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**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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1 Title

2 **Anaerobic digestates from sewage sludge used as fertilizer on a poor alkaline sandy**  
3 **soil and on a peat substrate: effects on tomato plants growth and on soil properties**

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25

26 **Abstract**

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

40

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

43

## 44 1. Introduction

45 Globally, the demand of the three primary plant nutrients used for soil fertilization (N, P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O) is  
46 increasing (Vanotti et al., 2019). In 2015, the total fertilizer nutrient demand was around 184 Mt and,  
47 by the end of 2020, it is expected to overcome 200 Mt (FAO, 2017). The production processes of  
48 these fertilizers are very expensive in terms of energy (ammonia) and non-renewable resources  
49 (phosphorus and potassium), with heavy environmental costs (Li et al., 2009). Ammonia production is  
50 mainly performed via the Haber-Bosch process which requires a large amount of fossil fuel (Basosi et  
51 al., 2014). Phosphate rock is the principal raw material exploited in the production of nearly all  
52 phosphate fertilizers (Fixen and Johnston, 2012; Reijnders, 2014). This non-renewable resource may  
53 contain many toxic heavy metals such as As, Hg, Ni, V (Mortvedt, 1995), Cd, Cr, Cu, Pb, Zn (Sabiha-  
54 Javied et al., 2009), fluorine (Mirlean and Roisenberg, 2007) and uranium (Schnug and Lottermoser,  
55 2013). The P<sub>2</sub>O<sub>5</sub> extraction can cause environmental pollution by contaminants accumulating in air,  
56 soil, and water bodies around the manufacturing place (Mirlean et al., 2008; Sabiha-Javied et al.,  
57 2009). It has been observed that these impurities can persist into phosphate fertilizers, provoking a  
58 subsequent accumulation in agricultural soils (De López Camelo et al., 1997). Potassium derives from  
59 non-renewable resources like minerals such as sylvite, sylvinitite, hartsalz and langbeinite (Fixen and  
60 Johnston, 2012). Furthermore, world distribution of phosphorous and potassium mines is not uniform:  
61 45% of global phosphate rock is concentrated in Morocco and the Western Sahara (Fixen and  
62 Johnston, 2012).

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

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Abbreviations: A<sub>N</sub>: assimilation; ANRE: Apparent Nitrogen Recovery Efficiency; ANUE: Agronomic Nitrogen Use Efficiency; C: centrifuged SSAD; CEC: cation exchange capacity; CCI: Chlorophyll Content Index; C<sub>i</sub>: CO<sub>2</sub> 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<sub>s</sub>: 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.

68 S, Fe, Mn, Cu, Zn and B) which are important for plant growth, but usually not included in most  
69 commercial fertilizers (Warman and Termeer, 2005). The percentage of the nutrients appears low, but  
70 it is important to underline that every year a huge amount of wastewater is produced. An empirical  
71 study revealed that approximately 330 km<sup>3</sup> of municipal wastewater are produced worldwide yearly  
72 (Mateo-Sagasta et al., 2015). Therefore, also the SS production has dramatically risen thanks to  
73 policies dealing with the improvement of wastewater treatment and of standard quality of effluents,  
74 such as the E.U. directive 91/271/EEC (Council of the European Communities, 1991a).

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

82 Today, SS is classified as waste and its safe disposal represents a very important issue in waste  
83 management (Epstein, 2002; Singh and Agrawal, 2008). The four main destinations of SS are  
84 incineration, landfilling, composting and agricultural use. In Italy, according to data of 2010 (Eurostat,  
85 2019), the majority of SS is sent to landfill (50.8 %), while 34.7% is reused in agriculture, 4% is  
86 incinerated and 10.4% is sent to other destinations. The Council Directive 86/278 (Council of the  
87 European Communities, 1986) regulates the agricultural SS reuse in Europe to prevent soil  
88 contamination. In fact, this practice has three principal problems that limit its unconditioned use:  
89 biological risk, heavy metal contamination and contamination by organic pollutants. The biological risk  
90 is principally represented by pathogens such as *Salmonella* spp., *Escherichia coli* (enterotoxigenic  
91 and enteropathogenic variants), *Campylobacter* spp., *Clostridium* spp., and *Yersinia* spp. (Arthurson,  
92 2008); stabilization treatments can reduce significantly their presence in SS and are mandatory before  
93 subsequent SS applications (Dumontet et al., 1999). For instance, one of the most diffused  
94 stabilization techniques is anaerobic digestion (Liu et al., 2012), in which the reduction of pathogens,  
95 putrescence and odor is coupled with biogas production, allowing energy recovery (Epstein, 2002).

