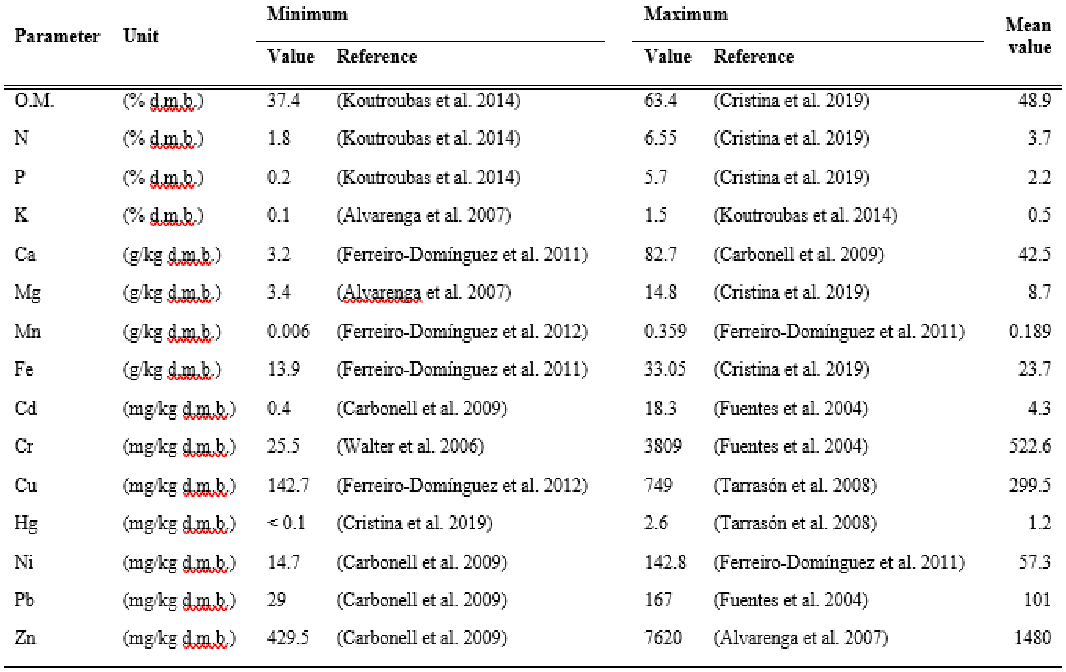
Anaerobic digestion (AD) is a fermentation process to reduce organic matter with the absence of oxygen. The technology is often used to manage SS and other types of putrescible waste, such as biomasses from agriculture and food-processing industries as well as the organic fraction of municipal solid waste. AD is the most common SS stabilization strategy in larger WWTPs. Despite the high initial investments and maintenance costs, this is currently the only technology that allows simultaneous stabilization and energy recovery. The main product is biogas, which is composed of about 50% to 80% by methane and from 30 to 50% by carbon dioxide (Lora Grando et al., 2017) with minor amounts of nitrogen, hydrogen, siloxanes and hydrogen sulphides. The biogas is combusted in dedicated power stations (in WWTP) for combined generation of heat and electricity (CHP). In the AD process, thanks to the absence of O_2 and mid-high temperatures, the OM of SS is biologically hydrolyzed, liquefied and gasified to CH_4 and CO_2. Not all OM is degraded by AD, in fact, some resistant components such as lignin or other cellulosic materials are not or scarcely decomposed. In the first phase, extracellular enzymes are synthesized and secreted by hydrogenotrophic bacteria which decompose the organic matter and make it more soluble. The anaerobic bacteria use oxygen from the OM and from soluble compounds of nitrogen. Carbon dioxide (CO_2), hydrogen (H_2), hydrogen sulfide (H_2S) methane (CH_4), and volatile organic acids (acetic, propionic, and butyric acid) are produced by the decomposition process. After that, there is a digestion process with acidogenic bacteria at pH 5-6. Afterward, the acidity lightly decreased together with the formation of CO_2 and H_2S; on the contrary, the decomposition process makes the smell very stinky. Next to the acidogenic phase (mature period), pH values increase 6.8-7.0. Thereafter starts the methanogenic phase: the most resistant substances (such as organic acids and proteins) are transformed into gases. The process becomes alkaline, nitrogen is converted in NH_3, propionic, acetic, and butyric acids are decomposed, and the fatty acids formed in the previous phase are decomposed in CO_2 and CH_4. After that, the pH decreases until the neutrality. This phase finishes at 10-12°C and sludge is removed by the digestion space (Gurjar and Tyagi, 2017).In WWTPs, SS is exploited as pure or mixed (in the case of co-digestion with other kinds of wastes) fed for anaerobic digesters. Prior to this, the fermentation process is initiated by thickening and warming up the SS. Inside the digester, SS must remain heated through the mesophilic or thermophilic phases, to activate different microorganisms. Mesophilic digestion is performed by microorganisms living at moderate-warm temperatures (~35°C), while thermophilic one involves microorganisms normally active at higher temperatures (~50°C). The advantages of thermophilic digestion are higher digestion rates, biogas production, and pathogen removal, while the main drawback is higher energy demand. Once biogas is produced, it is stored in gasometers which are water-sealed gas holders. Due to the low purity of the methane and presence of corrosive agents, biogas cannot be directly injected into the gas network.

Recent efforts to purify biogas into biomethane has resulted in a renewable and high added-value fuel that can be directly injected in gas networks and motor vehicles (Gurjar and Tyagi, 2017; Peirce et al., 2007).

The main by-product from this process is sewage sludge anaerobic digestate (SSAD). Once the AD process is terminated, SSAD is recovered from the digester, thickened and dewatered for further handling. Anaerobic digestates and SSAD are, in the broadest sense, a biomass rich in valuable macro- and micro-nutrients for plant growth. For its richness in terms of nutrients, this type of sludge was selected for the experimental part.

1.3.4. Beneficial compounds contained in SSAD

Sewage sludges contain a range of chemical substances: some of which are nutrients for plant growth and other phytotoxic, such as heavy metals. Nutrients in excess can become toxic to plants, while a deficiency usually damages their metabolism and physiological functions. **Table 1** gives an overview of key elements for plant growth that are present in SSADs, from 10 WWTPs in the Mediterranean area, i.e. Spain, Portugal, Italy, France and Greece (Alvarenga et al., 2007; Bourioug et al., 2015; Carbonell et al., 2009; Cristina et al., 2019; De Andres et al., 2010; Ferreiro-Domínguez et al., 2012, 2011; Fuentes et al., 2004; Koutroubas et al., 2014; Tarrasón et al., 2008; Walter et al., 2006).



Parameter	Unit	Minimum		Maximum		Mean value
		Value	Reference	Value	Reference	
O.M.	(% d.m.b.)	37.4	(Koutroubas et al. 2014)	63.4	(Cristina et al. 2019)	48.9
N	(% d.m.b.)	1.8	(Koutroubas et al. 2014)	6.55	(Cristina et al. 2019)	3.7
P	(% d.m.b.)	0.2	(Koutroubas et al. 2014)	5.7	(Cristina et al. 2019)	2.2
K	(% d.m.b.)	0.1	(Alvarenga et al. 2007)	1.5	(Koutroubas et al. 2014)	0.5
Ca	(g/kg d.m.b.)	3.2	(Ferreiro-Domínguez et al. 2011)	82.7	(Carbonell et al. 2009)	42.5
Mg	(g/kg d.m.b.)	3.4	(Alvarenga et al. 2007)	14.8	(Cristina et al. 2019)	8.7
Mn	(g/kg d.m.b.)	0.006	(Ferreiro-Domínguez et al. 2012)	0.359	(Ferreiro-Domínguez et al. 2011)	0.189
Fe	(g/kg d.m.b.)	13.9	(Ferreiro-Domínguez et al. 2011)	33.05	(Cristina et al. 2019)	23.7
Cd	(mg/kg d.m.b.)	0.4	(Carbonell et al. 2009)	18.3	(Fuentes et al. 2004)	4.3
Cr	(mg/kg d.m.b.)	25.5	(Walter et al. 2006)	3809	(Fuentes et al. 2004)	522.6
Cu	(mg/kg d.m.b.)	142.7	(Ferreiro-Domínguez et al. 2012)	749	(Tarrasón et al. 2008)	299.5
Hg	(mg/kg d.m.b.)	< 0.1	(Cristina et al. 2019)	2.6	(Tarrasón et al. 2008)	1.2
Ni	(mg/kg d.m.b.)	14.7	(Carbonell et al. 2009)	142.8	(Ferreiro-Domínguez et al. 2011)	57.3
Pb	(mg/kg d.m.b.)	29	(Carbonell et al. 2009)	167	(Fuentes et al. 2004)	101
Zn	(mg/kg d.m.b.)	429.5	(Carbonell et al. 2009)	7620	(Alvarenga et al. 2007)	1480

Table 1. Range (minimum, maximum and mean values) of SSAD chemical components that are essential for plant growth. O.M.: organic matter; d.m.b.: dry matter basis.

The plant nutrients in SSADs include both macronutrients and micronutrients, depending on their presence in plant tissues and metabolic needs of the plant. The macronutrients present in SSADs include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) (Jones Jr., 2012), and typical amounts are found in **Table 1**. Nitrogen, in its forms of ammonium (NH_4^+) and nitrate (NO_3^-), is involved in essential plant processes and functions, such as amino acid synthesis and protein formation. It is also a fundamental compound of the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and present in chlorophyll and B vitamins. In SSADs, nitrogen can be present in high concentrations, which favors the use of SSAD as fertilizer. Phosphorus is a basic compound of adenosine triphosphate (ATP), which is the most important metabolic "energetic carrier" in plants. Moreover, it is present in nucleic acids and in enzymes and proteins as post-translational modifications. Potassium frequently features in formulated chemical NPK-fertilizers (Jones Jr., 2012), where it regulates the water balance in plants by changing cell turgor pressure and by opening or closing stomata leaf. Lastly, it is essential for the accumulation and translocation of carbohydrates. Calcium is important for the cell wall structure, cell membrane, cell permeability, and signal transduction. Moreover, it improves pollen germination and growth, and activates the enzymes required for cell mitosis, division, and elongation. In plant cells, magnesium ions activate enzymes involved in respiration, photosynthesis, and DNA and RNA synthesis. Furthermore, magnesium is a fundamental element in the chlorophyll structure. Sulphur is present in two amino acids of plants, cysteine and methionine, and it is a constituent of several compounds essential for metabolism, such as Coenzyme A and Vitamin B1. Although none of the SSADs in **Table 1** reported sulphur, this element is likely present in many SSAD compounds (Dewil et al., 2008).

Micronutrients, such as boron (B), chlorine (Cl), molybdenum (Mo), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn), appear in SSADs and are required in relatively low concentrations in plants compared to the macronutrients. Presence of boron, chlorine and molybdenum in SSAD has been reported, but their quantification is unusual in scientific studies (Epstein, 2001). Boron deals with plant cell elongation and nucleic acid synthesis, and its presence in SSADs is certain (Chu and Poon, 1999). Chlorine is necessary for photosynthesis reaction. Its presence in SSADs is not confirmed but, if present, it is in low concentrations. Molybdenum is a component of enzymes, such as nitrate reductase, which promotes cell assimilation through the reduction of nitrate to nitrite, and nitrogenase which is responsible for molecular nitrogen fixation. Manganese ions are present in different enzyme families. It is required by decarboxylases and dehydrogenases taking part in the tricarboxylic acid cycle of the energetic metabolism, and superoxide dismutase, which protects from damage by reactive oxygen species. Last, manganese appears in a catalytic cluster in the oxygen evolving-complex, which allows the water-splitting activity, that is the first step of the photosynthesis (Nelson and Cox, 2013). Iron is a component of chemical groups, such as heme groups, associated with proteins like cytochromes. For instance, cytochrome b_6f complex is involved in electron transfer of reactions occurring during the photosynthesis (Willows, 2006). Copper and zinc are both considered micronutrients at low concentrations and heavy metal contaminants when in excess. Similar to iron, copper is associated with enzymes that are responsible of redox reactions, while zinc is as cofactor of other several enzymes.

Around 50% of dry matter of SSAD is composed by organic carbon. Carbon atoms in SS are in forms of organic compounds which have chemical bonds especially with other Corg atoms, hydrogen, oxygen, nitrogen, phosphorous and halogens. The two main types of organic compounds in SSAD are organic matter (OM) and organic pollutants, which have opposite effects on plant growth. Beneficial properties of OM on soil quality (such as acting as a depository for nutrients, reducing soil compaction, enhancing micro and macropores, increasing the microbial population and activity, rising cation exchange capacity (CEC) and soil water retention, which ameliorate the root environment and water uptake (Jones et al., 2005)) was explained in paragraph 1.2.3.1. OM soil decreasing is one of the causes of soil degradation and consequently of desertification but SS richness in OM give the possibility of increase OM in soils: this possibility will be explored in the experimental part. The downside of organic carbon in SSAD is represented by the presence of toxic organic pollutants which will be explained in the subsequent paragraph.

1.3.5. Pathogens and contaminants contained in SSAD

The Achilles' heel in SSAD composition is represented by pathogenic microorganisms and by inorganic and organic pollutants.

Wastewaters contain pathogens derived from human wastes and are discharged into the sewer system. Moreover, surface runoff can add to the sewer system mammalian and avian pathogens. Environmental and social conditions affect the quantity and types of pathogens in wastewaters (Epstein, 2001). Most of the pathogens are removed from wastewater thanks to the primary, secondary, and tertiary treatments (Paragraph 1.1.2.). Consequently, primary and secondary sludges are rich in pathogens that must be abated by stabilization processes such as AD (Paragraph 1.3.3.). Seven categories of pathogens could be present in SS before stabilization: bacteria, viruses, protozoa, nematodes, cestodes, helminths, and fungi. The most important bacteria that can be found in wastewater and consequently in SS are: Salmonella spp. (causing in humans salmonellosis and gastroenteritis), *Salmonella typhi* (causing typhoid fever), *Mycobacterium tuberculosis* (causing tuberculosis), *Escherichia coli* (there are pathogenic strains that can cause gastroenteritis), Yersinia spp. (causing yersinosis), *Campylobacter jejuni* (causing gastroenteritis), *Vibrio cholerae* (causing cholera) etc. For what it concerns viruses, it is possible to found in wastewaters and SS:

Adenoviruses (they can cause conjunctivitis, respiratory infections and gastroenteritis), Polio virus (causing poliomyelitis), Hepatitis viruses (causing hepatitis), etc. Concerning protozoa, it is possible to found *Giardia lamblia* (causing giardiasis), *Toxoplasma gondii* (causing toxoplasmosis), etc. Concerning nematodes, cestodes and helminths it is possible to found *Toxocara cati* and *T. canis* (known also as cat and dog roundworm: they can cause toxocariasis), *Taenia saginata* and *T. solium* (known also as beef tapeworm and pork tapeworm: they can cause teniasis) and many other species. Finally, concerning fungi, it is possible to found *Aspergillus fumigatus* (that can cause respiratory infections), *Candida ablicans* (causing candidiasis), Trichosporon spp. (causing infection of hair follicles), etc. How previously cited, all these pathogens can be abated by stabilization processes (Paragraph 1.3.3.): AD can cause a decrease of 0.5–4.0 units (Log-reduction) for bacteria, 0.5–2.0 for viruses and 0.5 for protozoa and helminths (where 1 Log-reduction is equal to 90% of reduction) (Epstein, 2001). Furthermore, successive heat treatments can even more increase this percentage. It is important to have an abatement of pathogenic microorganisms because potential risks of land disposal of SSADs containing pathogens are: firstly, the plant uptake and the possible pathogens ingress into the food-chain; secondly, the contamination of groundwater and, hence, the possible contamination of drinking water; thirdly, the direct exposure of people and animals to surface contaminated water by runoff and erosion of the soils treated with SSADs (Epstein, 2001). Thanks to the characteristic of fecal coliforms (in particular *Escherichia coli)* of reacting to the environmental conditions in a similar way to intestinal pathogenic bacteria, nowadays the presence of coliforms is utilized as an indicator for the potential presence of other pathogens (Epstein, 2001). Moreover, in 1988 Yanko demonstrated a strong correlation between fecal coliforms densities and Salmonella detection (Yanko, 1988). For these reasons and for Salmonellas potentially dangerous effects on human health, the abatement of Salmonella spp. is required by laws in case of land application of SS or the land application of other products derivate from SS (Italian Decree Law 75/2010, 2010; Italian Decree Law 99/1992, 1992) (Paragraph 1.3.9.).Typically, the category of inorganic pollutants in SS is referred to heavy metals (Wong et al., 2004), a group of elements with a relatively high molecular weight (density >5.0 mg/m^3) (Pais and Jones Jr, 1997). For what it concerns heavy metals in SS it is customary to refer to cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn). In SS their presence can range between 0.5% and 4% of total dry weight (Pathak et al., 2009). Example mean values contained in SSAD are summarized in **Table 1.** Some of them (Zn and Cu) are at the same time essential microelements essential for plant nutrition (paragraph 1.3.4.). Others (Cd, Ni, Pb), if they are present at very low concentrations, are considered essential to some animal species (Van Campen, 1991). The same author identifies Cu, Zn, Cr and Zn as essential trace elements also for human nutrition. The effects of heavy metals in plants and soils are complex. Whether they accumulate or become bioavailable depend on e.g. soil pH, oxidation number, and the presence of other chemicals. When absorbed by plants at low levels, simple bioaccumulation occurs, and often without any specific effect. When the concentration of heavy metals increases, phytotoxic effects emerge, such as growth retardation and inhibition of iron translocation (caused by excess of copper, nickel and zinc), and reduced root development (due to hexavalent chromium).

Organic contaminants (OC) can be very dangerous for life health (depending on the quantity) due to their environmental persistence, bioaccumulation, toxicity and endocrine disruption (Clarke and Smith, 2011). Examples of very popular OC are antibiotics and pesticides. Wastewater can contain OC due to the polluted soil runoff and to the industrial, commercial and domestic discharges (Epstein, 2001). Although wastewater and sludge treatments can remove several OC, many of them may persist in residual concentration in sludge. Due to lipophilic and hydrophobic properties of OC, they are mostly transferred to SS and hence, the cleaned water can be reused. Depending on the initial amount, their lipophilicity and the degree of destruction during treatments, OC can be present in SS in concentrations ranging from <ng kg^{-1} to percentages (on dry matter basis) (Clarke and Smith, 2011). From a meta-analysis conducted on Chinese sewage sludge of the lasts 30 years emerges that the classes of OC (between the selected 13 classes of OC) more abundant in SS are phthalate esters > alkylphenol polyethoxylates > synthetic musks > antibiotics > PAHs > ultraviolet stabilizers > bisphenol analogs > organochlorine pesticides > polybrominated diphenyl ethers > pharmaceuticals > hormones > perfluorinated compounds > polychlorinated biphenyls (Meng et al., 2016). Basing on the quantity of selected OC in SS, human health impacts persistence, ecotoxicity and bioaccumulation a study of 2011 selected two classes of OC that need particular attentions (Clarke and Smith, 2011): perfluorochemicals (PFCs) and polychlorinated alkanes (PCAs). Compared to other contaminants, due to their water solubility, PFCs have an elevated probability to pass in food chains. Differently, PCAs were selected for their relatively high concentration in sludge compared to other OC (1.8 g kg^{-1} on dry wet basis). Indeed, there is the class of emerging contaminants (EmC) in wastewater that is increasingly gaining more interest within the OC. EmCs include molecules such as endocrine-disrupting compounds (EDC, e.g. hormones), pharmaceutically active compounds (e.g. antibiotics), illicit drugs and pesticides (Fijalkowski, 2019). About OC contained in SS dangerousness, with the actual concentrations, persistence and possibility of bioaccumulation, researchers are in contrast between each other: if someone thinks that they can be dangerous on soil system (and then on all trophic chain) (Harrison et al., 2006) others think the opposite (Clarke and Smith, 2011; Smith, 2009). Surely, more researches on OC in SS are needed: although many studies were conducted, a lack in knowledge is revealed for what it concerns OC identification in SS, their effects on humans and the environment and their degradability in WWTPs (Gadupudi et al., 2019; Harrison et al., 2006; Kolpin et al., 2002; Laturnus et al., 2007).

1.3.6. Disposal of sewage sludge

Today, SS is classified as waste and its safe disposal represents a very important issue in waste management (Epstein, 2002; Singh and Agrawal, 2008). Actually, principal SS destinations include agriculture, composting, landfill, incineration and other treatments. Below are briefly described these SS disposals and, at least, some European country are provided as examples of the strategy:

- Agricultural use: the use of SS for agricultural purposes has been promoted in Europe since the 1980s (Council of the European Communities, 1986). The legislation on the agricultural use will be furthered in paragraph 1.3.9. It is quite common in Ireland, Spain, Portugal, and regulated by national and international guidelines and policies. Other countries remain reluctant, mainly due to the potentially damaging effects of pollutants on soil health.
- Composting: can be done together with other feedstocks, i.e. co-composting, primarily for agricultural purposes (Paragraph 1.3.9.). While other types of stable SS (e.g. SSAD, lime stabilized SS) are used as fertilizers, compost is mainly exploited as soil conditioner (Kacprzak et al., 2017). Composting as SS destination is common in Finland, Estonia and Hungary.
- Incineration: thermal treatments are primarily done to recover energy and reduce SS volume. Conventional incineration and co-incineration are heat treatment processes where SS is burnt alone or with e.g. coal, fuel oil or natural gas, returning by-products such as exhaust gases, slag and fly ash. The last ones may be further recovered to produce cementitious materials. Incineration is common in the Netherlands and Switzerland (Cieślik et al., 2015).
- Landfilling: is an old waste management strategy to store waste on ground. The negative environmental impacts are becoming more known, e.g. risk of soil and water contamination from leachate and carbon dioxide emissions (Kacprzak et al., 2017). Furthermore, since no recycling takes place, some countries are reaching a definite space limit (Council of the European Union, 1999). Nevertheless, landfills remain the principal disposal solution in many poorer countries, including European countries such as Serbia, Croatia, Romania, Bosnia and Herzegovina.
- Other destinations: other types of thermal treatments include vitrification (above 1000°C in presence of silica) and pyrolysis (in absence of oxygen). These technologies are effective but expensive (Cieślik et al., 2015).

In Italy, according to Eurostat data (Eurostat, 2021), the majority of SS is sent to landfill (50.8%), while 34.7% is reused in agriculture, 4% is incinerated and 10.4% is sent to other destinations. Concerning Italian legislation on SS disposals, it is important to highlight that SS is considered a “special waste” for the Italian Decree Law 152/2006, art. 184 (Italian Decree Law 152/2006, 2006). In the meantime, the same law affirms that sludges must be reused all time that it is possible their reuse (Art 127) in coherence with the circular economy hierarchy of wastes (Directive 98/2008/EC (European Commission, 2008)) that sustains that landfill disposal is the last option for the waste fate (Reduce → Reuse → Repair → Recycle → Recover → Landfill). Moreover, in article number 6 of this directive, there is the definition of “End Of Waste”. A waste ceases to be a waste when it has undergone recovery in accordance with specifics criteria: (a) the substance or object is commonly used for specific purposes; (b) a market or demand exists for such a substance or object; (c) the substance or object fulfills the technical requirements for the specific purposes and meets the existing legislation and standards applicable to products; (d) the use of the substance or object will not lead to overall adverse environmental or human health impacts. In Italy, Law n. 128/2019 affirms that in absence of specific criteria adopted by other laws, the authorizations for recovery operations are issued in the respect of these last conditions. As a consequence, local authorities can authorize the recovery of a specific waste case by case basis (Italian Law 128/2019, 2019). Furthermore, from the Art. 177 of the Italian Decree Law 152/2006, it is possible to assume that SS has got special law regarding its reuse in agriculture (Italian Decree Law 99/1992, 1992), and, if all threshold are respected its use is allowed (Paragraph 1.3.9.). Concerning landfilling and incineration disposal, urban SS is categorized with the EWC code 190805 (European Waste Catalogue (European Commission, 2000)). In accordance with the Italian legislation, this code allows to confer SS to landfills for non-hazardous wastes if specific values (such as heavy metals) are below specific thresholds (Italian Decree Law, 2020).

1.3.7. Current origin of fertilizers

Globally, the demand for the three primary plant nutrients used for soil fertilization (N, P_2O_5 and K_2O) is increasing (Vanotti et al., 2019). In 2015, the total fertilizer nutrient demand was around 184 Mt and, by the end of 2020, it is expected to overcome 200 Mt (FAO, 2017). The production processes of these fertilizers are very expensive in terms of energy (ammonia) and non-renewable resources (phosphorus and potassium), with heavy environmental costs (Li et al., 2009). Ammonia production is mainly performed via the Haber-Bosch process which requires a large amount of fossil fuel (Basosi et al., 2014) with an average energy consumption of 3-11 $\mathrm{kWh\,kg^{-1}\,NH_3}$ (Rouwenhorst et al., 2019). Furthermore, a recent study indicates that the Haber-Bosh process is responsible for 1% – 2% of global energy consumption and 1.44% of CO_2 emissions (Kyriakou et al., 2020). Phosphate rock is the principal raw material exploited in the production of nearly all phosphate fertilizers (Fixen and Johnston, 2012; Reijnders, 2014). This non-renewable resource may contain many toxic heavy metals such us As, Hg, Ni, V (Mortvedt, 1995), Cd, Cr, Cu, Pb, Zn (Sabiha-Javied et al., 2009), fluorine (Mirlean and Roisenberg, 2007) and uranium (Schnug and Lottermoser, 2013). The P_2O_5 extraction can cause environmental pollution by contaminants accumulating in air, soil, and water bodies around the manufacturing place (Mirlean et al., 2008; Sabiha-Javied et al., 2009). It has been observed that these impurities can persist into phosphate fertilizers, provoking a subsequent accumulation in agricultural soils (De López Camelo et al., 1997). Potassium derives from non-renewable resources like minerals such as sylvite, sylvinite, hartsalz and langbeinite (Fixen and Johnston, 2012). Furthermore, world distribution of phosphorous and potassium mines is not uniform: 45% of global phosphate rock is concentrated in Morocco and Western Sahara (Fixen and Johnston, 2012). Within a circular economy perspective, the recovery of SS as fertilizer represents an interesting scenario for many reasons: there is a global and large production of SS and it can substitute the use of fertilizers deriving from non-renewable resources (with high environmental costs of extraction and transportation).

1.3.8. The pros and cons of SS reuse directly on the soil

In this paragraph will be briefly described the pros and cons of the direct application of SS. It is important to underline that not all SS are equals and, in this thesis, attention is focused on SSADs. In fact, how it was shown in paragraph 1.3.1. many types of SS exist and each of these will have its own peculiarities. As consequence, their effect on soil and plants will be not equal. For example, differences in terms of solid particles, nutrients, HM, and organic contaminants are present between primary and secondary sludges (paragraph 1.1.2): usually, nutrients are higher in secondary sludge and solid particles, HM and organic contaminants are higher in primary sludges (Alvarez et al., 2002; Gianico et al., 2013; Gurjar and Tyagi, 2017; Katsoyiannis and Samara, 2005; Radjenović et al., 2009).

As first positive factor of SS reuses, there is the possibility of reusing a waste that is constantly in increasing production and that, alternatively, it would have high costs of disposal. Secondly, SS is a great substance in terms of macro and micronutrients useful for plant nutrition: in paragraph 1.3.4. it was shown how it could be rich in particular of two essential macronutrients (nitrogen and phosphorous) and micronutrients that, normally, are not present in commercial fertilizers. These nutrients addition to soil could combat chemical degradation of soils (paragraph 1.2.3.1.). Furthermore, SS has got high values of organic carbon and its addition to soil can also struggle soil chemical degradation, in particular against soil organic matter depletion. Due to its chemical and physical characteristics it can partially substitute the use of chemical fertilizers and conditioners reducing the current environmental impacts of their production and transportation (paragraph 1.3.7.).

As negative consequences of SS soil application, there are possible contaminations of the environment (and consequently of the food chain) by pathogens, HM and OC. The negative effects of these were exposed in paragraph 1.3.5. It is important to note that chemical, physical and biological treatments and SS stabilization can reduce these problems (paragraph 1.3.10.); AD seems to be one of the best strategies for their reduction in particular against pathogens and OC and, its effects are more effective when paired with others pre-treatments processes (Neumann et al., 2016). Another great limit for SS addition to soils is represented by the odor. Although stabilization technologies can reduce bad smell, it persists, and represents one of the most diffused reasons for the deficiency of its public acceptance (Lu et al., 2012). As well as stabilization technologies, other arrangements can be taken in order to reduce it smell: firstly, to incorporate SS in soil, secondly, to avoid SS distribution on windy days and thirdly, to choose only remote places to applicate it (Rynk and Goldstein, 2003). Another adverse effect is that, as well as in synthetic fertilizers, a SS improper and unplanned soil application can cause leaching of nutrients (N and P) in groundwater causing the phenomenon of eutrophication. To prevent this effect could be make a nutrients balance that estimates nutrient losses and additions along with a consequently planned SS addition in function of SS nutrient content, plants requirements, type of soil, water addition etc.

In the subsequent parts of the present thesis, these consequences will be deeply explored with experiments in order to arrive at a final judgment on soil application of SS, because if it is true that it contains many nutrients it is equally true that it also contains contaminants.

1.3.9. Current legislation on sewage sludge soil application

Legislation on the reuse of SS on soils is complex, different between each country and sometimes absent. For simplicity and proximity, it will be summarized the European situation and, in particular, the Italian one. In Europe, the reuse of SS is ruled by the Directive 86/278 EEC (Council of the European Communities, 1986). How previously described in Paragraph 1.3.6., in Italy, SS is classified as waste (Italian Decree Law 152/2006, 2006) (Paragraph 1.3.6.); in the meantime, the same law affirms that sludges must be reused all time that it is possible their reuse. With this principle, in Italy today it is possible to use SS on fields in accordance with the Directive 86/278 EEC that was transposed into the Italian Decree Law 99/1992 (Italian Decree Law 99/1992, 1992). This law states that SS can be spread only if it is previously treated, if it is guaranteed the fertilizer, stimulant or corrective effect on soil and, if it does not contain hazardous, persistent and bio-cumulable substances in a proportion that could be dangerous for life. In order to contain the presence of these potentially dangerous substances (such as HM and pathogens) were provided limits that SS must respect before the use (**Table 2**). Furthermore, there are also prohibitions regarding soil conditions, topography, land use, techniques of spreading, etc.: for example, it is not allowed to apply SS on fields that are subjected to floods, on soils with a pH < 5.0 and it is not allowed to apply SS with the technique of sprinkler irrigation. At the European level, these limits have remained the same until now (Directive 86/278 EEC (Council of the European Communities, 1986)), but it is from the end of the '90s that exists a European working document on SS management, with actualized rules and thresholds (Alvarenga et al., 2015; Hafidi et al., 2008). This document remained just a draft form, but some European countries have started to introduce stricter rules. In Italy, in 2018, the parliament approved some more stringent limits for contaminants content in SS for land application (Italian Law 130/2018, 2018). With this law, new thresholds on some organic compounds were added: for example, analysis on PCB (polychlorinated biphenyl) and PAH (polycyclic aromatic hydrocarbons) have become mandatory (**Table 2**).

In parallel with Directive 86/278 EEC, in Europe, the reuse of SS is ruled by fertilizers' legislation. Actually, fertilizers are regulated by the EU regulation 2003/2003 and 1069/2009 and no clarification about the use of SS in fertilizer composition it is provided (European Commission, 2003; European Parliament and Council, 2009). In Italy, the law 75 of 2010 ruled the possibility of SS of being a constituent part of two kinds of fertilizers: defecation gypsum and defecation calcium carbonate (Italian Decree Law 75/2010, 2010). In other European countries exist other categories of fertilizers deriving from SS, but due to the different national rules, they can't be commercialized in all EU. To make more harmonious the European laws on fertilizers, from July 16th 2022 it will enter into force the new Regulation 1009/2019 (European Parliament and Council, 2019). This law clarifies (in Annex 2 c.m.c. 5, 1 ii) that a fertilizer, a soil conditioner, or a biostimulant cannot derive from any kind of SS. This regulation does not provide the possibility of commercializing fertilizers derived from SSAD between European countries, but in the meantime, all national rules on fertilizers remain in force. It means that from July 16th 2022, two parallel markets of the fertilizing products will be active in each European country. From this date, in Italy, the products that derive from SSADs can be commercialized only in Italy.

Parameter	Unit of measure	Italian threshold for SS agricultural use (D. Lgs 99/92)	Additional Italian thresholds for SS agricultural use (L. 130/18)
N - Tot	% d.m.b.	> 1.5	
TOC	% d.m.b.	> 20	
P	% d.m.b.	> 0.4	
Cu	mg/kg d.m.b.	< 1000	
Zn	mg/kg d.m.b.	2500	
Pb	mg/kg d.m.b.	< 750	
Cd	mg/kg d.m.b.	< 20	
Ni	mg/kg d.m.b.	< 300	
Hg	mg/kg d.m.b.	< 10	
Salmonella	MPN/g d.m.b.	*< 103*	
As	mg/kg d.m.b.		*<20*
Cr	mg/kg d.m.b.		*<200*
Cr^{6+}	mg/kg d.m.b.		*<2*
PCB	mg/kg d.m.b.		≤ 0.8
Be	mg/kg d.m.b.		≤ 2
Se	mg/kg d.m.b.		≤ 10
Toluene	mg/kg d.m.b.		≤ 100
PCDD/PCDF + PCB-DL	mg/kg d.m.b.		≤ 25
PAH	mg/kg d.m.b.		≤ 6
Hydrocarbons (C10-C40)	mg/kg w.m.b.		≤ 1000

Table 2. SS contaminants thresholds allowed for agricultural use in the Italian legislation; MPN/g: most probable number; PCDD: polychlorinated dibenzodioxins; PCDF: polychlorinated dibenzofurans; PAH: polycyclic aromatic hydrocarbons; PCB-DL: polychlorinated biphenyl - Dioxin Like; PAH calculated as the sum of benzo(a)pyrene + benz(a)anthracene + benzo(b,k)fluoranthene + benzo(g,h,i)perylene + chrysene + dibenzo(a,h,i,e,l)pyrene + dibenzo(a,h)anthracene + indenopyrene + pyrene; d.m.b.: dry matter basis; w.m.b.: wet matter basis;

1.3.10. Technologies for contaminants removal

The implementation of technologies in contaminants removal from wastewater caused a rise in the accumulation of pollutants in SS. Due to the limits of contaminants imposed by national legislation on the reuse of SS in agricultural soils, also technologies for contaminants reduction in SS were increased (Stehouwer et al., 2000).

