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Evaluation of anaerobic digestates from sewage sludge as a potential solution for improvement of soil fertility

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(Article begins on next page)

1 **Title**

2 EVALUATION OF ANAEROBIC DIGESTATES FROM SEWAGE SLUDGE AS A POTENTIAL
3 SOLUTION FOR IMPROVEMENT OF SOIL FERTILITY

4

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27 **Declaration of Interest**

28 Declaration of interest: none.

29

30 **Abstract**

31 Sewage sludge production in European countries has widely raised in the last decade and its
32 fate is currently landfilling, incinerators, composting or land application. To explore its agronomic
33 potential, the main target of this work is to understand the effects of anaerobic digestates from
34 sewage sludge (SSAD). To this aim, four different SSADs (two liquids and two dewatered) were
35 characterized. On the liquid ones, Germination Index was evaluated through a plate bioassay
36 with *Lepidium sativum* L. seeds; low concentrations of SSAD (2.5%) improved GI in one case,
37 while at higher concentrations phytotoxic effects occurred in both. Then, pot experiments were
38 set in climate chamber with *Cucumis sativus* L. grown for 30 days on two different substrates: a
39 sandy, alkaline and poor soil, and peat substrate. All SSADs and a mineral fertilizer were used
40 at three increasing dosages: 85, 170, 255 kg of nitrogen per hectare (kg N/ha). Results in terms
41 of germination, dry biomass, chlorophyll content, net photosynthesis, stomatal conductance,
42 CO₂ concentration in substomatal cavity and root development were compared to a not treated
43 control.

44 All treatments gave results significantly higher or similar to control on all the parameters
45 evaluated. Moreover, the intermediate nitrogen dosage (170 kg N/ha) generally showed the
46 highest results compared to other dosages, especially for dewatered SSADs. All these results
47 were much more evident for cucumber plants grown on an the alkaline, sandy and poor soil
48 than on peat substrate, such demonstrating that SSADs have a fertilizing effect for plants
49 growing on this kind of soil.

50

51 Keywords: soil improver; nutrient-deficient soil; circular economy; pot experiment; climate
52 chamber; nitrogen content.

53

54 **1. Introduction**

55

56 The treatment of wastewater is a process dealing with different issues, such as the engineering
57 of innovative purification techniques, the environmental impact assessment and the effects on
58 society and the economy (EEA, 2016). In the last thirty years in Europe, many institutions and
59 authorities have devoted particular attention to this topic and this aspect has been sustained by
60 European Union through the adoption of directives, e.g. Directive 91/271/EEC E.U. (European
61 Council Directive, 1991a), which have been transposed to national laws by the different member
62 states, such as Italian Decree Law 152/1999 and Italian Decree Law 152/2006. As a result, the
63 quality of treated water received by groundwater bodies has improved substantially. However,
64 this implied a considerable drawback: the dramatic increase of sewage sludge (SS), that is the
65 principal waste coming from wastewater treatment process. The sewage sludge production of
66 European countries raised from 5.5 (European Commission Web Sources) to nearly 10
67 Mtonnes of dry solid matter of sludge per year (Milieu Ltd, 2008), divided in 8.7 Mtonnes from
68 EU-15 countries and 1.2 Mtonnes from EU-12 (Bianchini et al., 2016). According to the reports
69 of European Commission (European Commission, 2017), these values correspond to a mean
70 production of 17 kg per capita of dry sludge per year, but in some countries, including Italy, this
71 ratio is below to 10 kg per inhabitant, suggesting an insufficient level of collection and treatment.
72 SS is currently classified as a putrescible waste, which requires a proper stabilisation before its
73 disposal or reuse. For this purpose, anaerobic digestion (AD) is one of the most exploited
74 techniques in last generation wastewater treatment plants (WWTP), yielding biogas and
75 anaerobic digestate from sewage sludge (SSAD). This one shows the typical pros and cons of
76 sewage sludge: interesting agronomic features due to appreciable macronutrient content versus

Abbreviations: A_N: assimilation; C: centrifuged digestate; C-85: centrifuged digestate at 85 kg N/ha; C-170: centrifuged digestate at 170 kg N/ha; C: centrifuged digestate; C-255: centrifuged digestate at 255 kg N/ha; CCI: Chlorophyll Content Index; C_i: CO₂ concentration in substomatal cavity; D: dried digestate; D-85: dried digestate at 85 kg N/ha; D-170: dried digestate at 170 kg N/ha; D-255: dried digestate at 255 kg N/ha; EC50: half maximal effective concentration; GI: Germination Index; g_s: stomatal conductance; M: mineral fertilizer; M-85: mineral fertilizer at 85 kg N/ha; M-170: mineral fertilizer at 170 kg N/ha; M-255: mineral fertilizer at 255 kg N/ha; P: primary digestate; P-85: primary digestate at 85 kg N/ha; P-170: primary digestate at 170 kg N/ha; P-255: primary digestate at 255 kg N/ha; RDI: Root Development Index; S: secondary digestate; S-85: secondary digestate at 85 kg N/ha; S-170: secondary digestate at 170 kg N/ha; S-255: secondary digestate at 255 kg N/ha; SS: sewage sludge; SSAD: anaerobic digestate from sewage sludge; T: no treated, control thesis; WWTP: wastewater treatment plant.

77 the presence of organic and inorganic contaminants (Antonkiewicz et al., 2018). Land and
78 agronomic application as soil improver is currently regulated in Europe by an obsolete directive,
79 Council Directive 86/278/EEC (European Council Directive, 1986), and, until 2015, it was mostly
80 diffused in countries like Portugal and Spain, which adopted more stringent legislation for
81 exploitation in agriculture (Alvarenga et al., 2015). However, according to Eurostat data updated
82 on 2015, in other states SSAD is poorly reused and it is mostly disposed of as a solid waste
83 through incineration (e.g. in the Netherlands and Switzerland) or landfilling (Italy, Serbia,
84 Croatia; source: Eurostat, 2017). This solution, in particular, should be strictly limited whenever
85 possible and exploited only as last resort, according to the Landfill Council Directive 1999/31/EC
86 (European Council Directive, 1999).

87 Many works in literature have focused on this waste in order to propose different kinds of
88 solutions both in terms of treatment and application. Contaminants removal has been shown to
89 be possible through heavy metal leaching with physical, chemical and biological techniques
90 (Camargo et al., 2016). However, these procedures are often expensive in terms of cost, time
91 and sustainability. On the other hand, recovery of valuable compounds such as nitrogen (e.g.
92 ammonia stripping, struvite precipitation or advanced processes) (Monfet et al., 2018; Siciliano
93 et al., 2017) and phosphorus (e.g. recovery from ashes, struvite precipitation) (Ohtake and
94 Tsuneda, 2019) seems to be quite promising, especially for what concerns the preservation of
95 natural resources and the support of the circular economy perspective (Nesme and Withers,
96 2016). Other engineering approaches are based on pyrolysis, for production of syngas, bio-oil
97 and biochar (Schulz and Glaser, 2012), or composting (Perez-Murcia et al., 2006; Xu et al.,
98 2012) to yield soil organic conditioners. Besides these studies, many papers on the soil
99 application of the sewage sludge (McGrath et al., 1995) and its derivatives (Andrés et al., 2011;
100 Tarrasón et al., 2008) have also been published: in general, the main focus has been the
101 evaluation of fertilising effects depending on the dose, species and soil studied, with
102 experiments scaling from pots in greenhouses (Wong et al., 1996; Perez-Murcia et al., 2006) to
103 pots in outdoor (Singh and Agrawal, 2009; Alvarenga et al., 2016), to open field applications
104 (Hussein, 2009; Rigueiro-Rodríguez et al., 2010). On the other hand, many works have
105 investigated the phytotoxic effects of SS and SSAD, which are principally related to the excess

106 of heavy metals (Singh and Agrawal, 2007; Belhaj et al., 2016), organic pollutants (Erhardt and
107 Prüeß, 2001) and ammonia nitrogen (Gulyás et al., 2012).