96 Heavy metal content (normally represented by Cd, Cr, Cu, Hg, Ni, Pb, Zn) can be abated by means of  
97 chemical (e.g. chelating addition), physical (e.g. electroremediation) or biological (e.g.  
98 vermicomposting) treatments (Camargo et al., 2016). Finally, some organic pollutants (e.g. pesticides,  
99 antibiotics and hormones) can be volatilized or degraded through biotic or abiotic processes (Harrison  
100 et al., 2006). “Concerning organic pollutants, their abatement is trickier. Indeed, class of emerging  
101 contaminants (EmC) in wastewater is increasingly gaining more interest within the organic  
102 compounds. EmC include molecules such as endocrine disrupting compounds (e.g. hormones),  
103 pharmaceutically active compounds (e.g. antibiotics), illicit drugs and pesticides (Fijalkowski, 2019).  
104 EmC abatement is becoming even more required both on the effluent of WWTPs with advanced  
105 treatments (e.g. activated carbon absorption, advanced oxidation processes, reverse osmosis) and on  
106 sewage sludge (Gadupudi et al., 2019). Some studies affirmed that anaerobic digestion is the  
107 stabilization strategy ensuring the best EmC removal, especially when the sludge is pretreated (e.g.  
108 via ozonation) (Neumann et al., 2016). However, further studies are still required to improve the  
109 performances and reduce the costs of these techniques, which nowadays are rarely applied at WWTP  
110 level since they are money and/or time consuming (Camargo et al., 2016). The abovementioned EU  
111 directive regulates the SS soil application in the EU and establishes threshold values of some of these  
112 pathogens and pollutants in SS.

113 On the basis of these opportunities and threats related to SS, this work aims to deepen the  
114 knowledge about SS fertilizing effects over time in terms of nutrients and OM on a poor alkaline sandy  
115 soil. This kind of soil was selected because: i) nutrient depletion constrains plant growth to depend on  
116 treatment application; ii) a high pH both hinders the nutrient adsorption and reduce the metal  
117 bioavailability (Alvarenga et al., 2016); iii) sandy-textured soil lacks nutrients and has low water-  
118 holding capacity. These results were compared to the one obtained with a richer peat substrate. Pot  
119 experiments were performed on tomato plants (*Solanum lycopersicum* L.) in a greenhouse to  
120 evaluate nutrient provision of anaerobic digestates from sewage sludge (SSADs). Tomato plant was  
121 chosen because: i) it is one of the most exploited vegetables crop (Jones Jr, 2008); ii) there is an  
122 increasing interest on alternative nutrient sources for this crop (Zucco et al., 2015); iii) it has a high  
123 fertilizer requirements (Zucco et al., 2015); iv) plenty of scientific literature is available for this crop

124 (Jones Jr, 2008). In this work, no analysis on pathogens was carried out since anaerobic digestion is  
125 considered one of the safest technologies for pathogen reduction in SS (Epstein, 2002).  
126 Nevertheless, this aspect may be taken into consideration in future researches. Concerning the use of  
127 SS in agronomic experiments, a lack in details about SS typology is provided. Indeed, in many work  
128 no detail on stabilization strategy is provided (Bakshi et al., 2019), or the kind of SS digestion is not  
129 specified (Hossain et al., 2015). In the present work, the digestates used derived from the same  
130 WWTP and were obtained with consequent treatments (Cristina et al., 2019). As far as we know, this  
131 is the first example of use of four different and consequent SSADs to fertilize tomato plants. The  
132 paper examines agronomic parameters of tomato plants, the nutrient distribution in soil and nutrient  
133 absorption by plants after the application of SSADs. Furthermore, numerous plants physiological  
134 parameters were evaluated over a span of three months in order to better understand the effects in a  
135 time course approach.

## 136 2. Materials and methods

### 137 2.1. Characterizations

#### 138 2.1.1. Anaerobic digestates from sewage sludge

139 Four SSADs were used in the experiment: two liquid (primary (P) and secondary (S)) and two solid  
140 (centrifuged (C) and dried (D)). Physical and chemical characterization of the SSADs is described in a  
141 previous work (Cristina et al., 2019); characterization of the four SSADs is reported in Supplementary  
142 Material (Section I - **Table S1**).

#### 143 2.1.2. Cultivation substrates

144 Two types of substrates were used: a sandy soil and a commercial peat substrate (**Table 1**). The  
145 sandy soil was sampled within 20 and 100 cm depth in Grugliasco (TO), Italy (45°03'58.4"N,  
146 7°35'32.9"E). Analytical methods used for characterization of the sandy soil and the peat substrate  
147 are specified in Supplementary materials (Section II). Based on the distribution of the particle size  
148 (sand: 94% ± 2; silt: 3% ± 1; clay: 3% ± 1), the selected soil was classified as sandy (Buol et al.,  
149 2011). Based on ARPAV soil analysis (Arpa Veneto, 2007), the soil was considered alkaline (8.2 ±

150 0.16), very poor in OM ( $0.38 \pm 0.12\% < 0.8\%$ ) and very poor in macronutrients such as nitrogen ( $0.29$   
151  $\pm 0.09$  g/kg  $< 0.5$  g/kg), phosphorous ( $1.8 \pm 1.3$  mg/kg  $< 7$  mg/kg), potassium ( $18 \pm 1$  mg/kg  $< 40$   
152 mg/kg) and magnesium ( $15 \pm 5$  mg/kg  $< 50$  mg/kg). On the other hand, content of calcium ( $675 \pm 27$   
153 mg/kg  $< 1000$  mg/kg) and some microelements such as iron ( $2.5$  mg/kg  $< 6.7 \pm 1.1$  mg/kg  $< 20$   
154 mg/kg) and manganese ( $2$  mg/kg  $< 6.5 \pm 3.0$  mg/kg  $< 10$  mg/kg) 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 ( $\varnothing$  17  
159 cm, height 20 cm, surface area 0.227 m<sup>2</sup>). 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