For what it concerns heavy metal content in SS, it can be abated by means of chemical, physical or biological treatments (Camargo et al., 2016).

- Chemical treatments are efficient, easy and do not require long time of contact between reagent and sludge. The disadvantages of these kinds of treatments are the risk of secondary pollutants, the difficulties for the disposal of the new polluted stream and sometimes the high cost. The principle that characterizes these treatments is that the substrate's pH is directly related to the balance between metal absorption or complexation and its solubility; in fact, heavy metals can be found in many forms that generally are pH-dependent. The acidification method uses organic (oxalic acid and citric acid) and inorganic acids (nitric acid, sulfuric acid, and hydrochloric) to separate metals; the effectivity of removal depends on the acid applied and on the target metal to remove. The ion exchange treatment is another chemical method that replace the selected metal with another (non/less-pollutant) ion. Thirdly, has been demonstrated that the use of chelators such as EDTA, NTA or GLDA for the extraction of HM are valid methods that have the advantage of metal recovery after the reaction.

- Physical treatments: heat treatment is the most diffused physical treatment due to the short time of exposure (less than one hour) and the possibility of metal recovery. Higher is the temperature applied, higher is the HM abatement, but also higher will result the nutrient and organic matter depletion. Another physical treatment is the electrokinesis that could be based on three main mechanisms: electrophoresis, electrosmosis and electromigration.
- Biological treatments: due to the low cost and low risk of secondary pollutants, biological treatments represent a potential greener alternative for HM abatement in SS. They are not really spread mostly because the large time required for the treatment. Vermicomposting is a biological treatment that allows to concentrate metals in earthworm tissues (the most diffused is *Eisenia fetida*). Although it had good results in terms of HM reduction, this technique is not really used due to the large time of treatment (≃ 100 days). Bioleaching is a biological treatment that use the microbial activity of oxidizing iron and sulfur for the purpose of change metals form. The most used species are Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans.

Concerning organic pollutants, their abatement is even more challenging. Some of OC can be degraded with the above-cited treatments, while others require a longer time, so that, when they accumulate in soils, they may have adverse effects at various trophic levels (Kolpin et al. 2002; Harrison et al. 2006; Díaz-Cruz et al. 2009). Finally, some organic pollutants (e.g. pesticides, antibiotics and hormones) can be volatilized or degraded through biotic or abiotic processes (Harrison et al., 2006). Treatments exposed for HM removing often can degrade at the meantime OC. Particular attention must be given to heat treatments that, even if it reduces nutrients and organic carbon in SS, it can support also OC abatement. Some studies affirmed that anaerobic digestion is the stabilization strategy ensuring the best OC removal, especially when the sludge is pretreated (e.g. via ozonation) (Neumann et al., 2016). Regarding the EmCs abatement is becoming even more required both on the effluent of WWTPs with advanced treatments and on sewage sludge. The most diffused advanced treatments are the Activated Carbon Adsorption (ACA), the Advanced Oxidation Processes (AOPs), Reverse Osmosis (RO) and ozonation (Gadupudi et al., 2019).

However, further studies are still required to improve the treatment performances (both on HM and OC abatement) and to reduce the costs of these techniques, which nowadays are rarely applied at WWTP level since they are money and/or time-consuming (Camargo et al., 2016; Gadupudi et al., 2019).

1.4. Extraction of high-value compounds from SS

Due to the extremely variegate composition of SS, another strategy for the reuse of SS (different from soil application) is the extraction of high-value compounds (HVC). The objective of future researches will be to find and extract these HVC. Nowadays, SSAD production is mainly chosen because of its capacity of having as HVC the biogas (paragraph 1.3.3.). Moreover, biogas productivity through AD, can be further advanced by reducing by-products, such SSAD. One of the most promising technologies is thermal hydrolysis, a pretreatment of sewage sludge that increases biogas production while reducing pathogens and some organic pollutants (Taboada-Santos et al., 2019).

In the previous paragraph were exposed some technologies for HM reduction in SSAD, that, with the already cited chemical, physical and biological methods, can be potentially reused. Moreover, also rare metals (e.g. silver, tellurium, thallium, bismuth, antimony, indium, gallium, tin, germanium, and lead) can be removed by incineration with plasma furnaces under oxidative or reduction conditions and then used in industrial processes (Cieślik et al., 2015).

To advance the use of SSAD as sustainable practice, some critical research should include the improvement of the extraction of compounds with high fertilizing qualities from SS and SSAD. For example, phosphorus can be extracted from SS and SSAD using acid washing and alkali extraction, as well as electrodialysis, which also can remove heavy metals (Cieślik et al., 2015). Another highly promising technology is struvite precipitation. Struvite is a mineral composed by ammonia, phosphate and magnesium ($NH_4MgPO_4 \times 6H_2O$), showing excellent fertilizing properties. Struvite can be obtained by mixing, in basic conditions, selected chemical compounds (e.g. magnesium chloride) to mixtures rich in ammonia and phosphate such as SS and SSAD. Through struvite precipitation, two macronutrients (N and P) are recovered simultaneously and directly formulated as fertilizer (Yu et al., 2017). Another interesting compound that could be extract in WWTP is nitrogen: in fact, thanks to the ammonia stripping process it is possible to reduce the nitrogen presence in wastewater. As a byproduct of this process, there is ammonium sulphate, a compound with high fertilization properties (Kinidi et al., 2018). Finally, SSAD can contain others HVC: humic substances and in particular humic acids (Adani and Tambone, 2005; Réveillé et al., 2003). On these compounds, also defined as "the black gold of agriculture"

(Asing et al., 2009) due to their beneficial effects on soil quality and plant growth, will be dedicated all Chapter V.

The extraction of HVCs does not necessarily exclude the land application of SSAD; instead, they can be applied together. These technologies have been designed and applied in some WWTPs. Two examples that are paving the way for the evolution of WWTP schemes include one in Athens (Greece), which uses thermal hydrolyzation of SS before anaerobic digestion, and a WWTP in Carbonera (Italy), which is equipped with a biological phosphorous removal system that allows P-recovery as struvite. In fact, the WWTP concept is gradually changing from a simple treatment plant to a biorefinery, which is defined as "a network of facilities that integrates biomass conversion processes and equipment to produce biofuels, energy and chemicals from biomass" (Moncada et al., 2016). This means that the future of WWTPs is not only wastewater purification and waste management, but also a model for circular economy and sustainable agriculture.

1.5. Release of nutrients in soil

The release of nutrients after a SS application on soil depends on many factors: type of nutrient, quantity and quality of SS, soil characteristics (texture, the quantity of OM, pH, etc.), water and oxygen availability in soil, soil temperatures, rate of microbial activity, etc. In order to better understand which are the actions that occur in soil when SS is applied, it will be briefly explained the nitrogen and phosphorus cycles in soil.

Nitrogen cycle is very complex precisely because the nitrogen atom can become part of a large number of molecules: molecular nitrogen, ammonia and ammonium salts, nitrites, nitrates and organic nitrogen (the most diffused form in soil). The chemical processes involved in their formation can be divided into six types: ammonification, nitrification, denitrification, mineralization, immobilization and volatilization.

Ammonification is the conversion of organic nitrogen into ammonium by specific decomposing bacteria and fungi that, by degrading amminic nitrogen, release ammonia in the soil, where it can react with different compounds to form ammonium salts.

The soil ammonia molecules can be oxidized to nitrite and then to nitrate by free bacteria and archaea, with the process called nitrification. Firstly, ammonia-oxidizing bacteria (mainly Nitrosomonas Spp.) and ammonia-oxidizing archaea oxidize ammonia to nitrite (NO_2) and then bacteria such as Nitrobacter Spp. Nitrospira Spp. can oxidize nitrite to nitrate (NO_3^-). It is important to underline that nitrate is readily available by plants, but not for nitrite that can easily percolate into groundwater by leaching.

Differently, denitrification consists in the process of reduction of nitrate to gaseous nitrogen (usually N_2O and N_2) that returns to the atmosphere by closing the nitrogen cycle. This process is extremely rapid, and it is made mainly from bacteria of Pseudomonas Spp. and Clostridium Spp. under anaerobic conditions (poorly aerated soils and waterlogged soils). Denitrification is a form of anaerobic breathing that uses nitrate as an electron accepter in the absence of oxygen. Studies revealed that denitrification can be the major cause of nitrogen loss after SS application (Epstein, 2001).

The combination of ammonification and nitrification is called mineralization: organic nitrogen is firstly converted in NH_4^+ (ammonification), secondly NH_4^+ is oxidized to NO_2 and, thirdly, NO_2 is oxidized in NO_3^-(nitrification).

Nitrogen immobilization is the process in which nitrogen is temporally captured by soils microorganisms. These incorporate it as a protein and creates a stock of nitrogen that will be release (and disponible for plant nutrition) after their decomposition.

Ammonia can be lost in the air by a process called volatilization. It is a very diffused phenomenon that can loss most of $N\text{-}NH_4$ applied to soil, causing air pollution and nitrogen loss in soil at meantime. A study revealed that the 85% of $N\text{-}NH_4$ contained in a dewatered SS was lost in the first 3 weeks by volatilization (Robinson and Polglase, 2000).

Furthermore, in nitrogen cycle in soil is important to mention the nitrogen fixation that is a process by which the air molecular nitrogen (N_2) is converted into ammonia (NH_3) by means of bacteria such as Azotobacter, Clostridium, Rhizobium, etc.

Phosphorus availability in soil is normally low, in fact, P deficiency has been indicated as one of the most important soil fertility problems of the world (Weil and Brady, 2017). In soil this element is present as phosphates ions and the most easily accessible forms of phosphorus from plants are orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) whose availability depends on the pH of the soil. Concerning plant nutrition, it is possible to divide phosphates in three fractions: soluble phosphates, labile phosphates and non-labile phosphates.

Soluble phosphates include dissolved inorganic phosphorus in water/soil solution readily available for plant absorption. The P-labile fraction is composed of inorganic phosphorus attached to clay surfaces, iron oxides, aluminum and calcium in the soil. The phosphorus in this pool is released slowly for the absorption of plants. The non-labile pool is composed by mineral phosphorus which in turn is composed of primary and secondary phosphate minerals in the soil. Examples of primary phosphorus minerals include apatite, strengite and variscite. Secondary minerals of phosphorus include calcium, iron and aluminum phosphates. The release of phosphorus from this pool is extremely slow and occurs when the mineral dissolves in soil water.

Processes that increase soil available-P are mineralization, desorption, dissolution and weathering; on the contrary, processes that decrease plant available-P are immobilization, adsorption, precipitation, leaching, runoff and erosion.

Mineralization is the process in which soil organic-P is converted into inorganic-P by means of microbial. Immobilization, on the other hand, is the opposite of mineralization. During immobilization, inorganic phosphorus forms are converted into organic forms and absorbed into the living cells of soil microbes.

Desorption is a process in which the adsorbed-P (bind on clay surfaces or on the oxides and hydroxides of iron and aluminum) is released into the soil solution. On the contrary, adsorption is a process in which the phosphorus present in the soil solution is attached/tied to the surface of the soil particles.

Dissolution is a process of mineral alteration and appears when phosphate minerals dissolve and release phosphate into the soil solution. Precipitation, on the other hand, is a process in which metal ions such as Al^{3+} and Fe^{3+} (in acidic soils) and Ca^{2+} (in basic soils) react with the phosphate ions present in the soil solution forming aluminum, iron or calcium phosphates.

Weathering is a process in which through the slow chemical, physical and biological degradation of primary P-minerals (apatite), phosphorus became available for plant nutrition. Differently, in erosion and runoff phenomena the available-P is lost because of superficial flow of water. Finally, also in leaching process the available-P present in soil solution is translocated into groundwater and lost (causing eutrophication).

The phosphorus cycle in soil and its availability will be deeply investigated in Chapter IV.

1.6. Aim and structure of the work

Aim of the work is to understand if SSADs can effectively combat desertification against soil chemical degradation and soil organic matter depletion. Two strategies of the reuse of SSADs will be evaluated: land application of the entire SSAD and the extraction of some added-value compounds. Finally, the goal of the present study is to define if, and with which practice, it is possible to fight desertification with the use of SSADs. In the next chapters, the report of the experiment results will enable us to answer all these questions at the end of the work.

In Chapter II are exposed preliminary works that allowed to test the direct use of SSAD on poor soils. From the results obtained, it was possible to perform other experiments which will be presented in Chapter III. After having conducted these experiments, with all limitations and conditions that will be explained in that chapter, it was possible to affirm that the direct use of SSAD can combat desertification. From results obtained by the prementioned researches emerges the importance of phosphorus as a nutrient, and, for this reason, in Chapter IV it was investigate the phosphorus release in soil. After that, Chapter V illustrates possible reuse of an added value compound derived from SSAD: it will be proposed the formulation of a biostimulant derived from humic acids extracted from SSAD. Finally, in the conclusions, are exposed and summarized the principal results that emerged from these studies.

The thesis is mainly based on the three following papers and to the following book chapter:

- Cristina, G., Camelin, E., Pugliese, M., Tommasi, T., & Fino, D. (2019). Evaluation of anaerobic digestates from sewage sludge as a potential solution for improvement of soil fertility. Waste Management, 99, 122-134; DOI:10.1016/j.wasman.2019.08.018.
- Cristina, G., Camelin, E., Ottone, C., Garofalo, S. F., Jorquera, L., Castro, M., Fino, D., Schiappacasse M.C. & Tommasi, T. (2020). Recovery of humic acids from anaerobic sewage sludge: Extraction, characterization and encapsulation in alginate beads. International Journal of Biological Macromolecules, 164, 277-285; DOI:10.1016/j.ijbiomac.2020.07.097.
- Cristina, G., Camelin, E., Tommasi, T., Fino, D., & Pugliese, M. (2020). Anaerobic digestates from sewage sludge used as fertilizer on a poor alkaline sandy soil and on a peat substrate: Effects on tomato plants growth and on soil properties. Journal of Environmental Management, 269, 110767; DOI:10.1016/j.jenvman.2020.110767.
- Camelin, E., Cristina, G., Simelton, E., Fino, D. & Tommasi, T., (2021) The bioenergy-fertilizer nexus: a challenge achievable from municipal wastewater, UNIPA SPRINGER SERIES, in: Innovations in land, water and energy for Vietnam's sustainable development; Springer International Publishing, ISBN:978-3-030-51259-0; DOI:10.1007/978-3-030-51260-6_12.

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Chapter II

2. Preliminary studies on the effects of SSADs on plants

2.1. Introduction

This chapter represents the first part of the demonstration of the possible reuse of SSADs on soils in order to combat the desertification phenomenon. After listing in chapter I all physical, chemical and biological qualities (and problems) of SSADs, in this, and in the subsequent chapter, the possible beneficial use of SSADs will be verified. For the confirmation of positive effects, it was first necessary to characterize SSAD and then to test it on soil and plants. Hence, it was found four different kinds of SSADs with the purpose of also comparing different typologies of digestates deriving from different treatments of wastewater. Results and considerations obtained in previous experiments were used as a starting point for this work. For example, the choice of the substrates and of the species used in these two chapters derives from results obtained in other works carried out in my Ph.D. career and here not reported. With the purpose of demonstrating the possible beneficial use of SSADs, in this work it was evaluated the eventual fertilizing or phytotoxic effect of mixing SSADs with different types of substrates, growing plants on it. The parameters that will be evaluated in this chapter (and compared to a non-treated control) are mainly physiological. In order to provide a more detailed and completed overview, not only dry biomass was evaluated, but, for example, also chlorophyll content in leaves (CCI) was estimated. Undoubtedly, many are the external conditions that can have an impact on this typology of experiments: for this reason, it was chosen to maintain constants many parameters such as temperature, soil water content, illumination, etc. The selected variables in this experiment were the four typologies of SSADs used, two substrates (sand and peat) and concentrations of SSADs added to the soil. The main outcome of this study is thus the understanding of the reuse dynamics of this waste of our society, which is increasing more and more over the years (Hong et al., 2009; Mateo-Sagasta et al., 2015). This work represents the first example of a comparison of the fertilizing effects between two liquid SSADs (derived from separated anaerobic digestion of primary and secondary sludges) and two dewatered ones (centrifuged and dried SSADs) deriving from the same WWTP. Hence, as final outcomes, after statistical data analysis and interpretation of the results obtained, it is expected to determine: firstly, if SSADs have positive or phytotoxic effects on plants growth, secondly, which is the best type SSAD for plant growth and at which concentration, thirdly, if there are differences in effects between the application of digestates on the different substrates. It is essential to underline that, positive results with the sandy alkaline soil represent an excellent response to the question: "SSADs can contrast desertification?" Indeed, this kind of soil, thanks to its physical and chemical characteristics (paragraph 2.3.2.), is an exemplification of degraded soil under desertification process due to chemical degradation. Finally, the results of this work highlight the benefits that may derive from the application of SSADs on nutrient-deficient soils. To a brother extent, this approach could be not only a way to recycle SSAD, but also represent a potential solution to combat desertification.

2.2. Material and methods

2.2.1. Origin and characterization of digestates

Anaerobic digestates used in this study came from a large-scale wastewater treatment plant (3,800,000 population equivalents) located in north-west Italy. Four different types of digestates were used: a primary liquid digestate (P), a secondary liquid digestate (S), a centrifuged solid digestate (C) derived from a mix between P (55%) and S (45%) and a dried pulverulent digestate (D), obtained by the thermal treatment at 200°C of C (**Figure 4**). In the WWTP that produced these SSADs, the wastewater treatment process includes four steps: preliminary, primary, secondary and tertiary treatments. Preliminary treatments consist of the removal of sand, oil and coarse material. Primary treatment consists of primary sedimentation in 8 circular basins of 52 meters of diameter and 2.4 hours of average retention time. As a result, the primary sludge is produced. Secondary treatments include denitrification (12 rectangular basins; total volume: of 110000 m^3), biological oxidation (24 rectangular basins; total volume: 210000 m^3; average time of retention: 5.1 hours) and sedimentation (24 circular basins of 54 meters of diameter; total volume: 175600 m^3); thus, the secondary sludge is obtained. In this WWTP, tertiary treatments are performed with chemical phosphorus removal, chlorination and filtration prior to the release of clean water in the Po river. The obtained sludges undergo sewage sludge treatment processes, which include AD and dewatering. In order to activate the thermophilic anaerobic bacteria, primary and secondary sludges are thickened and preheated before the fermentation. The AD takes place in 6 full-scale digestors of 26 meters of diameter and 30 meters of height (total volume of 72000 m^3) with an average retention time of 15-20 days at 37-40°C. To reach higher biogas production (40000 Nm^3/d), the anaerobic digestion of primary and secondary sludges is performed separately. After AD, primary (P) and secondary (S) digestates are thickened and mixed. After the addition of a polyelectrolyte, the new mix is dewatered with drum centrifuges until reaching 25-27% of dry matter. The new solid is the centrifuged SSAD (C) used in this thesis. A part of centrifuged SSAD is dried in paddle driers at 200°C for 6 hours until reaching 91% of dry matter. In this way, the dried digestate (D) is obtained.

After the sampling from WWTP, digestates were stored at 4°C until use and characterized. The chemical analyses were performed according to “Analytical Methods for Fertilizers” by the Italian Ministry of Agriculture and Forestry (M.P.A.A.F., 2006) and “Methods for Analysis of Sewage Sludge by Water Research Institute of National Council of Researches (IRSA-CNR, 1985), unless specified differently. pH and electrical conductivity were measured on distilled water extracts (1:10 m/v) by potentiometry and conductometry, respectively. Dry matter content and humidity were measured by gravimetry, drying the samples at 105°C until constant weight. Ashes were determined with calcination at 550°C for 5 hours. Total organic carbon was evaluated as reported on “Official methods of soil analysis” by the Italian Ministry of Agriculture and Forestry (Italian Ministerial Decree, 1999), exploiting the Walkley-Black method: sample digestion with potassium dichromate and sulphuric acid is followed by titration with iron(II) sulfate heptahydrate. Organic matter content was calculated with the Van Bemmelen conversion factor (1.724) (Pribyl, 2010). Total nitrogen (N_{Tot}) was measured with the Kjeldahl method, which allows to measure both organic and inorganic forms of nitrogen (but not including nitrites and nitrates). Ammonium nitrogen (NH_4^+) was evaluated through distillation with magnesium oxide followed by titration with sulphuric acid, while nitrates ($N\text{-}NO_3^-$) were determined by the means of ionic chromatography. Organic nitrogen (N_{Org}) was then calculated by subtraction: $N_{Org} = N_{Tot} - (N\text{-}NH_4^+)$. Other macronutrients (K and P), micronutrients (Ca, Mg, Na, Fe, Mn, B, Zn) and heavy metals (Pb, Cr, Ni, Cu) were extracted with mineral acid digestion and then analyzed by the means of inductively coupled plasma optical emission spectrometry (ICP-OES). Other contaminants such as Cd and As were extracted with the same digestion protocol, but analyzed with graphite furnace atomic absorption spectroscopy (GF-AAS). Hg was evaluated with hydride generation atomic absorption spectroscopy (HGAAS) after microwave mineralization, while Cr^{6+} was determined by colorimetry after complexation with diphenylcarbazide.

Figure 4. The four SSADs used in this work. **a**: primary SSAD; **b**: secondary SSAD; **c**: centrifuged SSAD; **d**: dried SSAD.

2.2.2. Characterization of substrates

Two different growth substrates were used: a sandy soil and a peat substrate. The soil used in this study was sampled in Grugliasco (TO), Italy (45°03'58.4"N, 7°35'32.9"E). It was collected within 20 and 100 cm depth, sieved at 2 mm and not previously sterilized. Physical and chemical analyses were performed according to the official methods of soil analysis of Italian Ministry of Agriculture and Forestry (Italian Ministerial Decree, 1999), except for available Fe, Mn, Cu and Zn. Stones were evaluated by sieving (2 mm) (Method II.1) while soil texture was determined by granulometry (wet sieve analysis; Method II.6). Measures of pH and electrical conductivity, organic matter, nitrogen forms, and phosphorous were conducted on an aqueous extract obtained following the Sonneveld method (Sonneveld and Voogt, 2009). pH and electrical conductivity were measured by potentiometry (Method III.1) and conductometry (Method IV.1). Organic carbon was measured with the Walkley-Black method (Method VII.3). Organic matter content was calculated with the Van Bemmelen conversion factor (1.724) (Pribyl, 2010). Total nitrogen was measured with the Kjeldahl method (Method XIV.3). Mineral forms of nitrogen were extracted with an aqueous solution of KCl 2M (Method XIV.4); ammonium was measured through distillation (Method XIV.6), while nitrate and nitrite were quantified through continuous flux colorimetry (Griess-Ilosvay reaction; Method XIV.12 and XIV.13). Organic nitrogen and C/N ratio were obtained by calculation. Available phosphorous was determined by Olsen method (Method XV.3). Measures of cation exchange capacity (C.E.C.) and exchangeable bases (Na, K, Mg, Ca) were performed on an extract obtained with an aqueous solution of BaCl2 – triethanolamine (pH 8.2); C.E.C. was determined through complexometric titration (Method XIII.2) while exchangeable bases were measured by the means of flame atomic absorption spectroscopy (FAAS) (Method XIII.5). Analysis of available Fe, Mn, Cu and Zn was performed according to Italian Ministerial Decree (Italian Ministerial Decree, 1992) Method 37, which exploits the Lindsay-Norwell method, that is an extraction through an aqueous solution of DTPA, CaCl2 and triethanolamine (pH 7.3) followed by quantification with flame atomic absorption spectroscopy (FAAS).

Differently, peat substrate was mixed with perlite and then sterilized before each application. Chemical characterization of peat substrate was performed on an aqueous extract 1:2 (v/v water/peat substrate) according to Sonneveld method (Sonneveld and van den Ende, 1971). The analytical methods for peat analysis were all internal methods. pH and electrical conductivity were measured by potentiometry and conductometry. Total Nitrogen was evaluated with Kjeldahl method while organic nitrogen was calculated. Inorganic forms of nitrogen (ammonium, nitrite, nitrate) were measured by colorimetry (indophenol-blue method, diazotization method and dimethylphenol method respectively). Phosphorous were measured by colorimetry (molybdovanadate method). K, Mg, Ca, Na, Fe, Mn, Cu and Zn were measured through flame atomic absorption spectroscopy (FAAS).

2.2.3. Experimental set-up

2.2.3.1. Climatic chamber

The experiment took place in a climate chamber (**Figure 5**) with controllable photoperiod and temperature, which were set at 28°C for 14 hours during the day (07:00 - 21:00) and to 20°C for 10 hours during the night (21:00 - 07:00). During the first week after sowing, shoots were irrigated from the top one time a day; after this time water level in flowerpot saucer was kept constantly between 1 and 3 cm for the purpose of guarantee always water availability. Commercial plastic pots were used with a total volume of 1250 cm^3 and a surface area of 144 cm^2; consequently, each pot was filled with approximately 250 g of peat substrate and 2000 g of sandy soil. Ten not treated seeds of cucumber (*Cucumis sativus* L.), cv. Marketmore (Four company, Italy) were sown in each pot. The experimental trials lasted thirty days. The position of all plants in the cell was changed every week to minimize location effects. The cultivations on peat substrate and on sandy soil were performed by using the substrate mixed with different treatments: four types of anaerobic digestates from sewage sludge (P, S, C, D), one commercial fertilizer (M) (NPK 22-5-6 + 2MgO, “Osmocote Topdress”, ICL, Israel) and one not treated control (T). With the exclusion of T, all treatments were tested at three increasing doses (85, 170, 255 kg N/ha and they will be called as mentioned above), with four replicates per each. The intermediate nitrogen dosage was selected according to the Nitrates Directive which allows to apply yearly at least 170 kg N/ha of livestock manure to the nitrate vulnerable zones (ZVN) (Council of the European Communities, 1991). As lowest dosage was selected the half of 170 kg N/ha (85 kg N/ha). As highest dosage was selected 255 kg N/ha in order to keep the same difference between the application rates (85 + 85 + 85 kg N/ha).

Figure 5. A detail of the climatic chamber 5 days after sowing cucumber seeds in pots filled with the sandy soil.

2.2.3.2. Measures

Physiological measures were performed on tomato leaves using an Infrared Gas Analyzer (IRGA, ADC, Hoddesdon, UK). This is an instrument that allows to measure, in real time and in a non-destructive way for the plant, the gaseous exchanges that occur inside the leaf. Basically, it is constituted by an infra-red source, a gas chamber, a detector and gas filters (**Figure 6.a**). It measures the reduction in transmission of infra-red wavebands caused by the presence of gas in the chamber. The reduction in transmission is a function of the concentration of the gas. In this thesis it was used to record two days before the end of the experiment the following parameters: Assimilation (A_N), stomatal conductance (g_s) and CO_2 concentration in substomatal cavity (C_i).

A_N represents the net assimilation of CO_2 in a selected area of a single leaf (µmol CO_2 m^{-2} s^{-1}). It is calculated as:

$$A_N = \mu_s * \Delta C$$

where μ_s is the mass flow of air per m^2 of leaf area (mol m^{-2} s^{-1}) and ΔC is the difference in CO_2 concentration through the chamber (µmol mol^{-1}):

$$\Delta C = C_{ref} - C'_{an}.$$ C_{ref} is the CO_2 flowing into the leaf chamber (µmol mol^{-1}) and C'_{an} represents the CO_2 flowing out from the leaf chamber (µmol mol^{-1}).

G_s is the stomatal conductance of water vapor (mol m^{-2} s^{-1}). It is calculated as:

$$G_s = \frac{1}{R_s}$$

R_s represents the stomatal resistance to water vapor (m^2 s^{-1} mol^{-1}) and it is calculated as follows:

$$R_s = \frac{(W_{leaf} - wm_{an})}{\frac{\Delta eu_s}{p}} - r_b$$

W_{leaf} is the saturated water vapor concentration at leaf temperature (mol mol^{-1}) and is calculated as:

$$W_{leaf} = \frac{e_x}{p}$$

e_x is the saturated vapor pressure at leaf surface temperature (mbar); wm_{an} is the water vapor concentration out of leaf chamber (mol mol^{-1}); Δe is the differential water vapor concentration between in and out of the leaf chamber (mbar); u_s is the mass flow of air per m^2 of leaf area (mol m^{-2} s^{-1}); p is the atmospheric pressure (mbar); r_b is the boundary layer resistance to water vapor (m^2 s mol^{-1}).

C_i represents the concentration of CO_2 in the sub-stomatal cavity of the leaf. It is calculated as follows:

$$C_i = \frac{\left(\left(g_c - \frac{E}{2}\right) c'_{an}\right) - A_N}{g_c + \frac{E}{2}}$$

were:

$$g_c = \frac{1}{1.6\, r_s + 1.37\, r_b}$$

E is the transpiration rate (mol m^{-2} s^{-1}) and it derives from:

$$E = \frac{\Delta e\, u_s}{p}$$

Δe is the differential water vapor concentration between in and out of the leaf chamber (mbar); u_s is the mass flow of air into leaf chamber per square meter of leaf area (mol s^{-1} m^{-2}); p is the atmospheric pressure (mbar); c'_{an} is the CO_2 flowing out from leaf chamber (mol mol^{-1}); A_N is the photosynthetic rate of CO_2 exchange in the leaf chamber measured as described before (µmol m^{-2} s^{-1}); r_b is the boundary layer resistance to water vapor (m^2 s^{-1} mol^{-1}); r_s is the stomatal resistance to water vapor (m^2 s^{-1} mol^{-1})

These measures were performed on three leaves of each sample treated with the 170 kg N/ha dosage. The selected leaves were the second or the third from the top and they were the best developed and directly exposed to artificial light.

The day before the end of the test, Chlorophyll Content Index (CCI) was evaluated with a chlorophyll content meter (CCM-200, Opti Sciences, Inc., Hudson, NH, USA) (**Figure 6.b**). In fact, chlorophyll has distinct optical absorbance characteristics that the CCM-200 exploits to non-destructively measure relative chlorophyll concentrations. By measuring the amount of energy absorbed in the red band (653 nm) an estimate of the amount of chlorophyll present in the tissue is given. Absorbance in the infrared band (931 nm) can be used to quantify and account for leaf thickness, so providing a more accurate CCI value. The CCI is automatically calculated by the CCM using the following formula:

$$CCI = \frac{\%\, trasmittance\ \ at\ 931\, nm}{\%\, trasmittance\ \ at\ 653\, nm}$$

After the ordinary calibration, it was used on 5 different fully formed leaves per pot. CCI was used as an indicator of the healthy state and the photosynthetic potentiality of plants; to compare values obtained with results of studies that used SPAD-meter, the equations proposed by Parry and colleagues (Parry et al., 2014) were considered.

At the end of the experiment, all plants were cut and immediately weighed to measure the fresh biomass of single pots (replicates). Determination of dry biomass was carried out weighting these samples after thermal treatment (105°C for 72 hours). In order to compare the yields of each treatment, dry biomass ratio was calculated as ratio between the mean dry biomass of each treatment and control. Besides the related-to-control biomass values, even absolute dry biomasses were analyzed and compared. Per each concentration, each treatment was compared to the other ones, including the control.

Root Development Index (RDI) was assigned with a newly proposed method for the evaluation of root apparatus. This index is based on the soil compactness and cohesion, and on the coverage intensity by the roots over the pot-shaped soil. A score between 0 (no developed) and 4 (very well developed) was given to the apparent root expansion, inspecting the upside-down soil contained in each pot.

Figure 6. On the left side the Infrared Gas Analyzer (IRGA); On the right side the Chlorophyll Content Meter (CCM-200).

2.2.3.3. Statistical analysis

All data about pot phytotoxicity experiment with cucumber were analyzed by one-way ANOVA with a Tukey’s post-hoc test ($P \leq 0.05$), after the assessment of the fundamental assumptions of ANOVA: the normality of distributions (Shapiro-Wilk test, p-value > 0.05) and the homogeneity of the variances of the residuals (Levene’s test with $P(>F) > 0.05$). The statistical software R (version 3.5.1 - Feather Spray - 2018) was used for all statistical analysis.

2.3. Results and discussion

2.3.1. Sewage sludge analyses

Results of characterization of the digestates are shown in **Table 3**. Dry matter content in liquid digestates was 4.4% and 4.8% (for P and S, respectively), while it reached 25.8% and 88.8% (for C and D, respectively) after dewatering processes. pH decreased throughout the different digestates from 7.7 to 6.8. High was the content of total nitrogen that ranged from 7.5% (S) to 5% (D), while the mean content of SSADs in literature was estimated at 3.7% **(Table 1)**. Usually, secondary sludges have a higher content of nitrogen than primaries sludges (Gianico et al., 2013; Gurjar and Tyagi, 2017). In this work, they have comparable percentages (even if the statistical analysis has not been done) probably because of denitrification treatment of secondary sludge (paragraph 2.2.1.). The higher content in liquid digestates of NH_4^+ (up to six times higher in liquid than in solid SSADs), can represent a potential problem for their land application because a high content can be volatilized or leached and consequently lost after land application: this potentially dangerous characteristic will be considered more in-depth in the next chapter. Plant macronutrients such P and K had opposite behaviors: the first one showed appreciable concentrations, with a growing trend from liquid to solid digestates (4.16 to 6.26%); the latter revealed a decreasing trend from liquid to solid digestates (0.55 to 0.18%). P content results higher in the secondary digestate than in the primary one: this issue is well-known in literature (Gianico et al., 2013; Gurjar and Tyagi, 2017). Furthermore, P content in digestates was in all cases higher than the mean value found in other studies on SSADs (2.2%) and comparable to the content of P in some NPK fertilizers. On the contrary, despite K levels were a little bit low if compared to other studies (0.5%), the applied dosage in this work is sufficient for the early growth stages of plants. However, this aspect can negatively affect the proper potassium supply when SSAD is applied as fertilizer, especially in the phase of fruit maturation (Hawkesford et al., 2012). High values of organic matter were found in all digestates (>63.9%) and no consistent variation in organic matter levels were observed through the four digestates; as a consequence, C/N ratio increased from liquid to dewatered SSADs. It will be interesting to deeper analyze the OM: in fact, other works sustain that organic contaminants and pharmaceutical are lower in secondary sludges due to the higher biological degradation (Gianico et al., 2013; Katsoyiannis and Samara, 2005; Radjenović et al., 2009). Meso- and micronutrients (Ca, Mg, B, Zn) and some metals (Na, Cd, Ni, As) exhibited decreasing concentrations from liquid to solid digestates; the only metals which showed a diametrically opposed behavior were Fe and Cu. Lower content of HM was found in secondary SSAD if compared to the primary SSAD: this is in line with other literature works (Alvarez et al., 2002; Gianico et al., 2013) (paragraph 1.3.8.). Surely, one of the main disadvantages of these digestates revealed by this analysis was the presence of heavy metals. Despite all the analyzed ones complied with the limits imposed by the Italian Law on Sewage Sludge Land Application (Italian Decree Law 99/1992, 1992), in some cases (i.e. Zn, Cu and Ni) the thresholds imposed by Italian Discipline on Fertilizers (Italian Decree Law 75/2010, 2010) were overcome. This means that these SSADs cannot be considered as fertilizers (for the Italian law), but they can be used on land application. Moreover, it is important to note that heavy metals concentrations were generally lower than other studies on SSADs (**Table 1**). With the purpose of combating soil chemical degradation, these digestates appear with the correct chemical characteristics: high content of the fertilizer elements (especially in P and N), presence of other macro and micro-nutrients, and, high content of organic matter.