108 The targets of the present work are mainly three: 1) understanding the applicability of anaerobic
109 digestates from sewage sludge with an agronomic approach, exploiting nitrogen dosages
110 commonly used in field operations; 2) evaluation of their effects on sandy and poor soils; 3)
111 studying their phytotoxicity on cress seeds (plate bioassay with *Lepidium sativum* L.) and on
112 cucumber plants (pot experiment with *Cucumis sativus* L.) to assess the correct range for their
113 application as soil improvers.

114 The main outcome of this study is thus the understanding of the reuse dynamics of this waste of
115 our society, which is increasing more and more over years. The use of SSAD as soil improver is
116 already known in literature (Alvarenga et al., 2016). Nevertheless, to the best of our knowledge,
117 this is the first example of comparison of the fertilizing effects between two liquid SSADs
118 (derived from separated anaerobic digestion of primary and secondary sludges) and two
119 dewatered ones (centrifuged and dried SSADs) deriving from the same WWTP. Moreover,
120 another aspect of novelty is the study of physiological parameters of cucumber plants grown in
121 presence of different type of SSADs. with particular focus on physiological parameters.

122 Finally, the results of this work highlight the benefits that may derive from the application of
123 SSADs on nutrient-deficient soils. To a brother extent, this approach could be not only a way to
124 recycle SSAD, but also represent a potential solution to combat nutrient depletion in soils.

125 .

126 **2. Materials and methods**

127

128 **2.1 Characterization of the digestates**

129

130 Anaerobic digestates used in this study came from a large-scale wastewater treatment plant
131 (3,800,000 population equivalents) located in north-west Italy. Four different types of digestates
132 were used: a primary liquid digestate (P), a secondary liquid digestate (S), a centrifuged solid
133 digestate (C) derived from a mix between P and S and a dried pulverulent digestate (D),
134 obtained by the thermal treatment at 200 °C of C. After the sampling from WWTP, digestates

135 were stored at 4°C until use and characterized. All analyses were performed according to
136 “Analytical Methods for Fertilizers” by the Italian Minister of Agriculture and Forestry (M.P.A.A.F.
137 ed., 2006) and “Methods for Analysis of Sewage Sludge” by Water Research Institute of
138 National Council of Researches (IRSA-CNR, 1985). Moreover, all analyses were performed in
139 a laboratory meeting the requirements imposed by normative on quality management systems
140 applied to laboratories of analysis and testing (UNI EN ISO 9001: 2008 e UNI EN ISO/IEC
141 17025: 2005).
142

143 **2.2. Germination test on cress**

144

145 The protocol of DIVAPRA et al. (1998) was used to evaluate effects on germination of liquid
146 separates of P and S digestates which were obtained by centrifugation (15 minutes, 4000 rpm).
147 The same tests were not conducted on C and D since it was not possible to follow the same
148 experimental procedure, neither to obtain a proper aqueous extract due to excessive water
149 soaking by the solid digestates.

150 The supernatant was used at 10 different concentrations (2.5, 5, 7.5, 10, 15, 20, 25, 50, 75, 100
151 %) obtained by dilution of pure supernatant (100%) in distilled water. A control with pure distilled
152 water was prepared to compare results. Per each concentration, four replicates were set as
153 follow: one Whatman n°1 filter paper was placed in a sterile plastic Petri dish (Ø 90 mm) where
154 afterwards 5 ml of the abovementioned solutions were poured. At the same time, common cress
155 (*Lepidium sativum* L.) seeds (Green Paradise Srl, Italy) were sterilized in sodium hypochlorite
156 for 30 seconds and then rinsed abundantly with deionized water. Afterwards, seeds were
157 incubated for 1 hour in deionized water to reach an adequate imbibition; before use, they were
158 examined and selected, discarding discoloured, damaged or abnormally small ones (Pavel et
159 al., 2013). Finally, each Petri dish was firstly sown with ten seeds and secondly sealed with
160 parafilm. Plates were incubated for 72 hours at 25°C in absence of light. After 72 hours roots
161 length (as root + hypocotyl + epicotyl) was measured (Lencioni et al., 2016) and germination
162 index (GI) was calculated (Zucconi et al., 1981):

163
$$GI = (Lt * Gt / Lc * Gc) * 100$$

164 (where *Lt* is the treated seed average root length, *Gt* is the average number of treated
165 germinated seeds, *Lc* is the average root length of control seeds and *Gc* is the average number
166 of germinated seeds in the control). Seeds were considered germinated when emerging roots
167 were longer than seed size (Bae et al., 2014). Finally, also EC50, defined as the concentration
168 value determining a germination reduction of 50% over untreated control, was calculated.

169

170

171 **2.3. Pot experiment on cucumber**

172 **2.3.1 Substrates**

173 **Table 1.**

174 Physical and chemical analysis of the sandy soil and peat substrate used in this work.

175 C.E.C.: Cation-Exchange Capacity; AAS: Atomic Absorption Spectroscopy.

Parameter	Unit of measure	Sandy soil		Peat	
		Value	Method [*]	Value	Method
Stones	-	absent	Method II.1		
Sand (2.0 - 0.020 mm)	%	94%	Method II.6		
Silt (0.020 - 0.002 mm)	%	3%	Method II.6		
Clay (< 0.002 mm)	%	3%	Method II.6		
Texture	-	sandy	Method II.6		
pH	-	8.7	Method III.1	6.2	Potentiometry
Electrical conductivity	mS/cm	0.08	Method IV.1	0.722	Conductometry
Total limestone	%	36.9	Method V.1		
Active limestone	%	1	Method V.2		
Organic matter	%	0.2	Method VII.3		
N - Tot (Kjeldahl)	%	0.021	Method XIV.3	0.42	Kjeldahl
N - NO₂⁻	mg/kg	<1.0	Method XIV.9	<0.05	Colorimetry
N - NO₃⁻	mg/kg	1.6	Method XIV.9	30.4	Colorimetry
N - NH₄⁺	mg/kg	22.3	Method XIV.7	1.3	Colorimetry
N - Org	mg/kg	186	Calculation	4000	Calculation
P	mg/kg	2	Method XV.3	8.1	Colorimetry
Fe	mg/kg	5.6	Method XII.1	0.79	AAS
Mn	mg/kg	4.2	Method XII.1	0.15	AAS
Ca	mg/kg	950	Method XIII.4	36	AAS
Mg	mg/kg	54	Method XIII.4	28	AAS
Na	mg/kg	20	Method XIII.4	16	AAS
K	mg/kg	53	Method XIII.4	41.1	AAS
C.E.C.	meq/100 g	5.48	Method XIII.1		
As	mg/kg	1.9	USEPA 2007b		
Cd	mg/kg	0.1	USEPA 2007b		
Cr	mg/kg	64.5	USEPA 2006		
Hg	mg/kg	<0.1	Direct mercury analyser		
Ni	mg/kg	57.5	USEPA 2007b		
Pb	mg/kg	8.5	USEPA 2007b		
Cu	mg/kg	17	USEPA 2007b	<0.03	AAS
Zn	mg/kg	45.5	USEPA 2007b	0.02	AAS

* Methods for sandy soil analysis are referred to "Italian Ministerial Decree, 1999", unless differently specified.