176 month after sowing, it was not possible to measure CCI on the sand specimen because the minimum  
177 leaves size was not satisfied. At the end of the second month, assimilation ( $A_N$ ), stomatal  
178 conductance ( $g_s$ ) and  $CO_2$  concentration in substomatal cavity ( $C_i$ ) were measured by the means of  
179 an Infrared Gas Analyzer (IRGA, ADC, Hoddesdon, UK). These measurements were performed on  
180 three fully formed leaves in each replicate. The selected leaves had to be non-senescing, at the same  
181 physiological age (in the middle part of the plant, considering the third to fourth leaf from the shoot  
182 apex) and directly exposed to sunlight. After all the measurements were taken, each plant was  
183 subsequently cut and immediately weighed to record the fresh biomass value. In order to evaluate the  
184 mean dry biomass, each plant was dried at 105°C for at least 72 hours. Subsequently, agronomic  
185 nitrogen use efficiency (ANUE) was calculated as:

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

## 186 2.4. Chemical analysis

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

193 Chemical analyses of the leaves were conducted at the end of the second month, after the biomass  
194 measurement, in order to assess the content of nitrogen, phosphorus and potassium in the leaves. In  
195 the case of the samples from the sandy soil, the measurements were performed on samples treated  
196 with one liquid digestate (P), one solid digestate (D) and the mineral fertilizer (M). It was not possible  
197 to analyze samples from the negative control (T) due to the low biomass production. On the peat  
198 substrate, it was possible to evaluate N-P-K content not only in the P, D, and M samples, but also in  
199 the negative control ones (T). The plant samples were firstly processed with a humid digestion  
200 protocol (Mills and Jones Jr, 1996). Then, nitrogen was measured through the Kjeldahl method,

201 phosphorus was evaluated through colorimetry (molybdovanadate method) and potassium was  
202 quantified by Atomic Absorption Spectroscopy (AAS). Finally, the N, P and K percentages were used  
203 to calculate the mean total element present in the epigeal part of the plant using the following  
204 formula:

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

## 205 2.5. Statistical analysis

206 The experimental data were subjected to statistical analyses. Two-way ANOVA was used to compare  
207 the average results of different treatments on plant measurements. Differently, one-way ANOVA was  
208 used to compare the mean results of different treatments on the chemical analyses of soils and leaf  
209 nutrient content. After the ANOVA, Tukey's post-hoc test ( $P < 0.05$ ) was performed. The statistical  
210 software R (version 3.5.1 - Feather Spray - 2018) was used for all statistical analysis.

## 211 3. Results

### 212 3.1. Plant measurements

#### 213 3.1.1. Dry biomass and ANUE

214 On the sandy soil at the end of the first month, the dry biomass of the tomato plants grown with  
215 digestates did not show any difference between each other. Despite the absence of significant  
216 differences, it must be pointed out that biomass of S was 28.7 and 12.7-folds higher than control (T)  
217 and mineral fertilizer (M), respectively. At the end of second month, all digestates (P, S, C, D) showed  
218 a dry biomass production significantly higher (26.7, 33, 35.3 and 37.5-folds, respectively) than control.  
219 At the same time, S, C and D showed a higher biomass than mineral fertilizer (2.9, 3.1 and 3.3-folds,  
220 respectively).. At the end of third month, dewatered SSADs proved to be the most productive  
221 treatments, with C and D displaying the highest yields (10.23 g and 10.97 g). Their biomasses  
222 doubled mineral fertilizer one (5.13 g), which was only comparable to the biomass produced by plants  
223 treated with SSADs after two months. Furthermore, C and D yields were 16 and 17-folds higher than  
224 T (0.64 g), respectively (**Figure 1.A**).

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

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

### 234 3.1.2.Height

235 On the sandy soil, no differences in plant height were present at the end of the first month.  
236 Nevertheless, S treatment revealed the tallest tomato plants, up to 2.6 and 2.5-folds higher than T  
237 and M. In the second month, all SSADs-treated plants were significantly taller than control and  
238 mineral fertilizer, with D treatment displaying a height 6 and 2.1-folds higher than T and M,  
239 respectively. After three months, the mean height of T was still the lowest. The mean height of the  
240 plants grown on P and D was comparable to plants grown on mineral fertilizer. Plants grown with S  
241 and C treatments had a statistically higher height than mineral fertilizer (M). It is worth highlighting that  
242 the mean heights of the plants grown on all digestates was at least 3.5-folds higher than the control  
243 ones (**Figure 2.A**).

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

### 247 3.1.3.Leaves and inflorescences

248 After the first month, the plants grown on sandy soil in presence of D and C treatments showed a  
249 number of leaves comparable to control and minerally fertilized plants. On the other hand, samples  
250 from liquid SSADs (P and S) revealed a higher mean leaf number than control. After two months, the

251 leaves number on plants grown with digestates was significantly higher only than negative control  
252 plants. At the end of the experiment, samples from S and D treatments showed the highest number of  
253 leaves, which were not statistically different from samples from C treatment. Plants grown with P had  
254 similar number of leaves than C and mineral fertilizer, while leaves number in negative control was  
255 still the lowest one (**Figure 2.B**).

