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Title

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

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Abstract

Anaerobic digestates from sewage sludge (SSADs) are a by-product of the wastewater treatment process that still preserves a certain agronomic interest for its richness in plant nutrients and organic matter. Fertilizing properties of two liquid and two dewatered SSADs were tested on tomato plants (*Solanum lycopersicum* L.). Pot experiments were performed on sandy soil and peat substrate under greenhouse conditions with a SSADs application rate of 170 kg N/ha over a period of three months. Beneficial effects of SSADs were reported on different growth parameters, revealing an increase in biomass and height up to 37.5 and 6-folds over untreated control. No phytotoxic effect occurred on SSAD-exposed plants. Chemical analysis of soils treated with SSADs showed enrichment of macro- and micro-nutrients as well as organic matter. In some cases, the chemical characterization of leaves revealed an enhancement of uptaken macronutrients. This study contributed in general to deepen the knowledge on the short-term growing season fertilizing effects of SSAD. Despite the treatment dosage was calculated only on nitrogen requirements, the study highlighted the importance of the other nutrients and organic matter on plant growth.

Keywords: waste management; nitrogen; soil organic matter; nutrient recycling; sewage sludge; tomato plants.

1. Introduction

Globally, the demand of 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). 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 the Western Sahara (Fixen and Johnston, 2012).

Within a circular economy perspective, the reuse of sewage sludge (SS) as fertilizer is an interesting scenario. SS can be defined as “the residue generated from the treatment of wastewater” (Smith et al., 2009). This matrix is a valuable source in terms of plant nutrients: a study conducted on 240 dried samples from Pennsylvania revealed an average N, P and K content of 4.74%, 2.27%, and 0.31%, respectively (Stehouwer et al., 2000). Furthermore, SS can contain many micronutrients (e.g. Ca, Mg,

Abbreviations: A_N: assimilation; ANRE: Apparent Nitrogen Recovery Efficiency; ANUE: Agronomic Nitrogen Use Efficiency; C: centrifuged SSAD; CEC: cation exchange capacity; CCI: Chlorophyll Content Index; C_i: CO_2 concentration in substomatal cavity; CRF: controlled release fertilizer; D: dried SSAD; D.M.: dry matter; EC: electrical conductivity; EmC: Emerging Contaminants; EU: European Union; g_s: stomatal conductance; IRGA: infra-red gas analyzer; M: mineral fertilizer; OM: organic matter; P: primary SSAD; QL: quantification limits; S: secondary SSAD; SS: sewage sludge; SSAD: anaerobic digestate from sewage sludge; T: non-treated, control thesis; WWTP: wastewater treatment plant.

S, Fe, Mn, Cu, Zn and B) which are important for plant growth, but usually not included in most commercial fertilizers (Warman and Termeer, 2005). The percentage of the nutrients appears low, but it is important to underline that every year a huge amount of wastewater is produced. An empirical study revealed that approximately 330 km^3 of municipal wastewater are produced worldwide yearly (Mateo-Sagasta et al., 2015). Therefore, also the SS production has dramatically risen 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, 1991a).

The considerable presence of organic carbon and organic matter in SS is another strength of its reuse (Alvarenga et al., 2015; Mateo-Sagasta et al., 2015). In fact, land application of organic matter (OM) improves soil physical properties such as cation exchange capacity (CEC), soil structure, soil moisture content and retention (Epstein, 2002). Furthermore, the addition of SS can enhance the amount of organic carbon in soils (Kladivko and Nelson, 1979; Perez-Espinosa et al., 1999) and thus reverse the current reduction of organic matter in soils (known as *SOM decline*) (Schulze and Freibauer, 2005).