176

177 Two different growth substrates were used: a sandy soil and a peat substrate.

178 The soil used in this study was sampled in Grugliasco (TO), Italy (45°03'58.4"N, 7°35'32.9"E). It
179 was collected within 20 and 100 cm depth, sieved at 2 mm and not previously sterilized.
180 Physical and chemical soil properties (**Table 1**) were measured before the application of
181 treatments. This soil can be classified as sandy (IUSS, 94% sand, 3% silt, 3% clay), alkaline
182 (pH 8.7), really poor in organic matter (0.2%), high in carbonates (36.9%) but low in terms of
183 active carbonates (1.0%) and with normal salinity (E.C. 0.080 mS/cm).
184 Physical and chemical analysis were performed according to the methods accepted by Italian
185 law, which are published on Gazzetta Ufficiale n.248 of 21/10/1999 (Italian Ministerial Decree,
186 1999). Sonneveld method (Sonneveld et al., 2009) was used to obtain an aqueous extract, on
187 which chemical characterization was conducted. Extraction of soil heavy metals was performed
188 with microwave extraction method, according to U.S. Environmental Protection Agency
189 (USEPA, 2007a); determination of heavy metals was executed according to USEPA (see **Table**
190 **1** for each method). Detection of Hg was conducted with a direct mercury analyser.
191 Peat substrate was mixed with perlite and then sterilised before each application. Chemical
192 characterization (**Table 1**) of peat substrate was performed on an aqueous extract 1:2 (v/v
193 water/peat substrate) according to Sonneveld method (Sonneveld and van den Ende, 1971). All
194 the analytical methods exploited are specified in **Table 1**.

195

196 **2.3.2 Phytotoxicity**

197

198 The experiment took place in a climate chamber with controllable photoperiod and temperature,
199 which were set at 28°C for 14 hours during the day (07:00 - 21:00) and to 20°C for 10 hours
200 during the night (21:00 - 07:00). During the first week after sowing, shoots were irrigated from
201 the top one time a day; after this time water level in flowerpot saucer was kept constantly
202 between 1 and 3 cm for the purpose of guarantee always water availability.

203 Commercial plastic pots were used with a total volume of 1250 cm³ and a surface area of 144
204 cm²; consequently, each pot was filled with approximately 250 g of peat substrate and 2000 g of
205 sandy soil. Ten nontreated seeds of cucumber (*Cucumis sativus* L.), cv. Marketmore (Four
206 company, Italy) were sown in each pot. The experimental trials lasted thirty days. The position

207 of all plants in the cell was changed every week to minimize location effects. The cultivations on
208 peat substrate and on sandy soil were performed by using the substrate mixed with different
209 treatments: four types of anaerobic digestates from sewage sludge (P, S, C, D), one commercial
210 fertilizer (M) (NPK 22-5-6 + 2MgO, "Osmocote Topdress", ICL, Israel) and one not treated
211 control (T). All of them were tested at three increasing doses (85, 170, 255 kg N/ha and they will
212 be called as mentioned above), with four replicates per each. The intermediate nitrogen dosage
213 (170 kg N/ha) was selected according to the Nitrates Directive (European Council Directive,
214 1991b), and the lowest (85 kg N/ha) and highest (255 kg N/ha) ones were chosen to keep the
215 same difference between the application rates.

216

217 **2.3.3. Agronomic and physiologic parameters**

218

219 Germination was evaluated counting germinated seeds after three to ten days; then,
220 germination was calculated as: (germinated seeds/sown seeds) x 100

221 Assimilation (A_N), stomatal conductance (g_s) and CO_2 concentration in substomatal cavity (C_i)
222 were recorded two days before the end of the experiment using an Infrared Gas Analyzer
223 (IRGA, ADC, Hoddesdon, UK). The measurement was performed on three leaves of each
224 sample treated with the 170 kg/ha dosage. The selected leaves were the second or the third
225 from the top and they were the best developed and directly exposed to artificial light.

226 The day before the end of the test, Chlorophyll Content Index (CCI) was evaluated with SPAD
227 502 chlorophyll meter (CCM-200, Opti Sciences, Inc., Hudson, NH, USA). After the ordinary
228 calibration, it was used on 5 different fullyformed leaves per pot. CCI was used as an indicator
229 of the healthy state and the photosynthetic potentiality of plants. With the purpose of evaluating
230 CCI, SPAD (Minolta) and CCM (Opti-Science) meters can be exploited and the second one was
231 utilised in our investigation; to compare values obtained with results of studies that used SPAD-
232 meter, the equations proposed by Parry and colleagues (2014) were considered.

233 At the end of the experiment, all plants were cut and immediately weighed to measure the fresh
234 biomass of single pots (replicates). Determination of dry biomass was carried out weighing
235 these samples after thermal treatment (105°C for 72 hours). In order to compare the yields of

236 each treatment, dry biomass ratio was calculated as ratio between mean dry biomass of each
237 treatment and control. Besides the related-to-control biomass values, even absolute dry
238 biomasses were analysed and compared. Per each concentration, each treatment was
239 compared to the other ones, including the control.

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

245

246 **2.4 Statistical analysis**

247

248 For the germination tests on Petri dishes, the statistically significant differences between treated
249 and untreated samples were identified with Student's *t* test, specifying the different levels of
250 significance ($p \leq 0.05 = *$, $p \leq 0.01 = **$; $p \leq 0.001 = ***$). All data about pot phytotoxicity experiment
251 with cucumber were analysed by one-way ANOVA with a Tukey's post-hoc test ($P \leq 0.05$), after
252 the assessment of the fundamental assumptions of ANOVA: the normality of distributions
253 (Shapiro-Wilk test, $p\text{-value} > 0.05$) and the homogeneity of the variances of the residuals
254 (Levene's test with $P(>F) > 0.05$). The statistical software R (version 3.5.1 - Feather Spray -
255 2018) was used for all statistical analysis.

256

257 **3. Results**

258

259 **3.1. Characterization of the digestates**

260

261 Results of characterization of the digestates are shown in **Table 2**. Dry matter content in liquid
262 digestates was 4.4% and 4.8% (for P and S, respectively), while it reached 25.8% and 88.8%
263 (for C and D, respectively) after dewatering processes. pH decreased throughout the different
264 digestates from 7.7 to 6.8; total nitrogen levels ranged from 7.5% (S) to 5% (D), while NH_4^+ was

265 up to six times higher in liquid than in solid SSADs. No consistent variation in organic matter
 266 levels was observed through the four digestates; as a consequence, C/N ratio increased from
 267 liquid to dewatered SSADs. Plant macronutrients such P and K had opposite behaviours: the
 268 first one showed appreciable concentrations, with a growing trend from liquid to solid digestates;
 269 the latter revealed highly low levels (<1%), with a slight decrease in C and D SSADs. Meso- and
 270 micronutrients (Ca, Mg, B, Zn) and some metals (Na, Cd, Ni, As) exhibited decreasing
 271 concentrations from liquid to solid digestates; the only metals which showed a diametrically
 272 opposed behaviour were Fe and Cu. No consistent difference in Pb, Cr and Hg concentrations
 273 was reported across the four digestates.