256 With regards to the number of inflorescences, no plant on sandy soil showed flowers one month after  
257 sowing. At the end of the second month, plants in T and M were still not revealing any flower.  
258 Differently, P, S, C and D had some inflorescences, but no significant difference between treatments  
259 was present. At the end of the experiment, negative control plants still did not show any flower. Plants  
260 treated with P and S had a number of inflorescences statistically similar to mineral fertilizer. The  
261 highest number of inflorescences was found on C and D treatments (**Figure 2.C**).

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

#### 264 3.1.4. Chlorophyll Content Index (CCI)

265 On sandy soil, leaves dimension after one month was too small to measure CCI. At the end of second  
266 month leaves of plants treated with P, S and C showed a CCI higher than control and comparable to  
267 mineral fertilizer. The mean CCI value of plants grown with D digestate was statistically higher than  
268 mineral fertilizer (M) but comparable to the others SSADs. CCI measures performed at the end of  
269 third month revealed a substantial decrease in CCI values registered in all SSADs and in mineral  
270 fertilizer, whose values were not significantly different from the control. The only significant difference  
271 emerging at the endpoint was between P and mineral fertilizer measure. (**Figure 2.D**).

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

#### 274 3.1.5. Infra-red gas analyzer (IRGA)

275 As regards IRGA measurements, on sandy soil the lowest  $A_N$  value was found in control, where  
276 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 ( $\text{NH}_4^+$ ), 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).

356 SSAD application on tomato crops resulted also in an augmented number of leaves and  
357 inflorescences with respect to control and mineral fertilizer. Moreover, inflorescences number of  
358 SSAD-treated plants increased from 2 to 3-folds over the last month. These findings were in general  
359 agreement with other results reported on tomato grown in presence of SS (Bakshi et al., 2019),  
360 despite the higher treatment dosages.

361 Number of leaves and inflorescences are developmental parameters considered also with other plant  
362 species when testing the fertilizing effects of SSAD. For instance, Eid and colleagues (2017)  
363 registered on cucumber a boost in the number of leaves of more than 2-folds, which is in line with the  
364 results of the present work. Similar outcomes have been reported in terms of number of flowers in  
365 common bean (*Phaseolus vulgaris* L.)(Fernández-Luqueño et al., 2010) and marigold (*Tagetes erecta*  
366 L.)(Solanki et al., 2017) grown in SS dosages lower and higher, respectively, than the present work.  
367 In contrast with these results, Tariq and co-workers (2012) described a decrease up to 60% in flowers  
368 number in *Dahlia x hortensis*, whose growth had probably been compromised by an excessive SS  
369 dosage.

370 Results of the present work confirmed the positive effects of SS application on net photosynthesis  
371 (Bouriouq et al., 2018; Pascual et al., 2008) and chlorophyll content. Leaf chlorophyll content was  
372 directly correlated with indirect chlorophyll measurements such as readings through SPAD and CCI-  
373 meters (Xiong et al., 2015), whose value can be compared to each other with the equations proposed  
374 by Parry and colleagues (2014). Application of SSAD improved chlorophyll content values of tomato  
375 plants grown on sandy soil at the end of second month, as well as dry biomass and net  
376 photosynthesis ( $A_N$ ). This beneficial effect has been already observed also in sunflower (Bouriouq et  
377 al., 2018), sorghum (Alvarenga et al., 2016) and triticale (Kchaou et al., 2018). On the other hand,  
378 literature provides examples of reduction of leaf chlorophyll content in tomato after treatment with SS  
379 (Elloumi et al., 2016), which is probably due to the excessive heavy metals presence in the used SS  
380 (Singh and Agrawal, 2007). However, this aspect was likely not linked with the reduction of chlorophyll  
381 content over time observed in the present study. Indeed, this phenomenon has been already  
382 observed in other SS-treated plant species, such as common bean (Fernández-Luqueño et al., 2010).  
383 A possible explanation of this reduction in CCI at the end of the third month could be the deficiency of

384 nutrients in soil. A second hypothesis for CCI decrease has been proposed by de Oliveira and co-  
385 workers (2017): after the initial blooming of the plant, gradual degradation of chlorophyll occurs due to  
386 the beginning of the fruit development phase, which induces a metabolic change in the plant, with a  
387 more sustained nutrients accumulation in the fruit. Taking into account the relationship between leaf  
388 nitrogen and chlorophyll content (Xiong et al., 2015), a third justification for CCI decrease can be  
389 provided by the so-called nitrogen dilution curve. In fact, biomass increase in tomato plant was  
390 accompanied by a reduction in nitrogen concentration (and, consequently in chlorophyll content)  
391 because the structural compartment (lower in N%) becomes proportionally more massive than  
392 metabolic active one (higher in N%) (Tei et al., 2002).

## 393 4.2. Chemical analysis

### 394 4.2.1. Substrates analyses

395 The application of SS on soil can affect different physical and chemical soil characteristics (Epstein,  
396 2002). Likewise, many changes were documented in this experiment (both on sandy soil and on peat  
397 substrate) two months after treatments application. Although peat substrate was low in nutrient  
398 content, it showed a consistently higher amount of microelement than sandy soil. Moreover, peat  
399 substrate has many other advantages such as lightweight, high water holding capacity and high air  
400 space (Gruda et al., 2016). All these peculiarities most probably contributed to the minor differences  
401 registered on peat substrate.