Today, SS is classified as waste and its safe disposal represents a very important issue in waste management (Epstein, 2002; Singh and Agrawal, 2008). The four main destinations of SS are incineration, landfilling, composting and agricultural use. In Italy, according to data of 2010 (Eurostat, 2019), 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. The Council Directive 86/278 (Council of the European Communities, 1986) regulates the agricultural SS reuse in Europe to prevent soil contamination. In fact, this practice has three principal problems that limit its unconditioned use: biological risk, heavy metal contamination and contamination by organic pollutants. The biological risk is principally represented by pathogens such as *Salmonella* spp., *Escherichia coli* (enterotoxigenic and enteropathogenic variants), *Campylobacter* spp., *Clostridium* spp., and *Yersinia* spp. (Arthurson, 2008); stabilization treatments can reduce significantly their presence in SS and are mandatory before subsequent SS applications (Dumontet et al., 1999). For instance, one of the most diffused stabilization techniques is anaerobic digestion (Liu et al., 2012), in which the reduction of pathogens, putrescence and odor is coupled with biogas production, allowing energy recovery (Epstein, 2002).

Heavy metal content (normally represented by Cd, Cr, Cu, Hg, Ni, Pb, Zn) can be abated by means of chemical (*e.g.* chelating addition), physical (*e.g.* electroremediation) or biological (*e.g.* vermicomposting) treatments (Camargo et al., 2016). Finally, some organic pollutants (*e.g*. pesticides, antibiotics and hormones) can be volatilized or degraded through biotic or abiotic processes (Harrison et al., 2006). "Concerning organic pollutants, their abatement is trickier. Indeed, class of emerging contaminants (EmC) in wastewater is increasingly gaining more interest within the organic compounds. EmC include molecules such as endocrine disrupting compounds (e.g. hormones), pharmaceutically active compounds (e.g. antibiotics), illicit drugs and pesticides (Fijalkowski, 2019). EmC abatement is becoming even more required both on the effluent of WWTPs with advanced treatments (e.g. activated carbon absorption, advanced oxidation processes, reverse osmosis) and on sewage sludge (Gadupudi et al., 2019). Some studies affirmed that anaerobic digestion is the stabilization strategy ensuring the best EmC removal, especially when the sludge is pretreated (e.g. via ozonation) (Neumann et al., 2016). However, further studies are still required to improve the performances and 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). The abovementioned EU directive regulates the SS soil application in the EU and establishes threshold values of some of these pathogens and pollutants in SS.

On the basis of these opportunities and threats related to SS, this work aims to deepen the knowledge about SS fertilizing effects over time in terms of nutrients and OM on a poor alkaline sandy soil. This kind of 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. These results were compared to the one obtained with a richer peat substrate. Pot experiments were performed on tomato plants (*Solanum lycopersicum* L.) in a greenhouse to evaluate nutrient provision of anaerobic digestates from sewage sludge (SSADs). Tomato plant 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, 2002). 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 work 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 (Cristina et al., 2019). As far as we know, this is the first example of use of four different and consequent SSADs to fertilize tomato plants. The paper examines agronomic parameters of tomato plants, the nutrient distribution in soil and nutrient absorption by plants after the application of SSADs. Furthermore, numerous plants physiological parameters were evaluated over a span of three months in order to better understand the effects in a time course approach.

2. Materials and methods

2.1. Characterizations

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 a previous work (Cristina et al., 2019); characterization of the four SSADs is reported in Supplementary Material (Section I **- Table S1**).

2.1.2. Cultivation substrates

Two types of substrates were used: a sandy soil and a commercial peat substrate (**Table 1**). The sandy soil was sampled within 20 and 100 cm depth in Grugliasco (TO), Italy (45°03'58.4"N, 7°35'32.9"E). Analytical methods used for characterization of the sandy soil and the peat substrate are specified in Supplementary materials (Section II). 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 ±

150 0.16), very poor in OM (0.38 ± 0.12% < 0.8%) and very poor in macronutrients such as nitrogen (0.29
151 ± 0.09 g/kg < 0.5 g/kg), phosphorous (1.8 ± 1.3 mg/kg < 7 mg/kg), potassium (18 ± 1 mg/kg < 40
152 mg/kg) and magnesium (15 ± 5 mg/kg < 50 mg/kg). On the other hand, content of calcium (675 ± 27
153 mg/kg < 1000 mg/kg) and some microelements such as iron (2.5 mg/kg < 6.7 ± 1.1mg/kg < mg/kg 20)
154 and manganese (2 mg/kg < 6.5 ± 3.0 mg/kg < mg/kg 10) resulted normal.