274

275 **Table 2.**

276 Physicochemical properties of the four anaerobic digestates from sewage sludge used in this
 277 work; last three columns on right specify analysis methods for sewage sludge, Italian law limits
 278 for Land application of sewage sludges (Italian Decree Law 99/1992), and Italian law limits for
 279 heavy metals in fertilizers (Italian Decree Law 75/2010).

280 d.m.b.: dry matter basis; E.C.: Electrical conductivity; HGAAS: Hydride Generation Atomic

281 Absorption Spectrometry; TOC: Total Organic Carbon.

Parameter	Unit of Measure	Anaerobic digestates				Method of analysis	Italian Law Land application of sewage sludge (D. Lgs. 99/92)	Italian Law Discipline on fertilizers (D.Lgs. 75/2010)
		Primary (P)	Secondary (S)	Centrifuged (C)	Dried (D)			
pH (1:10)		7.7	7.5	7.3	6.8	M.P.A.A.F ed.2006 Method III.3		
E.C.	mS/cm	0.378	0.36	1069	1.575	M.P.A.A.F ed.2006 Method III.4		
N - Tot (Kjeldahl)	% d.m.b.	7.4	7.5	6.3	5	IRSA-CNR, 1985 Issue 64, vol 3, Method 6	>1.5	
N - Org	% d.m.b.	5.84	6.16	5.33	4.75	M.P.A.A.F ed.2006 Method IV.12		
N - NO ₃ ⁻	% d.m.b.	<0.01	<0.01	<0.01	<0.01	M.P.A.A.F ed.2006 Method IV.12		
N - NH ₄ ⁺	% d.m.b.	1.56	1.34	0.97	0.25	M.P.A.A.F ed.2006 Method IV.12		
N - org / N - Tot	%	79	82	84	94	Calculation		
Dry matter	%	4.4	4.8	25.8	88.8	Calculation		
Humidity	%	95.6	95.2	74.2	11.2	M.P.A.A.F ed.2006 Method III.1		
Organic matter	% d.m.b.	64.7	68.5	63.9	64.4	Italian Ministerial Decree, 1999 Method VII.3		
TOC	% d.m.b.	37.5	39.7	37.1	37.3	Calculation	>20	
C/N		5.1	5.3	5.9	7.4	Calculation		
Ashes	% d.m.b.	35.3	31.5	36.1	35.6	Calculation		
Ca	% d.m.b.	6.46	4.69	5.02	4.64	M.P.A.A.F ed.2006 Method VIII		
Mg	% d.m.b.	1.78	1.53	1.45	1.16	M.P.A.A.F ed.2006 Method VIII		
Na	% d.m.b.	1.05	1.03	0.34	0.19	M.P.A.A.F ed.2006 Method VIII		
K	% d.m.b.	0.55	0.69	0.39	0.18	M.P.A.A.F ed.2006 Method VIII		
P	% d.m.b.	4.16	5.75	6.74	6.26	M.P.A.A.F ed.2006 Method VIII	>0.4	
Fe	% d.m.b.	2.43	3.32	3.99	3.48	M.P.A.A.F ed.2006 Method IX		
Mn	mg/kg d.m.b.	255	190	268	228	M.P.A.A.F ed.2006 Method IX		
Cu	mg/kg d.m.b.	357	340	406	396	M.P.A.A.F ed.2006 Method IX	1000	230

Zn	mg/kg d.m.b.	918	650	849	719	M.P.A.A.F ed.2006 Method IX	2500	500
B	mg/kg d.m.b.	51	60	52	41	M.P.A.A.F ed.2006 Method IX		
Pb	mg/kg d.m.b.	92	70	92	79	M.P.A.A.F ed.2006 Method IX	750	140
Cr	mg/kg d.m.b.	245	210	245	217	M.P.A.A.F ed.2006 Method IX	<200*	
Cd	mg/kg d.m.b.	1	0.6	0.8	<0.1	M.P.A.A.F ed.2006 Method IX	20	1.5
Ni	mg/kg d.m.b.	163	120	155	137	M.P.A.A.F ed.2006 Method IX	300	100
As	mg/kg d.m.b.	2.8	2.1	0.9	<0.1	M.P.A.A.F ed.2006 Method IX	<20*	
Hg	mg/kg d.m.b.	<0.1	<0.1	<0.1	<0.1	HGAAS	10	1.5
Cr⁶⁺	mg/kg d.m.b.	<0.1	<0.1	<0.1	<0.1	M.P.A.A.F ed.2006 Method IX	<2*	0.5

* Values introduced with Italian Law 130/18

282

283

284 3.2. Germination test on cress

285

286 **Table 3.** Germination Index (GI) on *Lepidium sativum* L. using primary and secondary
 287 digestates. Observed significance levels (p values) from Student's t test ($p < 0.05 = *$, $p < 0.01 = **$;
 288 $p < 0.001 = ***$) comparing treated with untreated seeds. Error is expressed as standard deviation
 289 (SD).

Concentration (%)	Liquid SSAD			
	Primary (P)		Secondary (S)	
	GI	SD	GI	SD
0.0	1.000	0.000	1.000	0.000
2.5	1.144 **	0.061	1.067	0.379
5.0	1.017	0.195	1.049	0.279
7.5	0.781 **	0.096	1.036	0.409
10.0	0.815	0.188	0.640 **	0.164
15.0	0.620 ***	0.076	0.459 ***	0.051
20.0	0.245 ***	0.077	0.183 ***	0.134
25.0	0.093 ***	0.023	0.081 ***	0.106
50.0	0.000 ***	0.000	0.000 ***	0.000
75.0	0.000 ***	0.000	0.000 ***	0.000
100.0	0.000 ***	0.000	0.000 ***	0.000

290 Results of germination test on Petri dishes are shown in **Table 3**. In both P and S, GI in 50%,
 291 75% and 100% concentrations were 0 due to absence germination. On P the highest GI was
 292 obtained at 2.5% concentration, with a gradual decrease at higher concentrations. The 10%
 293 concentration case deserved particular attention because it showed a significantly higher value
 294 than previous and following points. Moreover, the calculated EC50 was at 17.5%.

295 On S, GI at 2.5%, 5% and 7.5% was slightly higher than control, but not enough to affirm that GI
296 was significantly increased from control dosage. Then, the index decreased to 0% more rapidly
297 than P for concentrations higher than 7.5%. However, this GI value was reached at the same
298 concentration of P treatment (50%). The calculated EC50 was at 12.5%.