402 Soil analysis results revealed a change in soil pH after the treatments application. Many works  
403 reported an increase (Bayoumi Hamuda et al., 2009; Ferreiro-Domínguez et al., 2011) or a decrease  
404 (Mosquera-Losada et al., 2016; Singh and Agrawal, 2007) in soil pH. In the present work, acidification  
405 occurred in treated sandy soil samples, probably due to both the lower pH of SSADs and the nitrogen  
406 mineralization (Rasouli-Sadaghiani and Moradi, 2014). In particular, the nitrification process ( $\text{NH}_4^+ \rightarrow$   
407  $\text{NO}_3^-$ ) (Stamatiadis et al., 1999) induces the release of  $\text{H}^+$  in soil solution media and the leaching of  
408  $\text{NO}_3^-$  by water (Whitehead, 1995). Another conceivable theory for soil acidification in SSAD-treated  
409 samples could be the generation of organic acids during sewage sludge mineralization (Angin et al.,

410 2012; Bourioug et al., 2018). Additionally, the low buffering capacity might be yet another conceivable  
411 effect occurring in the sandy soil case.

412 Electrical conductivity values (both on sandy soil and on peat substrate) did not statistically change  
413 after treatments application unlike many other works (Bourioug et al., 2018; Singh and Agrawal,  
414 2007), likely due to the consistently lower SSAD application rates. Nevertheless, it must be pointed  
415 out that, concerning sandy soil, EC values in M were approximatively doubled compared to SSAD  
416 ones, which in turns were somewhat higher than control. High EC of M might be due to the  
417 particularly higher concentration of nitrates, likely released as bioavailable form nitrogen by the  
418 commercial fertilizer. However, these relatively elevate nitrate amounts were likely not necessary, as  
419 confirmed by the better growth parameters and ANUE values of tomato plants growing on SSAD  
420 amended soil. On the contrary, excess of nitrates may result in undesired drawbacks such as  
421 leaching and hyperaccumulation in plant tissues, feature in agreement with the foliar analyses. EC  
422 values in SSAD treatments was probably influenced by sodium presence in the digestates, which  
423 however did not affect the physiological parameters of tomato plants as confirmed by IRGA  
424 measurements.

425 The thesis of a possible increasing of soil OM in soils treated with SSADs (Kladivko and Nelson,  
426 1979; Perez-Espinosa et al., 1999) was confirmed by the present work. Despite the OM percentage  
427 was very low in all samples, the value in SSADs treated theses was higher than control and mineral  
428 fertilizer. This may partially justify the better performances of treated samples in term of biomass and  
429 height, according to the well-known soil OM benefits on plants growth (Bot and Benites, 2005).

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

433 Total N, available P, exchangeable Ca and Na and available Fe and Zn concentrations increased in  
434 the sandy soil amended with SSADs due to their higher concentration in SS (Singh and Agrawal,  
435 2007).

436 Two months after treatments application,  $N_{Tot}$  (Kjeldahl) was higher in C and D than liquid SSADs (P  
437 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

466 some cases in treated soils, confirming the results of Ferreiro Dominguez and Singh (Ferreiro-  
467 Domínguez et al., 2011; Singh and Agrawal, 2007).

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

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

#### 475 4.2.2. Leaf analysis

476 In some cases, in literature the use of SS enhanced the percentage of macronutrients in leaves  
477 (Angin et al., 2012; Zuo et al., 2019), in other ones no change took place (Kotecki et al., 2014; Pinna  
478 et al., 2009) and still in other ones concentration increased only for some nutrients (Bakshi et al.,  
479 2019; De Andres et al., 2010). This work belongs to the third category, since only foliar P% and total  
480 uptaken P of control plants grown on peat substrate were significantly lower than SSADs ones. On  
481 sandy soil, content of uptaken P was significantly higher in D and P treatments, which was likely  
482 influenced by the phosphorous amount in the initial application. Nevertheless, no significant  
483 differences emerged in foliar P% despite the difference in uptaken P content between SSADs and  
484 mineral fertilizer: probably, the controlled nutrient release of the mineral fertilizer compensated the  
485 higher quantity of P in the SSADs. Moreover, it could be inferred that differences in foliar  
486 macronutrient content could have been appreciated between control and treated samples on sandy  
487 soil. However, the too low biomass of untreated samples made impossible this investigation.

488 The total amount of N and K uptaken in leaves had varied results. On sandy soil, D samples revealed  
489 a significantly higher N content than P ones due to the different biomass production. Concerning  
490 plants grown on peat substrate, P and D showed a nitrogen plant uptake similar to negative control,  
491 but lower than mineral fertilizer, likely due to the characteristics of the fertilizer, such as the controlled  
492 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  $K_{\text{Extractable}}$  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

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

527 Despite reserves and resources for N, P and K appear adequate for the near future, it is necessary to  
528 find less impactful solutions to produce fertilizers in the short term. In this way, the reuse of SS can  
529 reduce the negative effects connected by the extraction, manufacturing and the use of mineral  
530 fertilizers derived from non-renewable resources. Furthermore, this experiment showed how the  
531 positive effects of SSADs are emphasized if applied on a poor alkaline soil.