Parameter	Unit of measure	Anaerobic digestates				Italian threshold for SS agricultural use (D. Lgs 99/92)	Italian threshold of fertilizers (D.Lgs 75/10)
		Primary (P)	Secondary (S)	Centrifuged (C)	Dried (D)		
pH (1:10)		7.7	7.5	7.3	6.8		
E.C.	mS/cm	0.378	0.36	1069	1.575		
N - Tot (Kjeldahl)	% d.m.b.	7.4	7.5	6.3	5	>1.5	
N - Org	% d.m.b.	5.84	6.16	5.33	4.75		
N - NO_3^-	% d.m.b.	<0.01	<0.01	<0.01	<0.01		
N - NH_4^+	% d.m.b.	1.56	1.34	0.97	0.25		
N - org / N - Tot	%	79	82	84	94		
Dry matter	%	4.4	4.8	25.8	88.8		
Humidity	%	95.6	95.2	74.2	11.2		
Organic matter	% d.m.b.	64.7	68.5	63.9	64.4		
TOC	% d.m.b.	37.5	39.7	37.1	37.3	>20	
C/N		5.1	5.3	5.9	7.4		
Ashes	% d.m.b.	35.3	31.5	36.1	35.6		
Ca	% d.m.b.	6.46	4.69	5.02	4.64		
Mg	% d.m.b.	1.78	1.53	1.45	1.16		
Na	% d.m.b.	1.05	1.03	0.34	0.19		
K	% d.m.b.	0.55	0.69	0.39	0.18		
P	% d.m.b.	4.16	5.75	6.74	6.26	>0.4	
Fe	% d.m.b.	2.43	3.32	3.99	3.48		
Mn	mg/kg d.m.b.	255	190	268	228		
Cu	mg/kg d.m.b.	357	340	406	396	1000	230
Zn	mg/kg d.m.b.	918	650	849	719	2500	500
B	mg/kg d.m.b.	51	60	52	41		
Pb	mg/kg d.m.b.	92	70	92	79	750	140
Cr	mg/kg d.m.b.	245	210	245	217	*<200**	
Cd	mg/kg d.m.b.	1	0.6	0.8	<0.1	20	1.5
Ni	mg/kg d.m.b.	163	120	155	137	300	100
As	mg/kg d.m.b.	2.8	2.1	0.9	<0.1	*<20**	
Hg	mg/kg d.m.b.	<0.1	<0.1	<0.1	<0.1	10	1.5
Cr^{6+}	mg/kg d.m.b.	<0.1	<0.1	<0.1	<0.1	*<2**	0.5

** Values introduced with Italian L. 130/18 (Italian Law 130/2018, 2018)*

Table 3. Physicochemical properties of the four anaerobic digestates from sewage sludge used in this work; last two columns on right specify Italian law limits for Land application of sewage sludges (Italian Decree Law 99/1992, 1992) and Italian law limits for heavy metals in fertilizers (Italian Decree Law 75/2010, 2010). d.m.b.: dry matter basis; E.C.: Electrical conductivity; TOC: Total Organic Carbon.

2.3.2. Soil analyses

Physical and chemical soil properties (**Table 4**) were measured before the application of treatments.

Based on the distribution of the particle size (sand: 94% ± 2; silt: 3% ± 1; clay: 3% ± 1), the selected soil was classified as sandy (Buol et al., 2011). Based on ARPAV soil analysis (Arpa Veneto, 2007), the soil was considered alkaline (8.2 ± 0.16), very poor in OM (0.38 ± 0.12% < 0.8%) and very poor in macronutrients such as nitrogen (0.29 ± 0.09 g/kg < 0.5 g/kg), phosphorous (1.8 ± 1.3 mg/kg < 7 mg/kg), potassium (18 ± 1 mg/kg < 40 mg/kg) and magnesium (15 ± 5 mg/kg < 50 mg/kg). On the other hand, content of calcium (675 ± 27 mg/kg < 1000 mg/kg) and some microelements such as iron (2.5 mg/kg < 6.7 ± 1.1mg/kg < mg/kg 20) and manganese (2 mg/kg < 6.5 ± 3.0 mg/kg < 10 mg/kg) resulted normal. Due to the poorness in macronutrients and organic matter this soil was selected for this experiment: it can perfectly represent a chemically degraded soil.

The peat substrate used consisted of a commercial blend of blond and black peat (15:85, Turco Silvestro, Italy), mixed with perlite (80:20 v/v). The substrate was steamed at 90°C for 30 minutes before use. From chemical analysis resulted that the used peat substrate had an acid pH (6.2 ± 0.1), higher electrical conductivity than the sandy soil (0.722 ± 0.146 dS/m > 0.131 ± 0.018 dS/m), and, in most of the cases it was richer in terms of macro and micronutrients. Surely, the most important characteristic of peat substrate was the high content in terms of organic matter. This data was provided by the producer and it reaches 32% d.m.b. Hence, the peat substrate could be reasonably considered a good cultivation substrate, satisfying the requirements as a benchmark to be compared with the poor sandy soil.

Sandy soil			Peat substrate		
Parameter	**Unit**	**Value**	**Parameter**	**Unit**	**Value**
Stones	-	absent	**Stones**		-
Sand (2.0 - 0.020 mm)	%	94 ± 2	**Sand (2.0 - 0.020 mm)**		-
Silt (0.020 - 0.002 mm)	%	3 ± 1	**Silt (0.020 - 0.002 mm)**		-
Clay (< 0.002 mm)	%	3 ± 1	**Clay (< 0.002 mm)**		-
Texture	-	sandy	**Texture**		-
pH	-	8.2 ± 0.16	**pH**	-	6.2 ± 0.1
Electrical conductivity	dS/m	0.131 ± 0.018	**Electrical conductivity**	dS/m	0.722 ± 0.146
Organic matter	%	0.38 ± 0.12	**Organic matter**		-
Organic carbon	%	0.22 ± 0.07	**Organic carbon**		-
N - Tot (Kjeldahl)	g/kg	0.29 ± 0.09	**N - Tot (Kjeldahl)**	%	0.42 ± 0.06
N - NO_2^-	mg/kg	< 0,2	**N - NO_2^-**	mg/l	< QL
N - NO_3^-	mg/kg	6.33 ± 1.53	**N - NO_3^-**	mg/l	30.4 ± 7.2
N - NH_4^+	mg/kg	3 ± 1	**N - NH_4^+**	mg/l	1.3 ± 0.3
N - Org	g/kg	0.29 ± 0.09	**N - Org**	%	0.4 ± 0.40
C/N		7.6 ± 0.2	**C/N**		-
P - Olsen	mg/kg	1.8 ± 1.3	**P $_{\text{extractable}}$**	mg/l	8.1 ± 2.3
K $_{\text{exchangeable}}$	mg/kg	18 ± 1	**K $_{\text{extractable}}$**	mg/l	41.1 ± 6.8
Mg $_{\text{exchangeable}}$	mg/kg	15 ± 5	**Mg $_{\text{extractable}}$**	mg/l	28 ± 7
Ca $_{\text{exchangeable}}$	mg/kg	675 ± 27	**Ca $_{\text{extractable}}$**	mg/l	36 ± 8
Na $_{\text{exchangeable}}$	mg/kg	6 ± 3	**Na $_{\text{extractable}}$**	mg/l	16 ± 11
Fe $_{\text{available}}$	mg/kg	6.7 ± 1.1	**Fe $_{\text{extractable}}$**	mg/l	0.79 ± 0.21
Mn $_{\text{available}}$	mg/kg	6.5 ± 3.0	**Mn $_{\text{extractable}}$**	mg/l	0.15 ± 0.04
Cu $_{\text{available}}$	mg/kg	0.69 ± 0.29	**Cu $_{\text{extractable}}$**	mg/l	< QL
Zn $_{\text{available}}$	mg/kg	0.47 ± 0.29	**Zn $_{\text{extractable}}$**	mg/l	0.02 ± 0.00
CEC	cmol/kg	3.65 ± 0.35	**CEC**		-

Table 4. Physical and chemical analysis of soil and peat used in the present work. CEC: Cation-Exchange Capacity.

2.3.3. Climatic chamber test

2.3.3.1. Biomass

On sandy soil, all treatments, except for P255, overcame the yields of the control: C255 and D255 were considerably higher than others doubling the control biomass. The increase of biomass production was proportional with the dosages of C and D digestates as well as M; the highest dosage of the last one did not seem to cause further increase. On the other hand, P and S digestates had the highest yields at intermediate dosages (P170 and S170), while dry biomasses at the lowest dosages (P85 and S85) were comparable to the highest ones (P255 and S255) and were not significantly different from control (**Figure 7.a**).

For what concerns the biomass yield on peat substrate, the common biomass trend is an increase going from 85 to 170 kg N/ha dosages, and a decrease moving from 170 to 250 kg N/ha. However, P digestate is the only one displaying decreasing biomass values for higher application rate of treatment. The highest biomass yield was found in C170, even doubling the control one. Moreover, P85, S170 and S255 were the only ones showing a significantly higher biomass than control. (**Figure 7.b**). Very important differences were found in 170 kg N/ha treatments: all yielded significantly more biomass (1.10 to 1.21 g) than the control (0.75 g) on sandy soil (**Figure 8.a**); on peat substrate, P, S and C treatments provided more biomass (2.92 g, 3.61 g and 3.95 g, respectively) than control (2.07 g), with S and C showing the top production, while D (2.82 g) and M (2.51 g) behaved similarly to the control (**Figure 8.b**).

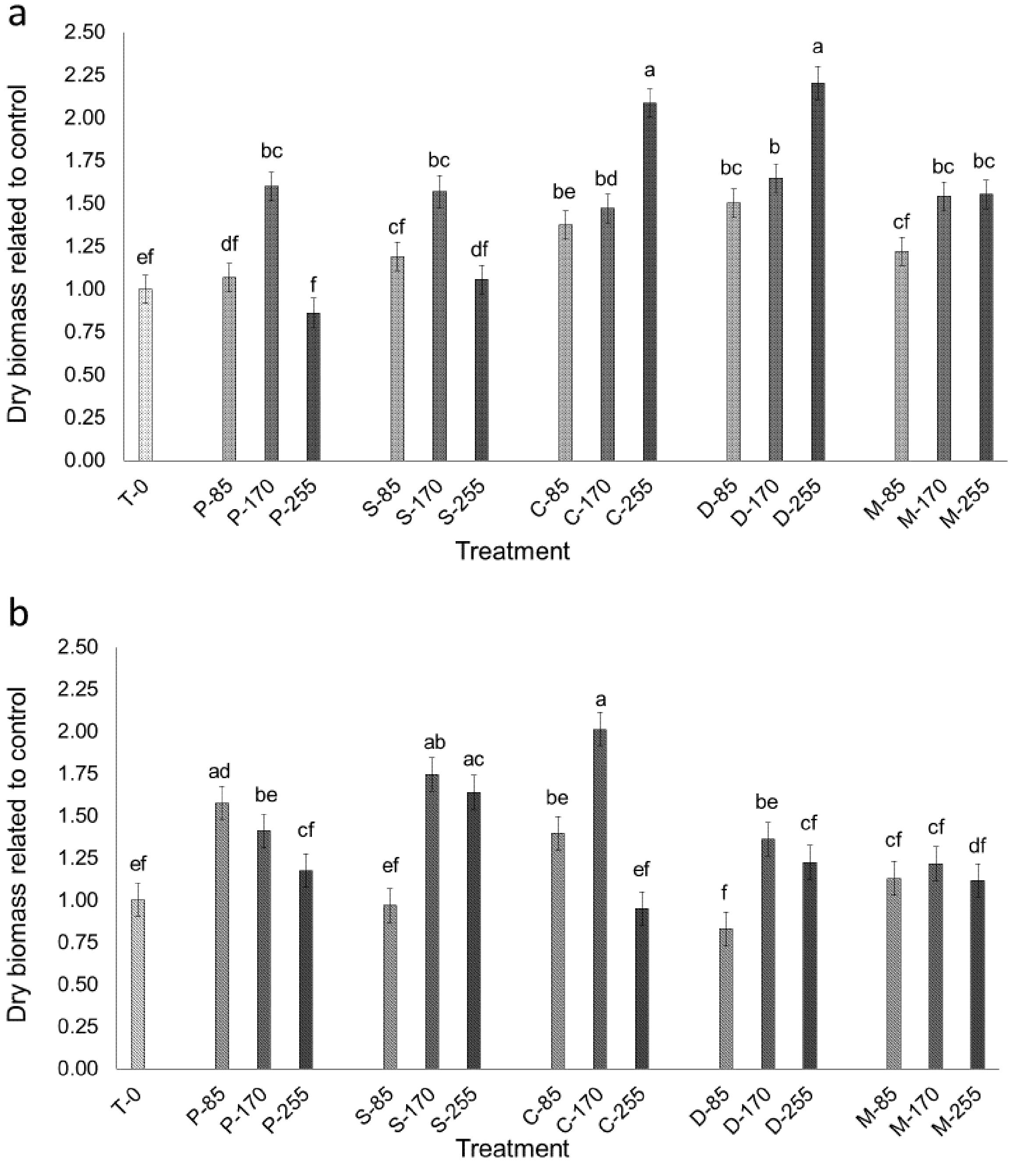


Figure 7. Mean dry biomass related to control of *C. sativus* grown on sandy soil (**a**) and peat substrate (**b**). Each data point represents mean of replicates to mean of control replicates ratio ± standard error;

different letters indicate differences between treatments and concentrations of N that are significant at P < 0.05 (Tukey HSD).

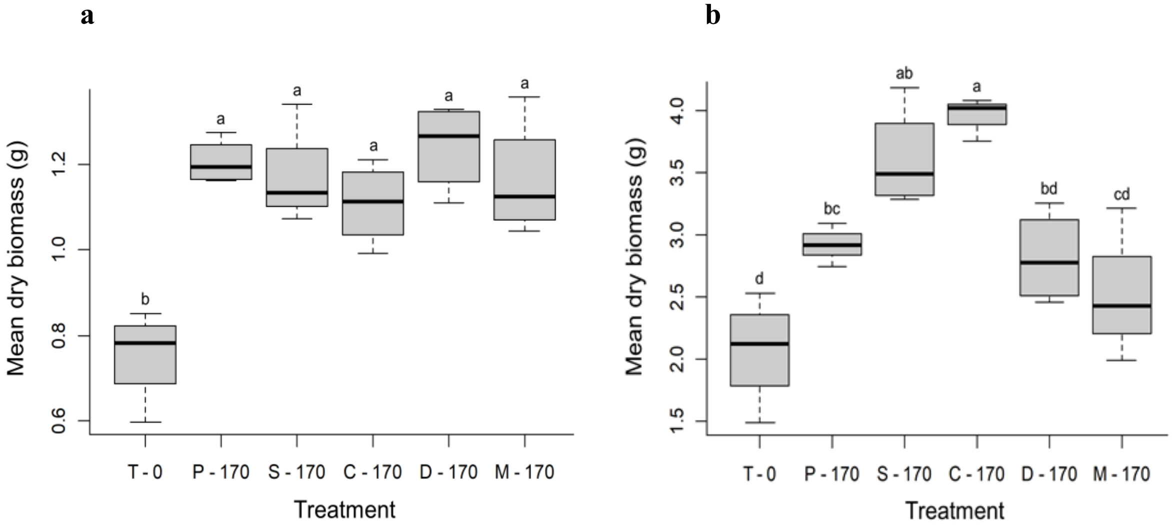


Figure 8. Mean dry biomass of *C. sativus* grown on sandy soil (**a**) and peat substrate (**b**) with 170 kg N/ha treatments. Different letters indicate differences between treatments that are significant at P < 0.05 (Tukey HSD).

The fertilizing effects of the digestates on cucumber were studied in previous works. However, the ones dealing with sewage sludges and derived products are mostly focused on the toxic effects derived from organic and inorganic pollutants present in this waste (Waqas et al., 2014; Wyrwicka et al., 2014). In this experiment, higher biomass yields were recorded for the plants grown on peat substrate than on sandy soil due to the richness in organic matter and macronutrients of the first one. Nevertheless, this aspect likely contributed to the lower degree of differences between control and treated samples; indeed, all treatments on sandy soil at 170 kg N ha^{-1} were significantly different from the control, while the same conditions on peat substrate revealed results, for D and M, slightly comparable to T. In general, it could be inferred that fertilizing effects occurred at different levels both in terms of soil and treatment concentration. In fact, dry biomass overcame the control in all cases except four (P255 on sandy soil; S85, C255 and D85 on peat substrate). These biomass-promoting effects on cucumber grown on sandy soil have already been reported by Hussein (2009): despite the higher application rate (up to ten times greater, in terms of total nitrogen), the authors observed a crop yield improvement over control around 70%, which is in good agreement with our results. Moreover, cucumber was utilized to test the effects of sewage sludge compost applied on a sandy soil. Even in this case, the dry weight of shoot biomass almost doubled the control one (Xu et al., 2012), similarly to C255 and D255 conditions on sandy soil of the present study. Moving to a broader perspective, other works designed with a pot experiment approach assessed the fertilizing effect of sewage sludge on different species. Asagi and Ueno (2008) and Shaheen and co-workers

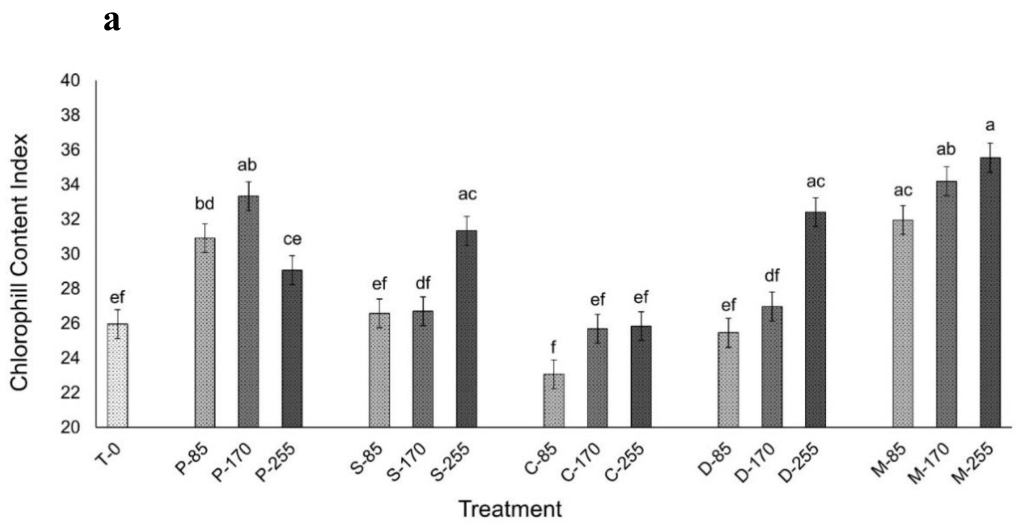
(2014) reported examples of komatsuna (*Brassica rapa* L. var. perviridis) grown on sandy soil, and rocket (*Eruca sativa* Mill.), grown on calcareous soil, which quintupled and doubled their dry biomass yield, respectively. Furthermore, relevant outcomes have been described on sunflower (*Heliantus annuus* L.) (Belhaj et al., 2016) and kenaf (*Hibiscus cannabinus* L.; De Andres et al., 2010) grown in presence of dewatered anaerobic digestates similar to C and D treatments, providing well comparable results with this study. Qasim and colleagues (2001) and Alvarenga and co-workers (2016) provided examples of cereal crops (maize and sorghum, respectively) fertilized with an unstabilized sewage sludge and a yield increase of 40% and 400%, respectively, over untreated control was reported. Even if it's difficult to compare the behavior of different plants exposed to diversely treated sludges, it is conceivable that weaker performances of digestates of this study may be due not only to lower application rates, but also to the nitrogen fractionation. In fact, in the present work, this is skewed in favor of organic nitrogen (N-Org/N-Tot ranging from 79% to 94%), with lower concentrations of "readily-available" nitrogen (i.e. NH^{4+} and NO^{3-}; Paragraph 1.5).

Nevertheless, the main drawbacks of sewage sludge land application are the phytotoxic effects occurring at higher application rates, preventing the optimal growth of the plant. Indeed, this aspect has been deeply investigated as regards the presence of organic and inorganic pollutants, such as heavy metals. These ones can interfere with the biomass yield as widely reported in literature (Nagajyoti et al., 2010; Singh and Agrawal, 2007). In the present work, the decrease of dry weight with higher application rates was observed only in few cases (e.g. P255 and S255 on sandy soil, and P255, C255 and D255 on peat substrate). These reductions can be justified in part with the metal-derived toxicity, especially in the case of peat substrate. Its slightly acidic conditions maybe allowed a more sustained metal bioavailability, which was instead down modulated by high pH in sandy soil (belhaj et al., 2016; Sukreeyapongse et al., 2002). On the other hand, another conceivable hypothesis is the ammonia-connected toxicity occurring in alkaline conditions: increasing soil pH induces a higher NH_3 percentage of total ammoniacal nitrogen (Masoni and Ercoli, 2010), according to the NH^{4+}/NH_3 acid-base equilibrium (Gay and Knowlton, 2009). Thus, at the pH of sandy soil exploited in this work (8.7), around 20-25% of ammoniacal nitrogen is represented by NH_3, which can negatively affect the plant growth under different aspects as described by van der Eerden (1982). This aspect has been observed mainly on plants exposed to liquid digestates, which revealed ammonia-nitrogen concentrations up to six times higher than dewatered ones. On the contrary, dehydration of SSAD might have had a positive effect on the ammonia abatement, which resulted in an overall slighter phytotoxicity exhibited by solid SSADs (C and D, in this study). In this respect, this aspect is confirmed by Alvarenga et al. (2016) and de Andrés Parlorio et al. (2010). Moreover, the last one devoted particular attention to the treatment formulation (pelletization, in this case), which can be an aspect to take into account even for future works.

2.3.3.2. Chlorophyll Content Index (CCI)

CCI control mean level of plants grown on sandy soil was 26.0 (**Figure 9.a**); the chlorophyll concentration significantly higher than control were obtained in M255 (35.5), M170 (34.2), P170 (33.3), D255 (32.4) and M85 (31.95), S255 (31.3) and P85 (30.9). Moreover, the chlorophyll content was higher with the increase of the SSAD application rate. However, this behavior was not detected for P digestate, where the increase of treatment dosage was related firstly to a CCI increment in P170, then to a CCI reduction in P255 (29.1).

On peat substrate **(Figure 9.b),** control mean level of CCI (28.2) was higher than on sandy soil. Similarly to CCI of cucumber grown on sandy soil, mineral fertilizer in M255 (36.4) and M170 (33.3) gave high results and, together with C170 (37.2), were significantly higher than control. Moreover, C170 was significantly different from other dosages within same treatment, while no significative differences among concentrations were found on P, S and D treatments.



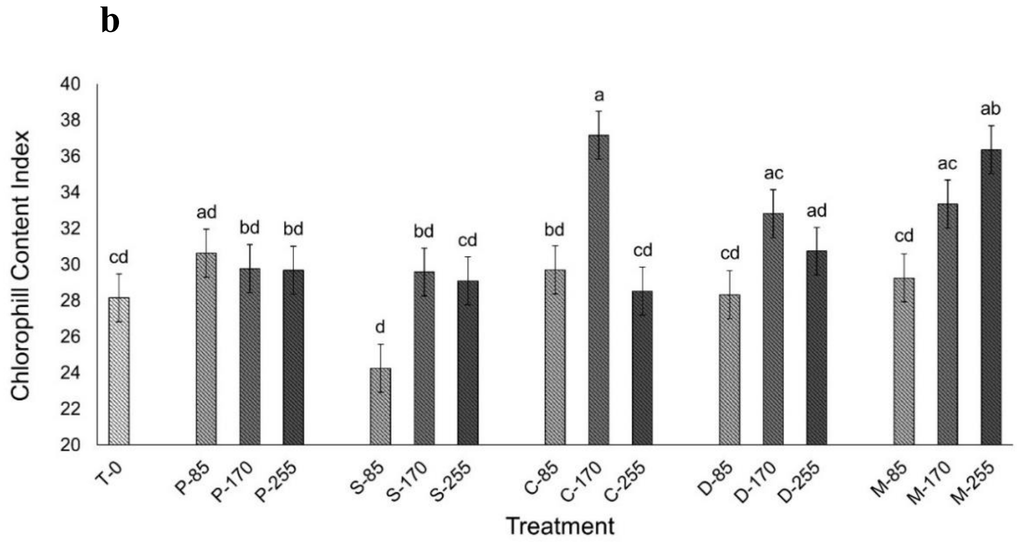


Figure 9. Mean Chlorophyll Content Index (CCI) measured on the leaves of *C. sativus* grown on sandy soil (**a**) and on peat substrate (**b**). Different letters indicate differences between the treatments with the different concentrations of N at 85, 170 and 250 kg N/ha, which are significant at $P < 0.05$ (Tukey HSD).

Chlorophyll content can be strongly correlated to crop nitrogen content and can be sensitive to differential nitrogen nutrition in vegetable crops (Padilla et al., 2017). Nitrogen nutrition index (NNI) is an indicator of plant nitrogen status, and NNI =1 values correspond to optimal N nutrition (Lemaire and Gastal, 1997); in the case of cucumber, it was matched to CCI values between 24 and 36. In the present work, the CCI values obtained on peat substrate were in this range, likely due to the better capacity of peat substrate to retain nutrients, while on sandy soil they were lower. These values are in agreement with the ones reported by Shaaban and El-Bendary (1999), Güler and Büyük (2007), Jahromi et al. (2012) and Xu et al. (2012). High values of CCI did not coincide necessarily to high biomass yields: in fact, on sandy soil C255 had middle-low CCI, but its biomass yield was the highest. Latare and co-workers (2014) reported a similar behavior for wheat and rice, in which yield increase was not accompanied by a significative rise in SPAD values. Moreover, M255 showed the highest CCI value on sandy soil: this result is probably linked to the mineral fertilizer formulation which ensures a long-lasting nitrogen release. Considering the typologies of treatment, many works show a general improvement of CCI values upon application of sewage sludge and its derivatives. Improvements of chlorophyll content compared to untreated controls have been recorded on cereals (Alvarenga et al., 2016; Koutroubas et al., 2014), edible plants (Asagi and Ueno, 2008) and trees (Han et al., 2004). This general behavior indicates that sewage sludge provides a good amount of nutrients, which is an aspect that clearly emerges even in all this work.

2.3.3.3. Infra-Red Gas analyzer (IRGA)

Treated and control cucumber plants grown on sandy soil showed significative differences in Net photosynthesis (A_N): control value (1.83 CO_2 $m^{-2}s^{-1}$) was lower than all other treatments, which however did not differ from each other (**Figure 10.a**). Therefore, it is worth underlining the value measured on P treatment (3.75 µmol CO_2 $m^{-2}s^{-1}$), which doubled control value. In order to stomatal conductance (g_s), all digestate treatments at least doubled the one of control thesis (0.098 mmol H_2O $m^{-2}s^{-1}$), while S even trebled this result (0.333 H_2O $m^{-2}s^{-1}$) (**Figure 10.b**). On the other hand, while M showed an intermediate behavior between digestates and control as regards stomatal conductance, it reached the highest concentration of CO_2 (536 ppm) in substomatal cavity (C_i) (**Figure 10.c**).

Moving to peat substrate, differences in net assimilation of CO_2 (**Figure 10.a**) between treatments and control were few: C (4.83 CO_2 $m^{-2}s^{-1}$) and D (4.78 CO_2 $m^{-2}s^{-1}$) had a higher A_N than all other treatments (including T). However, it is important to point up that only C (0.383 mmol H_2O $m^{-2}s^{-1}$) displayed also a significantly greater value in terms of stomatal conductance (**Figure 10.b**). CO_2 concentration in substomatal cavity revealed two different groups: the first gathering the highest C_i values, S and D (586 ppm), and the second collecting all other treatments (T included), which showed lower results (**Figure 10.c**).

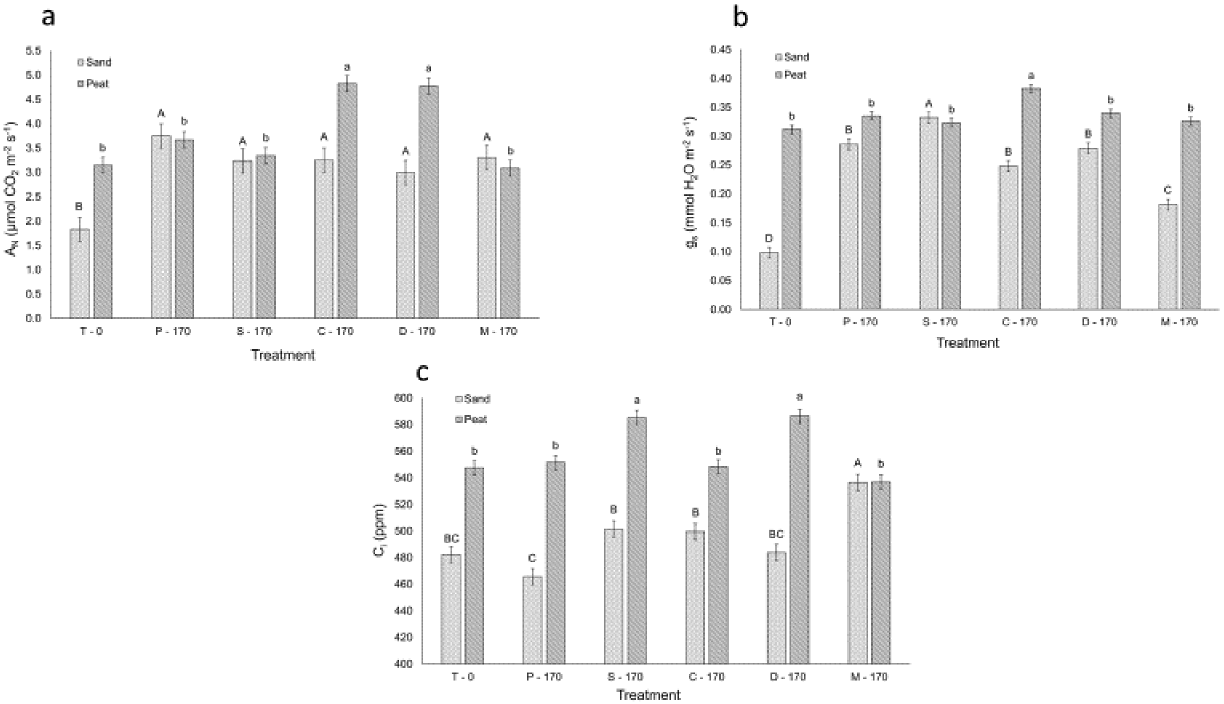


Figure 10. IRGA measurements on *C. sativus* grown on sandy soil and peat substrate with 170 kg N/ha treatments. (**a**): Net assimilation (A_n in µmol CO_2 m^{-2} s^{-1}) ± mean standard error, (**b**) Stomatal conductance (g_s in mmol H_2O m^{-2} s^{-1}) ± mean standard error and (c) CO_2 concentration in substomatal cavity (C_i in ppm) ± mean standard error. Different letters indicate differences between treatments that

are significant at $P < 0.05$ (Tukey HSD); upper-case letters refer to sandy soil and lower-case letters refer to peat substrate.

The results of gas analysis measurements were not directly comparable to other values in the literature because these are strictly depending on environmental conditions (light, temperature, irrigation and phenological phase). On peat substrate, almost no difference was appreciable; just in C case, A_N and g_s values were higher than T; anyway, these differences reflect values obtained in biomasses and CCI measurements. To the best of my knowledge, no measurements of physiologic parameters and gas exchange have been performed on cucumber exposed to sewage sludge treatments with pot experiments. However, some comparisons can be done with studies on physiologic parameters of plants exposed to sewage sludge and studies on physiologic parameters of cucumber. Antolín et al. (2010) and Bourioug et al. (2015) carried out pot experiments with alfalfa (*Medicago sativa* L.) and European larch (*Larix decidua* L.), applying both sewage sludge rates like the ones of this study. The significative differences reported in the case of cucumber grown on sandy soil are in good agreement with A_N and g_s values of the first work, while in the second study only with A_N ones. Furthermore, similar results of A_N and g_s have been assessed using two different dosages of sewage sludge in field on rice crop (*Oryza sativa* L.) (Singh and Agrawal, 2010). On the other hand, studies with sewage sludge on beet (*Beta vulgaris* L.) (Singh and Agrawal, 2007) and okra (*Abelmoschus esculentus* L.) (Singh and Agrawal, 2009) showed lower results in terms of A_N and g_s, probably due to the higher SS doses, provoking phytotoxic effects. Physiologic parameters of cucumber plants were studied mainly as regards metals stress, such as toxicity derived from copper (Alaoui-Sossé et al., 2004) and sodium (Chartzoulakis, 1994): their increasing concentration caused the decrease of the physiologic parameters. Anyway, in the present study, concentrations of copper and sodium were lower and, consequently, A_N and g_s values were higher. Moreover, an increase of stomatal conductance in presence of heavy metals was explained by Singh and Agrawal (2010), claiming that it may be due to high nutrient availability through SS amendment which nullified the heavy metal toxicity.

2.3.3.4. Root Development Index

Root apparatus was mostly developed in plants grown on C and D treatments on sandy soil (**Figure 11**). Indeed, C255 (3.625), D85 (3.375), D255 (3.000), D170 (2.500), C85 (2.500) and C170 (2.375) revealed an RDI significantly higher than control. Data on peat substrate did not respect the homogeneity of variances (P-value = 0.0449) (data not shown).