299

300 3.3. Germination and phytotoxicity on cucumber

301

302 3.3.1. Germination

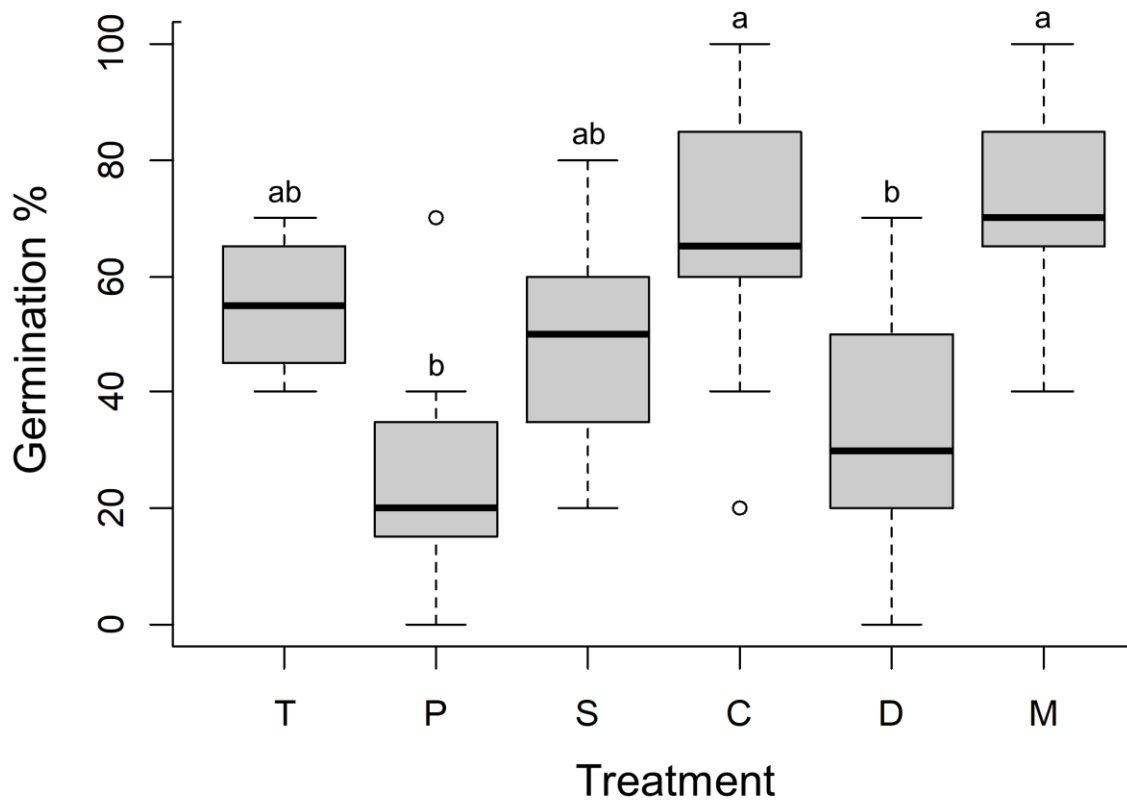
303

304 Figure 1.

305 Mean germination (%) after three days of *C. sativus* grown on sandy soil under each treatment.

306 Different letters indicate differences between treatments that were significant at $P < 0.05$ (Tukey

307 HSD).



308

309 Germination on sandy soil revealed significant differences only at the third day after sowing.

310 More in detail, these differences were found only between the typology of treatment, highlighting

311 a greater germination on C and M than on P and D (**Figure 1**). This treatment was the only one
312 displaying significant differences even between treatment concentrations, with D255
313 presenting the smallest germination value (16%). Overall germination (all germinated seeds to
314 all sown seeds ratio) after 3 days was 43% on sandy soil, while at the end of experiment it
315 reached 80% (data not shown). On the other hand, no significant difference in germination on
316 peat substrate emerged during the 10 days after sowing. Even in this case overall germination
317 increased along the experiment, shifting from 83% at 3 days to 90% at 30 days after sowing.

318

319 **3.3.2. Biomass**

320

321 On sandy soil, all treatments, except for P255, overcame the yields of the control: C255 and
322 D255 were considerably higher than others doubling the control biomass. The increase of
323 biomass production was proportional with the dosages of C and D digestates as well as M; the
324 highest dosage of the last one did not seem to cause further increase. On the other hand, P and
325 S digestates had the highest yields at intermediate dosages (P170 and S170), while dry
326 biomasses at lowest dosages (P85 and S85) were comparable to the highest ones (P255 and
327 S255) and were not significantly different from control (**Figure 2**).

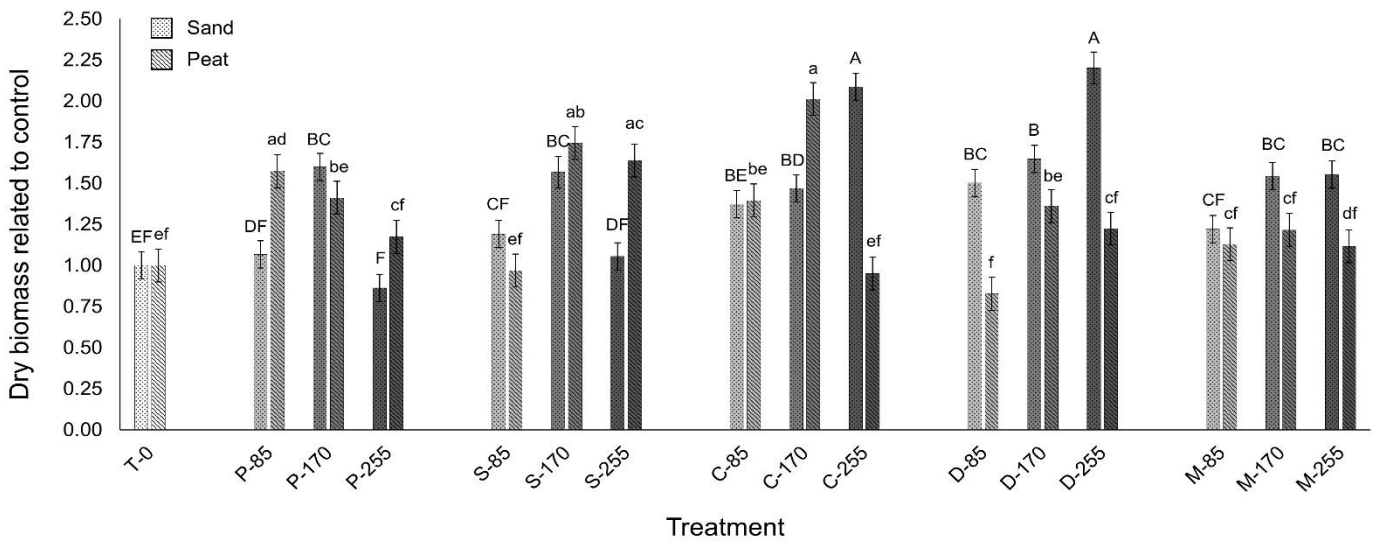
328 For what concerns the biomass yield on peat substrate, the common biomass trend is an
329 increase going from 85 to 170 kg N/ha dosages, and a decrease moving from 170 to 250 kg
330 N/ha. However, P digestate is the only one displaying decreasing biomass values for higher
331 application rate of treatment. The highest biomass yield was found in C170, even doubling the
332 control one. Moreover, P85, S170 and S255 were the only ones showing a significantly higher
333 biomass than control. (**Figure 2**). Very important differences were found in 170 kg N/ha
334 treatments: all yielded significantly more biomass (1.10 to 1.21 g) than the control (0.75 g) on
335 sandy soil (**Figure 3.a**); on peat substrate, P, S and C treatments provided more biomass (2.92
336 g, 3.61 g and 3.95 g, respectively) than control (2.07 g), with S and C showing the top
337 production, while D (2.82 g) and M (2.51 g) behaved similarly to the control (**Figure 3.b**).

338

339 **Figure 2.** Mean dry biomass related to control of *C. sativus* grown on sandy soil and peat
 340 substrate.

341 Each data point represents mean of replicates to mean of control replicates ratio \pm standard
 342 error; different letters indicate differences between treatments and concentrations of N that are
 343 significant at $P < 0.05$ (Tukey HSD); upper-case letters refer to samples from sandy soil and
 344 lower-case letters refer to samples from peat substrate.