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753 **Table 1.** Physiscal and chemical anlysis 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         |
| <b>Stones</b>                         | -       | absent        | <b>Stones</b>                         | -    | -             |
| <b>Sand (2.0 - 0.020 mm)</b>          | %       | 94 ± 2        | <b>Sand (2.0 - 0.020 mm)</b>          | -    | -             |
| <b>Silt (0.020 - 0.002 mm)</b>        | %       | 3 ± 1         | <b>Silt (0.020 - 0.002 mm)</b>        | -    | -             |
| <b>Clay (&lt; 0.002 mm)</b>           | %       | 3 ± 1         | <b>Clay (&lt; 0.002 mm)</b>           | -    | -             |
| <b>Texture</b>                        | -       | sandy         | <b>Texture</b>                        | -    | -             |
| <b>pH</b>                             | -       | 8.2 ± 0.16    | <b>pH</b>                             | -    | 6.2 ± 0.1     |
| <b>Electrical conductivity</b>        | dS/m    | 0.131 ± 0.018 | <b>Electrical conductivity</b>        | dS/m | 0.722 ± 0.146 |
| <b>Organic matter</b>                 | %       | 0.38 ± 0.12   | <b>Organic matter</b>                 | -    | -             |
| <b>Organic carbon</b>                 | %       | 0.22 ± 0.07   | <b>Organic carbon</b>                 | -    | -             |
| <b>N - Tot (Kjeldahl)</b>             | g/kg    | 0.29 ± 0.09   | <b>N - Tot (Kjeldahl)</b>             | %    | 0.42 ± 0.06   |
| <b>N - NO<sub>2</sub><sup>-</sup></b> | mg/kg   | < 0,2         | <b>N - NO<sub>2</sub><sup>-</sup></b> | mg/l | < QL          |
| <b>N - NO<sub>3</sub><sup>-</sup></b> | mg/kg   | 6.33 ± 1.53   | <b>N - NO<sub>3</sub><sup>-</sup></b> | mg/l | 30.4 ± 7.2    |
| <b>N - NH<sub>4</sub><sup>+</sup></b> | mg/kg   | 3 ± 1         | <b>N - NH<sub>4</sub><sup>+</sup></b> | mg/l | 1.3 ± 0.3     |
| <b>N - Org</b>                        | g/kg    | 0.29 ± 0.09   | <b>N - Org</b>                        | %    | 0.4 ± 0.40    |
| <b>C/N</b>                            |         | 7.6 ± 0.2     | <b>C/N</b>                            |      | -             |
| <b>P<sub>Olsen</sub></b>              | mg/kg   | 1.8 ± 1.3     | <b>P<sub>extractable</sub></b>        | mg/l | 8.1 ± 2.3     |
| <b>K<sub>exchangeable</sub></b>       | mg/kg   | 18 ± 1        | <b>K<sub>extractable</sub></b>        | mg/l | 41.1 ± 6.8    |
| <b>Mg<sub>exchangeable</sub></b>      | mg/kg   | 15 ± 5        | <b>Mg<sub>extractable</sub></b>       | mg/l | 28 ± 7        |
| <b>Ca<sub>exchangeable</sub></b>      | mg/kg   | 675 ± 27      | <b>Ca<sub>extractable</sub></b>       | mg/l | 36 ± 8        |
| <b>Na<sub>exchangeable</sub></b>      | mg/kg   | 6 ± 3         | <b>Na<sub>extractable</sub></b>       | mg/l | 16 ± 11       |
| <b>Fe<sub>available</sub></b>         | mg/kg   | 6.7 ± 1.1     | <b>Fe<sub>extractable</sub></b>       | mg/l | 0.79 ± 0.21   |
| <b>Mn<sub>available</sub></b>         | mg/kg   | 6.5 ± 3.0     | <b>Mn<sub>extractable</sub></b>       | mg/l | 0.15 ± 0.04   |
| <b>Cu<sub>available</sub></b>         | mg/kg   | 0.69 ± 0.29   | <b>Cu<sub>extractable</sub></b>       | mg/l | < QL          |
| <b>Zn<sub>available</sub></b>         | mg/kg   | 0.47 ± 0.29   | <b>Zn<sub>extractable</sub></b>       | mg/l | 0.02 ± 0.00   |
| <b>CEC</b>                            | cmol/kg | 3.65 ± 0.35   | <b>CEC</b>                            |      | -             |

755 **Table 2.** Chemical characterization performed two months after treatments application on sandy soil (A) and on peat substrate (B). Data are  
 756 expressed as mean  $\pm$  standard deviation. Asterisks mean significant differences according to ANOVA test (\*, \*\*, \*\*\* differences between means  
 757 significant at  $P \leq 0.05$ , 0.01 and 0.001, respectively). CEC, cation exchange capacity; QL, quantification limit.