155 2.2. Experimental set-up

156 A greenhouse experiment was performed over three months during the summer season in a
157 greenhouse of the Centre of Competence AGROINNOVA – University of Torino, located in Grugliasco
158 (TO), Italy. The experimental campaign was carried out with commercial plastic pots of 2.5 L (Ø 17
159 cm, height 20 cm, surface area 0.227 m^2). Four types of SSADs (P, S, C, D) were applied as
160 treatments, and compared to a commercial fertilizer (M) (NPK 22-5-6 + 2MgO, “Osmocote Topdress”,
161 ICL, Israel) and an untreated control (T). The experiment was designed in a completely randomized
162 block, with 15 replications per each thesis. The same experimental set-up was adopted on the two
163 cultivation substrates (sandy soil and peat substrate). Each treatment was applied at the dosage of
164 170 kg N/ha, in line with the European Nitrates Directive (Council of the European Communities,
165 1991b). Moreover, this application rate was chosen as it showed the best results in a preliminary
166 study (Cristina et al., 2019).Three untreated seeds of tomato (*Solanum lycopersicum* L. cv. Beefsteak,
167 “Furia sementi”, Parma, Italy) were sown in each pot. Automatic sprinkler irrigation was set three
168 times a day. Ten days after sowing a thinning was conducted and the best plant from each pot was
169 kept. At the end of each month, five pre-selected replicates of each treatment were removed to carry
170 out all the measurements.

171 2.3. Measurement of plant parameters

172 At the end of every month, the five removed replicates were examined. Firstly, height was measured,
173 then, leaves, inflorescences and fruits were counted, if present. After that, the Chlorophyll Content
174 Index (CCI) was evaluated with a SPAD 502 chlorophyll meter (CCM-200, Opti Sciences, Inc.,
175 Hudson, NH, USA) using the method described in the previous work (Cristina et al., 2019). 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. Subsequently, agronomic nitrogen use efficiency (ANUE) was calculated as:

$$ANUE = \frac{(Dry\ biomass\ treated\ samples - Dry\ biomass\ control\ samples)}{Amount\ of\ nitrogen\ applied\ in\ treated\ samples}$$

2.4. 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 (see Supplementary material - Section II).

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:

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

2.5. 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. The statistical software R (version 3.5.1 - Feather Spray - 2018) was used for all statistical analysis.

3. Results

3.1. Plant measurements

3.1.1. Dry biomass and ANUE

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. Despite the absence of significant differences, it must be pointed out that biomass of S was 28.7 and 12.7-folds higher than control (T) and mineral fertilizer (M), respectively. At the end of second month, all digestates (P, S, C, D) showed a dry biomass production significantly higher (26.7, 33, 35.3 and 37.5-folds, respectively) than control. At the same time, S, C and D showed a higher biomass than mineral fertilizer (2.9, 3.1 and 3.3-folds, respectively).. 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 was 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 1.A**).

On the peat substrate, no significant differences between treatments were appreciable within the same month. The only significant differences emerged between biomass values between three different months (**Figure 1.B**).

Results of ANUE (**Table S2 and S3**) did not satisfy the requirements of Levene test during ANOVA, hence no significant differences could be reported reasonably. However, it must be pointed out that ANUE values of SSADs in sandy soil were up to 23, 3.5 and 2.3-folds higher than mineral ones after one, two and three months after sowing, respectively. In the case of substrate, the value did not follow any trend, also considering that in some cases control samples resulted more massive than treated ones.

3.1.2. Height

On the sandy soil, no differences in plant height were present at the end of the first month. Nevertheless, S treatment revealed the tallest tomato plants, up to 2.6 and 2.5-folds higher than T and M. In the second month, all SSADs-treated plants were significantly taller than control and mineral fertilizer, with D treatment displaying a height 6 and 2.1-folds higher than T and M, respectively. 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 2.A**).