The trend of biomass production did not match always with a sustained root development (RDI). This mismatch between shoot and roots biomass in cucumber has been already reported in literature (Xu et al., 2012). Root development results clearly revealed that C and D gave best outcomes, with an RDI similar between them and higher than liquid digestates and M. Furthermore, these findings demonstrate that the kind of treatment had a greater effect on roots growth than the nitrogen amount (except for the case of C255). This observation is in contrast to the study of Gulyás and co-workers (2012), which described a root reduction in ryegrass (*Lolium perenne* L.) treated with the same dosages of SSAD, probably due to excessive ammonium content. Despite comparable nitrogen application rate, root development was not inferior than control presumably because of a lower NH^{4+}/N-Tot ratio of the SSADs used in the present experiment.

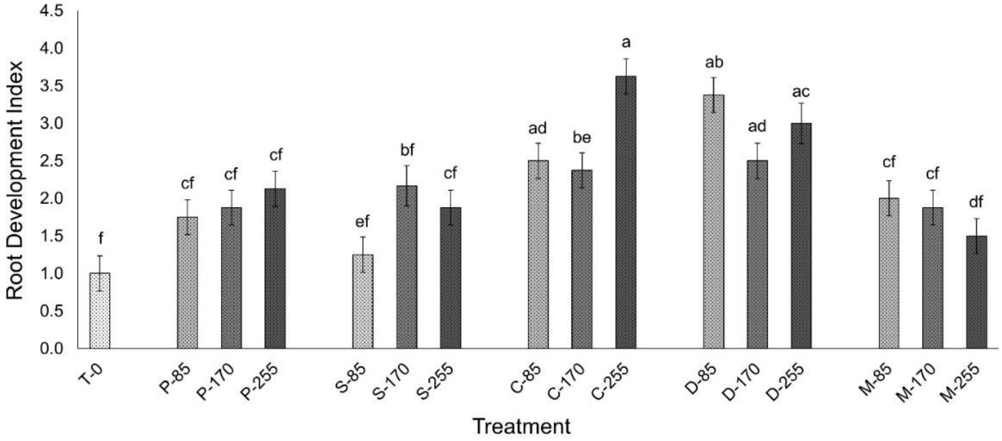


Figure 11. Mean Root Development Index of *C. sativus* grown on sandy soil. Each data point represents mean of replicates to mean of control replicates ratio ± mean standard error; different letters indicate differences between treatments and concentrations of N that are significant at $P < 0.05$ (Tukey HSD).

2.4. Conclusion

Four different SSADs (two liquid and two dewatered) from the same WWTP were characterized and exploited as a soil improver for promoting plant growth in pot experiments. To the best of my knowledge, this is the first study that has conducted a systematic comparison of the fertilizing and phytotoxic effects of anaerobic digestates from primary, secondary, centrifuged and dried sludges. As outlined in Chapter I, the stabilization treatment applied to the sludge (anaerobic digestion) gave new physico-chemical characteristics to the digestate, which are similar to those of fertilizers. These characteristics were confirmed by the analysis performed on the SSADs, which revealed a high presence of N and P and the presence of micronutrients. Furthermore, the differences found between the SSADs are possibly due to how the digestates were treated at WWTP level and had an effect not only on their chemical peculiarities, but also on their agronomic potential. In fact, from the statistical analysis it emerged that, in most cases, solid SSADs (in this case C and D treatments) had better results. Due to the incomplete response, this aspect will be further investigated in the subsequent chapter. The application of SSADs improved plant growth by exploiting the nitrogen dosages commonly used in field operations. In general, the intermediate nitrogen dosage of 170 kg N/ha (the maximum dosage permitted yearly in the nitrate vulnerable zones by the Nitrates Directive (Council of the European Communities, 1991) showed the best results in terms of biomass, chlorophyll content, net photosynthesis, stomatal conductance and root development. All these results were much more evident for cucumber plants grown on alkaline, sandy and poor (concerning organic matter and nutrients) soil than on an acid and rich cultivation substrate, such as peat substrate. However, in some cases phytotoxicity effects occurred, probably due to the excessive presence of ammonia nitrogen and heavy metals (paragraph 2.3.3.1.). This possible phytotoxicity obtained at high dosages confirmed once again that 170 kg N/ha should be considered the highest dosage possible for single applications. The effects revealed in longer periods are also important. In the next chapter, the effects of a single application will be evaluated over a longer time period. Due to the partial results obtained with this experiment, the final judgment about the reuse of SSADs on depleted soils will be given at the end of the next chapter, in which more details will be presented.

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Chapter III

3. Effects of SSAD on soils and plants growth

3.1. Introduction

This chapter has the same main objective of Chapter II: to understand if the direct application of SSADs on soil can be a factor that could contrast some of the desertification causes. Reasons, possibilities, and problems connected with this theme were widely listed and discussed in Chapter I. In this section will be evaluated as exhaustively as possible effects of SSADs on plants and soils. Particular emphasis will be given to the balance of macro-nutrients, micro-nutrients, and organic matter in order to understand if effectively SSADs can combat desertification processes through: the reduction of soil chemical degradation process (Lal, 1990) and, concurrently, through the contrast to the decrease of organic matter (OM) in soils (known as *SOM decline*) (Henry et al., 2018; Lal et al., 2007; Schulze and Freibauer, 2005) (Paragraph 1.2.3.1.). Since the estimated quantity of these substances added with treatments in the soil system was already commented in the previous chapter, in this second part it will be quantified the presence of these substances in soil and in plant systems after a period of controlled environmental conditions. In this way, it will be possible to create a balance of nutrients (with input and output in soil and plant systems) and it will be possible to theoretically estimating the chemical transformations of nutrients (Paragraph 1.5.). Nutrients quantification after a selected period from treatments application is necessary in order to prove if all theoretical bases listed in this thesis are effectively true. With that data, it will be possible to affirm (or in some cases just to suppose) if they nutrients were absorbed by plants, immobilized in soil, lixiviate, free in soil solution etc. If at the end of experiment some parameter (such as OM) will be improved, then the thesis of the contrast to desertification with SSADs could be deemed acceptable, but valid just for the conditions applied. Concerning the selected experimental set-up, in order to see effects on larger time it was chosen a longer trial (3 months) and, with the purpose of being a more realistic experiment, were chosen less controlled environmental conditions with a greenhouse instead of climatic chamber as location for the experiment. In order to be in continuity with the previous test, the same soil typologies were chosen: a poor alkaline sandy soil compared to a rich peat substrate. This alkaline sandy soil was selected because: i) nutrient depletion constrains plant growth to depend on treatment application; ii) a high pH both hinders the nutrient adsorption and reduce the metal bioavailability (Alvarenga et al., 2016); iii) sandy-textured soil lacks nutrients and has low water-holding capacity. Regarding the selection of the plant for the experiment, *Solanum Lycopersicum* L. was chosen because: i) it is one of the most exploited vegetables crop (Jones Jr, 2008); ii) there is an increasing interest on alternative nutrient sources for this crop (Zucco et al., 2015); iii) it has a high fertilizer requirements (Zucco et al., 2015); iv) plenty of scientific literature is available for this crop (Jones Jr, 2008). In this work, no analysis on pathogens was carried out since anaerobic digestion is considered one of the safest technologies for pathogen reduction in SS (Epstein, 2001). Nevertheless, this aspect may be taken into consideration in future researches. Concerning the use of SS in agronomic experiments, a lack in details about SS typology is provided. Indeed, in many works no detail on stabilization strategy is provided (Bakshi et al., 2019), or the kind of SS digestion is not specified (Hossain et al., 2015). In the present work, the digestates used derived from the same WWTP and were obtained with consequent treatments (Paragraph 2.2.1). As far as I know, this is the first example of the use of four different SSADs to fertilize tomato plants.

3.2. Materials and methods

3.2.1. Characterizations

3.2.1.1. Anaerobic digestates from sewage sludge

Four SSADs were used in the experiment: two liquid (primary (P) and secondary (S)) and two solid (centrifuged (C) and dried (D)). Physical and chemical characterization of the SSADs is described in the previous chapter (Paragraph 2.3.1) and results are summarized in **Table 3**.

3.2.1.2. Cultivation substrates

The same types of substrates described in the previous chapter were used in this experiment: a sandy soil and a commercial peat substrate. Methods of analysis of the substrates were described in Paragraph 2.2.2. and their results were summarized in **Table 4**.

3.2.1.3. Experimental set-up

A greenhouse experiment was performed over three months during the summer season in a greenhouse of the Centre of Competence AGROINNOVA – University of Torino, located in Grugliasco (TO), Italy. The experimental campaign was carried out with commercial plastic pots of 2.5 L (Ø 17 cm, height 20 cm, surface area 0.227 m^2). Four types of SSADs (P, S, C, D) were applied as treatments, and compared to a commercial fertilizer (M) (NPK 22-5-6 + 2MgO, “Osmocote Topdress”, ICL, Israel) and an untreated control (T). The experiment was designed in a completely randomized block, with 15 replications per each thesis. The same experimental set-up was adopted on the two cultivation substrates (sandy soil and peat substrate). How announced at the end of the previous chapter, due to the best results obtained with the medium dosage of N adopted in that experiment and in line with the European Nitrates Directive (Council of the European Communities, 1991), the four types of SSADs and the mineral fertilizer were mixed to the soil calculating an apport of 170 kg N/ha. Three untreated seeds of tomato (*Solanum lycopersicum* L. cv. Beefsteak, “Furia sementi”, Parma, Italy) were sown in each pot. Automatic sprinkler irrigation was set three times a day. **Figure 12** shown the greenhouse with all tomato plants grown on sandy soil and peat substrate one week after sowing. Ten days after sowing a thinning was conducted and the best plant from each pot was kept. At the end of each month, five pre-selected replicates of each treatment were removed to carry out all the measurements.

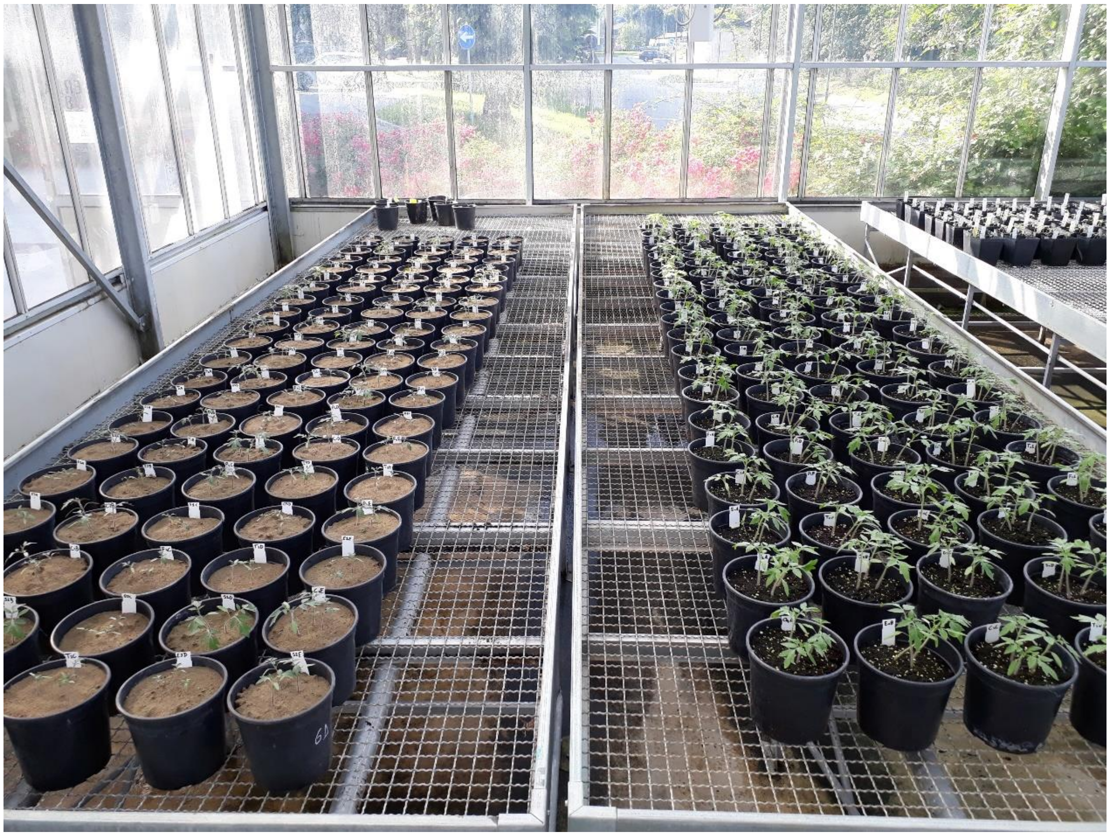

Figure 12. Greenhouse with tomato plants one week after sowing. On the left side pots filled with sandy soil; on the right side pots filled with peat substrate.

3.2.2. Measurement of plant parameters

At the end of every month, the five removed replicates were examined. Firstly, height was measured, then, leaves, inflorescences and fruits were counted, if present. After that, the Chlorophyll Content Index (CCI) was evaluated with a CCM 200 chlorophyll meter (CCM-200, Opti Sciences, Inc., Hudson, NH, USA) using the method described in the previous chapter. One month after sowing, it was not possible to measure CCI on the sand specimen because the minimum leaves size was not satisfied. At the end of the second month, assimilation (A_N), stomatal conductance (g_s) and CO_2 concentration in substomatal cavity (C_i) were measured by the means of an Infrared Gas Analyzer (IRGA, ADC, Hoddesdon, UK). These measurements were performed on three fully formed leaves in each replicate. The selected leaves had to be non-senescing, at the same physiological age (in the middle part of the plant, considering the third to fourth leaf from the shoot apex) and directly exposed to sunlight. After all the measurements were taken, each plant was subsequently cut and immediately weighed to record the fresh biomass value. In order to evaluate the mean dry biomass, each plant was dried at 105°C for at least 72 hours.

3.2.3. Chemical analysis

Substrates were chemically characterized at the end of the second month, once the aerial plant part had been cut. Chemical analyses were performed on samples from the treatment with SSADs (P, S, C, D) as well as on minerally fertilized ones (M) and untreated control (T). The samples were collected excluding the upper 3 cm of topsoil and the rhizosphere area. The analyses were performed with the same methods exploited for the chemical characterization of substrates prior to the experiment (Paragraph 2.2.2.).

Chemical analyses of the leaves were conducted at the end of the second month, after the biomass measurement, in order to assess the content of nitrogen, phosphorus and potassium in the leaves. In the case of the samples from the sandy soil, the measurements were performed on samples treated with one liquid digestate (P), one solid digestate (D) and the mineral fertilizer (M). It was not possible to analyze samples from the negative control (T) due to the low biomass production. On the peat substrate, it was possible to evaluate N-P-K content not only in the P, D, and M samples, but also in the negative control ones (T). The plant samples were firstly processed with a humid digestion protocol (Mills and Jones Jr, 1996). Then, nitrogen was measured through the Kjeldahl method, phosphorus was evaluated through colorimetry (molybdovanadate method) and potassium was quantified by Atomic Absorption Spectroscopy (AAS). Finally, the N, P and K percentages were used to calculate the mean total element present in the epigean part of the plant using the following formula:

$$\frac{dry\ sample\ biomass\ (g) * N,P,K\ in\ sample\ (\%)}{100 * 1000} = total\ N,P,K\ presence\ in\ single\ sample\ (mg)$$

Data of N content in leaves and soil were used to calculate the apparent nitrogen balance. This index was calculated according to Yang and co-workers (Yang et al., 2020), with some modifications. Total nitrogen was considered as the sum of Kjeldahl nitrogen, nitrites and nitrates per pot. Total initial nitrogen was calculated as the sum of N present in nude soil and N added with treatments. Stored nitrogen in soil was the N still present after two months; stored nitrogen in plants was the N measured in plants (epigean part) two months after sowing. N loss was calculated as the difference between initial and stored nitrogen.

3.2.4. Statistical analysis

The experimental data were subjected to statistical analyses. Two-way ANOVA was used to compare the average results of different treatments on plant measurements. Differently, one-way ANOVA was used to compare the mean results of different treatments on the chemical analyses of soils and leaf nutrient content. After the ANOVA, Tukey's post-hoc test ($P < 0.05$) was performed. In order to evaluate if there were statistically significant differences between N loss between P and D, Student's t-test was used. The statistical software R (version 3.5.1 - Feather Spray - 2018) was used for all statistical analyses.

3.3. Results

3.3.1. Effects on plants

3.3.1.1. Dry biomass

On the sandy soil at the end of the first month, the dry biomass of the tomato plants grown with digestates did not show any difference between each other and compared to control (T) and mineral fertilizer (M). At the end of second month, all digestates (P, S, C, D) showed a dry biomass production significantly higher than control. At the same time, S, C and D showed a higher biomass than mineral fertilizer. The biomass production in D resulted 37.5-folds higher than in untreated control. At the end of third month, dewatered SSADs proved to be the most productive treatments, with C and D displaying the highest yields (10.23 g and 10.97 g). Their biomasses doubled mineral fertilizer one (5.13 g), which is only comparable to the biomass produced by plants treated with SSADs after two months. Furthermore, C and D yields were 16 and 17-folds higher than T (0.64 g), respectively (**Figure 13.A**).

On the peat substrate, no significant differences between treatments were appreciable within the same month. Significant differences emerged between biomass values obtained on the first month and biomass values obtained on the second and third month. Significant differences in biomass production between second and third month emerged only in M (**Figure 13.B**).

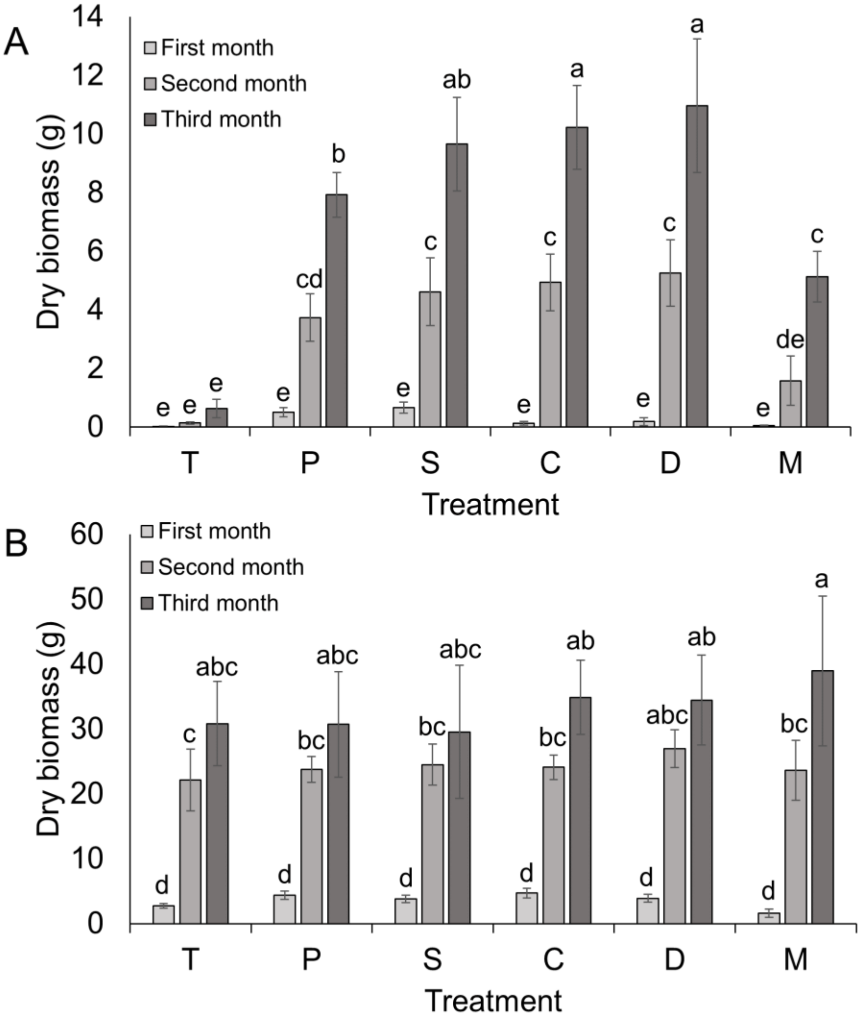

Figure 13. Mean dry biomasses of *S. lycopersicum* L. grown on sandy soil (**A**) and peat substrate (**B**) with different treatments among three months. Different letters indicate differences between treatments that are significant at $P < 0.05$ (Tukey HSD). Each error bar represents one standard deviation. T: non-treated, control thesis; P: primary digestate; S: secondary digestate; C: centrifuged digestate; D: dried digestate; M: mineral fertilizer.

3.3.1.2. Height

On the sandy soil, no differences in plant height were present at the end of the first month. In the second month, all SSADs-treated plants showed higher height than control and mineral fertilizer. After three months, the mean height of T was still the lowest. The mean height of the plants grown on P and D was comparable to plants grown on mineral fertilizer. Plants grown with S and C treatments had a statistically higher height than mineral fertilizer (M). It is worth highlighting that the mean heights of the plants grown on all digestates was at least 3.5-folds higher than the control ones (**Figure 15.A**). In order to give the reader a concrete idea about differences in plant height, a picture of a sample of each treatment at the end of the second month was added (**Figure 14**).

On the peat substrate, no significant differences were observed between the different treatments within the same month. Significant differences emerged only between the height of the samples collected on different months (data not shown).

Figure 14. Picture of tomato plants grown on sandy soil. One replica of each treatment is shown. From the left side: Control, Primary SSAD, Secondary SSAD, Centrifuged SSAD, Dried SSAD and Mineral fertilizer.

3.3.1.3. Leaves and inflorescences

After the first month, the plants grown on sandy soil in presence of D and C treatments showed a number of leaves comparable to control and minerally fertilized plants. On the other hand, samples from liquid SSADs (P and S) revealed a higher mean leaf number than control. After two months, the leaves number on plants grown with digestates was significantly higher only than negative control plants. At the end of the experiment, samples from S and D treatments showed the highest number of leaves, which were not statistically different from samples from C treatment. Plants grown with P had similar number of leaves than C and mineral fertilizer, while leaves number in negative control was still the lowest one (**Figure 15.B**).

With regards to the number of inflorescences, no plant on sandy soil showed flowers one month after sowing. At the end of the second month, plants in T and M were still not revealing any flower. Differently, P, S, C and D had some inflorescences, but no significant difference between treatments was present. At the end of the experiment, negative control plants still did not show any flower. Plants treated with P and S had a number of inflorescences statistically similar to mineral fertilizer. The highest number of inflorescences was found on C and D treatments (**Figure 15.C**).

As regards the number of leaves and inflorescences of plants grown on peat substrate, no differences between treatments at the same month were highlighted by statistical analysis (data not shown).

3.3.1.4. Chlorophyll Content Index (CCI)

On sandy soil, leaves dimension after one month was too small to measure CCI. At the end of second month leaves of plants treated with P, S and C showed a CCI higher than control and comparable to mineral fertilizer. The mean CCI value of plants grown with D digestate was statistically higher than mineral fertilizer (M) but comparable to the others SSADs. CCI measures performed at the end of the third month revealed a substantial decrease in CCI values registered in all SSADs and in mineral fertilizer, whose values were not significantly different from the control. The only significant difference emerging at the endpoint was between P and mineral fertilizer measure. (**Figure 15.D**).

On peat substrate, the only differences were recorded between the CCI measure of D and P at the end of second month, and S and control at the end of the third month (data not shown).

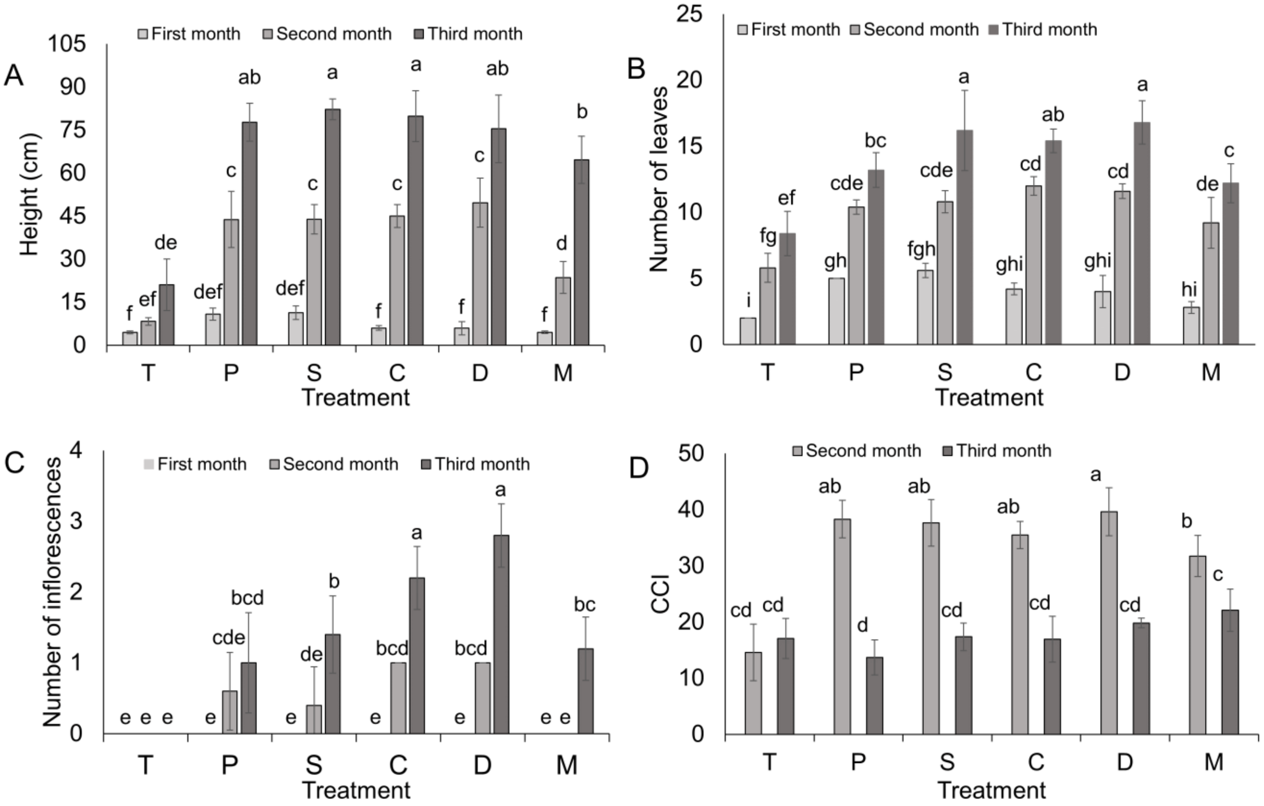

Figure 15. (A) Mean heigh of *S. lycopersicum* L. grown on sandy soil with different treatments among three months; (**B**) Mean number of leaves of *S. lycopersicum* L. grown on sandy soil with different treatments among three months; (**C**) Mean number of flower of *S. lycopersicum* L. grown on sandy soil with different treatments among three months. (**D**) Mean Chlorphyll Content Index (CCI) of leaves of *S. lycopersicum* L. grown on sandy soil with different treatments among 3 months. Different letters indicate differences between treatments that are significant at $P < 0.05$ (Tukey HSD). Each error bar represents one standard deviation. T: non-treated, control thesis; P: primary digestate; S: secondary digestate; C: centrifuged digestate; D: dried digestate; M: mineral fertilizer.

3.3.1.5. Infra-red gas analyzer (IRGA)

As regards IRGA measurements, on sandy soil the lowest A_N value was found in control, where significantly higher values were recorded on C and S. Net photosynthesis (A_N) of tomato plants leaves grown on sandy soil showed significant differences between treatments (**Table 5**). The lowest A_N value was found in control (4.08 µmol CO_2 $m^{-2}s^{-1}$), while the significantly highest values were recorded on C (10.56 µmol CO_2 $m^{-2}s^{-1}$) and S (10.21 µmol CO_2 $m^{-2}s^{-1}$). P, D and mineral fertilizer (M) did not display values statistically different to the lowest (T) and highest ones (T and S). Moving to the stomatal conductance (g_s) and CO_2 concentration in substomatal cavity (C_i), no statistically significant difference was registered. The overall mean g_s value was 0.25 mmol H_2O $m^{-2}s^{-1}$ while the overall mean C_i value was 276.22 ppm.

As regards IRGA measurements on peat substrate, A_N did not show any significative difference between the treatments and overall mean calculated value was 8.64 µmol CO_2 $m^{-2}s^{-1}$. The stomatal conductance of the digestates and of the control was higher than on mineral fertilizer. Concerning C_i, no significant difference between the treatments was found; the overall mean of C_i value was 280.89 ppm.

Treatment \ Parameter	A_N ($\mu mol\ CO_2\ m^{-2}s^{-1}$)		g_s ($mmol\ H_2O\ m^{-2}s^{-1}$)	C_i (ppm)
Control (T)	4.08 ± 1.87	b	0.17 ± 0.09	300.11 ± 10.19
Primary digestate (P)	9.96 ± 1.12	ab	0.27 ± 0.06	264.00 ± 11.35
Secondary digestate (S)	10.21 ± 1.48	a	0.28 ± 0.02	269.56 ± 8.18
Centrifuged digestate (C)	10.56 ± 2.37	a	0.28 ± 0.04	260.22 ± 20.72
Dry digestate (D)	8.29 ± 3.37	ab	0.26 ± 0.07	281.56 ± 27.84
Mineral fertilizer (M)	7.30 ± 1.97	ab	0.24 ± 0.07	281.89 ± 12.36

Table 5. Results of infra-red gas analyzer (IRGA) on plants grown on sandy soil. Different letters indicate differences between treatments that are significant at $P < 0.05$ (Tukey HSD). Data are expressed as mean ± standard deviation. A_N: net assimilation ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$); g_s: stomatal conductance ($mmol\ H_2O\ m^{-2}\ s^{-1}$); C_i: CO_2 concentration in substomatal cavity (ppm).

3.3.2. Chemical analysis

3.3.2.1. Substrates analyses

Results of chemical analyses performed on the sandy soil after two months from treatments application are summarized in **Table 6.A**. SSADs showed all an intermediate mean pH included between control (8.3) and mineral fertilizer samples (8.0). OM was significantly higher in P, C and D treatments than in S, mineral fertilizer and control. As expected, values of organic carbon showed a trend similar to OM. Total nitrogen (Kjeldahl) was lower in control, mineral fertilizer and S than P, C and D treatments. All results of nitrite analysis were below quantification limits (QL). Nitrates were detectable only in S, C and D treatments, showing very low concentrations (between 1 and 4 mg/kg) with respect to M sample (60 mg/kg). Organic nitrogen values were roughly similar to total Kjeldahl nitrogen ones. Regarding C/N ratio, the lowest value was calculated in control and mineral fertilizer, while all SSADs revealed higher values. Olsen phosphorus was below QL in T and M samples; differently, phosphorous content in samples treated with SSADs was higher. The lowest value of exchangeable calcium was observed in S samples followed by negative control, D, P, C and mineral fertilizer. A great difference in exchangeable sodium content was found between negative control samples and all the treatments. Available zinc ranged between 0.21 mg/kg in control samples, and 1.00 mg/kg in D ones, with samples treated with liquid SSADs and mineral fertilizer showing an intermediate behavior. Digestates showed intermediate values of CEC, included between control (2.81 cmol/kg) and mineral fertilizer samples (3.58 cmol/kg). Values of electrical conductivity, ammonia nitrogen (NH^{4+}), exchangeable K, exchangeable Mg, available Mn and available Cu did not show any significant difference between treatments on sandy soil.

Results of chemical analyses performed on peat substrate two months after treatments application are summarized in **Table 6.B**. pH values ranged from a minimum of 6.6 (M) to a maximum of 7.4 (D). Total Kjeldahl nitrogen was lower in control samples and gradually increased along with the dry matter of SSADs; the highest value was displayed by mineral fertilizer samples. Organic nitrogen values were analogous to total Kjeldahl nitrogen in terms of values, trend and differences between treatments. Nitrites, nitrates, extractable Mn, Cu and Zn were below detection limits. All other parameters did not show any significant difference.

A

Parameter	Unit of measure	Control (T)		Primary (P)		Secondary (S)		Centrifuged (C)		Dried (D)		Mineral fertilizer (M)	
pH	-	8.3 ± 0.1	***	8.2 ± 0.1	***	8.2 ± 0.1	***	8.1 ± 0.1	***	8.1 ± 0.1	***	8.0 ± 0.1	***
Electrical conductivity	dS/m	0.155 ± 0.020		0.219 ± 0.032		0.201 ± 0.010		0.197 ± 0.023		0.198 ± 0.025		0.399 ± 0.146	
Organic matter	%	0.16 ± 0.01	***	0.24 ± 0.01	***	0.18 ± 0.02	***	0.25 ± 0.02	***	0.26 ± 0.01	***	0.16 ± 0.02	***
Organic carbon	%	0.09 ± 0.00	***	0.14 ± 0.00	***	0.11 ± 0.01	***	0.14 ± 0.01	***	0.15 ± 0.00	***	0.10 ± 0.01	***
N - Tot (Kjeldahl)	g/kg	0.17 ± 0.01	***	0.19 ± 0.01	***	0.15 ± 0.01	***	0.20 ± 0.00	***	0.22 ± 0.01	***	0.17 ± 0.01	***
N - NO_2^-	mg/kg	< QL		< QL		< QL		< QL		< QL		< QL	
N - NO_3^-	mg/kg	< QL		< QL		1 ± 1	*	4 ± 4	*	2 ± 1	*	60 ± 40	*
N - NH_4^+	mg/kg	< QL		< QL		< QL		1 ± 1		2 ± 0		1 ± 0	
N - Org	g/kg	0.17 ± 0.01	***	0.19 ± 0.01	***	0.15 ± 0.01	***	0.20 ± 0.00	***	0.22 ± 0.01	***	0.17 ± 0.01	***
C/N	-	5.5 ± 0.2	**	7.3 ± 0.6	**	6.9 ± 0.5	**	7.3 ± 0.5	**	7.1 ± 0.3	**	5.8 ± 1.0	**
$\mathbf{P}_{\text{Olsen}}$	mg/kg	< QL		4.2 ± 0.5	**	10.3 ± 0.8	**	15.4 ± 4.6	**	36.2 ± 11.4	**	< QL	
$\mathbf{K}_{\text{exchangeable}}$	mg/kg	14 ± 3		11 ± 3		12 ± 1		12 ± 2		9 ± 1		13 ± 2	
$\mathbf{Mg}_{\text{exchangeable}}$	mg/kg	11 ± 2		21 ± 7		26 ± 3		25 ± 4		22 ± 4		25 ± 2	
$\mathbf{Ca}_{\text{exchangeable}}$	mg/kg	524 ± 26	*	594 ± 25	*	491 ± 62	*	626 ± 94	*	579 ± 48	*	646 ± 62	*
$\mathbf{Na}_{\text{exchangeable}}$	mg/kg	16 ± 1	***	35 ± 2	***	33 ± 3	***	26 ± 3	***	32 ± 3	***	27 ± 5	***
$\mathbf{Fe}_{\text{available}}$	mg/kg	5.7 ± 0.1	***	7.6 ± 0.5	***	9.3 ± 0.2	***	11.2 ± 0.7	***	12.5 ± 0.4	***	6.1 ± 0.21	***
$\mathbf{Mn}_{\text{available}}$	mg/kg	5.1 ± 0.4		14.8 ± 16.6		33.7 ± 2.5		35.8 ± 27.1		20.5 ± 28.1		5.2 ± 0.21	
$\mathbf{Cu}_{\text{available}}$	mg/kg	0.40 ± 0.08		0.47 ± 0.13		0.60 ± 0.06		0.85 ± 0.12		0.96 ± 0.29		0.40 ± 0.01	
$\mathbf{Zn}_{\text{available}}$	mg/kg	0.21 ± 0.03	***	0.36 ± 0.04	***	0.37 ± 0.02	***	0.73 ± 0.08	***	1.00 ± 0.07	***	0.43 ± 0.05	***
CEC	cmol/kg	2.81 ± 0.13	*	3.32 ± 0.17	*	2.83 ± 0.33	*	3.47 ± 0.43	*	3.24 ± 0.20	*	3.58 ± 0.32	*

QL: N - NO_2^- = 0.2 mg/kg; N - NO_3^- = 1 mg/kg; N - NH_4^+ = 1 mg/kg; P = 1 mg/kg.