345

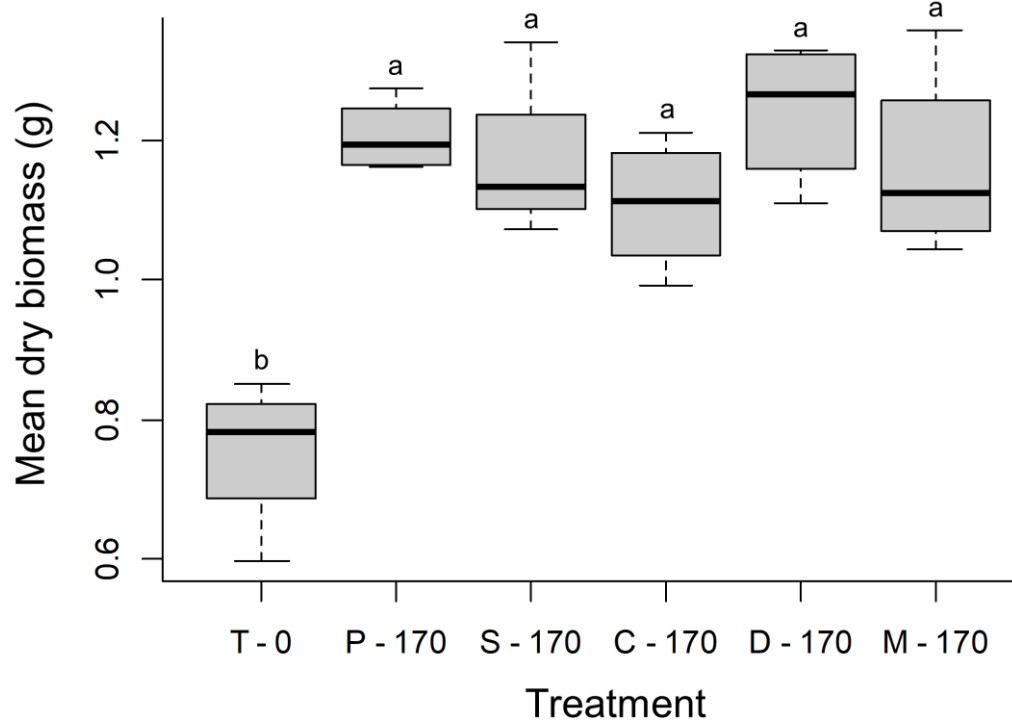


346

347

348 **Figure 3.a.** Mean dry biomass of *C. sativus* grown on sandy soil with 170 kg N/ha treatments.

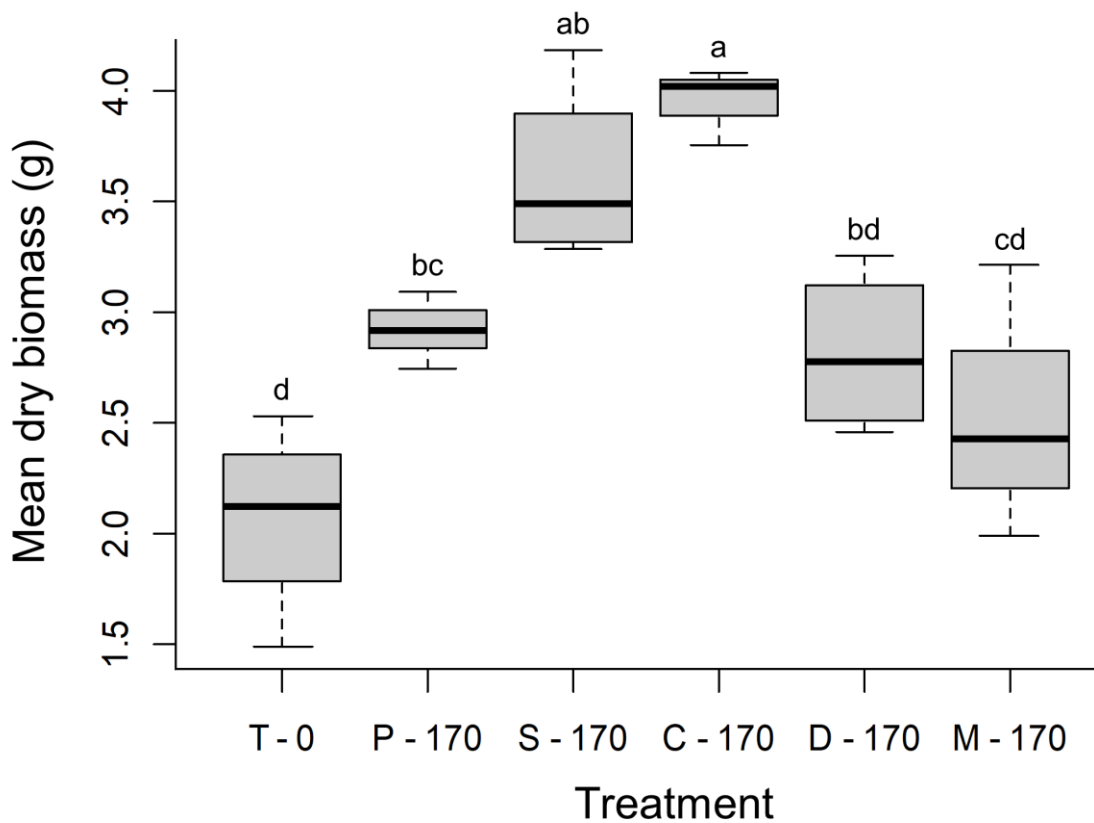
349 Different letters indicate differences between treatments that are significant at $P < 0.05$ (Tukey
 350 HSD).



351

352 **Figure 3.b.** Mean dry biomass of *C. sativus* grown on peat substrate with 170 kg N/ha
 353 treatments.

354 Different letters indicate differences between treatments that are significant at $P < 0.05$ (Tukey
 355 HSD).



356

357

358 3.3.3. Chlorophyll Content Index (CCI)

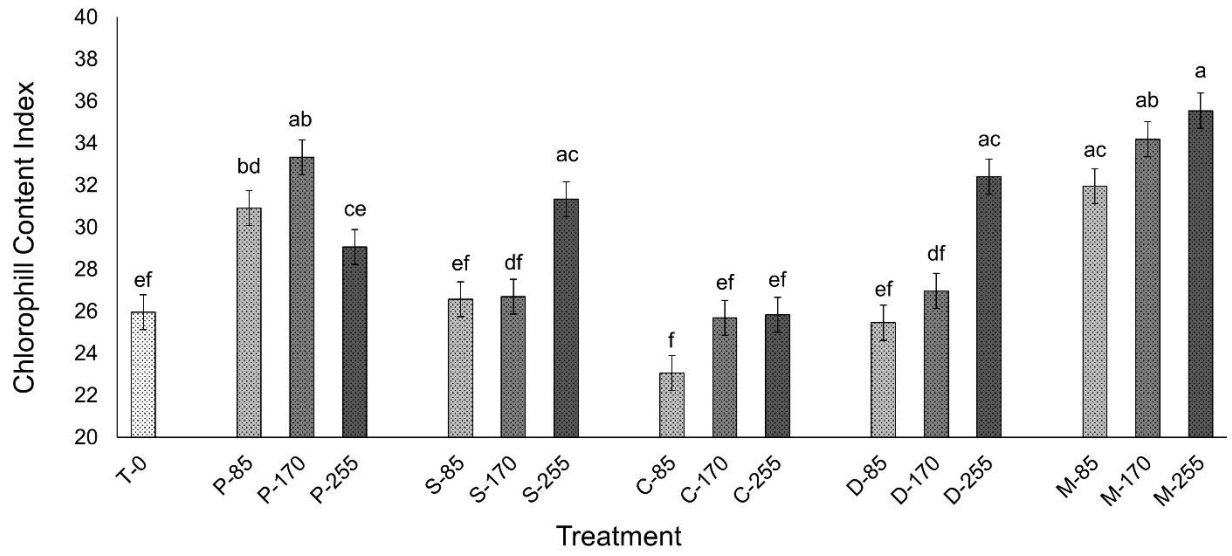
359

360 CCI control mean level of plants grown on sandy soil was 26.0 (**Figure 4.a**); the chlorophyll
 361 concentration significantly higher than control were obtained in M255 (35.5), M170 (34.2), P170
 362 (33.3), D255 (32.4) and M85 (31.95), S255 (31.3) and P85 (30.9). Moreover, the chlorophyll
 363 content was higher with the increase of the SSAD application rate. However, this behaviour was
 364 not detected for P digestate, where the increase of treatment dosage was related firstly to a CCI
 365 increment in P170, then to a CCI reduction in P255 (29.1).