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

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 2.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 2.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).

3.1.4. 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 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 2.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).

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. Detailed results are reported in **Table S4**.

3.2. Chemical analysis

3.2.1. Substrates analyses

Results of chemical analyses performed on the sandy soil after two months from treatments application are summarized in **Table 2A**. 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 2B**. 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

303 treatments. Nitrites, nitrates, extractable Mn, Cu and Zn were below detection limits. All other
304 parameters did not show any significant difference.

305 3.2.2. Leaf analysis

306 On sandy soil, chemical characterization of leaves showed a concentration of nitrogen and potassium
307 in P and D significantly lower than mineral fertilizer samples. As regards phosphorous, no significant
308 difference emerged. The total nitrogen accumulated in leaves in D plants was significantly higher than
309 in P ones. The mean phosphorous uptake by plants was significantly different across D, P and M
310 samples. Finally, the potassium uptaken in leaves did not show significant differences between thesis
311 (**Table 3A**).
312 On plants grown on peat substrate, concentrations and total uptake of both nitrogen and potassium
313 on control, P and D were statistically similar to each other, but they resulted lower in comparison with
314 mineral fertilizer ones. Concentration and total uptake of phosphorous in leaves, control showed the
315 lowest values while D samples the highest ones (**Table 3B**).

316 4. Discussion

317 4.1. Agronomic and physiological evaluations

318 For many years extensive studies and reviews have shown that soil and plant benefit from SS.
319 Indeed, SS is a good source of macro and micro nutrients as well as of OM; this enhances soil fertility
320 and, as a consequence, crop production even in a more effective way than commercial fertilizers
321 (Singh and Agrawal, 2008). The results of the present work were in agreement with literature and the
322 better performances of SS compared to inorganic fertilizers have been confirmed. **Table 4** shows
323 technical details and results of other works dealing with SS treatment of tomato plant with pot
324 experiments. It is important to notice that not only SSAD application rate was considerably lower in
325 the present work, but also that the results obtained were remarkably higher. For instance, biomass
326 and height of treated tomato plants at two months after sowing was up to 37.5 and 6-folds higher,
327 respectively, than control plants (corresponding to an increase of 3652% and 500%), results never
328 reached before in other works on tomato plants. Interestingly, fertilizing performances of SSAD also

329 overcame the ones of mineral fertilizer, especially one month after sowing, when S treatment revealed
330 biomass and height of tomato plants up to 12.7 and 2.5-folds higher than M. From here on out,
331 differences between SSAD treatments and M samples were less accentuated, probably because
332 nutrients release of the mineral fertilizer was faster after an initial “lag” phase. As a corollary, biomass
333 values were reflected by ANUE ones, which were higher than the ones reported in literature for
334 tomato plants grown in pot under greenhouse conditions treated with a 10-folds higher nitrogen
335 application (Wang et al., 2013). Improvement in terms of leaves number and chlorophyll content were
336 less intense, but still higher than the examples reported in literature (Bakshi et al., 2019; Elloumi et al.,
337 2016; He et al., 2016; Hossain et al., 2015).
338 To a broader extent, results of the present study in terms of biomass and plant height can be
339 compared to other works conducted with a similar experimental setup but exploiting different model
340 species. In order to biomass, the general trend was an increase in dry matter ranging usually between
341 4 (*Capsicum annuum* L.; Pascual et al., 2008) and 16-folds (*Triticum aestivum* L.*;* Eid et al., 2019)
342 more than untreated control. The findings of the present work confirmed and went beyond these
343 results, considering also that the most used SS application rates ranged between the dosage used in
344 this work and a 35-folds higher one (Eid et al., 2019). On the other hand, the improvements in plant
345 height were in line with the results obtained by Eid and colleagues on cucumber (*Cucumis sativus* L.)
346 (2017) and wheat (*Triticum aestivum* L.) (2019), reporting a stem length improvement up to 3 and 6-
347 folds, respectively, over untreated control. The only case with a striking higher biomass production
348 was described for the sunflower (*Heliantus annuus L.*)*,* whose production increased up to 125-folds
349 more than the untreated control. However, the SS dosage was up to 35-folds higher than the present
350 study. Moreover improvement in terms of height was comparable to the present work (Bourioug et al.,
351 2018). Taking into account the works using SS dosages comparable to 170 kg N/ha, the majority
352 were open field experiments. For instance, triticale (*X Triticosecale Wittmack*) (Kchaou et al., 2018)
353 revealed a biomass increase of 2-folds. Furthermore, results of the present work corroborate positive
354 effects on biomass of SS application on soils poor in nutrients (Walter et al., 2000) and strongly
355 alkaline (Zuo et al., 2019).