B

Parameter	Unit of measure	Control (T)	Primary SSAD (P)	Secondary SSAD (S)	Centrifuged SSAD (C)	Dried SSAD (D)	Mineral fertilizer (M)
pH	-	7.0 ± 0.4 *	6.7 ± 0.3 *	7.2 ± 0.2 *	6.9 ± 0.2 *	7.4 ± 0.3 *	6.6 ± 0.2 *
Electrical conductivity	dS/m	0.235 ± 0.040	0.436 ± 0.220	0.183 ± 0.038	0.495 ± 0.134	0.225 ± 0.074	0.523 ± 0.202
N - Tot (Kjeldahl)	% D.M.	0.23 ± 0.03 *	0.24 ± 0.03 *	0.25 ± 0.03 *	0.28 ± 0.04 *	0.31 ± 0.04 *	0.32 ± 0.04 *
N - NO$_2^-$	mg/l	< QL	< QL	< QL	< QL	< QL	< QL
N - NO$_3^-$	mg/l	1.0 ± 0.9	1.5 ± 0.5	1.0 ± 0.2	2.6 ± 0.4	1.2 ± 0.3	2.6 ± 1.7
N - NH$_4^+$	mg/l	< QL	< QL	< QL	< QL	< QL	< QL
N - Org	% D.M.	0.22 ± 0.03 *	0.24 ± 0.03 *	0.25 ± 0.03 *	0.28 ± 0.04 *	0.31 ± 0.04 *	0.32 ± 0.04 *
P $_{\text{extractable}}$	mg/l	0.4 ± 0.1	1.2 ± 0.4	1.2 ± 0.5	0.6 ± 0.3	1.1 ± 0.1	< QL
K $_{\text{extractable}}$	mg/l	2.9 ± 0.3	2.0 ± 0.3	2.4 ± 0.8	2.4 ± 0.4	5.0 ± 2.2	2.6 ± 0.2
Mg $_{\text{extractable}}$	mg/l	8 ± 2	22 ± 16	5 ± 2	24 ± 12	6 ± 3	26 ± 17
Ca $_{\text{extractable}}$	mg/l	13 ± 4	26 ± 16	12 ± 3	33 ± 14	14 ± 3	32 ± 17
Na $_{\text{extractable}}$	mg/l	24 ± 2	29 ± 7	17 ± 3	32 ± 4	23 ± 7	31 ± 4
Fe $_{\text{extractable}}$	mg/l	1.17 ± 0.26	0.52 ± 0.46	0.80 ± 0.13	0.52 ± 0.30	0.73 ± 0.06	0.28 ± 0.11
Mn $_{\text{extractable}}$	mg/l	< QL	< QL	< QL	< QL	< QL	0.03 ± 0.01
Cu $_{\text{extractable}}$	mg/l	< QL	< QL	< QL	< QL	< QL	< QL
Zn $_{\text{extractable}}$	mg/l	< QL	< QL	< QL	< QL	< QL	0.02 ± 0

QL: N - NO$_2^-$ = 0.05 mg/l; N - NH$_4^+$ = 0.06 mg/l; P = 0.3 mg/l; Mn = 0.03 mg/l; Cu = 0.03 mg/l; Zn = 0.02 mg/l.

Table 6. Chemical characterization performed two months after treatments application on sandy soil (**A**) and on peat substrate (**B**). Data are expressed as mean ± standard deviation. Asterisks mean significant differences according to ANOVA test (*, **, *** differences between means significant at *P* ≤ 0.05, 0.01 and 0.001, respectively). CEC, cation exchange capacity; QL, quantification limit.

3.3.2.2. Leaf analysis

On sandy soil, chemical characterization of leaves showed a concentration of nitrogen and potassium in P and D significantly lower than mineral fertilizer samples. As regards phosphorous, no significant difference emerged. The total nitrogen accumulated in leaves in D plants was significantly higher than in P ones. The mean phosphorous uptake by plants was significantly different across D, P and M samples. Finally, the potassium uptaken in leaves did not show significant differences between thesis (**Table 7.A**).

On plants grown on peat substrate, concentrations and total uptake of both nitrogen and potassium on control, P and D were statistically similar to each other, but they resulted lower in comparison with mineral fertilizer ones. Concentration and total uptake of phosphorous in leaves, control showed the lowest values while D samples the highest ones (**Table 7.B**).

A

Parameter	Unit of measure	Primary SSAD (P)		Dried SSAD (D)		Mineral fertilizer (M)	
N	%	1.10 ± 0.05	b	1.35 ± 0.28	b	2.95 ± 0.36	a
	Total (mg)	46.64 ± 7.45	b	81.08 ± 15.63	a	60.83 ± 9.26	ab
p	%	0.14 ± 0.01	a	0.16 ± 0.01	a	0.13 ± 0.02	a
	Total (mg)	5.97 ± 1.25	b	9.61 ± 1.26	a	2.56 ± 0.33	c
K	%	1.46 ± 0.38	b	1.40 ± 0.18	b	3.63 ± 0.57	a
	Total (mg)	61.61 ± 16.41		84.54 ± 10.95		74.24 ± 7.52	

B

Parameter	Unit of measure	Control (T)		Primary SSAD (P)		Dried SSAD (D)		Mineral fertilizer (M)	
N	%	1.26 ± 0.08	b	1.32 ± 0.04	b	1.29 ± 0.19	b	2.05 ± 0.36	a
	Total (mg)	319.01 ± 11.24	b	323.86 ± 20.48	b	360.41 ± 53.17	b	550.22 ± 102.49	a
p	%	0.23 ± 0.01	b	0.29 ± 0.02	ab	0.31 ± 0.04	a	0.27 ± 0.02	ab
	Total (mg)	58.02 ± 7.29	b	69.82 ± 2.92	ab	85.44 ± 9.77	a	72.95 ± 8.78	ab
K	%	1.83 ± 0.04	b	1.92 ± 0.04	b	1.74 ± 0.21	b	2.53 ± 0.27	a
	Total (mg)	465.11 ± 37.08	b	470.69 ± 24.49	b	486.97 ± 57.85	b	677.03 ± 53.15	a

Table 7. Results of leaves analyses performed after two months after treatments application on sandy soil (**A**) and on peat substrate (**B**). Different letters indicate differences between treatments that are significant at $P < 0.05$ (Tukey HSD). Data are expressed as mean ± standard deviation.

3.3.2.3. Nitrogen apparent balance

Statistical analysis of N loss highlighted no significant differences between treatments on sandy soil. Results are summarized in **Table 8**. On the other hand, in order to evaluate differences between the loss of nitrogen in P and D, the application of the T-

Test showed that with the use of primary SSAD there was a statistically higher loss of nitrogen (p = 0.008). With regards to peat substrate, nitrogen storage was not calculated since not even significant differences were observed in biomass analyses.

Treatment	Initial nitrogen			Nitrogen storage		Loss of nitrogen	
	Soil nitrogen storage (g)	Nitrogen added (g)	Total initial soil (g)	Soil (g)	Plant (mg)	(g)	%
Primary SSAD	1.16 ± 0.32	0.39	1.55 ± 0.32	0.76 ± 0.03	46.64 ± 7.45	0.75 ± 0.04	48.14 ± 2.51
Dried SSAD	1.16 ± 0.32	0.39	1.55 ± 0.32	0.87 ± 0.04	81.08 ± 15.63	0.60 ± 0.03	38.46 ± 2.24
Mineral fertilizer	1.16 ± 0.32	0.39	1.55 ± 0.32	0.91 ± 0.20	60.83 ± 9.26	0.58 ± 0.17	37.45 ± 11.17

Table 8. Results of the apparent balance of nitrogen in sandy soil. Nitrogen amounts are reported as mean values ± standard deviation.

3.4. Discussion

3.4.1. Evaluations on plants growth

For many years extensive studies and reviews have shown that soil and plant benefit from SS. Indeed, SS is a good source of macro and micro nutrients as well as of OM; this enhances soil fertility and, as a consequence, crop production even in a more effective way than commercial fertilizers (Singh and Agrawal, 2008). The results of the present work are in agreement with literature and the better performances of SS compared to inorganic fertilizers have been confirmed. **Table 8** shows technical details and results of other works dealing with SS treatment of tomato plant with pot experiments. It is important to notice that not only SSAD application rate was considerably lower in the present work, but also that the results obtained were remarkably higher. For instance, biomass (**Figure 13**) and height (**Figure 15.A**) of treated tomato plants at two months after sowing was up to 37.5 and 6-folds higher, respectively, than control plants (corresponding to an increase of 3652% and 500%), results never reached before in other works on tomato plants (**Table 9**); these impressive results were obtained with the use of dried digestate on sandy soil. Improvement in terms of leaves number and chlorophyll content were less intense, but still higher than the examples reported in the literature (Bakshi et al., 2019; Elloumi et al., 2016; He et al., 2016; Hossain et al., 2015). Surely, the use of sandy soil poor in nutrients and OM as a substrate has emphasized the good performances of the digestates. On the other hand, undoubtedly this was not the only reason for the good performances of these digestates: the good physico-chemical composition has affected final results. These performing recipes were probably due to the rich row material, the successful AD and effective treatments for solids abatements. These conditions allowed to have rich digestates in terms of macronutrients (in particular of phosphorus), micronutrients and OM. Phosphorus detailed analyses will be described in the next chapter; furthermore, it would be interesting to carry out a specific study on OM in order to evaluate its quality: in fact, effective biological treatments could improve OM quality.

To a broader extent, results of the present study in terms of biomass and plant height can be compared to other works conducted with a similar experimental setup but exploiting different model species. In order to biomass, the general trend is an increase in dry matter ranging usually between 4 (*Capsicum annuum* L.; (Pascual et al., 2008)) and 16-folds (*Triticum aestivum* L.; (Eid et al., 2019)) more than untreated control. The findings of the present experiment confirm and go beyond these results, considering also that the most used SS application rates range between the dosage used in this work and a 35-folds higher one (Eid et al., 2019). On the other hand, the improvements in plant height are in line with the results obtained by Eid and colleagues on cucumber (*Cucumis sativus* L.) (Eid et al., 2017) and wheat (*Triticum aestivum* L.) (Eid et al., 2019), reporting a stem length improvement up to 3 and 6-folds, respectively, over untreated control. The only case with a striking higher biomass production was described for the sunflower (*Heliantus annuus L.*), whose production increased up to 125-foldsmore than the untreated control. However, the SS dosage was up to 35-folds higher than the present study. Moreover, improvement in terms of height was comparable to the present work (Bourioug et al., 2018). Taking into account the works using SS dosages comparable to 170 kg N/ha, the majority are open-field experiments. For instance, triticale (*X Triticosecale Wittmack*) (Kchaou et al., 2018) revealed a biomass increase of 2-folds. Furthermore, the results of the present work corroborate positive effects on biomass of SS application on soils poor in nutrients (Walter et al., 2000) and strongly alkaline (Zuo et al., 2019).

SSAD application on tomato crops grown on the sandy soil resulted also in an augmented number of leaves (**Figure 15.B**) and inflorescences (**Figure 15.C**) with respect to control and mineral fertilizer. Moreover, inflorescences number of SSAD-treated plants increased from 2 to 3-folds over the last month. These findings are in general agreement with other results reported on tomato grown in presence of SS (Bakshi et al., 2019), despite the higher treatment dosages (**Table 9**). Number of leaves and inflorescences are developmental parameters considered also with other plant species when testing the fertilizing effects of SSAD. For instance, Eid and colleagues (Eid et al., 2017) registered on cucumber a boost in the number of leaves of more than 2-folds, which is in line with the results of the present work. Similar outcomes have been reported in terms of number of flowers in common bean (*Phaseouls vulgaris* L.) (Fernández-Luqueño et al., 2010) and marigold (*Tagetes erecta* L.)(Solanki et al., 2017) grown in SS dosages lower and higher, respectively, than the present work. In contrast with these results, Tariq and co-workers (Tariq et al., 2012) described a decrease up to 60% in flowers number in *Dahlia x hortensis*, whose growth had probably been compromised by an excessive SS dosage.

Results of the present work confirmed the positive effects of SS application to the sandy soil with regard to net photosynthesis (**Table 5**) (Bourioug et al., 2018; Pascual et al.,

2008) and to chlorophyll content (**Figure 15.D**). Leaf chlorophyll content is directly correlated with indirect chlorophyll measurements such as readings through SPAD and CCI-meters (Xiong et al., 2015), whose value can be compared to each other with the equations proposed by Parry and colleagues (Parry et al., 2014). Application of SSAD improved chlorophyll content values of tomato plants grown on sandy soil at the end of second month, as well as dry biomass and net photosynthesis (A_N). This beneficial effect has been already observed also in sunflower (Bourioug et al., 2018), sorghum (Alvarenga et al., 2016) and triticale (Kchaou et al., 2018) (**Table 9**). On the other hand, literature provides examples of reduction of leaf chlorophyll content in tomato after treatment with SS (Elloumi et al., 2016), which is probably due to the excessive heavy metals presence in the used SS (Singh and Agrawal, 2007a). However, this aspect is likely not linked with the reduction of chlorophyll content over time observed in the present study. Indeed, this phenomenon has been already observed in other SS-treated plant species, such as the common bean (Fernández-Luqueño et al., 2010). A possible explanation of this reduction in CCI at the end of the third month could be the deficiency of nutrients in soil. A second hypothesis for CCI decrease has been proposed by de Oliveira and co-workers (Maria et al., 2017): after the initial blooming of the plant, gradual degradation of chlorophyll occurs due to the beginning of the fruit development phase, which induces a metabolic change in the plant, with a more sustained nutrients accumulation in the fruit. Taking into account the relationship between leaf nitrogen and chlorophyll content (Xiong et al., 2015), a third justification for CCI decrease can be provided by the so-called nitrogen dilution curve. In fact, biomass increase in tomato plant is accompanied by a reduction in nitrogen concentration (and, consequently in chlorophyll content) because the structural compartment (lower in N%) becomes proportionally more massive than metabolic active one (higher in N%) (Tei et al., 2002).

Cultivar	SS typology	Dosage	Kind of experiment	Cultivation substrate	Differences with respect to untreated control				Reference
					Biomass increase	**Plant height**	**Leaves and inflorescences**	**Chlorophyll content**	
Cherry	Digested[a]	10 t/ha (2X)	Pot experiment Greenhouse 16 weeks	Chromosol	*Dry biomass:* + 20%	10 weeks: + 50% 13 weeks: + 20% 15 weeks: + 7%	n.a.	n.a.	Hossain et al., 2015
Red Robin	n.a.	SS:soil 1:10 (65X)	Pot experiment Growth chamber 120 days	Loamy soil	*Fresh biomass:* Stem: + 70% Leaves: + 142%	+ 43%	*Leaves:* + 33% *Flowers:* +130%	*Chlorophyll*[b]*:* *a:* + 18.3% *b:* + 34.8%	Bakshi et al., 2019
Rio Grande	Aerobically digested	2.5%; 5.0%; 7.5% (11X; 22X; 33X)	Pot experiment Greenhouse 30 days	Sandy soil	*Dry biomass:* + 180% + 280% +140%	n.a	n.a.	*Chlorophyll a+b*[b]*:* + 17.5% - 40% - 68.5%	Elloumi et al., 2016
n.a.	Aerobically digested	400 - 800 kg N/ha (2.35X - 4.7X)	Pot experiment Greenhouse 90 days	Clay soil	*Dry biomass:* + 18.6% + 29.6%	+ 19.2% + 24.5%	n.a.	n.a.	He et al. 2016
Beefsteak	Anaerobically digested (4 typologies: P, S, C, D)	170 kg N/ha	Pot experiment Greenhouse 120 days	Sandy soil	*Dry biomass* up to + 3652% (D treatment, II month)	up to + 500% (D treatment, II month)	*Leaves:* up to + 180% (S treatments, I month) *Flowers:* not observed in untreated control	*CCI:* up to + 172% (D treatment, II month)	This work
				Peat substrate	*Dry biomass:* up to + 70% (C treatment, I month)	up to + 24% (P treatment, I month)	n.a.	*CCI:* up to + 64% (D treatment, III month)	

[a] In this work, no details about the typology of digestion are provided.

[b] In these works, leaf chlorophyll content was evaluated with methods based on extraction with organic solvents followed by spectrophotometrical quantification.

Table 9. Comparison of the results from other works in the literature on the effects of treatment with sewage sludge on tomato plants. Application dosages are shown as reported in the original works; values in brackets indicate how many folds more is the SS application rate with respect to the present study. n.a., not available.

3.4.2. Chemical analysis

3.4.2.1. Substrates analyses

The application of SS on soil can affect different physical and chemical soil characteristics (Epstein, 2002). Likewise, many changes were documented in this experiment (both on sandy soil and on peat substrate) two months after treatments application. Despite peat substrate is low in nutrient content, it showed a consistently higher amount of microelement than sandy soil. Moreover, peat substrate has many other advantages such as lightweight, high water holding capacity and high air space (Gruda et al., 2016). All these peculiarities most probably contributed to the minor differences registered on peat substrate.

Soil analysis results revealed a change in soil pH after the treatments application (**Table 6**). Many works reported an increase (Bayoumi Hamuda et al., 2009; Ferreiro-Domínguez et al., 2011) or a decrease (Mosquera-Losada et al., 2016; Singh and Agrawal, 2007a) in soil pH. In the present work, acidification occurred in treated sandy soil samples, probably due to both the lower pH of SSADs and the nitrogen mineralization (Rasouli-Sadaghiani and Moradi, 2014). In particular, the nitrification process ($NH_4^+ \rightarrow NO_3^-$) (Stamatiadis et al., 1999) induces the release of H^+ in soil solution media and the leaching of NO_3^- by water (Whitehead, 1995). Another conceivable theory for soil acidification in SSAD-treated samples could be the generation of organic acids during sewage sludge mineralization (Angin et al., 2012; Bourioug et al., 2018).

Electrical conductivity values (both on sandy soil and on peat substrate) did not statistically change after treatments application unlike many other works (Bourioug et al., 2018; Singh and Agrawal, 2007a), likely due to the consistently lower SSAD application rates.

The thesis of a possible increase of soil OM in soils treated with SSADs (Kladivko and Nelson, 1979; Perez-Espinosa et al., 1999) was confirmed by the present work. Despite the OM percentage was very low in all samples, the value in SSADs treated theses was higher than control and mineral fertilizer. This may partially justify the better performances of treated samples in term of biomass and height, according to the well-known soil OM benefits on plants growth (Bot and Benites, 2005).

CEC significantly increased in SSADs-treated soil, which was probably caused by the OM increment. This effect is even more pronounced on alkaline soils (Bohn et al., 2001) and similar results were found in other works (Angin et al., 2012; Ferreiro-Domínguez et al., 2011).

Total N, available P, exchangeable Ca and Na and available Fe and Zn concentrations increased in the sandy soil amended with SSADs due to their higher concentration in SS (Singh and Agrawal, 2007a).

Two months after treatments application, N_{Tot} (Kjeldahl) was higher in C and D than liquid SSADs (P and S), probably due to their solid form that plausibly induced a slower release, both on sandy soil and peat substrate. Other studies revealed that total soil nitrogen can persist in higher concentrations also for longer periods after SSAD treatment application (Bourioug et al., 2015). Anyway, all samples showed a total N content lower than before digestates application. It means that a remarkable part of nitrogen both already present in sandy soil and added with digestates was absorbed, transformed or leached after two months.

The significant variation in N and OM content in treated sandy soils changed C/N ratio. The results obtained with SSADs were still low (< 9; (Arpa Veneto, 2007)), but higher than in control and mineral fertilizer, emphasizing again the importance of carbon addition to soil.

In all SSADs treated soils, the available P was higher than control and mineral fertilizer. Considering that the different dosages were normalized on N dosage per each thesis, the difference in P content between the samples treated with SSADs can be explained by the different percentages of P in the four SSADs. This diversity could also explain the differences among different treatments on physiological parameters of tomatoes. Moreover, the addition of OM probably enhanced the availability of P in soil treated with SSADs (Fekri et al., 2011). In fact, this can increase the abundance and the activity of microorganisms, favoring P capture (Nobile et al., 2019). Similar results in the increase of soil P were obtained by Singh and Agrawal (Singh and Agrawal, 2007b) and Walter and colleagues (Walter et al., 2000).

For what it concerns K, no differences were registered in soil after digestates application, probably due to their low concentration in this macronutrient. These results agree with other works (Bourioug et al., 2015; Walter et al., 2000).

Many SS are rich in Ca due to the stabilization by means of liming (Epstein, 2002). Although the SSADs exploited in this work did not undergo Ca addition at WWTP level, its content was pretty high (> 4.64% D.M.). Considering the medium content in the initial soil, exchangeable Ca increased in some cases in treated soils, confirming the results of Ferreiro Dominguez and Singh (Ferreiro-Domínguez et al., 2011; Singh and Agrawal, 2007a).

A significant increase of exchangeable Na was measured in all treated soils due to the sodium percentage in SSAD and confirmed by the two abovementioned works (Ferreiro-Domínguez et al., 2011; Singh and Agrawal, 2007a). The excess of Na is a well-known limiting factor for plants growing (Jones Jr., 2012) but Na has been recently defined as a "new beneficial element" (Morgan, 2000) that, in small quantities, can increase tomato yields (Jones Jr., 2012).

The consistent presence of Fe and Zn in SSADs likely provoked the increase in their concentration in sandy soil, confirming the results of Angin and colleagues (Angin et al., 2012).

3.4.2.2. Leaf analysis

In some cases, in literature, the use of SS enhanced the percentage of macronutrients in leaves (Angin et al., 2012; Zuo et al., 2019), in other ones no change took place (Kotecki et al., 2014; Pinna et al., 2009) and still in other one's concentration increased only for some nutrients (Bakshi et al., 2019; De Andres et al., 2010). This work belongs to the third category since only foliar P% and total uptaken P of control plants grown on peat substrate were significantly lower than SSADs ones (**Table 7.B**). On sandy soil, the content of uptaken P was significantly higher in D and P treatments, which was likely influenced by the phosphorous amount in the initial application (**Table 7.A**). Nevertheless, no significant differences emerged in foliar P% despite the difference in uptaken P content between SSADs and mineral fertilizer: probably, the controlled nutrient release of the mineral fertilizer compensated the higher quantity of P in the SSADs. Moreover, it could be inferred that differences in foliar macronutrient content could have been appreciated between control and treated samples on sandy soil. However, the too low biomass of untreated samples made impossible this investigation. The total amount of N and K uptaken in leaves had varied results. On sandy soil, D samples revealed a significantly higher N content than P ones due to the different biomass production. Concerning plants grown on peat substrate, P and D showed a nitrogen plant uptake similar to the negative control, but lower than mineral fertilizer, likely due to the characteristics of the fertilizer, such as the controlled nutrient release and the presence of readily bioavailable nitrogen forms. As regards K, despite its higher amount in mineral fertilizer, total K uptaken in leaves did not result significantly different between the treatments applied on sandy soil, due to the different aboveground biomass production. On the other hand, on peat substrate, the $K_{Extractable}$ content of plant with mineral fertilizer was the highest considering the similar biomass production.

3.5. Conclusion

The present work confirmed the possibility of increasing nutrients and OM in soils after just one SSAD application. In fact, the thesis of the enhancement of soil OM and the increments of some macro- (nitrogen, phosphorous, and calcium) and micro-nutrients (iron and zinc) in sandy soil were both demonstrated by soil chemical analyses. Nevertheless, some of the registered values were low and it can be reasonably assumed that most of the nutrients had already been assimilated to let the plant grow. This aspect was confirmed by leaves analysis, which showed a remarkable uptake in N, P, and K by tomato plants. With respect to these macronutrients, it is worth emphasizing that the experiment was designed to administer plants, across the different treatments, the same nitrogen dosage as sludge application rate is usually based on plants nitrogen requirements. However, the differences in SSADs composition implied a remarkable imbalance in terms of other nutrients and OM. Hence, we can assume that these differences likely influenced plant growth, providing consistent differences between different treatments. The last Chapter II did not arrive to the solution of: "which is the best SSAD?" but it highlighted that solid SSADs had better results on plant growth than liquid ones. The work of Chapter III confirmed the same results adding that nitrogen losses with primary SSAD were higher than with dried digestate: in a broader sense, it means that with the use of liquid SSADs instead of solid ones, more nitrogen can be lost by volatilization. Less probable appeared the loss of nitrogen by denitrification, because of the needing of anaerobic conditions that hardly appear in this sandy soil. Once again, it was not possible to define which solid digestate was the best for plant growth. In fact, despite the higher results were obtained with the use of dried digestate, statistical analysis did not reveal any statistically significant difference between C and D. Indeed, a consideration that should be better examined in future studies is to evaluate on costs basis: if centrifuged digestate required a thermal treatment less than the dried one (it means lower costs for the treatment), at the meantime the transport costs for centrifuged digestate will be higher due to the major quantity of water enclosed within. Furthermore, another factor that could determine the choice of one or the other digestate is the effective good applicability of them to the soil: C could be easily spread but it could form clumps of solid digestate causing inhomogeneity in its spatial distribution; on the other hand D, due to its powdery composition, could be uniformly distributed but, at the same time, it could be easily moved by the wind. Another interesting difference between the two solid treatments that was observed after two months from the application was the higher level of bio-available phosphorus in the soil treated with the dried digestate. This consideration has given rise to another research that will be synthetized in the next chapter.

The application of all these digestates clearly highlighted beneficial effects on different growth parameters of tomato plants, especially when cultivated on sandy, alkaline, and poor soil. For instance, it is essential to point out that plant biomass and height reached values up to 37.5 and 6-folds, respectively, higher than the untreated control. These results are equally important in fighting desertification because, how it was described in Paragraph 1.1.1., another strategy for the same purpose is reforestation. With this experiment, it was demonstrated that plants could have a fertilizing effect (even greater than mineral fertilizer) by the application of this kind of waste (SSAD) that from now I recommend defining it as a renewable resource. This resource can substitute mineral fertilizers in reforestation projects of degraded lands significantly reducing their environmental and economic costs. Despite reserves and resources for N, P and K appear adequate for the near future. It is necessary to find less impactful solutions to produce fertilizers in the short term. In this way, the reuse of SS can reduce the negative effects connected by the extraction, manufacturing, and use of mineral fertilizers derived from non-renewable resources.

Clearly, this work will not mark the end of the vision of SSADs as a waste, but it tries to support the thesis of addressing a problem such as a un opportunity. In the meantime, it is not possible to affirm that the solution against desertification was found, but surely this work could represent a base study for the development of future strategies for combating desertification. Finally, it is important to affirm that the only method for having an effective demonstration of the SSADs capability of fighting desertification is to perform full-scale long-term experiments in desertified areas.

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Chapter IV

4. Time-based evaluation of phosphorus forms and behaviors in a calcareous soil after SSAD application

4.1. Introduction

In previous chapters, it has been theoretically demonstrated (on a laboratory scale) that SSADs can combat actively 2 of the causes of desertification: nutrients and organic matter depletion. The experiments were based on nitrogen dosages according to both the Nitrates Directive (Council of the European Communities, 1991) and the fertilization habits, which are usually balanced using the plant's nitrogen requirements. On the other hand, considering only nitrogen plant's needs, soils resulted unbalanced in terms of phosphorus (Huang et al., 2012). For this reason (and others described later), this chapter is focused on the study of this macronutrient. In fact, P is fundamental in plant nutrition and, in general, in many vital life processes (paragraph 1.3.4.). Nowadays, phosphorus reserves are being depleted (Gilbert, 2009), while P fertilizers demand increases (Tóth et al., 2014). Furthermore, the phosphorus used in fertilizers is mainly derived from non-renewable resources (such as phosphate rocks (Fixen and Johnston, 2012; Reijnders, 2014)) with heavy environmental costs of extraction (Li et al., 2009). Also, it is well contained in renewable resources such as SSADs (paragraph 1.3.4.). To better explore this feedstock as a phosphorus source, this chapter will explain how and how much of the phosphorus contained in SSADs can effectively increase phosphorus availability in soils. In chapter III, it has already been demonstrated that an increase in soil P can happen with SSAD addition, but in this new case study, the experiment is entirely focused on phosphorus changing over time. Before that, it is necessary to further describe some soil-P characteristics. Total phosphorus (P-Tot) in soil surfaces is less abundant than nitrogen and potassium and it can range from about 0.005 to 0.15% (Fekri et al., 2011). Moreover, the quantity of P-Tot in soils has little or no relationship with the availability for plant nutrition (Havlin et al., 2016) and just a little part of this percentage (1-3%) is bioavailable (Yadav et al., 2012). In fact, plants can uptake only the inorganic forms of P as anions, namely dihydrogen phosphate ($H_2PO_4^-$) and monohydrogen phosphate (HPO_4^{2-}). The ionic form of P depends on the pH of soil solution: if pH is 7, they are in equilibrium; in acid soil solution pH, $H_2PO_4^-$is the predominant form; in alkaline pH, the most frequent form is HPO_4^{2-} (Jones Jr., 2012). P-bioavailabilty is related to many soil characteristics: soil texture, organic matter content, moisture content, pH, diffusion rates and relative rates of adsorption-desorption, precipitation dissolution reactions (Yang et al., 2002), soil chemical composition, amount of silicate clays, $CaCO_3$, presence of Fe or Al oxides, P addition rates and times (Afif et al., 1993) and soil temperature (Sinegani and Sedri, 2011). As already described in Paragraph 1.5., soil P can be considered as accruing in three compartments:

Solution-P ↔ Labile-P ↔ Non-labile-P

This subdivision is usually used to describe phosphorus pools in soils in relation with plant nutrition: Solution-P is the pool with readily available P, with soluble phosphates in soil solution; Labile-P (or Active-P) is the pool with solid inorganic and organic phosphates that are relatively easy to release in the soil solution; Non-Labile-P pool (or Fixed-P) is a source of very slow release phosphates and contains very insoluble inorganic phosphates and organic compounds resistant to mineralization. The reactions ruling this process are many: mineralization, desorption, dissolution, weathering, adsorption, precipitation, leaching, runoff and erosion (Paragraph 1.5.). The attempt of the experiment described in this chapter is to determine which reactions are the most important in a controlled environment. Surely, great importance is due to the method of P quantification. Many methods are available to measure phosphorus forms in soils, but in the last years the SMT methods (Standards Measurements and Testing) (Ruban et al., 2001, 1999) has increased in popularity due to the repeatability, the easiness in procedures (compared to other methods) and the non-sequential extraction of P (Wang et al., 2013). In particular, this last quality drove the choice for the use in this work. In fact, with three independent procedures, it is possible not only to measure P-Tot, but also to estimate organic phosphorus (P-org), inorganic phosphorus (P-inorg), apatite phosphorus (AP; the form associated with calcium) and non-apatite phosphorus (NAIP; the form associated with oxides and hydroxides of Al, Fe and Mn). There are many other methods for P fractionation (Hedley et al., 1982; Moore Jr et al., 1994; Williams et al., 1967) but this was the only one proposed and selected by the European Community in order to have comparable results: in fact, a lack of uniformity in methods makes difficult to compare results (García-Albacete et al., 2012). This technique is normally applied for P-fractionation in freshwater sediment (Pardo et al., 2004; Ruban et al., 2001), but it can be easily adapted to make it work also on soils and sewage sludges (García-Albacete et al., 2012; González Medeiros et al., 2005; Xie et al., 2011; Zhang et al., 2016). As regards bioavailable-P, many methods are well-known for quantification. Olsen method (Olsen et al., 1954) is considered the benchmark of P bioavailability due to its suitability to be used with calcareous and alkaline soil (Yang et al., 2002). Finally, this chapter aims to determine and understand all these different P fractions' changes over time. Although soil P chemistry has been studied more intensively than that of any other element (excluding nitrogen), quantitative predictions of phosphate concentrations in soil are still lacking (Bohn et al., 2001). Nevertheless, some kinetic studies exist, and, taking advantage of models and kinetic already determined and supported by literature, the experimental data of this work will be fitted with the equations of the models. Kinetics of P-related phenomena are certainly key to designing fertilizers and optimizing their efficiency and, hence, reducing drawbacks (e.g., eutrophication) (Hosseinpur and Pashamokhtari, 2008). Although kinetic can suggest retention mechanisms, soil-P behavior's complexity makes this prospect difficult to obtain (Bohn et al., 2001). Nevertheless, it will be tried to understand and interpret the physic-chemical processes that took part in this experimental campaign.

4.2. Materials and methods

4.2.1. Sewage sludge and soil

Sewage sludges used in this experiment are the centrifuged (C) and dried (D) SSADs used in previous experiments and described in Paragraph 2.3.1. The soil adopted is the same calcareous sandy soil used in previous experiments and described in paragraph 2.3.2. A new evaluation of dry matter and P-Tot were performed on SSADs.