366 On peat substrate (**Figure 4.b**), control mean level of CCI (28.2) was higher than on sandy soil.
 367 Similarly to CCI of cucumber grown on sandy soil, mineral fertilizer in M255 (36.4) and M170
 368 (33.3) gave high results and, together with C170 (37.2), were significantly higher than control.
 369 Moreover, C170 was significantly different from other dosages within same treatment, while no
 370 significative differences among concentrations were found on P, S and D treatments.

371

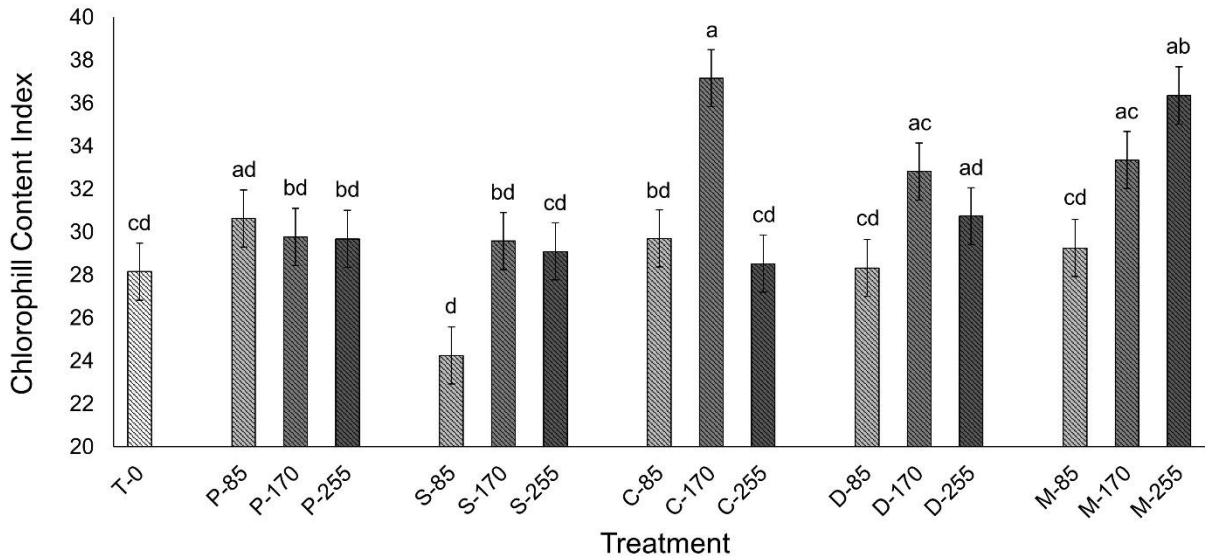
372 **Figure 4.a.** Chlorophyll Content Index (CCI) of leaf of *C. sativus* grown on sandy soil.
 373 Different letters indicate differences between the treatments with the different concentrations of
 374 N at 85, 170 and 250 kg N/ha, that are significant at $P < 0.05$ (Tukey HSD).



375

376

377 **Figure 4 b.** Chlorophyll Content Index (CCI) of leaf of *C. sativus* grown on peat substrate.
 378 Different letters indicate differences between treatments and concentrations of N that are
 379 significant at $P < 0.05$ (Tukey HSD).



380

381

382 3.3.4. Infra-Red Gas analyser (IRGA)

383

384 Treated and control cucumber plants grown on sandy soil showed significant differences in
 385 Net photosynthesis (A_N): control value ($1.83 \text{ CO}_2 \text{ m}^{-2}\text{s}^{-1}$) was lower than all other treatments,
 386 which however did not differ from each other (**Figure 5.a**). Therefore, it is worth underlining the
 387 value measured on P treatment ($3.75 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$), which doubled control value. In order to
 388 stomatal conductance (g_s), all digestate treatments at least doubled the one of control thesis
 389 ($0.098 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), while S even trebled this result ($0.333 \text{ H}_2\text{O m}^{-2}\text{s}^{-1}$) (**Figure 5.b**). On
 390 the other hand, while M showed an intermediate behaviour between digestates and control as
 391 regards stomatal conductance, it reached the highest concentration of CO_2 (536 ppm) in
 392 substomatal cavity (C_i) (**Figure 5.c**).

393 Moving to peat substrate, differences in net assimilation of CO_2 (**Figure 5.a**) between treatments
 394 and control were few: C ($4.83 \text{ CO}_2 \text{ m}^{-2}\text{s}^{-1}$) and D ($4.78 \text{ CO}_2 \text{ m}^{-2}\text{s}^{-1}$) had a higher A_N than all other
 395 treatments (including T). However, it is important to point up that only C ($0.383 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$)
 396 ¹⁾ displayed also a significantly greater value in terms of stomatal conductance (**Figure 5.b**).

397 CO₂ concentration in substomatal cavity revealed two different groups: the first gathering the
398 highest C_i values, S and D (586 ppm), and the second collecting all other treatments (T
399 included), which showed lower results (**Figure 5.c**).

400

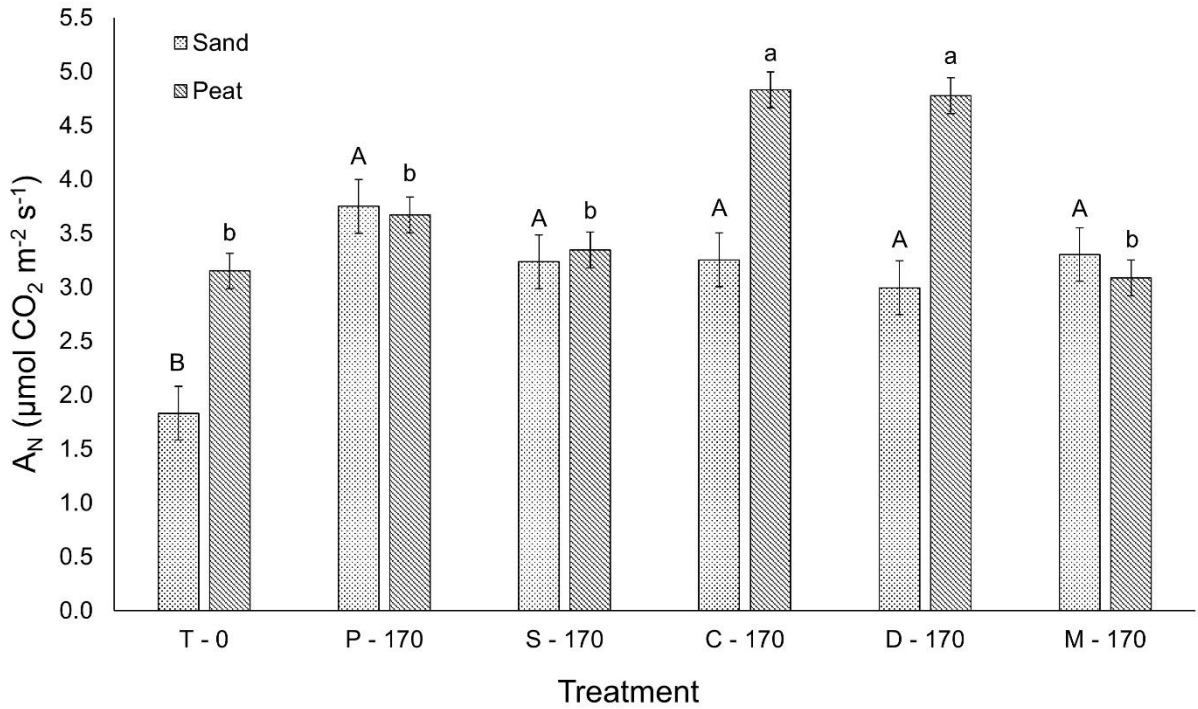
401 **Figure 5.** IRGA measurements on *C. sativus* grown on sandy soil and peat substrate with 170
402 kg N/ha treatments. **5.a.** Net assimilation (A_n in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) \pm mean standard error, **5.b.**
403 Stomatal conductance (g_s in $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) \pm mean standard error and **5.c.** CO₂
404 concentration in substomatal cavity (C_i in ppm) \pm mean standard error.