SSAD application on tomato crops resulted also in an augmented number of leaves and inflorescences 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 were in general agreement with other results reported on tomato grown in presence of SS (Bakshi et al., 2019), despite the higher treatment dosages.

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 (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 (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 on net photosynthesis (Bourioug et al., 2018; Pascual et al., 2008) and chlorophyll content. Leaf chlorophyll content was 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 (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). 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, 2007). However, this aspect was 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 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 (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 was 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).

4.2. Chemical analysis

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. Although peat substrate was 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. 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, 2007) 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). Additionally, the low buffering capacity might be yet another conceivable effect occurring in the sandy soil case.

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, 2007), likely due to the consistently lower SSAD application rates. Nevertheless, it must be pointed out that, concerning sandy soil, EC values in M were approximatively doubled compared to SSAD ones, which in turns were somewhat higher than control. High EC of M might be due to the particularly higher concentration of nitrates, likely released as bioavailable form nitrogen by the commercial fertilizer. However, these relatively elevate nitrate amounts were likely not necessary, as confirmed by the better growth parameters and ANUE values of tomato plants growing on SSAD amended soil. On the contrary, excess of nitrates may result in undesired drawbacks such as leaching and hyperaccumulation in plant tissues, feature in agreement with the foliar analyses. EC values in SSAD treatments was probably influenced by sodium presence in the digestates, which however did not affect the physiological parameters of tomato plants as confirmed by IRGA measurements.

The thesis of a possible increasing 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, 2007).

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

438 and peat substrate. Other studies revealed that total soil nitrogen can persist in higher concentrations
439 also for longer periods after SSAD treatment application (Bourioug et al., 2015). Anyway, all samples
440 showed a total N content lower than before digestates application. It means that a remarkable part of
441 nitrogen both already present in sandy soil and added with digestates was absorbed, transformed or
442 leached after two months.
443 The significant variation in N and OM content in treated sandy soils changed C/N ratio. The results
444 obtained with SSADs were still low (< 9; Arpa Veneto, 2007), but higher than in control and mineral
445 fertilizer. The small changes in C/N and the relatively low values across treatments likely indicated
446 that nitrogen mineralization could have prevailed over microbial immobilization. Therefore, nitrogen in
447 SSAD treated samples was surely bioavailable and used efficiently plants, as also confirmed by
448 ANUE values. However, it should be also noticed that mineralisation was likely a slow nitrogen
449 release process, as evidenced by soil nitrate soil and leaf nitrogen analyses. Indeed, these evidenced
450 that nitrogen was much more bioavailable in M treatments, but not efficiently utilizable, according to
451 ANUE values.
452 In all SSADs treated soils, the available P was higher than control and mineral fertilizer. Considering
453 that the different dosages were normalized on N dosage per each thesis, the difference in P content
454 between the samples treated with SSADs can be explained by the different percentages of P in the
455 four SSADs. This diversity could also explain the differences among different treatments on
456 physiological parameters of tomato. Moreover, the addition of OM probably enhanced the availability
457 of P in soil treated with SSADs (Fekri et al., 2011). In fact, this can increase the abundance and the
458 activity of microorganisms, favoring P capture (Nobile et al., 2019). Similar results in increase of soil P
459 were obtained by Singh and Agrawal (2007) and Walter and colleagues (2000).
460 For what it concerns K, no differences were registered in soil after digestates application, probably
461 due to their low concentration in this macronutrient. These results agree with other works (Bourioug et
462 al., 2015; Walter et al., 2000).
463 Many SS are rich of Ca due to the stabilization by means of liming (Epstein, 2002). Although the
464 SSADs exploited in this work did not undergo Ca addition at WWTP level, its content was pretty high
465 (> 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, 2007).