4.2.2. Methods of phosphorus analysis in soil and sewage sludge

Phosphorus in SSADs and soil was extracted using the SMT method (Standards, Measurements and Testing Programme), a standardized method developed and approved by the European Commission (Ruban et al., 2001, 1999). Basically, it is a modified version of the Williams protocol (Williams et al., 1980) and it allows, with three independent procedures, to extract five P fractions: total phosphorus (P-Tot), inorganic phosphorus (P-inorg), organic phosphorus (P-org), non-apatite inorganic phosphorus (NAIP) and apatite phosphorus (AP). For P-Tot extraction, 200 mg of dry sample were calcinated in porcelain crucible at 450°C for three hours. Once the porcelain crucible was cooled down, ashes were transferred into centrifuge tubes and 20 ml of 3.5 mol L^{-1} HCl were added. After that, centrifuge tubes were covered and stirred overnight (all overnight stirrings were performed for 16 hours). After 16 hours, samples were centrifuged at 2000 g for 15 minutes. The extract was collected and analyzed for P-Tot determination. Inorganic phosphorus was determined by adding 20 ml of 1 mol L^{-1} HCl to a centrifuge tube containing 200 mg of dried sample of SSADs or, alternatively, of treated sand. After an overnight stirring, the tubes were centrifuged at 2000 g for 15 minutes. The extract was used to determine P-inorg and the residue was used for P-org extraction. In fact, 12 ml of demineralized water were added for two times to wash the residue, centrifuged at 2000 g for 15 minutes and discarded the supernatant. The residue was dried at 80°C and then transferred to a porcelain crucible.

After calcination of 3 hours at 450°C, ashes were put into centrifuge tubes with 20 ml of 1 mol L^{-1} HCl. Centrifuge tubes were closed and stirred overnight. After an overnight stirring of 16 hours, the tubes were centrifuged at 2000 g for 15 minutes and the extract was collected and analyzed for P-org determination. In order to extract AP and NAIP a sequential extraction procedure was applied: firstly, 20 ml of 1 mol L^{-1} NaOH were added to 200 mg of dried sample. After stirring overnight, samples were centrifuged at 2000 g for 15 minutes. From this point, 10 ml of the extract were collected and used for NAIP extraction while the cake of centrifugation was used for AP extraction. To the 10 ml of the extract were added 4 ml of 3.5 mol L^{-1} HCl and then stirred for 20 seconds. The samples were let stand for 16 hours in a covered centrifuge tube, centrifugation at 2000 g for 15 minutes was applied. NAIP was measured on the supernatant. The cake of extraction previously obtained was used for AP extraction. For two times were added 12 ml 1 mol L^{-1} NaCl then stirred for 5 minutes, then centrifuged at 2000 g for 15 minutes and finally discarded the supernatant. After that, it was added 20 ml of 1 mol L^{-1} HCl to the solid part and the tube was covered and stirred overnight. After centrifugation at 2000 g for 15 minutes, the extract was used for apatite phosphorus measuring. The accuracy of results obtained with the protocol was tested by comparing the sum of single fractions as suggested by Xie and colleagues (Xie et al., 2011):

P-tot = P-inorg + P-org and P-inorg = NAIP + AP

Available-P (Olsen-P) was extracted using Olsen method (Olsen et al., 1954), a procedure suitable for both acidic and alkaline soils, and also for soils with a high presence of $CaCO_3$. Following the method, two grams of dried sample were injected into a plastic container. Then were added 0.5 g of activated carbon and 40 mL of 0.5 mol L^{-1} of $NaHCO_3$ (with a pH of 8.5). Previously, the activated carbon was washed with the solution of sodium bicarbonate in order to eliminate residual P on the activated carbon. Afterward, the plastic container was kept in agitation for 30 minutes and the solution was filtered with Whatman® n° 42 filters. 20 ml of the liquid solution were collected in a plastic tube and 10 ml of H_2SO_4 1 N were slowly added. After 24 hours from the addition of H_2SO_4, the bioavailable-P contained into the partially covered tube were determined. The method relies on the presence of carbonate ions in the solution that reduces the activity of Ca^{2+} and Al^{3+} with a consequent increase of phosphorus solubility. In calcareous soils, the addition of carbonate ions reduces the activity of Ca^{2+} which precipitates in the form of $CaCO_3$ and, in this way, the solubility of the calcium phosphate increases with the decrease of the Ca^{2+} in solution.

Phosphorus quantification, in the form of orthophosphate PO_4^{3-}, was determined spectrophotometrically at 430 nm using a Hach DR5000 spectrophotometer through the Hach molybdovanadate method (Yellow method - 8114) adapted from Standard methods for the examination of water and wastewater (Federation and Association, 2005). With this method, the PO_4^{3-}, reacts with molybdate in an acid medium to give a phosphomolybdate complex. Vanadium in the sample causes a yellow vanadomolybdophosphoric acid to form. The intensity of the yellow color is proportional to the phosphate concentration. All reagents used had high purity (>99.5%) and all glassware, plasticware and laboratory tools were cleaned with HCl 6M, rinsed 3 times with deionized water, one time with milliQ water in order to remove all impurities and finally air-dried at 60°C.

4.2.3. Climatic chamber

In order to have a constant temperature, a climatic chamber was built (**Figure 16**). A box made of insulating material was used to maintain isolated the system. With the purpose of maintaining the chamber at a constant temperature of 25°C ± 0.2°C a Peltier cell was exploited. With the scope of maintaining an elevated relative humidity into the chamber, a full water baker was left into the chamber. A thermometer and a hygrometer were placed inside the chamber for monitoring data of temperature and humidity percentage. Every 2 minutes, all data of temperature and moisture were recorded into an SD-card.

Figure 16. The climatic chamber used in this experiment.

4.2.4. Experimental part

The soil was collected, sieved at 2 mm and air-dried at 105°C for 48 hours. After that, three different treatment were performed: in C case, 3 kg of sandy soil were mixed with centrifuged digestate (C); in D case, 3 kg of sandy soil were mixed with dried digestate (D); in T case 3 kg of sandy soil were used as control (T). After that, digestates treatments were thoroughly and accurately mixed. Finally, 70 g of the mixed sand were inserted into sterilized plastic tubes and milliQ water was added to each plastic tubes in order to reach field capacity according to Benton Jones (Jones Jr., 2012). The concentration of P-Tot added with treatments was estimated in about 100 ppm of P in order to be around 200 Kg of P_2O_5/ha application rate. Samples were incubated in the climatic chamber for 1, 3, 7, 14, 28, 45, 60 and 90 days. At these times, three replicates of each treatment were randomly selected and sampled. The central part of the sand contained in the plastic tubes was firstly air-dried at 105°C and then P-Tot, P-inorg, P-org, NAIP, AP and bioavailable-P were evaluated. A completely randomized disposition was used for plastic tubes in the climatic chamber (**Figure 17**). All samples stayed at 25°C ± 0.2°C and relative humidity was between 90.0% and 99.9%. Every seven days all samples were weighted and take back to the field capacity thanks to new milliQ water addition.

Figure 17. The sterilized plastic tubes filled with treated and no-treated sandy soil.

4.2.5. Kinetic modeling

Kinetics of bioavailable-P (kinetic of $NaHCO_3$ extractable-P) on soils with T, C and D treatments were analyzed using four kinetic models: first order, second order, Elovich and power function equations according to Sparks and Sinegani (Sinegani and Sedri, 2011; Sparks, 1989) and summarized in **Table 10**. Excluding the power function equation, in all other equations were applied the boundary conditions $t = 0$ to $t = t$ and $pt = p0$ to $pt = pt$ in which t represents the time and p the bioavailable phosphorus.

Model	Equation
First order	$\ln P_t = \ln P_0 - k_1 t$
Second order	$1/P_t = 1/P_0 + k_2 t$
Simple Elovich	$P_t = 1/\beta \ln(\alpha\beta) + (1/\beta) \ln t$
Power function	$P_t = a t^b$

Table 10. The kinetic equations tested in the present work.

Usually, first-order equation is expressed as (Sparks, 1989):

$D(P_t)/dt = - k_1 * (p_t)$

P_t represents the bioavailable phosphorus at time t expressed in PPM and k_1 (expressed in h^{-1}) represents the rate constant of first-order sorption. After integration, the integrated form becomes:

$\ln P_t = \ln P_0 - k_1 * t$

Second-order kinetic equation is expressed as (Sparks, 1989):

$d(P_t)/d_t = k_2 * (P_t)^2$

P_t represents the bioavailable phosphorus at time t expressed in PPM and k_2 represents the rate constant of second-order sorption. After integration, the integrated form becomes:

$1/(P_t) = 1/P_0 + k_2 * t$

Elovich kinetic equation is expressed as (Sparks, 1989):

$d(P_t)/d_t = \alpha e^{-\beta * (P_t)}$

P_t represents the bioavailable phosphorus at time t expressed in PPM; α represents the initial adsorption rate and β is the desorption constant (Sinegani and Sedri, 2011). Assuming that $\alpha \beta t >> 1$ (Chien and Clayton, 1980) becomes:

$P_t = 1/\beta * \ln(\alpha\beta) + (1/\beta) * \ln t$

Power function equation is expressed as (Sparks, 1989):

$(P_t) = a t^b$

P_t is the bioavailable phosphorus at time t; a and b are equation constant (Sinegani and Sedri, 2011). The linearized form is:

$\mathrm{Ln}(P_t) = \ln a + b \ln t$

All equations used to describe kinetics were evaluated according to their coefficient of determination (r^2) and standard error of the estimate (SE) (Chien and Clayton, 1980; Fekri et al., 2011; Hosseinpur and Pashamokhtari, 2008; Sparks, 1989). Due to the no systematic attempt of literature in the interpretation of the parameters resulted from P kinetics (Islas-Espinoza et al., 2014), these parameters data will be not showed.

4.2.6. Statistical analysis

One-way ANOVA was performed separately on P-tot and bioavailable-P results of control, centrifuged and dried treatments. After the ANOVA, Tukey's post-hoc test ($P < 0.05$) was performed. The statistical software R (version 3.5.1 - Feather Spray - 2018) was used for all statistical analyses.

4.3. Result and discussion

4.3.1. Total phosphorus

Total phosphorus was measured on the sandy soil and on SSADs before the experiment. P-tot in sandy soil was 478 ± 12 ppm, a normal value if referred to calcareous soils (Hosseinpur and Pashamokhtari, 2008). Total phosphorus in SSADs was similar to the values obtained in the previous experiments. Phosphorus in centrifuged SSAD resulted 2.82 ± 0.02% (d.m.b.). It means that the P_2O_5 contained was 6.45% because of the conversion factor of 2.291 (2.82*2.291 = 6.45). Phosphorus in dried SSAD was 2.98 ± 0.01% (d.m.b.), which means that the P_2O_5 contained was 6.84%. In **Figure 18** the time course of phosphorus in soil no treated (control) and treated with centrifuged and dried SSADs is shown. Firstly, it is possible to note that, from day 1, there was an increment of phosphorus of around 100 ppm in C and D thanks to the addition of 200 Kg of P_2O_5/ha of digestate treatments. Apparently, PPM mean values changed in different times of sampling. The statistical analysis allowed to reject this possibility (**Figure 19**). In fact, the Tukey test revealed that no significant changes occurred during experiment time. Total P remained constant for 90 days in control soil (**Figure 19.A)** in soil with centrifuged SSAD (**Figure 19.B**) and in soil with the dried digestate (**Figure 19.C**). This trend revealed as expected and in line with the literature (100-3000 ppm (Karamesouti and Gasparatos, 2017)). In fact, phosphorus has got a very low solubility in soil system (<0.01 mg P L^{-1} (Karamesouti and Gasparatos, 2017)). It could be lost by erosion and runoff or absorbed by plants, all phenomena that could not appear in a closed system such as the falcon tubes used in this experiment. In the closed system designed for this experiment it could be "lost" only for leaching, moving from the upper part of the tube to the lower part: anyway, statistical analysis disclaim this hypothesis.

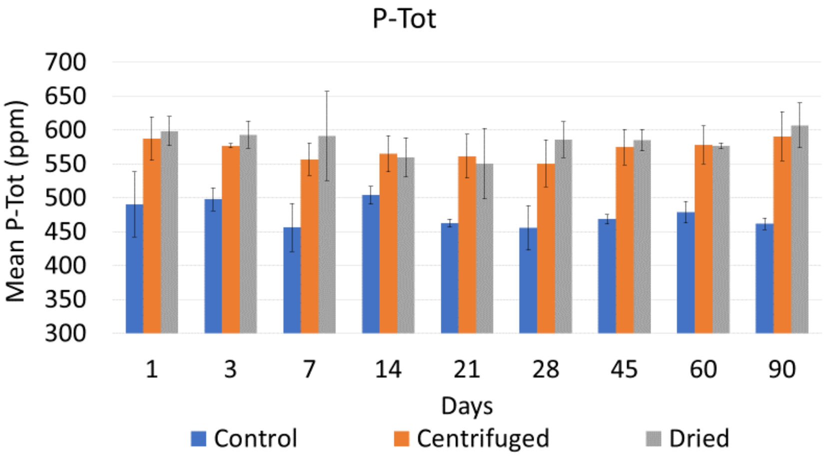

Figure 18. Mean P-tot (ppm) with different treatments among three months. Each error bar represents the standard deviation.

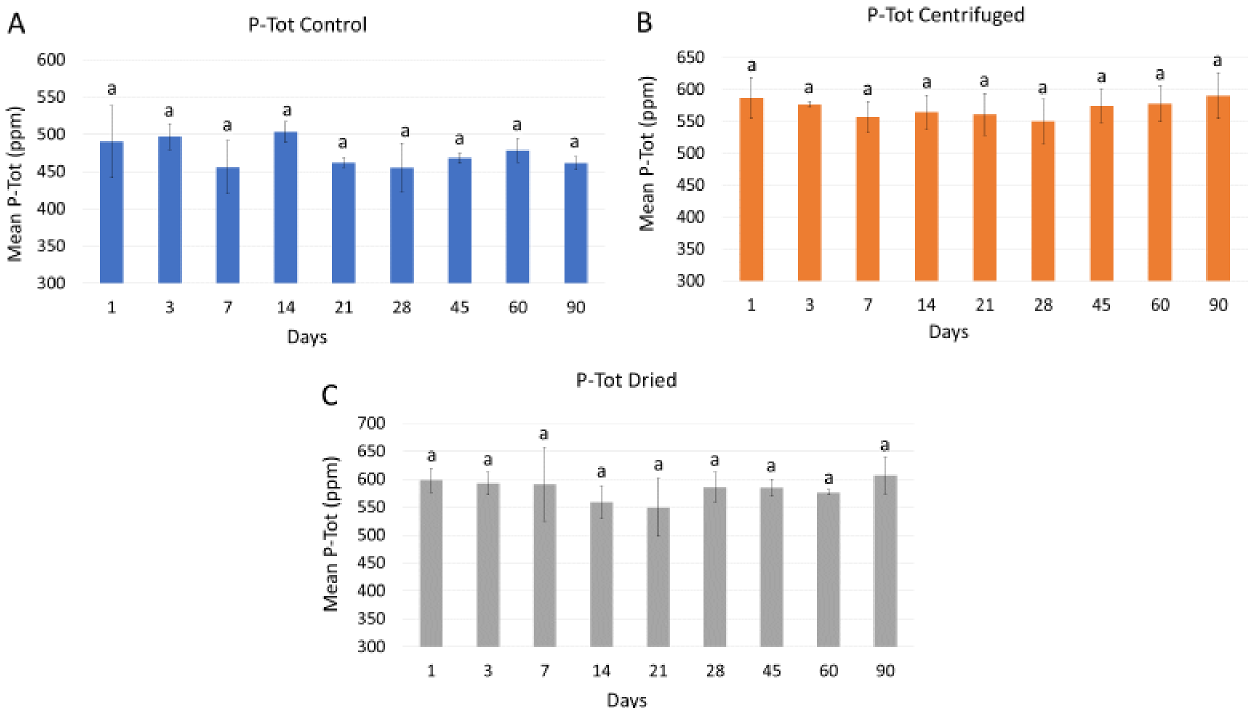

Figure 19. Mean P-tot (ppm) in soil of control (**A**), centrifuged (**B**) and dried (**C**) among three months. Different letters indicate differences between ppm levels that are significant at $P < 0.05$ (Tukey HSD). Each error bar represents one standard deviation.

4.3.2. Phosphorus fractionation

The fractionation of the P contained in SSADs revealed that most of it was in the inorganic form: 85.3% (24033 ppm) in centrifuged digestate and 83.9% (25050 ppm) in dried digestate. P-org was 9.9% (2779 ppm) in centrifuged and 7.1% (2107 ppm) in dried digestate (**Figure 20a**). In centrifuged digestate there was a missing percentage of 4.8% while in dried SSAD was about 9.0%. Due to the sequential extraction process of the method, the SMT usually gives not residual parts of P (Ruban et al., 2001). On the other hand, it is possible to broadly compare these results to the others in literature since many other studies on SS gave similar residual parts to the ones obtained in this study. A Chinese study on different forms of P contained in sewage sludge of 46 cities and measured with the SMT method, revealed that more than 60% of P-tot was inorganic and about 26% was organic (Wang et al., 2019). Another work that used SMT method (Xie et al., 2011) reported that in their sewage sludges P-inorg ranged from 75.9 and 87.6% of P-tot and P-org ranged from 11.5 and 22.1% of P-tot. Garcia-Albacete and coworkers analyzed heat SS with SMT method and revealed that 87.6% of P was inorganic and 2.56% was organic (García-Albacete et al., 2012). This comparison revealed that results obtained on SS are in line with other literature studies, with P-inorg >> P-org. The different proportions of P fractions may depend on the origin and the treatment process of sludge (Nobile et al., 2019). Taking into account this last assertion, the two SSADs differ for the heat treatment. It may have changed the proportion between P-Inorg and P-Org: in fact, probably because the heat treatment both the P-Org total content and the P-Org percentages were lower in Dried SSAD (Li et al., 2014; Nobile et al., 2019).

Concerning NAIP and AP in SSADs the application of SMT method gave an overestimation of results probably due to an underestimation of P-inorg: in fact, the sum of NAIP and AP should give P-inorg. In this study, there was an overestimation of 2.5% in centrifuged SSAD and 5.4% in dried SSAD. Mean percentages of NAIP referred to P-inorg were 68.6% (16497 ppm) and 79.4% (19909 ppm) in centrifuged and dried SSADs respectively. Mean percentages of apatite phosphorus related to the inorganic one were 33.9% (8137 ppm) and 25.9% (6499 ppm) in centrifuged and dried digestates respectively (**Figure 20b**). As before, it is possible to broadly compare this work to the ones in the literature, since other studies show similar residual parts. The Chinese research on the sludges of 46 cities cited above (Wang et al., 2019) mentioned that the mean percentage (referred to P-inorg) of NAIP and AP were about 78% and 26% respectively. Xie et al. (Xie et al., 2011) referred that in their SS NAIP ranged from 32.4% to 68.0% of P-tot and AP ranged from 37.1% to 69.7%. The work of Garcia-Albacete and colleagues revealed that 32.0% of inorganic P was NAIP and 51.5% was AP (García-Albacete et al., 2012). In Nobile and colleagues work (Nobile et al., 2019), a dewatered SSAD had 75.3% of AP and 4.8% of NAIP; in the same work the same SSAD received a heat treatment that changed that proportion to 63.4% of AP and 18.7% of NAIP. That different proportion of AP and NAIP could be due to different chemical and thermic treatments in WWTPs. For what it concerns chemical treatments, a large proportion of AP may be attributed to the addition of lime to improve dewatering efficiency during the sludge treatment process (Xie et al., 2011), while, on the contrary, a high presence of NAIP could be related to the iron content of the sludge (García-Albacete et al., 2012). Such as before, it is possible that the different percentages between centrifuged and dried SSADs in terms of NAIP and AP could be motivated by the heat treatment of the dried SSAD (Li et al., 2014; Nobile et al., 2019): it was demonstrated that in sludges the heat treatment between 120° and 220°C could increase NAIP % and decrease AP %. Above 220°C, NAIP and AP exchange rapidly their percentages (Li et al., 2014). SSADs used in this work derived from the same raw material; dried SSAD derived from centrifuged digestate thermally treated at around 180°C: this treatment probably increased P-inorg % and NAIP %.

Unfortunately, the application of SMT method to treated and non-treated soils did not give the expected results. Sum of P-inorg + P-org did not reach P-tot. If in SSADs there was an "acceptable" "missing part of P" (<9%), during three months of soil study the "missing P" had an average percentage of 23.7%, 26.0% and 19.9% in control, centrifuged and dried samples respectively. Although some authors revealed that is normal to have an underestimation of the values of P-inorg + P-org with respect to P-tot (between 0 and 22% (García-Albacete et al., 2012; Pardo et al., 2004)), it is not correct to find any variation on these P fractions. By the way, in **Figure 21** the time course of P-inorg and P-org fractions compared to P-tot is represented. Literature supports the thesis that, after the addition of sludge to the soil, the transformation of P-org contained in sludge into P-inorg can be occurred (Tibbert et al., 2003). Concerning NAIP and AP changes during the three months of the experiment, measures were recorded in day 1, 28, 60 and 90. Nevertheless, the sum of average values of NAIP + AP was 96.1%, 98.8% and 86.3% of P-inorg mean values measured on control, centrifuged and dried soils respectively, and, for that reason, it is not correct to comment these data. In fact, if there was an underestimation of P-inorg, it was also possible that NAIP or AP was underestimated. Anyway, a graphical representation of the sum of NAIP + AP compared to P-inorg is reported in **Figure 22**.

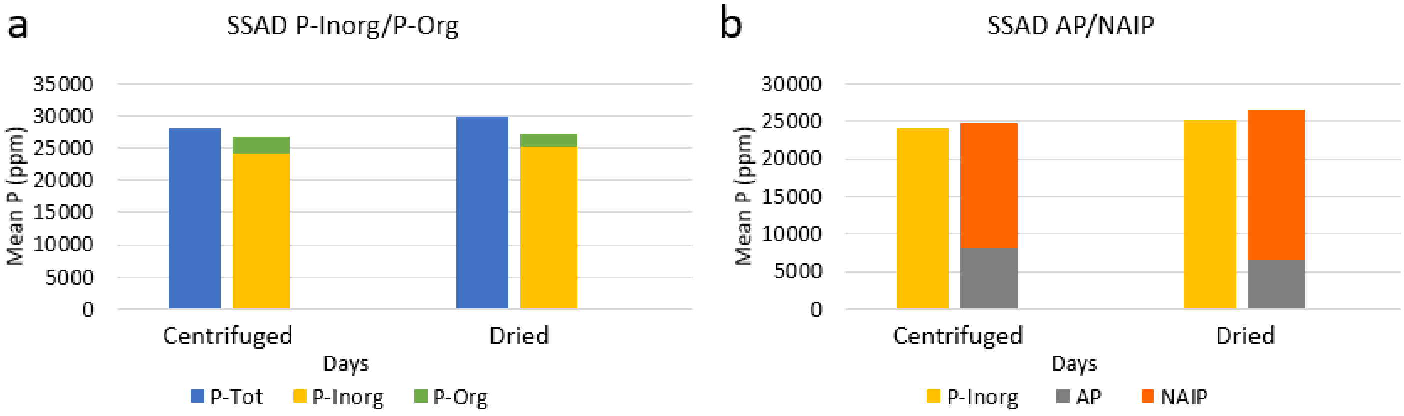

Figure 20. SSAD P fractionation. **a**: P-Tot fractionation in P-Inorg and P-Org; **b**: P-Inorg fractionation in Ap and NAIP.

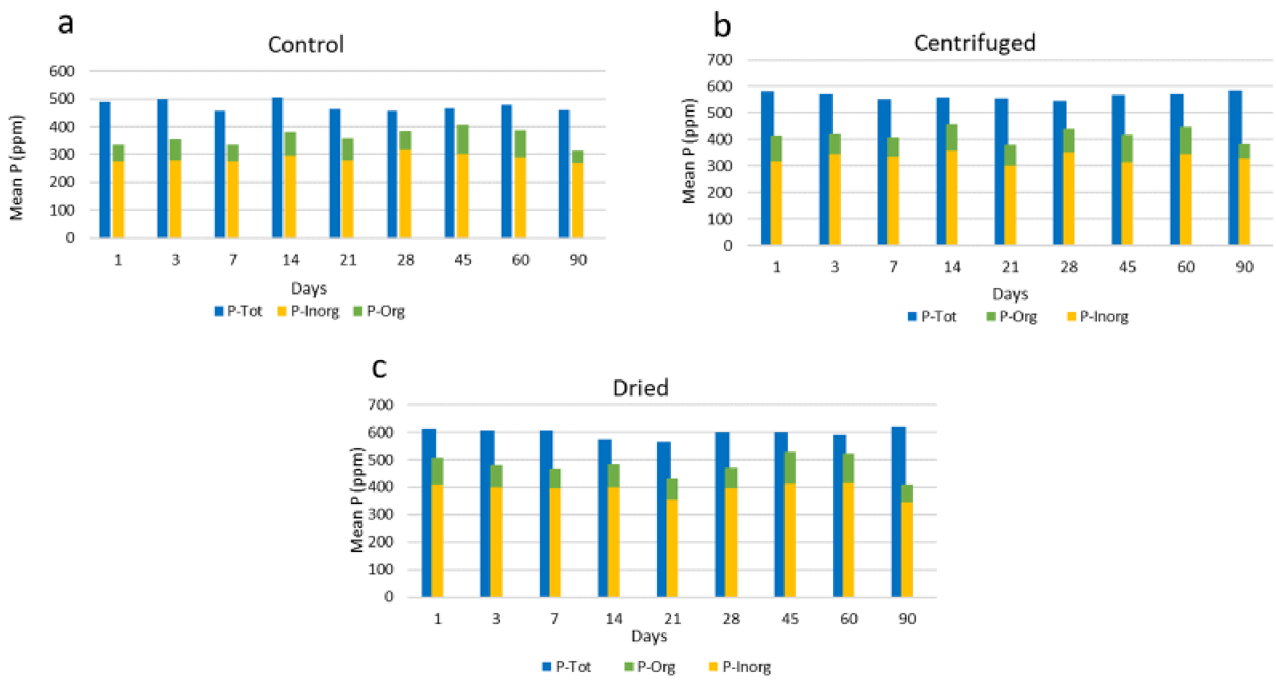

Figure 21. Sum of mean values of inorganic and organic P fractions compared to mean values of P-tot during 3 months of the experiment. **a**. The time course of control. **b**. The time course of C treatment. **c**. The time course of D treatment.

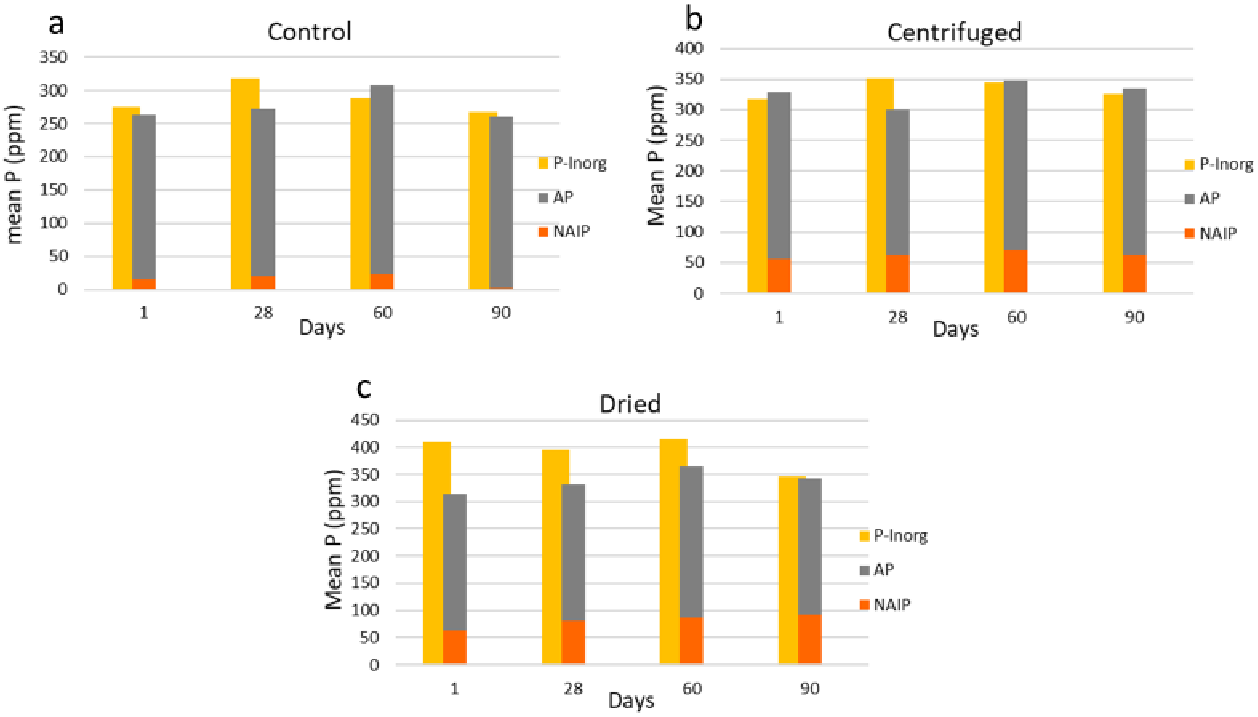

Figure 22. Sum of mean values of NAIP and AP fractions compared to mean values of P-inorg during 3 months of the experiment. **a**. The time course of control. **b**. The time course of C treatment. **c**. The time course of D treatment.

4.3.3. Bioavailable-P

Bioavailable-P (NaHCO$_3$ extractable P) in soil before the experiment was 4.4 ± 1.3 ppm. This is a very low value if compared to other soils (Arpa Veneto, 2007). Considering that the extraction of bioavailable-P with the Olsen method is appropriate and precise only in soils, bioavailable-P was not extracted on SSADs. On the other hand, some authors assume that the bioavailable-P can be represented by the sum of NAIP and P-org (only partially available) measured with SMT method (Pardo et al., 2003; Wang et al., 2013; Xie et al., 2011): in this way, the 68.5% and the 73.8% of P-tot of centrifuged and dried SSADs con be considered as potentially bioavailable. Surely, due to the residual-P not determined in P fractionation, it is not correct to compare these two percentages. It is only possible to observe that, using SMT method, most of the P contained in SSADs can be considered bioavailable. Furthermore, because of the different methods of analysis, this data can't be directly compared to Olsen ones. **Figure 23** shows the time course of average P-bioavailable during three months of the experiment. Data analysis revealed that the SSAD addition to soil increased the bioavailable-P. This result confirms the fertilizing properties of SSADs and it supports the results obtained in treated soils in the previous experiment (Chapter III). This increase is supported by other studies in the literature (Huang et al., 2012; Islas-Espinoza et al., 2014; Nobile et al., 2019). The day after the addition of SSADs (Day 1), the quantity of mean bioavailable-P increased from 4.4 ± 1.3 ppm of the not-treated soil before the addition of SSAD to 34.9 ± 2.9 ppm of centrifuged-treated soil and to 22.2 ± 1.5 ppm of dried-treated soil. Considering that 100 ppm of P-tot were added with SSADs application, a great percentage of that can be considered as bioavailable: around 30% and 18% in centrifuged and dried SSADs, respectively. These values were lower than was expected to be bioavailable-P calculated as the sum of NAIP and P-org. This difference can be due to two different reasons: firstly, they were extracted with two different methods and, secondly, they were extracted after one day from P addition and a loss of bioavailable-P could have happened meanwhile. In the following days, a decrease in bioavailable-P in control and centrifuged treatments seemed to happen. Statistical analysis confirmed these decreasing trends (**Figure 24**). **Figure 24.A** shows the bioavailable-P gradual decrease in control soil from 4.2 ± 1.2 ppm of day 1 to 0.3 ± 0.6 ppm of day 90. **Figure 24.B** shows the bioavailable-P decrease of soil treated with centrifuged SSAD: it decreased from 34.9 ± 2.9 ppm of day 1 to 23.8 ± 1.5 ppm of day 90. Similar decreasing of bioavailable-P over time is well supported in the literature: in fact, similar decreasing trends were found using organic soil improvers (such as farmyard manure, processed city waste and poultry litter (Bibi et al., 2018)) and using mineral fertilizers such as KH_2PO_4 (Javid and Rowell, 2002; Sinegani and Sedri, 2011). The decrease in P-bioavailability could be motivated by many processes (paragraph 1.5.): immobilization, adsorption, precipitation, leaching, runoff and erosion. Runoff and erosion must be excluded because of the closed system of the experiment. The measure of bioavailable-P in the upper, medium and lower parts of the samples demonstrated that leaching did not appear (data not shown). Immobilization, due to the sterilization of sandy soil at 105°C, should be excluded at least in control; on the other hand, it is possible that some microorganisms survived in centrifuged and dried SSADs. Moreover, net immobilization can occur if the substances added to soil have a C/P ratio greater than 300:1, while net mineralization is likely if the ratio is below 200:1 (Weil and Brady, 2017). The C:P ratio of SSADs was below 200. Hence, it was also possible that the mineralization process appeared causing an increase in P availability, but, due to the non-increase of bioavailable P, this option was not observed here. The most probable processes that could have happened are adsorption and precipitation. Different trend appeared in the bioavailable-P of dried SSAD **(Figure 24.C**). Data analysis highlighted that, from day day 1 (22.2 ± 1.5 ppm) to day 90 (the lowest mean value, 19.6 ± 0 ppm), there was a peak on day 14 in which the concentration of bioavailable-P was the highest (26.6 ± 3.0 ppm). This different trend could be due to the presence of organic matter (OM) in SSADs. It is known that OM can enhance P bioavailability: after addition of OM to soil, there is an inhibition of P sorption thanks to dissolved organic carbon compounds - DOC (humic acids, fulvic acids, tannic acids, aromatic acids, low molecular weight organic acids, polysaccharides, amines and others C compounds (Stevenson, 1994)). DOC can release phosphorus by decreasing sorption sites on colloid surfaces by metal complexation, dissolution reaction (on Fe and Al oxides), competitive sorption and metal bridging (Guppy et al., 2005). In soil treated with dried SSAD, probably the DOC contained in the sludge acted slowly with one or more of these modalities. However, this explication does not clarify why the same phenomenon did not appear in soil with centrifuged SSAD. It is possible that in C treatment the effects of OM addition were rapid (in the first 24 hours), while in D treatment, these reactions were postponed due to the heat treatment. Another conceivable hypothesis to justify this peak may be a possible anoxic condition in the sample analyzed: in fact, the release of P is favored under anaerobic conditions as a consequence of Fe reduction. All these hypotheses on bioavailable-P changed over time will be better explored in the next paragraph.