758 **A**

| Parameter                        | Unit of measure | Control (T) |             |     | Primary (P) |             |     | Secondary (S) |             |     | Centrifuged (C) |             |     | Dried (D) |             |     | Mineral fertiliser (M) |             |     |
|----------------------------------|-----------------|-------------|-------------|-----|-------------|-------------|-----|---------------|-------------|-----|-----------------|-------------|-----|-----------|-------------|-----|------------------------|-------------|-----|
| pH                               | -               | 8.3         | $\pm$ 0.1   | *** | 8.2         | $\pm$ 0.1   | *** | 8.2           | $\pm$ 0.1   | *** | 8.1             | $\pm$ 0.1   | *** | 8.1       | $\pm$ 0.1   | *** | 8.0                    | $\pm$ 0.1   | *** |
| Electrical conductivity          | dS/m            | 0.155       | $\pm$ 0.020 |     | 0.219       | $\pm$ 0.032 |     | 0.201         | $\pm$ 0.010 |     | 0.197           | $\pm$ 0.023 |     | 0.198     | $\pm$ 0.025 |     | 0.399                  | $\pm$ 0.146 |     |
| Organic matter                   | %               | 0.16        | $\pm$ 0.01  | *** | 0.24        | $\pm$ 0.01  | *** | 0.18          | $\pm$ 0.02  | *** | 0.25            | $\pm$ 0.02  | *** | 0.26      | $\pm$ 0.01  | *** | 0.16                   | $\pm$ 0.02  | *** |
| Organic carbon                   | %               | 0.09        | $\pm$ 0.00  | *** | 0.14        | $\pm$ 0.00  | *** | 0.11          | $\pm$ 0.01  | *** | 0.14            | $\pm$ 0.01  | *** | 0.15      | $\pm$ 0.00  | *** | 0.10                   | $\pm$ 0.01  | *** |
| N - Tot (Kjeldahl)               | g/kg            | 0.17        | $\pm$ 0.01  | *** | 0.19        | $\pm$ 0.01  | *** | 0.15          | $\pm$ 0.01  | *** | 0.20            | $\pm$ 0.00  | *** | 0.22      | $\pm$ 0.01  | *** | 0.17                   | $\pm$ 0.01  | *** |
| N - NO <sub>2</sub> <sup>-</sup> | mg/kg           | < QL        |             |     | < QL        |             |     | < QL          |             |     | < QL            |             |     | < QL      |             |     | < QL                   |             |     |
| N - NO <sub>3</sub> <sup>-</sup> | mg/kg           | < QL        |             |     | < QL        |             |     | 1             | $\pm$ 1     | *   | 4               | $\pm$ 4     | *   | 2         | $\pm$ 1     | *   | 60                     | $\pm$ 40    | *   |
| N - NH <sub>4</sub> <sup>+</sup> | mg/kg           | < QL        |             |     | < QL        |             |     | < QL          |             |     | 1               | $\pm$ 1     |     | 2         | $\pm$ 0     |     | 1                      | $\pm$ 0     |     |
| N - Org                          | g/kg            | 0.17        | $\pm$ 0.01  | *** | 0.19        | $\pm$ 0.01  | *** | 0.15          | $\pm$ 0.01  | *** | 0.20            | $\pm$ 0.00  | *** | 0.22      | $\pm$ 0.01  | *** | 0.17                   | $\pm$ 0.01  | *** |
| C/N                              | -               | 5.5         | $\pm$ 0.2   | **  | 7.3         | $\pm$ 0.6   | **  | 6.9           | $\pm$ 0.5   | **  | 7.3             | $\pm$ 0.5   | **  | 7.1       | $\pm$ 0.3   | **  | 5.8                    | $\pm$ 1.0   | **  |
| P <sub>Olsen</sub>               | mg/kg           | < QL        |             |     | 4.2         | $\pm$ 0.5   | **  | 10.3          | $\pm$ 0.8   | **  | 15.4            | $\pm$ 4.6   | **  | 36.2      | $\pm$ 11.4  | **  | < QL                   |             |     |
| K <sub>exchangeable</sub>        | mg/kg           | 14          | $\pm$ 3     |     | 11          | $\pm$ 3     |     | 12            | $\pm$ 1     |     | 12              | $\pm$ 2     |     | 9         | $\pm$ 1     |     | 13                     | $\pm$ 2     |     |
| Mg <sub>exchangeable</sub>       | mg/kg           | 11          | $\pm$ 2     |     | 21          | $\pm$ 7     |     | 26            | $\pm$ 3     |     | 25              | $\pm$ 4     |     | 22        | $\pm$ 4     |     | 25                     | $\pm$ 2     |     |
| Ca <sub>exchangeable</sub>       | mg/kg           | 524         | $\pm$ 26    | *   | 594         | $\pm$ 25    | *   | 491           | $\pm$ 62    | *   | 626             | $\pm$ 94    | *   | 579       | $\pm$ 48    | *   | 646                    | $\pm$ 62    | *   |
| Na <sub>exchangeable</sub>       | mg/kg           | 16          | $\pm$ 1     | *** | 35          | $\pm$ 2     | *** | 33            | $\pm$ 3     | *** | 26              | $\pm$ 3     | *** | 32        | $\pm$ 3     | *** | 27                     | $\pm$ 5     | *** |
| Fe <sub>available</sub>          | mg/kg           | 5.7         | $\pm$ 0.1   | *** | 7.6         | $\pm$ 0.5   | *** | 9.3           | $\pm$ 0.2   | *** | 11.2            | $\pm$ 0.7   | *** | 12.5      | $\pm$ 0.4   | *** | 6.1                    | $\pm$ 0.21  | *** |
| Mn <sub>available</sub>          | mg/kg           | 5.1         | $\pm$ 0.4   |     | 14.8        | $\pm$ 16.6  |     | 33.7          | $\pm$ 2.5   |     | 35.8            | $\pm$ 27.1  |     | 20.5      | $\pm$ 28.1  |     | 5.2                    | $\pm$ 0.21  |     |
| Cu <sub>available</sub>          | mg/kg           | 0.40        | $\pm$ 0.08  |     | 0.47        | $\pm$ 0.13  |     | 0.60          | $\pm$ 0.06  |     | 0.85            | $\pm$ 0.12  |     | 0.96      | $\pm$ 0.29  |     | 0.40                   | $\pm$ 0.01  |     |
| Zn <sub>available</sub>          | mg/kg           | 0.21        | $\pm$ 0.03  | *** | 0.36        | $\pm$ 0.04  | *** | 0.37          | $\pm$ 0.02  | *** | 0.73            | $\pm$ 0.08  | *** | 1.00      | $\pm$ 0.07  | *** | 0.43                   | $\pm$ 0.05  | *** |
| CEC                              | cmol/kg         | 2.81        | $\pm$ 0.13  | *   | 3.32        | $\pm$ 0.17  | *   | 2.83          | $\pm$ 0.33  | *   | 3.47            | $\pm$ 0.43  | *   | 3.24      | $\pm$ 0.20  | *   | 3.58                   | $\pm$ 0.32  | *   |