A significant increase of exchangeable Na was measured in all treated soils due to the sodium percentage in SSAD and confirmed by two abovementioned works (Ferreiro-Domínguez et al., 2011; Singh and Agrawal, 2007). 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 (2012).

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 ones 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. On sandy soil, content of uptaken P was significantly higher in D and P treatments, which was likely influenced by the phosphorous amount in the initial application. 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 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

493 higher amount in mineral fertilizer, total K uptaken in leaves did not result significantly different
494 between the treatments applied on sandy soil, due to the different aboveground biomass production.
495 On the other hand, on peat substrate, the $K_{Extractable}$ content of plant with mineral fertilizer was the
496 highest considering the similar biomass production.

497 5. Conclusions

498 In the present work, pot experiments under greenhouse conditions on two different substrates were
499 performed to evaluate fertilizing effects of four different SSADs over a time span of three months. The
500 application of these digestates clearly highlighted beneficial effects on different growth parameters of
501 tomato plants, especially when cultivated on a sandy, alkaline and poor (in nutrient and OM) soil. For
502 instance, it is important to point out that plant biomass and height reached values up to 37.5 and 6-
503 folds, respectively, higher than untreated control; additionally, SSAD-treated plants showed values of
504 biomass and height up to 12.7 and 2.5-folds, respectively, higher than mineral treatment, indicating
505 that SSAD could be a valuable alternative to mineral fertilizers to boost fertility in poor and sandy soils
506 . Moreover, the present work confirmed the thesis of the enhancement of soil OM with the use of
507 SSAD. Furthermore, it is important to notice the increments of some macro- (nitrogen, phosphorous
508 and calcium) and micro-nutrients (iron and zinc) in sandy soil, showing significant differences with
509 respect to untreated control. Nevertheless, some of the registered values were low and it can be
510 reasonably assumed that most of nutrients had already been assimilated to let the plant grow. This
511 aspect was confirmed by leaves analysis, which showed a remarkable uptake in N, P and K by
512 tomato plants. With respect to these macronutrients, it is worth emphasizing that the experiment was
513 designed to administer plants, across the different treatments, the same nitrogen dosage as sludge
514 application rate is usually based on plants nitrogen requirements. However, the differences in SSADs
515 composition implied a remarkable imbalance in terms of other nutrients and OM. Hence, we can
516 assume that these differences likely influenced plant growth, providing consistent differences between
517 different theses.
518 Future work should include on one side a deeper analysis of the issues tackled in the present paper,
519 and on the other hand it should consider also related aspects. Concerning the formers, chemical

characterization of the treated substrates and plants should be carried out in a time-course fashion, allowing to properly describe the mass balance of the elements and their release dynamics over time, and, consequently, a more detailed evaluation of fertilizing indexes such as ANRE and ANUE. As regards the latter ones, soil application of SSAD should be explored both analyzing the presence of organic pollutants (e.g. antibiotics, EDC) as well as considering microbiological aspects, such as the effects on microbial communities and the study of metagenomics and metatranscriptomics traits (e.g. antibiotics resistance genes).

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 the use of mineral fertilizers derived from non-renewable resources. Furthermore, this experiment showed how the positive effects of SSADs are emphasized if applied on a poor alkaline soil.