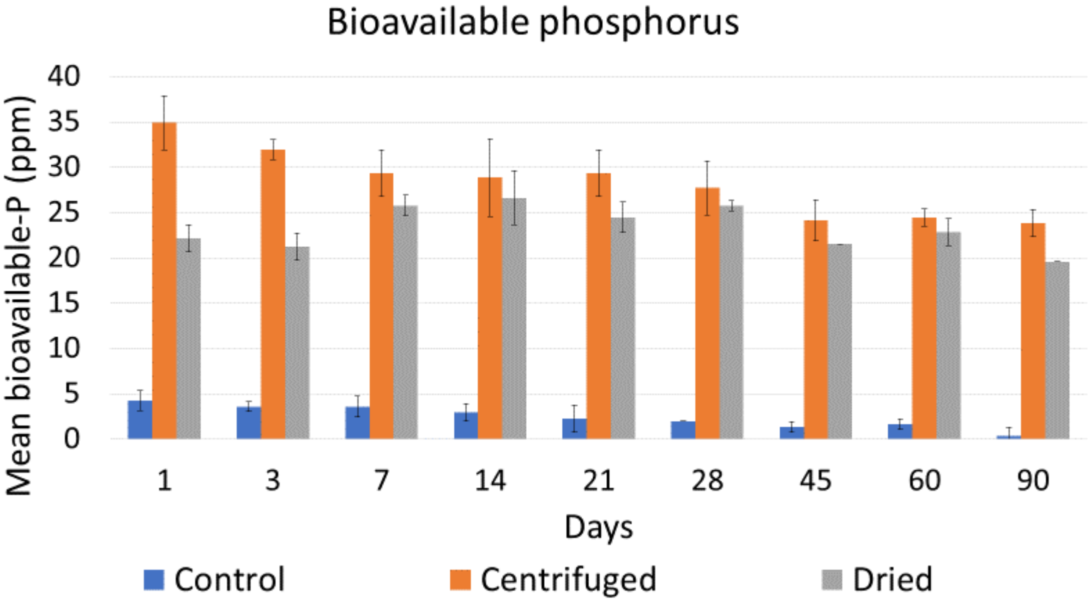

Figure 23. Mean bioavailable-P (ppm) with different treatments among three months. Each error bar represents one standard deviation.

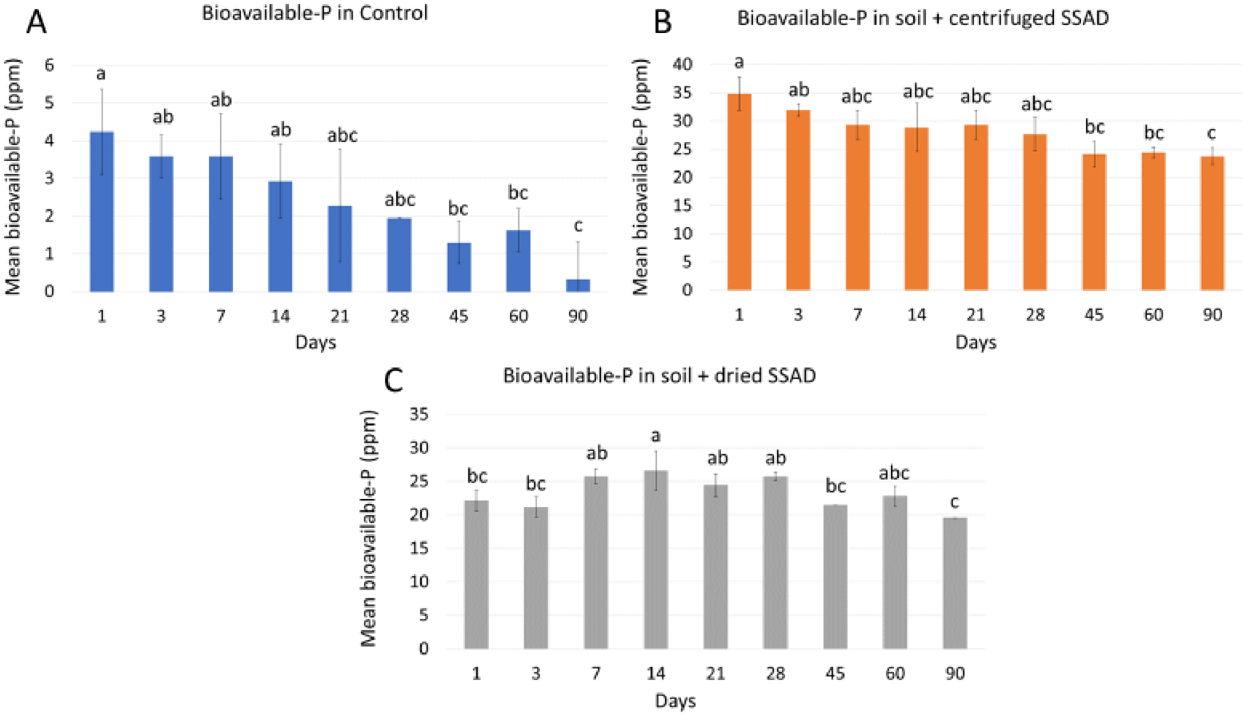

Figure 24. Mean bioavailable-P (PPM) in soil of control (**A**), centrifuged (**B**) and dried (**C**) among three months. Different letters indicate differences between PPM levels that are significant at $P < 0.05$ (Tukey HSD). Each error bar represents one standard deviation.

4.3.4. Decreasing kinetic of phosphorus bioavailability

Results of the coefficients of determination (r^2) and the standard error of estimate (SE) for kinetic equations, which were used to describe phosphorus bioavailability in the poor, alkaline and sandy soil are represented in **Table 11**. Due to the high r^2 and low SE values, kinetic of control bioavailable-P can be well described by the first-order equation firstly, and by simple Elovich equation, secondly. Concerning the kinetic of bioavailable-P in the soil treated with centrifuged SSAD, the Elovich equation seems to be the best model for fitting these data due to the high r^2 (0.93). On the other hand, this model had a high SE (1.05). As well, the power equation successfully described this kinetic, with a high r^2 (0.91) and very low SE (0.04). Similar results were obtained in the work of Sinegani and co-workers (Sinegani and Sedri, 2011): in that study, potassium dihydrogen phosphate (KH_2PO_4) was added to sterile soil and the Elovich kinetic equation was the equation that better described the processes. As introduced in the previous paragraph, the most probable processes that could cause a decrease in $NaHCO_3$ extractable-P were precipitation and adsorption. Precipitation can be caused by Ca^{2+}, Fe^{3+} and Al^{3+} ions. From previous analyses on the sandy soil, it resulted that total limestone was 369 g/kg, active limestone was 10 g/kg and the exchangeable calcium was 950 ppm. With these high values of Ca^{2+} and a soil pH > 8, Ca^{2+} resulted the predominant cation and the soluble phosphorus can reasonably precipitate forming Ca phosphates (Yadav et al., 2012; Fekri et al., 2011). As well the increase of pH in soil solution, for the principle of Le Chatelier, the formation of Ca phosphates is favored by the increasing of Ca^{2+} and P concentrations in soil solution. Many reactions could have occurred, but, at this soil pH, it is very probable that HPO_4^{2-} quickly reacted with calcium forming a sequence of products with decreasing solubility immediately after the addition of P to the soil (Weil and Brady, 2017). Firstly, the dicalcium phosphate (slightly soluble) was formed followed by the tricalcium phosphate (very low soluble):

$$HPO_4^{2-} \xrightarrow{CaCO_3} 2(CaHPO_4*2H_2O) + CO_{2\uparrow} \xrightarrow{CaCO_3} Ca_3(PO_4)_2 + CO_{2\uparrow} + 5H_2O$$

The solubility of these compounds and, consequently, bioavailable-P, decrease as the phosphorus changes from HPO_4^{2-} ion to tricalcium phosphate. After that, the formation of more insoluble compounds could have continued forming (in order of decreasing solubility): oxyapatite $[3Ca_3(PO_4)_2] \cdot CaO$, hydroxyapatite $[3Ca_3(PO_4)_2] \cdot Ca(OH)_2$, carbonate apatite $[3Ca_3(PO_4)_2] \cdot CaCO_3$ and finally fluorapatite $[3Ca_3(PO4)_2] \cdot CaCO_3$ (Weil and Brady, 2017).

As introduced before, also cations of Fe and Al can precipitate with P forming Fe and Al phosphates such as variscite and strengite (Penn and Camberato, 2019):

$$Al^{3+} + H_2PO_4^-{}_{(aq)} + 2H_2O \leftrightarrows Al(PO_4)*2H_2O_{(S)} + 2H^+ \text{ variscite}$$
$$Fe^{3+} + H_2PO_4^-{}_{(aq)} + 2H_2O \leftrightarrows Fe(PO_4)*2H_2O_{(S)} + 2H^+ \text{ strengite}$$

These reactions are common in acidic soils with P additions due to the instability of Fe and Al cations at low pH, but in alkaline soils are rare. On the other hand, the addition of Fe salts (such as $FeSO_4*7H_2O$) is adopted in WWTPs as a strategy to remove P from the water via precipitation and, hence, an accumulation of Fe phosphates in SS likely occurred (Epstein, 2001; Huang et al., 2007, 2012). In this way, the used SSADs were rich in terms of Fe phosphates and their addition to a moist alkaline soil could have caused the dissolution of Fe phosphates, enhancing P in the soil solution (Huang et al., 2007). Huang and colleagues (Huang et al., 2012) found that after some days from SSADs application (chemically stabilized with $FeSO_4*7H_2O$) to an alkaline soil, was observed an increase of bioavailable-P, due to P mineralization or mineral transformations. That could be another hypothesis explaining the enhancement of bioavailable-P after some days from the application of dried SSAD treatment. The other phenomenon that might have happened was the adsorption to Al and Fe oxides and hydroxides and the adsorption on the edges of alumino-silicate minerals. On the other hand, adsorption on oxides and hydroxides of Fe and Al and on alumino-silicate minerals tends to decrease with the increase of pH (Penn and Camberato, 2019). Differently, in a calcareous soil with a basic soil pH, rich in calcium carbonate it was highly possible that adsorption on calcite has occurred (Griffin R.A. and Jurinak J.J., 1973; Kuo and Loxse, 1972). Regarding bioavailable-P kinetic with dried SSAD addition, none of the kinetic models proposed had a satisfactory fitting. On the other hand, similar results were obtained in the work of Sinegani and colleagues: in that work, the same models tested here resulted satisfied for sterilized soil with P addition (Sinegani and Sedri, 2011). Furthermore, in that work there was a treatment that was not satisfied by any of the tested models: after around 20 days from P addition to an unsterilized soil there was an increase in P-bioavailability. This increase was very similar to what happened in the soil treated with dried SSAD. In that case, the authors motivated the increasing of P with the release of organic acids (e.g. α-keto glucorunic acid (Motsara et al., 1995)) by phosphate solubilizing microorganisms. These organic acids can chelate Ca, Fe and Al, causing an increase in the concentration of P in soil solution. This motivation can further explain the peak, but it is probably unrealistic for this case study due to the sterilization of soil combined with heat treatment of SSAD. In **Figure 25** the experimental mean values of bioavailable-P compared with values of bioavailable-P applying different kinetic models are graphically represented. It is possible to note that, especially in centrifuged-treated soil, the P decreasing occurred in two stages: firstly, a rapid stage of loss of bioavailable-P and secondly, a gradual decreasing stage in which it seems that bioavailable-P reach the equilibrium. Many works reported that after an addition of P, the equilibrium was reached in different times: in laboratory conditions from only 15 seconds (Kuo and Loxse, 1972) to 60 days (Chen et al., 1973) and until 15 years in field conditions (Ma et al., 2009). It was observed in other works the same two different stages of P-sorption by soils observed in this work (Sinegani and Sedri, 2011; Sparks, 1989). The first quick stage is attributed to adsorption (ligand exchange) and the second slow stage by precipitation into increasingly less soluble states as time increases (Bohn et al., 2001). Moreover, if P concentration is high (such as in centrifuged-treated soil), ligand exchange and surface precipitation can be considered as a continuum because of a rapid P precipitation (Afif et al., 1993; Chen et al., 1973; Penn and Camberato, 2019). These motivations can explain the bioavailable-P decreasing kinetic obtained in control and centrifuged-treated soil. Furthermore, the use of Elovich model to describe this kinetic is totally in line with previous motivations: in fact, the Elovich model is often used to describe soils reactions (such as PO_4^{3-} sorption and desorption) that could be described only by the combination of two or three first-order reactions (Chien and Clayton, 1980; Sparks, 1989). Hence, the Elovich equation can describe the probable adsorptive/precipitate process that appeared in control and centrifuged treated soils. Finally, in a study with similar SSADs added to a similar soil it was suggested that the rate-determining step was the precipitation of Ca-phosphates (Huang et al., 2012).

Treatment	First order		Second order		Simple Elovich		Power function	
	r^2	SE	r^2	SE	r^2	SE	r^2	SE
Control	0.91	0.25	0.73	0.49	0.90	0.42	0.64	0.50
Centrifuged	0.79	0.06	0.82	0.00	0.93	1.05	0.91	0.04
Dried	0.28	0.10	0.30	0.00	0.01	2.59	0.01	0.11

Table 11. Coefficient of determination (r^2) and standard error of estimate (SE) for kinetic equations used to describe the bioavailable-P data.

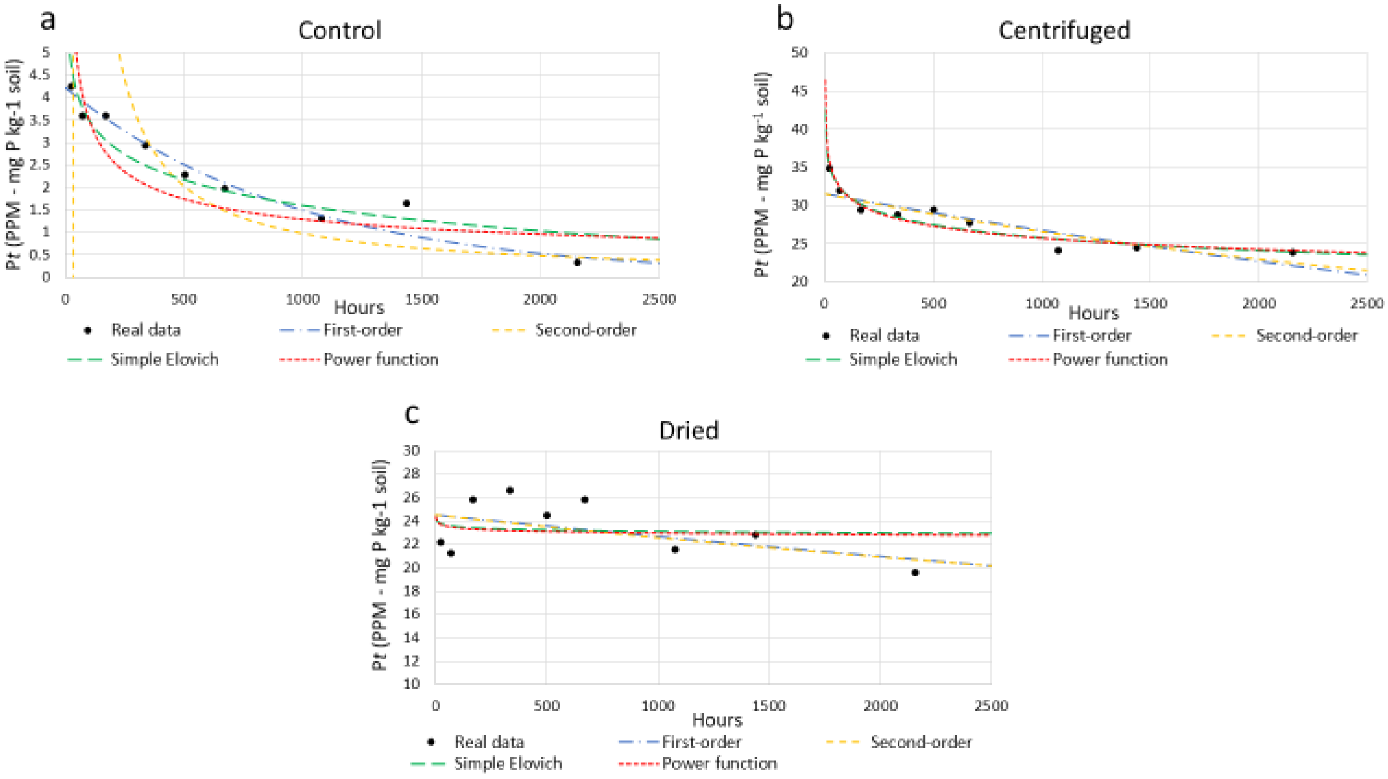

Figure 25. Kinetic of bioavailable-P in soils over 2160 hours (90 days) in: (**a**) not treated soil, (**b**) treated with centrifuged SSAD soil and (**c**) treated with dried SSAD soil. On the x-axis is represented the time course in hours and on the y-axis is represented the bioavailable-P content in ppm. Black points represent the mean values of bioavailable-P measured in the experiment. Fitted kinetic models of bioavailable-P are represented with colored lines.

4.4. Conclusion

In this experiment, phosphorus changings after the addition of SSADs to an alkaline soil were evaluated. Fractionation of P in SSADs revealed that most of it was in the inorganic form and the majority of this last was formed by NAIP, the more bioavailable fraction of P-inorg. After the addition of SSADs to soil, it was seen the increment of P-tot of around 100 ppm was supposed to be. The P-tot content remained constant for all three months of the experiment. Differently, the bioavailable-P decreased from day

1 to day 90 in control and in soil treated with centrifuged SSAD. This decrease could be due principally to the precipitation of P with calcium: in fact, at alkaline pH and with a great presence of Ca^{2+} ions, the formation of sequential calcium phosphates, even less soluble along time, appeared to be the principal phenomenon. Furthermore, it was even possible that P adsorption occurred at a lesser extent. Due to the high pH of soil solution, it unlikely occurred on Al and Fe oxides and hydroxides and alumino-silicates since it was probably adsorbed on calcite surfaces. These explications are in line with other literature studies and with some of the models tested. In particular, the Elovich model, due to the high r^2 and low SE, seems to best describe the adsorptive-precipitate process of bioavailable-P in control and centrifuged-treated soils. None of the models tested described satisfactorily the behavior of bioavailable-P in dried SSAD tested soil. In fact, on day 14 from treatment addition, there was an increment in P-bioavailability. The same behavior was also found in other works and could be caused by an anoxic environment, microorganisms, or organic matter. Precisely this latter topic will be at the center of the next chapter. Future works will be directed on improving the techniques for phosphorus fractionation with the SMT method in order to reduce the "missing parts" of P. In this way, it will be possible to create specifics time course for all P fractions. Another future perspective of this work could be the study of the kinetics parameter to compare these results with others in the literature. In order to confirm the hypothesis of precipitation of sequential calcium phosphates less soluble over time, the last future perspective could be the research of the specifics calcium phosphates in the soil samples through for example, the use of X-ray diffraction (XRD) technique. Finally, it would be also important to deeply investigate on the behavior of dried SSADs, in order to define unequivocally which was the cause of the enhancing of bioavailable-P 14 days after SSAD addition.

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Chapter V

5. Extraction and encapsulation of humic acids derived from SSAD

5.1. Introduction

As introduced in paragraph 1.4, this chapter is dedicated to the extraction of one high-value compound (HVC) from SSAD: humic acids (HAs). Hence, in addition to the direct use of SSADs in soils, the extraction of HVC is the other strategy that can be applied for the reuse of SSADs. HAs belong to the group of the humic substances (HS) that are defined as the highly transformed part of non-living natural organic matter (NOM), which is formed by organic compounds with structures that vary in their degree of complexity (Ghabbour and Davies, 2014). These substances are also defined as "the black gold of agriculture" (Asing et al., 2009) due to their beneficial effects on soil quality and plant growth, a concept present in the literature for many years (Tan and Nopamornbodi, 1979). HS are natural polymers with a highly heterogeneous structure and are traditionally classified as humic acids (HAs), fulvic acids (FAs) and humins according to their solubility. In fact, FAs are soluble at all pH, HAs are insoluble in acids, and humins are insoluble at all pH (Stevenson, 1994). The molecular sizes range typically between 5 to 100 kDa for HAs, and less than 10 kDa for FA (De Nobili and Chen, 1999; Stevenson, 1994). According to Grinhut and colleagues (Grinhut et al., 2007), the half-life time of HS in nature can reach thousands of years, attributable mainly to HA and humins, whose biodegradability is very slow. In sewage sludges from wastewater treatment plants, HS are present in concentrations ranging from 7.7 to 28.6%, expressed as volatile solids (Gonzalez et al., 2018). Typically, sludge HS are adsorbed to extracellular polymeric substances (Nouha et al., 2018). In particular, a percentage between 24% and 76% of HS consists of HAs, depending on the characteristics of the wastewater as well as the operational conditions of the wastewater treatment plant (Fernández et al., 2008; Liu et al., 2019; Réveillé et al., 2003; Zhang et al., 2015). The positive effects of HAs on plant growth usually depend on many factors (Rose et al., 2014), such as the HA concentration rate, plant species and origin of the raw material used as HA source. These effects include the improvement of the physico-chemical soil properties (such as water retention and soil structure), and the increase of enzymatic activity and soil microbial diversity (Li et al., 2019). Moreover, Chen and Aviad (Chen and Aviad, 1990) demonstrated that specific dosages of these substances can enhance seed germination, stimulate root initiation and lateral root development, and boost root and shoot growth. Many mechanisms are involved in plant growth and, amongst them all, the major role is mainly attributed to the HA/plant membrane interaction. Indeed, improved performance membrane transporters allow better absorption of soil nutrients (Canellas et al., 2015). A clear example is represented by phosphorus bioavailability in soils treated with HAs and P-fertilizer: HAs increases water-soluble phosphate, phosphorus plant uptake and plant biomass, retarding the formation of occluded phosphate (corresponding to the fraction of P-inorg and P-org adsorbed within surfaces of Al, Mn and Fe oxides of soil aggregates; the inorganic part is the NAIP in the SMT fractionation) (Wang et al., 1995). Hence, HAs cannot replace mineral fertilization, but they can provide more productive cropping systems with fewer negative impacts on the environment deriving from fertilizers' lower application. All these characteristics consent HAs to be used as biostimulants in horticulture (Canellas et al., 2015). Moreover, it is worth underlining that nowadays commercial HAs derive mostly from non-renewable resources, such as leonardite, coal and peat, while only in a few cases do they come from renewable sources, such as compost and vermicompost (Canellas et al., 2015).

Different biodegradable polymers have been studied as matrixes for the encapsulation of bioactive compounds for different applications with the aim of having a controlled release of the substances in time. The most used matrixes are chitosan, collagen, gelatin and alginate (Gombotz and Pettit, 1995; Reakasame and Boccaccini, 2018; Tavernini et al., 2020). In the field of agriculture, different bioactive capsules can be found for the purpose of releasing herbicides (Nörnberg et al., 2019), fertilizers (Baki and Abedi-Koupai, 2018; Yao et al., 2013), pesticides (Ni et al., 2010), or even complete cells that have a symbiotic effect with the plant growth (Young et al., 2006). Generally, the objective of having a controlled release is to reduce the amount of product that is added to the soil, which permits the operational costs to be reduced and, more importantly, ensures a constant and correct dose of each bioactive compound. As a consequence, the product is not released into the environment, avoiding the environmental issues cited above. Another attractive feature of encapsulation is the reduction of bioactive compound deterioration.

Among the principal commercial polymers, alginate has the advantage of being a cost-effective material, which is mainly extracted from brown algae. Alginate is a linear polysaccharide composed of two monosaccharide residues, (1,4)-β-D-mannuronic acid (M) and (1,4)-α-L-guluronic acid (G). The particularity of alginate is that it can form a physical hydrogel (insoluble form) in the presence of divalent cations such as Ca^{2+} and Ba^{2+}, which form an ionic cross-linking between the G monomers of two adjacent polymer chains (Reakasame and Boccaccini, 2018). The mechanism of the release of bioactive materials encapsulated in alginate beads can be divided in two steps, the leakage of the bioactive compound and the degradation of the matrix (Gombotz and Pettit, 1995). In fact, bioactive compounds with a size smaller than the matrix pores are leached upon water irrigation, while the bigger ones are released after the degradation of the matrix. The degradation occurs through alginate solubilization due to the action of chelating compounds or extracellular enzymes. Chelators sequestrate the divalent cations yielding to a disruption of the electrostatic interactions between the alginate chains and the Ca^{2+} cations. On the other hand, enzymatic degradation is carried out by alginate lyases, which hydrolyze the polysaccharide bonds. Thus, the degradation of alginate varies with the number of ionic bonds between the Ca^{2+} and G monomers and will depend on the presence of microorganisms able to produce alginate lyases enzymes in the soil (Kaneko et al., 1990).

Adopting a circular economy approach, the principal aim of this chapter work is to evaluate the valorization of anaerobic sewage sludge with the purpose of improving soil quality. More in detail, a process of extraction of HAs from SSAD and their encapsulation in alginate beads was implemented to obtain an added-value product free from heavy metals and contaminants. The quality of the extracted HAs was assessed through electron microscopy. Finally, the effect of the HA beads on plant growth and biomass was evaluated on lettuce plants with a pot experiment under greenhouse conditions.

5.2. Materials and methods

5.2.1. Materials

Anaerobic digestate from sewage sludge was sampled from a wastewater treatment plant in Chile (population equivalent: 3.7 million people). The SSAD was a digestate obtained by centrifugation of the SSAD derived from the AD of a liquid mix between primary and secondary sludge. The scheme of this WWTP is very similar to Italian WWTP previously described. Water treatment starts with preliminary treatments for grit and grease removal with 8 tanks measuring 8 m x 30 m. After, the primary settling is performed in 16 works measuring 20 m x 65 m with a depth of between 3.95 m and 4.6 m. Biological treatment is performed with 16 works, each measuring 11000 m^3 and then with 16 clarifiers with a diameter of 50 m. Finally, the disinfection treatment is performed using chlorine in 4 contact tanks with a total capacity of 21000 m^3. Sludge treatment begins with gravity-based thickening in 4 works with a diameter of 26 m. After that, there is the biological flotation in 6 works with a diameter of 20 m each. The AD of primary and secondary sludges mixed together takes place in 8 works with a diameter of 34 m and a water depth of 15.5 m each (here the is the major difference with the Italian WWTP in which the AD of primary and secondary sludges takes place separately). After that, there is a storage of biogas in 2 tanks for sulphur removal before the dewatering treatment (with 5 Guinard centrifuges). The WWTP average total flow rate is estimated in 766039 m^3/day. The average monthly sludge production is estimated at 11752 m^3/month. The centrifuged solid SSAD was used in this work. Commercial HAs, extracted from leonardite, were provided by Sanagro (Chile), Sodium alginate (food grade) was purchased from Merck. All other chemicals used (KOH, $K_4P_2O_7$, HCl, NaOH, $CaCl_2$, H_3BO_4, $CuSO_4*H_2O$, $FeSO_4*7H_2O$, $MnSO_4*H_2O$, $NaMoO_4*2H_2O$, $ZnSO_4*7H_2O$) were reagent-grade.

5.2.2. Extraction protocol

An extract of HAs was obtained from SSAD adapting the protocol of HS alkaline extraction from soil (Stevenson, 1994). The solid SSAD was firstly mixed with water to reach a humidity of 87% in order to obtain adequate liquefaction of the digestate. Successively, KOH and $K_4P_2O_7$ were added to reach a final concentration of 0.01 M each. Next, a steady and slow mixing was carried out for five hours at room temperature with a homogenizing mixer equipped with a paint mixing drill bit. Subsequently, the mixture was neutralized to pH 7 with H_3PO_4 6M. Finally, a centrifugation (40', 3500 g) was performed to separate humins (pellet) and the extract with HAs (supernatant). A scheme of the extraction process is provided in **Figure 26**.

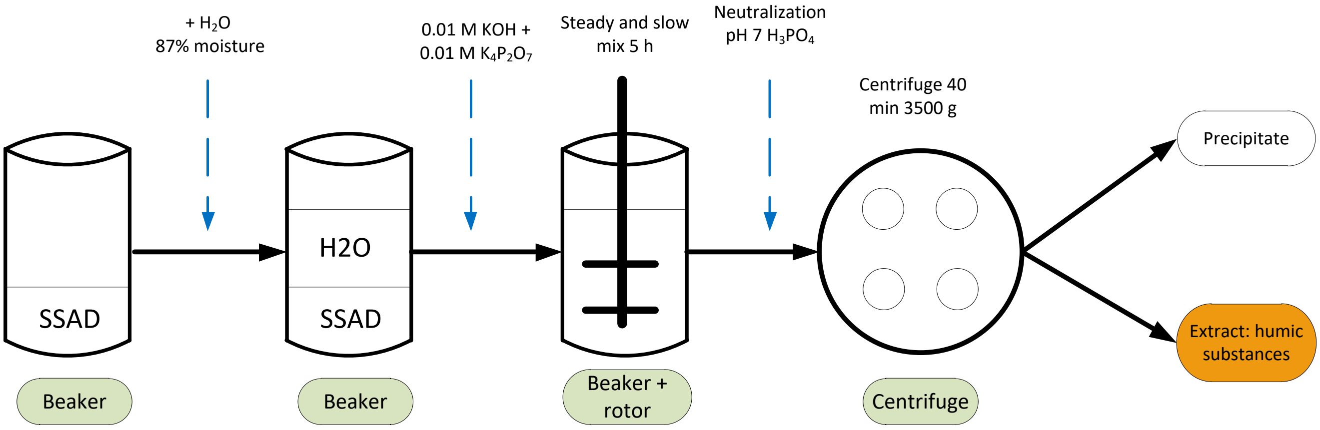

Figure 26. The adopted extraction procedure.

5.2.3. Chemical analysis and HA quantification

Methods of chemical analysis of SSAD are the same reported in paragraph 2.2.1. and their results are reported in **Table 12**. Quantification of HAs on SSAD and on HA extract was performed adapting the method proposed by Lamar and co-workers (Lamar et al., 2014) on the analyzed matrixes. This method is also adopted by the International Humic Substances Society (IHSS) and by the Humic Products Trade Association (HPTA). **Figure 27** schematize the method and afterword the methodology is briefly described. It started with an alkaline extraction with 0.1 M NaOH on the sample. After that, a stirring for 6 hours and a centrifuge of 20 minutes at 3500 g was applied in order to obtain: a precipitate containing humins and a liquid solution containing HAs + FAs. In order to flocculate the HAs, the addition of concentrated HCl (1:1) to the alkaline extract is required until reaching the pH of 1.0 ± 0.05. After, the flocculated HAs were covered with Parafilm and they were stirred for 1 hour. Then it was firstly checked pH and readjusted to 1.0 with HCL or NaOH and, secondly, it was centrifuged for 40 minutes at 3500 g. The centrifugation separated the FAs mix (in supernatant) to HA mix (in the precipitate). After that, the centrifuge tubes containing HAs were placed in a drying hoven at 90° C for more than 24 hours (until obtaining constant weight). After, the obtained residuals were combusted in a muffle oven for 6 h at 500°C. After the combustion, ashes were achieved. Quantification of Has was determined by the difference of the residuals previously obtained and the ashes quantified after the 6 hours in muffle hoven. Quantification was carried out with the same methods to the commercial HAs as a benchmark reference.

Parameter	UM	Digestate
pH (1:10)		8.0
Electrical conductivity	mS/cm	1.557
N - Tot (Kjeldahl)	% d.m.b.	5.78
N - Org	% d.m.b.	4.13
N - NO_3^-	% d.m.b.	<0.01
N - NH_4^+	% d.m.b.	1.65
N - org / N - Tot	%	71.5
Dry matter	%	25.4
Humidity	%	74.6
Organic matter	% d.m.b.	47.5
TOC	% d.m.b.	27.5
C/N		4.8
Ashes	% d.m.b.	52.5
Ca	% d.m.b.	9.41
Mg	% d.m.b.	1.91
Na	% d.m.b.	0.18
K	% d.m.b.	0.48
P	% d.m.b.	4.52
Fe	% d.m.b.	2.40
Mn	mg/kg d.m.b.	370
Cu	mg/kg d.m.b.	410
Zn	mg/kg d.m.b.	920
B	mg/kg d.m.b.	50
Pb	mg/kg d.m.b.	39
Cr	mg/kg d.m.b.	113
Cd	mg/kg d.m.b.	1.8
Ni	mg/kg d.m.b.	37
As	mg/kg d.m.b.	2.0
Hg	mg/kg d.m.b.	<0.1
Cr^{6+}	mg/kg d.m.b.	<0.1

UM: unit of measure

Table 12. Physicochemical properties of the anaerobic digestate from sewage sludge used in this work; UM: unit of measure; d.m.b.: dry matter basis; TOC: Total Organic Carbon.

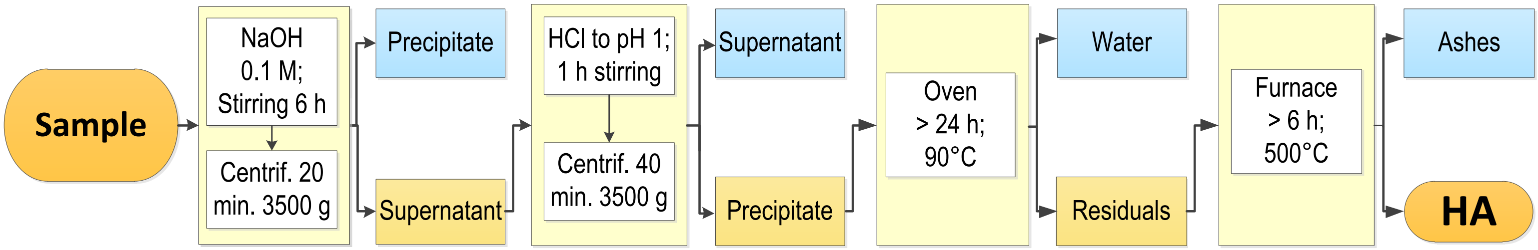

Figure 27. The schematization of the quantification method used for HA.

5.2.4. Encapsulation

The HS extract obtained from SSAD was firstly sieved (0.8 mm) to remove residual debris. After that, micronutrients were added to the solution in proportion inspired by Epstein and Bloom (Epstein and Bloom, 2005). The concentration of these elements was: B 0.57 g L^{-1}, Cu 0.57 g L^{-1}, Fe 7.36 g L^{-1}, Mn 4.34 g L^{-1}, Mo 0.13 g L^{-1} and Zn 1.12 g L^{-1}. After, the solution was mixed at 40°C with sodium alginate powder to a final concentration of 2.3% w/v, until the solution resulted homogeneous. Hence, the mixture was poured into a glass bottle and put under a nitrogen (N_2) pressure of 450 mbar to allow the injection in the encapsulator Buchi B-390 (**Figure 28**). The encapsulator was set at a frequency of 40 Hz and a voltage of 250 V. The solution extruded from the encapsulator was drop-shaped by a nozzle with a diameter of 1 mm. Drops fell in a hardening bath of $CaCl_2$ in the range of 0.06 - 6 M where the Na/Ca exchange took place. Finally, beads were air-dried. In order to measure the diameters of dry and wet beads, beads were photographed, and their pictures were analyzed with the ImageJ software (Abràmoff et al., 2006).