QL: N - NO<sub>2</sub><sup>-</sup> = 0.2 mg/kg; N - NO<sub>3</sub><sup>-</sup> = 1 mg/kg; N - NH<sub>4</sub><sup>+</sup> = 1 mg/kg; P = 1 mg/kg.

759

760 **B**

| Parameter                             | Unit of measure | Control (T)   | Primary SSAD (P) | Secondary SSAD (S) | Centrifuged SSAD (C) | Dried SSAD (D) | Mineral (M)   |
|---------------------------------------|-----------------|---------------|------------------|--------------------|----------------------|----------------|---------------|
| <b>pH</b>                             | -               | 7.0 ± 0.4 *   | 6.7 ± 0.3 *      | 7.2 ± 0.2 *        | 6.9 ± 0.2 *          | 7.4 ± 0.3 *    | 6.6 ± 0.2 *   |
| <b>Electrical conductivity</b>        | 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 |
| <b>N - Tot (Kjeldahl)</b>             | % 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 * |
| <b>N - NO<sub>2</sub><sup>-</sup></b> | mg/l            | < QL          | < QL             | < QL               | < QL                 | < QL           | < QL          |
| <b>N - NO<sub>3</sub><sup>-</sup></b> | 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     |
| <b>N - NH<sub>4</sub><sup>+</sup></b> | mg/l            | < QL          | < QL             | < QL               | < QL                 | < QL           | < QL          |
| <b>N - Org</b>                        | % 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 * |
| <b>P extractable</b>                  | mg/l            | 0.4 ± 0.1     | 1.2 ± 0.4        | 1.2 ± 0.5          | 0.6 ± 0.3            | 1.1 ± 0.1      | < QL          |
| <b>K extractable</b>                  | 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     |
| <b>Mg extractable</b>                 | mg/l            | 8 ± 2         | 22 ± 16          | 5 ± 2              | 24 ± 12              | 6 ± 3          | 26 ± 17       |
| <b>Ca extractable</b>                 | mg/l            | 13 ± 4        | 26 ± 16          | 12 ± 3             | 33 ± 14              | 14 ± 3         | 32 ± 17       |
| <b>Na extractable</b>                 | mg/l            | 24 ± 2        | 29 ± 7           | 17 ± 3             | 32 ± 4               | 23 ± 7         | 31 ± 4        |
| <b>Fe extractable</b>                 | 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   |
| <b>Mn extractable</b>                 | mg/l            | < QL          | < QL             | < QL               | < QL                 | < QL           | 0.03 ± 0.01   |
| <b>Cu extractable</b>                 | mg/l            | < QL          | < QL             | < QL               | < QL                 | < QL           | < QL          |
| <b>Zn extractable</b>                 | mg/l            | < QL          | < QL             | < QL               | < QL                 | < QL           | 0.02 ± 0      |

QL: N - NO<sub>2</sub><sup>-</sup> = 0.05 mg/l; N - NH<sub>4</sub><sup>+</sup> = 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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766 **Table 3.** Results of leaves analyses performed after two months after treatments application on sandy soil (A) and on peat substrate (B). Different  
 767 letters indicate differences between treatments that are significant at  $P < 0.05$  (Tukey HSD). Data are expressed as mean  $\pm$  standard deviation.

768 **A**

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

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770 **B**

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

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

<sup>a</sup>In this work, no details about the typology of digestion are provided.

<sup>b</sup>In these works, leaf chlorophyll content was evaluated with methods based on extraction with organic solvents followed by spectrophotometrical quantification.

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