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753 **Table 1.** Physiscal and chemical anlalysis of soil and peat used in the present work. CEC: Cation-Exchange Capacity; AAS: Atomic Absorption

754 Spectroscopy.

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 $_{extractable}$**	mg/l	8.1 ± 2.3
K $_{exchangeable}$	mg/kg	18 ± 1	**K $_{extractable}$**	mg/l	41.1 ± 6.8
Mg $_{exchangeable}$	mg/kg	15 ± 5	**Mg $_{extractable}$**	mg/l	28 ± 7
Ca $_{exchangeable}$	mg/kg	675 ± 27	**Ca $_{extractable}$**	mg/l	36 ± 8
Na $_{exchangeable}$	mg/kg	6 ± 3	**Na $_{extractable}$**	mg/l	16 ± 11
Fe $_{available}$	mg/kg	6.7 ± 1.1	**Fe $_{extractable}$**	mg/l	0.79 ± 0.21
Mn $_{available}$	mg/kg	6.5 ± 3.0	**Mn $_{extractable}$**	mg/l	0.15 ± 0.04
Cu $_{available}$	mg/kg	0.69 ± 0.29	**Cu $_{extractable}$**	mg/l	< QL
Zn $_{available}$	mg/kg	0.47 ± 0.29	**Zn $_{extractable}$**	mg/l	0.02 ± 0.00
CEC	cmol/kg	3.65 ± 0.35	**CEC**		-

Table 2. 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 $P \leq 0.05$, 0.01 and 0.001, respectively). CEC, cation exchange capacity; QL, quantification limit.

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Parameter	Unit of measure	Control (T)			Primary (P)			Secondary (S)			Centrifuged (C)			Dried (D)			Mineral fertiliser (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	**	
P_{Olsen}	mg/kg	< QL			4.2 ± 0.5	**		10.3 ± 0.8	**		15.4 ± 4.6	**		36.2 ± 11.4	**		< QL		
$K_{exchangeable}$	mg/kg	14 ± 3			11 ± 3			12 ± 1			12 ± 2			9 ± 1			13 ± 2		
$Mg_{exchangeable}$	mg/kg	11 ± 2			21 ± 7			26 ± 3			25 ± 4			22 ± 4			25 ± 2		
$Ca_{exchangeable}$	mg/kg	524 ± 26	*		594 ± 25	*		491 ± 62	*		626 ± 94	*		579 ± 48	*		646 ± 62	*	
$Na_{exchangeable}$	mg/kg	16 ± 1	***		35 ± 2	***		33 ± 3	***		26 ± 3	***		32 ± 3	***		27 ± 5	***	
$Fe_{available}$	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	***	
$Mn_{available}$	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		
$Cu_{available}$	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		
$Zn_{available}$	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.

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760 **B**

Parameter	Unit of measure	Control (T)	Primary SSAD (P)	Secondary SSAD (S)	Centrifuged SSAD (C)	Dried SSAD (D)	Mineral (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 $_{extractable}$	mg/l	0.4 ± 0.1	1.2 ± 0.4	1.2 ± 0.5	0.6 ± 0.3	1.1 ± 0.1	< QL
K $_{extractable}$	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 $_{extractable}$	mg/l	8 ± 2	22 ± 16	5 ± 2	24 ± 12	6 ± 3	26 ± 17
Ca $_{extractable}$	mg/l	13 ± 4	26 ± 16	12 ± 3	33 ± 14	14 ± 3	32 ± 17
Na $_{extractable}$	mg/l	24 ± 2	29 ± 7	17 ± 3	32 ± 4	23 ± 7	31 ± 4
Fe $_{extractable}$	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 $_{extractable}$	mg/l	< QL	< QL	< QL	< QL	< QL	0.03 ± 0.01
Cu $_{extractable}$	mg/l	< QL	< QL	< QL	< QL	< QL	< QL
Zn $_{extractable}$	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.

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Table 3. 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.

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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

773 **Table 4.** Comparison of the results from other works in literature on the effects of treatment with sewage sludge on tomato plants. Application
774 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
775 present study. n.a., not available.

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.

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