Figure 28. The encapsulator Buchi B-390.

5.2.5. Microscopy analysis of extract and beads

With the purpose of describing morphological features of HA and evaluating the elements present in the samples, the HA extract and alginate beads were investigated through Field Emission Scanning Electron Microscopy (FESEM, Zeiss MERLIN, Gemini-II column, Oberkochen, Germany) and Energy dispersive X-ray (EDX) analyses (AZTec, Oxford Instruments, Abingdon, UK). The EDX analysis was performed on a wide area (100 µm x 100 µm) in three different regions of the samples in order to have an average result of the elementary composition. Commercial HA were also analyzed in order to get qualitative information on chemical composition and as a standard of comparison. Beads without HAs were created and analyzed with FESEM in order to compare the structure of beads with HAs and without. Previously all FESEM analysis, samples were metalized with chromium. The liquid HA extract was previously dewatered to be analyzed by the means of FESEM. Therefore, lyophilization was performed instead of classical thermal drying in order to not compromise the structure of HA. Lyophilization was performed with an IlShin FD5518 Freeze Dryer with the following settings: temperature -60°C, pressure 5 mTorr, time 48 hours. As a result, a lyophilized HA extract with 82% in dry matter was obtained.

5.2.6. ATR-FTIR spectroscopy

Lyophilized HAs extract was analyzed employing Fourier transformed infrared (FTIR) spectroscopy, which was performed in attenuated total reflectance (ATR) mode. The instrument used was a Bruker Tensor 27 spectrometer equipped with a Platinum ATR and a KBr beamsplitter. The spectra were recorded in the range 4000-400 cm^{-1} (mid IR) with a resolution of 4 cm^{-1}, 32 scans per sample (measurement time: 15 s) and background correction with ambient air.

5.2.7. Pot experiment

In order to evaluate beads' effects on plant growth, a pot experiment was performed in a greenhouse located in Quillota, Valparaiso Region (Chile). Pots of 30 cm in diameter were filled with a sandy soil previously sieved at 2.5 mm. Before the transplanting of Chilean lettuce plants (*Lactuca sativa* L.), a basal dressing with a commercial NPK fertilizer for vegetables (N: 8%, P_2O_5: 15%, K_2O: 25%) and a fungicide treatment with Captan (5 g L^{-1}) was applied in each pot following manufacturer instructions. Three days after the transplanting, half of pots (9 replicates) were randomly treated with alginate-extract beads and the second half was no treated (control). The quantity of beads per-pot was of 8.7 kg ha^{-1} of HAs due to a commercially recommended dosage as reported in the datasheet of Humic plus®. A drip irrigation plant was installed, and plants were irrigated every 3 days. Finally, 70 days after transplanting, plants were cut at the basis, and epigean fresh biomass was immediately weighted. Fresh biomass of root apparatus was measured after washing with water to remove residual soil particles. Finally, dry matter of the epigean and hypogean part of plants was weighted after thermal treatment (105°C, 48 hours). Furthermore, the chemical characterization of soils was performed (Sadzawka et al., 2006).

5.2.8. Statistical analysis

Data of the pot experiment were analyzed by one-way ANOVA (analysis of variance) with a Tukey's post-hoc test ($P \leq 0.05$), after the assessment of the fundamental assumptions of ANOVA: the normality of distributions (Shapiro-Wilk test, p-value > 0.05) and the homogeneity of the variances of the residuals (Levene's test with P(>F) > 0.05). The statistical software R (version 3.5.1 - Feather Spray - 2018) was used for all statistical analysis.

5.3. Results and discussion

5.3.1. Quantification of HAs

The application of the Lamar method (Lamar et al., 2014) allowed the quantification of the HAs content in SSAD, HA extract and commercial HAs. The results are summarized in **Table 13**. The HA content in SSAD was 12.53 ± 1.60 % on dry matter basis (d.m.b.), a value comparable to other one reported in the literature (7.33%) (Li et al., 2017). The HA content in the extract was 26.87 ± 0.35% d.m.b., indicating that the process contributed to a more than two-fold enrichment in HAs. The percentage of HAs in the extract compared with HAs in SSAD is 7.33%. The quantification of the HAs in commercial HA powder revealed the highest content, estimated in 77.87 ± 1.46% d.m.b., in line with other commercial HAs derived from leonardite (80%) (Zhang and Ervin, 2004). Although the HA% of the extract is lower than commercial HAs, it is important to underline that normally HAs are extracted from non-renewable resources, such as peat, lignite and leonardite, while in this case the HAs came from a waste.

Parameter	Dry matter %		% HAs (d.m.b.)	
	Mean value	Standard deviation	Mean value	Standard deviation
SSAD	25.58	± 0.49	12.53	± 1.60
Extract	1.13	± 0.02	26.87	± 0.35
Commercial HAs	83.95	± 0.08	77.87	± 1.46

Table 13. Dry matter mean percentages and humic acids mean percentages measured in anaerobic sewage sludge (SSAD), extract of SSAD (Extract) and commercial humic acids (Commercial HAs). d.m.b.: dry matter basis.

5.3.2. Encapsulation of HA extract in calcium alginate beads

The HA extract was encapsulated to obtain a solid product with a slow release of the active components over time. Different concentrations of $CaCl_2$ (0.06 M – 6 M) were studied with the scope of reducing the presence of Ca and Cl in the beads. However, concentrations below 0.6 M did not provide a solid formation of beads and therefore 0.6 M was chosen to harden the beads for pot experiments. The beads were dried with the scope of increasing their lifetime by reducing the possibility of microbial degradation, since they were mainly composed of organic matter (Chan, 2011). After one year of storage at 25ºC in dry conditions, no visual damage neither degradation has been observed. In addition, HAs are known to be resistant to biodegradation, thus, the rate-limiting step of the release process can be reasonably attributed to the degradation of the alginate matrix (Gombotz and Pettit, 1995). The mean diameter of wet and dry beads was 2.4 ± 0.4 mm and 1.1 ± 0.1 mm, respectively (from picture analysis with ImageJ software). Considering that all the HA extract used during encapsulation was successfully entrapped by beads, the content of HAs in the dry beads represented the 6.09%, according to the mass balance calculation.

Data from the literature report that Ca-alginate beads show macro-porous in the order of 10 µm (Scherer et al., 1981) and mesoporous in the range of 8 -14 nm (Xu et al., 2020). This complex structure has been successfully used for the entrapment of complete cells on one hand, but on the other it has revealed enzymes leaching (Klein et al., 1983). Similarly to enzymes, HAs can be washed out from the Ca-alginate matrix because of their molecule size, which ranges between 2 and 1300 kDa (de Melo et al., 2016).

The FESEM images (**Figure 29**) show the morphology of the surface of the beads, which was made of micrometer sized aggregates. Pure alginate structure is visible in **Figure 29.a**. The cluster of smaller globular particles that protrude from the entrapment made by the calcium alginate reticular structure, shown in **Figure 29.b**, confirmed the correct encapsulation of the HA extract within the calcium alginate matrix. A similar cluster-like structure is observed with the commercial HA (**Figure 29.d)**. More compacted aggregates are observed from the lyophilized HA extract, which were due to the water elimination treatment (**Figure 29.c**).

The EDX analysis shown in **Figure 31** confirmed that the HA extraction process yielded to a product free of heavy metals. This was an interesting result, considering that the chemical analysis of the SSAD demonstrated the presence of As, Ni, Cd, Cr, Hg and Pb (**Table 12**). Carbon, nitrogen, and oxygen elements were not included in the EDX analysis because of the high errors associated with their low atomic weight. It is worth noting that the high standard deviation of the EDX results obtained with the beads is in line with the FESEM images, which demonstrated the low homogeneity of the samples, showing regions with the presence of crystals and regions that are clearer (**Figure 29**). The higher amount of K and P in the extract was attributed to the use of a solution containing potassium hydroxide and pyrophosphate during the extraction process. As expected, the content of these two components was considerably lower in the beads due to the dilution of the extract in the alginate solution during beads preparation. In contrast, there was a substantial increase of sodium, chlorine and calcium in the beads with respect to the extract, which was in line with FESEM images showing crystals of NaCl on the surface of the beads (**Figure 30**). Those elements came from the sodium alginate and calcium chloride solution used to harden the beads. However, further washing steps to reduce the amount of the contaminants were not conducted in order to avoid the premature leaching of the HA molecules from the beads.

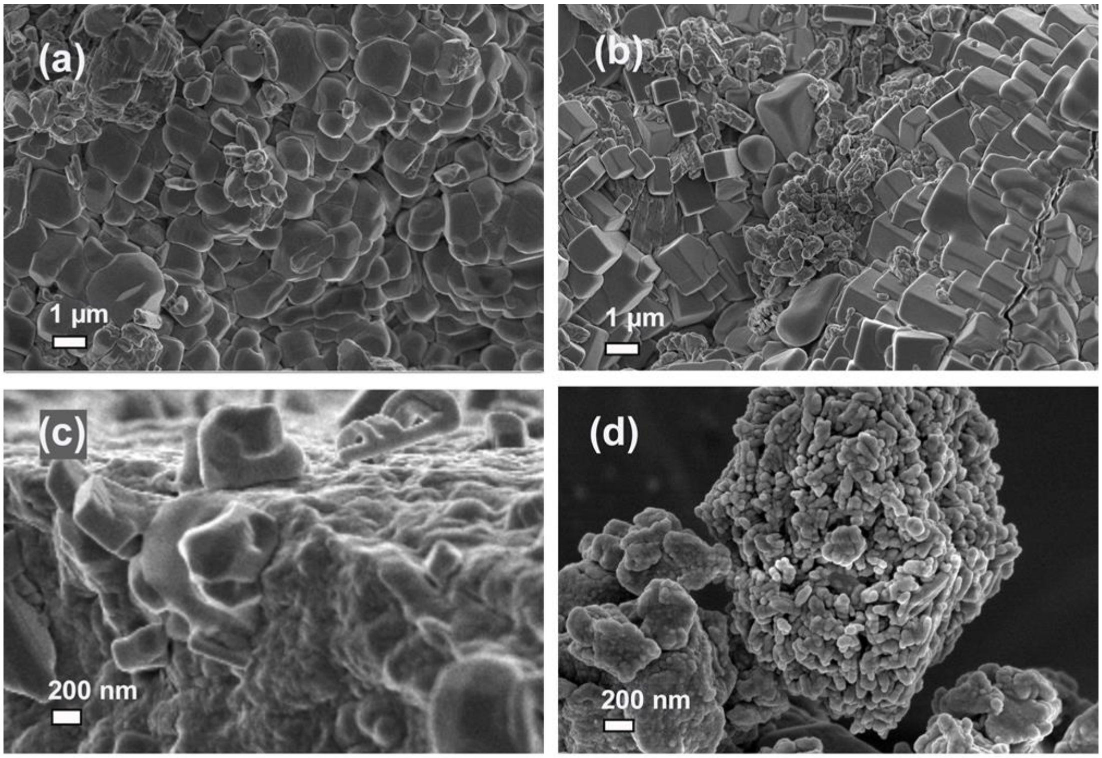

Figure 29. FESEM images of the empty (**a**) and with HA extract (**b**) calcium alginate beads and of the lyophilized HA extract (**c**) and commercial HA powder (**d**).

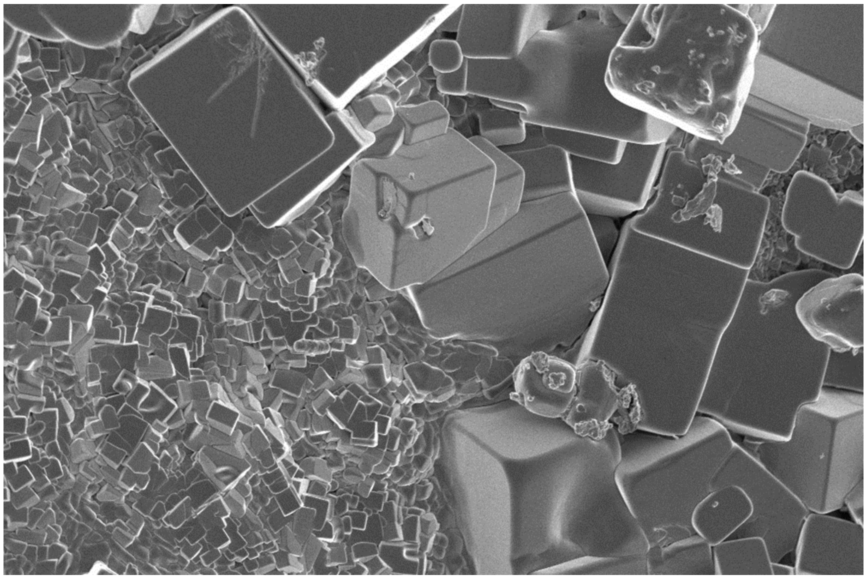

Figure 30. FESEM images showing crystals of NaCl on the beads surface.

Infrared spectroscopy analysis of the lyophilized HA extract confirmed the presence of chemical moieties peculiar of HS, as shown by **Figure 32**, displaying ATR-FTIR absorption spectra with bands typical of HS. The sample spectra presented a principal band around 3270 cm^{-1}, corresponding to the H-bonded O-H stretching of carboxylic acids, phenols, and alcohols and it was followed by a doublet at 2920-2850 cm^{-1} due to the C-H stretching of aliphatic structures (Helal et al., 2011; Martin et al., 2014; Mayans et al., 2019; Tatzber et al., 2007).
The peak around 1630 cm^{-1} was ascribable to the C=O stretching of carboxylic and ketonic groups, and to the aromatic C=C stretching (Martin et al., 2014; Mayans et al., 2019), followed by a peak around 1545 cm^{-1} due to the C=N stretching of amides (Mayans et al., 2019; Tatzber et al., 2007).

Peaks at 1450 and 1400 cm^{-1} were characteristic of the bending of aliphatic C-H (Martin et al., 2014; Tatzber et al., 2007) and of the O-H bending of carboxylic acid (Sigmaaldrich, 2020). The large peak around 1040 cm^{-1} corresponded to the C-O stretching of alcohols and aliphatic ethers (Tatzber et al., 2007), but it might be also assigned to the presence of Si–O silicate impurities as confirmed by EDX analysis (Helal et al., 2011; Martin et al., 2014). At lower wavelengths (900-600 cm^{-1}) the HA extract spectrum showed several peaks that could be reasonably attributed to the C=C bending (Sigmaaldrich, 2020). ATR-FTIR spectra were recorded also on HA-alginate beads (data not shown), but the signal of alginate was too much intense, hindering the proper characterization of HAs.

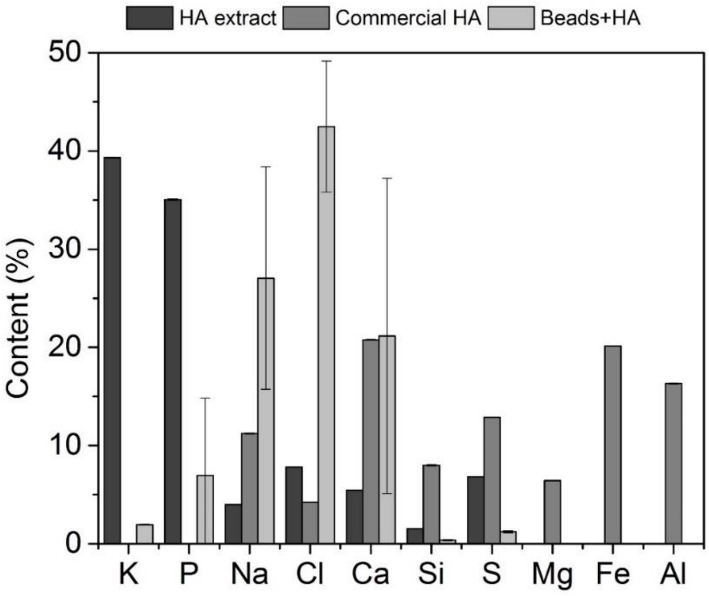

Figure 31. Elementary composition by EDX analysis of the elements present in the samples of HA extract, in commercial HAs and in calcium alginate beads with HAs.

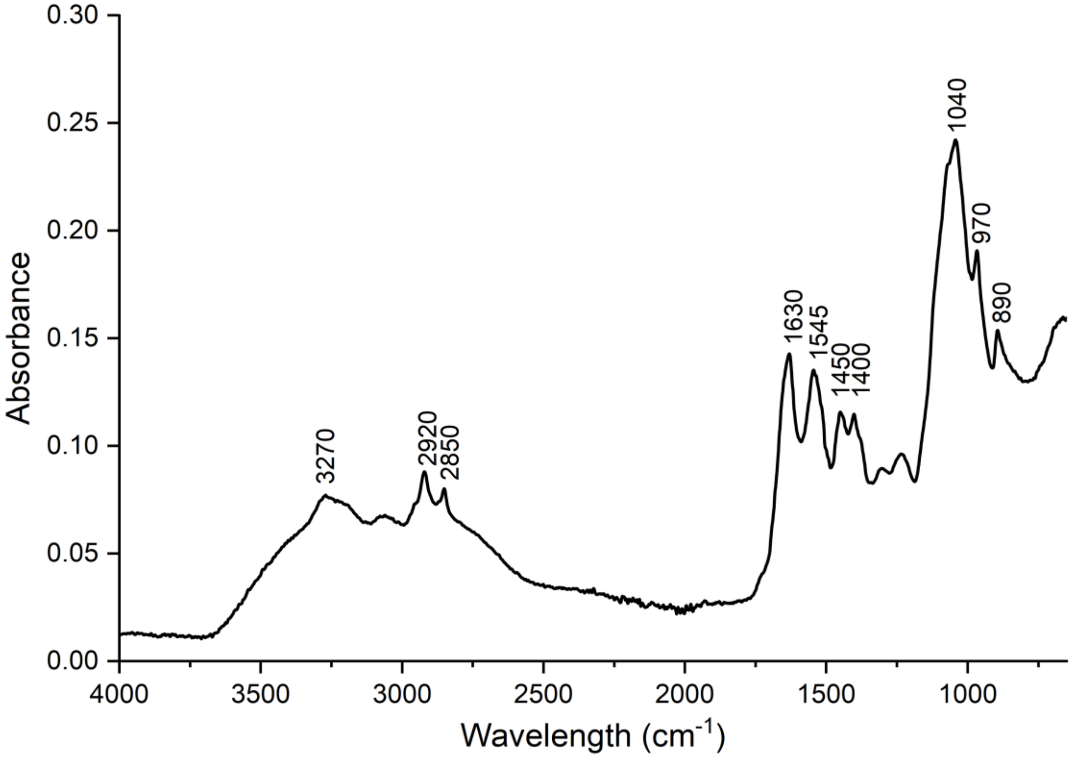

Figure 32. ATR-FTIR spectrum of lyophilized HA extract.

5.3.3. Effects on plants and soil

The results of the final dry biomasses of lettuce grown in the greenhouse experiment are represented in **Figure 33**. As regards the epigean dry biomass of the lettuce plants, the addition of HA beads did not provide significant differences with the untreated control. FESEM and EDX analyses (**Figure 30** and **Figure 31**) showed NaCl presence in beads which probably contributed to enhancing the soil electrical conductivity (E.C.) of treated samples (0.46 dS m^{-1}), which was higher than control (0.26 dS m^{-1}) (**Table 14**). Nevertheless, E.C. did not reach potentially dangerous levels for plants (>2 dS m^{-1}) (Arpa Veneto, 2007), and it likely did not affect biomass production. Many studies about the effects on shoot biomass have been conducted by adding HAs to soils and the results are discordant. In some cases, no difference was reported, in others ones shoot biomass production was enhanced (Canellas et al., 2015). For studies dealing with lettuce, no differences between treated and untreated samples were reported despite the high amounts of HAs applied (until 300 kg ha^{-1}) (Cimrin and Yilmaz, 2005). On the contrary, in another work lettuce plants had a statistically higher growth when compared to the untreated control at a dosage of 2000 mg of HAs kg^{-1} of soil (Tüfenkçi et al., 2006). In the present work, the estimated dosage of HAs used was 8.7 kg ha^{-1}, corresponding to approximately 2.7 mg of HA kg^{-1} of soil, a dosage 740-fold lower than that reported in the work of Tüfenkçi and colleagues (Tüfenkçi et al., 2006). On the other hand, in the present study the hypogean dry biomass of plants grown in the presence of the HA beads was significantly higher (+63%) than the negative control. Hence, this result confirmed the stimulation effect of HAs on root growth, already widely documented in the literature (Canellas et al., 2015; Chen and Solovitch, 1988). Moreover, this result supports the work of Young and Chen, who demonstrated root biomass enhancement by HAs in lettuce (Young and Chen, 1997).

It is important to underline that the enhancement of shoot biomass driven by HAs addition to soil is more unusual. A work on tomato showed that only one out of nine dosages of HAs applied promoted shoot biomass, but roots biomass production was promoted in all cases (Atiyeh et al., 2002). On the other hand, a recent meta-analysis of 89 papers on random-effects revealed that the dry weight of shoot and root increases of 22.4% and 21.6%, respectively, in response to HS application (Rose et al., 2014). Furthermore, this study elucidated which are the significant factors likely enhancing shoots and roots growth using HS. The type of HS (origin and chemical moieties) was the most important parameter in affecting both shoots and roots biomasses increase. After that, the HS application rate resulted the most important parameter influencing shoot growth promotion, followed by stressing conditions and plant type. In the present experiment, the not significant increase of shoot biomass could be due to some of these parameters but, as explained before, the low application rate was likely the most conceivable cause. As regards root growth promotion, the above-mentioned study revealed that, after the HS type, the growth media and plant species were the factors that mostly affected roots enhancement. Application rate, application location and stress did not affect roots growth. In this way, our experiment demonstrated that the HAs extracted and applied to the plants were adequate to increase the biomass of lettuce roots.

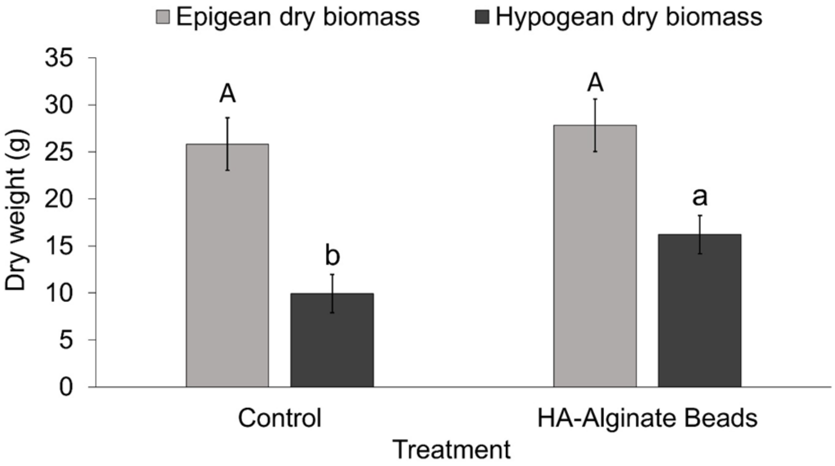

Figure 33. Mean dry epigean and hypogean biomasses of *L. sativa* L. grown on sandy soil with beads treatment and without beads treatment. Different letters indicate differences between treatments that are significant at $P < 0.05$ (Tukey HSD). Error bars represent standard error. Upper-case letters refer to statistical analysis applied to epigean dry biomass samples and lower-case letters refer to statistical analysis applied to hypogean dry biomass samples.

Parameter	U.M.	Control soil	Beads soil
pH (25°C)		7.11	6.84
E.C. (25°C)	dS/m	0.26	0.46
Organic matter	%	0.24	0.23
N	mg/kg	7.35	4.55
P	mg/kg	8.38	6.83
K	mg/kg	131	31.6
Ca	(cmol+/kg)	12	2.86
Mg	(cmol+/kg)	5.73	1.47
Zn	mg/kg	0.55	0.82
Mn	mg/kg	0.47	0.58
Fe	mg/kg	7.67	7.56
Cu	mg/kg	0.19	0.32

Table 14. Chemical characterization performed at the end of the experiment on not treated soil (Control) and on treated soil (Beads soil). U.M.: unit of measure; E.C.: Electrical conductivity.

5.4. Conclusion

Sewage sludge is a waste with a high recycling potential given the appreciable amount of valuable compounds it contains. However, the simultaneous presence of toxic elements strongly limits its reuse. Hence, an effective approach is the consideration of sewage sludge as a "raw material", a candidate for the selective "mining" of its added value and profitable compounds. This strategy is in line with the purposes of the present chapter, which has dealt with the extraction, quantification, characterization and greenhouse testing of HAs recovered from SSAD. One of the main advantages of the proposed process is that it uses waste found all over the world (and which is being increasingly produced). Furthermore, the use of HAs extracted from SS would reduce the need to use the non-renewable resources from which HAs are currently extracted (leonardite, peat, etc.). The protocol used allowed not only an HA extract with an HA concentration (26.87%), on dry matter basis, to be obtained, double the starting material (SSAD, 12.53%), but also eliminated heavy metals. These positive results could be improved even more with future research, optimizing the protocol for higher HA recovery: a higher KOH dosage, a different mixing speed and a higher centrifuge speed could enhance the HA percentage in the extract and, consequently, in the beads. Another great benefit of this kind of extraction is that, unlike the traditional extraction with NaOH, it uses KOH. This has a twofold advantage: if, on the one hand, the non-use of NaOH involves the absence of Na^+ (a potentially dangerous element for crop growth) in the extracted solution, on the other hand, the use of KOH instead of NaOH adds K^+ (one of the main macronutrients helpful for vegetable nutrition) to the extracted solution. Despite the absence of NaOH in the extracted solution, Na^+ was found in the beads due to the encapsulation process with sodium alginate. Despite the NaCl content in the beads, its presence did not negatively affect the lettuce growth. In fact, the HA-alginate beads benefitted the growth of the root apparatus of the lettuce cultivated on poor and sandy soil (+63% over untreated control). With the purpose of investigating this issue in greater depth, future perspectives should include the testing of the effectiveness of other crosslinking agents, in order to reduce the NaCl content in the beads, and the application of the slow-release HA product to different plant species. In this study a dehydrated digestate (centrifugated) was used as a raw material and then it was re-hydrated. In order to increase the efficiency of the entire process (from the WWTP to bead production), it is advisable to start from liquid digestate and then dehydrate it until the required percentage is obtained. Concerning the transition from laboratory to industrial scale, the encapsulator represents the main limit for the upgrading of the process (both economically and technically). It represents the highest investment cost of the entire process, has a low hourly production (600 ml/h of extract → corresponding to 30 g of dry beads/h), and only allows batch production. Although with a 4-times greater cost it is possible to apply 6 nozzles and increase production by up to 180 g beads/h, beyond this threshold, with current technologies it would be necessary to buy other encapsulators, with a great increase in investment costs. To reduce these high investment costs, increase hourly bead production and transform the process from batch to continuous, it would be necessary to invest in the research and development of a new encapsulator with many nozzles and a single unit machine. Electron microscopy and infrared spectroscopy provided insight into the peculiarities of HA extract, revealing features of isolated HAs comparable to those reported in the literature as far as concerns their morphology and chemical moieties. Despite this outcome, another future work on this field could be to better characterize the HAs contained in SSADs and beads. This improved characterization would be especially useful for understanding the different HA typologies, and consequently the different qualities of the HAs contained in SSADs and beads. Thus, it would be possible to understand if the extraction procedure changes the quality of HAs and if specific SSADs are more suitable for HA extraction. In this work, ATR-FTIR spectroscopy, which is one of the most common techniques for HA characterization, was used. Other methodologies that could be used for this purpose are nuclear magnetic resonance (NMR) spectroscopy and electron spin resonance (ESR) spectroscopy.

In conclusion, the encouraging results of this study suggest that HA extraction from SSAD is a promising strategy for the sustainable production of the commercial HS of tomorrow. While, with the current European legislation on fertilizers and biostimulants (Regulation 1009/2019 (European Parliament and Council, 2019)) this product could not be sold as a biostimulant on the European market (Paragraph 1.3.9.), this limitation does not exclude the possibility of production and commercialization on the markets of the individual EU member states or in non-EU countries. Consequently, the slow-release biostimulant containing HAs derived from waste obtained in this work can successfully fulfil the principles of the circular economy.

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Chapter VI

6. Conclusions

In this thesis the use of SSAD (sewage sludge anaerobically digested) to combat desertification was firstly theorized and, secondly, demonstrated. With this objective, the direct reuse of SSAD on soil and the extraction of a high-value compound (HVC) from SSADs were performed. At the beginning, chemical analyses were achieved on four SSADs derived from the same treatment plant: a primary liquid digestate, a secondary liquid digestate, a centrifuged digestate (obtained from a mix of primary and secondary SSADs) and a dried digestate (centrifuged digestate thermally treated). The analyses performed have highlighted the strong points of this waste: a high presence of nitrogen, phosphorus, organic matter and all the necessary micronutrients for plant growth (such as Ca, Fe, Mg etc.). On the other hand, these kinds of digestates have as the Achilles heel the presence of organic and inorganic contaminants. In first experiments, the SSADs were added to a soil similar to ones found in the desert (sandy, alkaline and poor in nutrients). Thanks to statistic tools it was demonstrated that, under certain conditions, the direct application of SSADs can contrast desertification processes. After the addition of SSADs and to a greater extent with centrifuged and dried SSADs, the data interpretation on soil analyses showed an effective improvement of soil characteristics. Because of the high presence of phosphorus in SSADs, an increment in plant tissues and in soil solution was determined. After studying this element in detail, the deeper causes were discovered that made the phosphorus have a very low bioavailability in soils. An experiment carried out regarding the changes of the phosphorus fractions over a three-month period showed that the total phosphorus in the soil remained constant, but the bioavailable-P decreased. The decreasing trend of the soil treated with dried digestate is well described by the Elovich equation and these results support this. According to the literature the most relevant phenomena which reduce the availability of phosphorus are the precipitation of calcium phosphates and, to a lesser extent, adsorption. This could explain the decrease of phosphorus observed.

Repeated addition of SSADs on soils could lead to an accumulation of heavy metals (HMs) and hardly degradable organic compounds. The literature is discordant about potential long-term negative effects of repeated addition of SSADs on soils. However, in accordance with the precautionary principle sustained by the European Union in Article 191 of the Treaty on the Functioning of the European Union (TFEU), it is deemed appropriate to use SSADs but to avoid continuative and repeated treatments with them on soils. It would indeed be a missed opportunity not to use these free products to provide base fertilizations to trees planted in degraded desertified areas. I believe a few initial applications could give a “starter effect” to plants, whose beneficial effects are much greater than the potential negative effects. Like the proven amelioration of soils and plants grown in desertified areas, this hypothesis should be confirmed by field trials in those areas.

In the last chapter, the other approach to the SSADs reuse was tested: the extraction of high-value compounds. Humic acids (HAs), known as “the black gold of agriculture”, were extracted from a Chilean centrifuged SSAD. Through the use of innovative methods, HAs were quantified, extracted and encapsulated in calcium alginate beads. The encapsulated HAs were tested and positive results were obtained (+63% of roots apparatus growth compared to untreated control). Consequently, it is possible to affirm that a biostimulant product was created starting from waste in the full compliance with circular economy principles. This approach to extraction has advantages and disadvantages if compared to soil application of SSADs. One advantage is given by the possibility of choosing the specific product to extract. In the present study, the specific products were HAs, but other HVCs could be contained in the SS. A useful application of this approach to extraction would make it possible to create biorefineries that, by successive extractions, can generate HVCs from SS. Moreover, in the extraction process of HAs proposed in this thesis, HMs were not found in beads (a great advantage in comparison with the direct soil application of SSADs); however, by using the biorefinery model, HMs could be previously or subsequently extracted with chemical, physical or biological treatments. With this approach, other HVCs such as struvite (fertilizer) and even rare metals could be also separated and extracted. In this way, SSADs disposal would effectively turn from a problem into an important resource with positive possible consequences from the environmental point of view (less waste in landfills and to incinerators and less consumption of fertilizers), from the social point of view (more occupation) and from the economical point of view (potential profits deriving from the sale of HVC extracted). On the other hand, this approach has the disadvantage of having higher costs due to the additional processes. Furthermore, technological knowledge is necessary but is not always available in all contexts and is necessary to have political will to reuse SS as a raw material for biofertilizer products (currently absent in EU policies – EU Regulation 1009/2019).

In order to quantify the amount of the two solid SSADs required for a fertilization of 170 kg N/ha, the direct application of the centrifuged and dried SSADs would require around 10.5 T/ha (w.m.b.) and 3.8 T/ha (w.m.b.) respectively. For beads, the recommended dosage of 8.7 kg HA/ha would require 143 kg of beads/ha. To minimize possible adverse effects and maximize beneficial effects of the soil treatment with SSADs and beads, it may be appropriate to carry out precise applications. Regarding SSAD fertilization, a precise application can reduce leaching, groundwater pollution (with nitrates and phosphates), contamination by HM and OC and costs of transportation. Regarding beads application, a precise application can reduce costs of production and lower environmental impacts due to a reduced use of chemicals. In both cases, it will result in higher efficiency of the products. With a precise application of SSAD and beads, the single plant dosage would correspond approximately to the dosage per single pot applied in previous experiments: 24 g/plant with centrifuged SSAD, 9 g/plant with dried SSAD and 1 g/plant with beads. Considering a planting density of 1 plant/m^2, the requirement would be 240 kg/ha of centrifuged SSAD, 90 kg/ha of dried SSAD and 10 kg/ha of beads. Finally, the great amount of SS production all over the world (in Italy alone SS production is estimated at about 1103 thousand tonnes/year (paragraph 1.1.2.)) and the increasing global production of SS, raw material for the SSADs and bead production will be easily available and can be used for the fight against desertification.

Future objectives will include the improvement of extraction yields of HAs, the implementation of the encapsulation techniques and the investigation of other possible HVCs that can be extracted from SSADs. Finally, considering the obtained results, it is possible to affirm that both strategies of SSADs valorization are potentially suitable for fighting desertification in agreement with the United Nations Sustainable Development Goal 15.3.