405 Different letters indicate differences between treatments that are significant at $P < 0.05$ (Tukey
406 HSD); upper-case letters refer to sandy soil and lower-case letters refer to peat substrate.

407

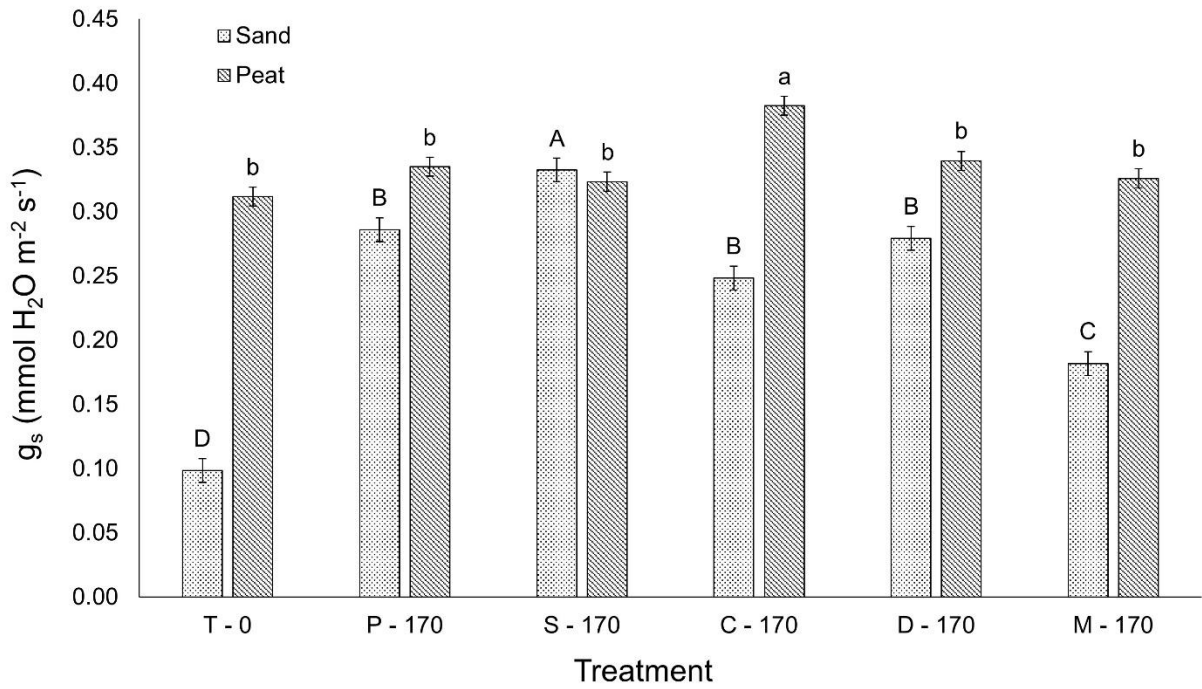
408

409 **Figure 5 a.** Net assimilation on sandy soil and peat substrate.



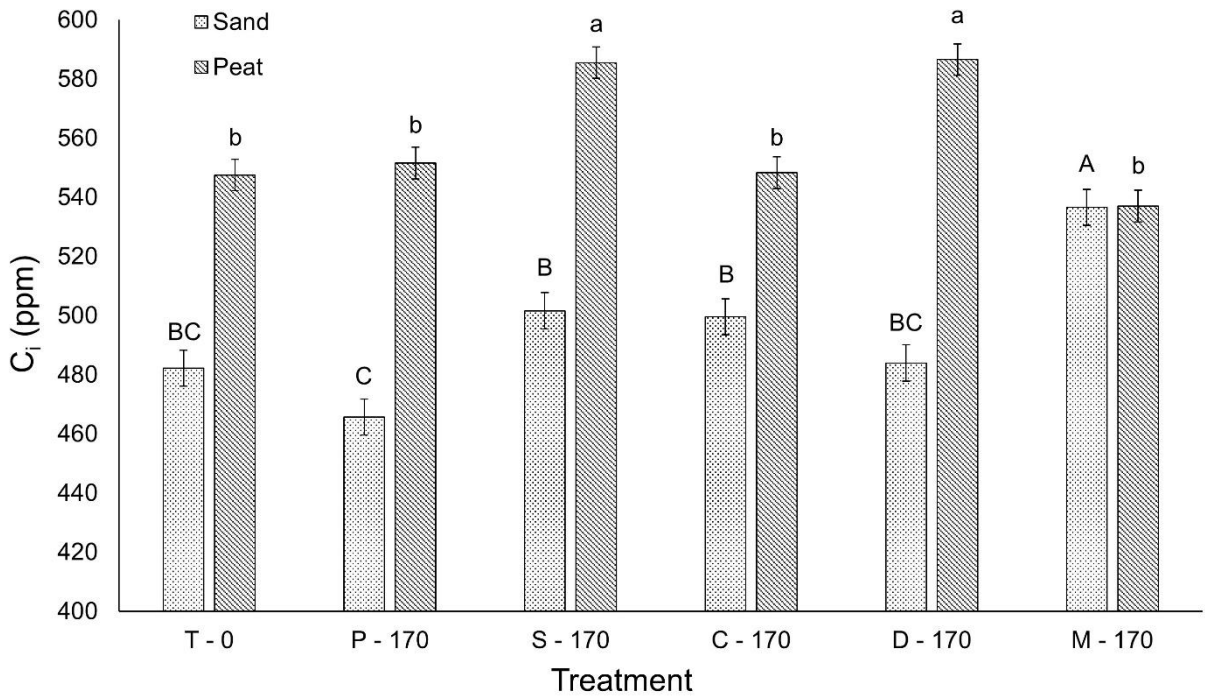
410
411

Figure 5 b. Stomatal conductance on sandy soil and peat substrate.



412
413

414 **Figure 5.c.** CO₂ in substomatal chamber on sandy soil and peat substrate.



415

416 **3.3.5 Root Development Index**

417

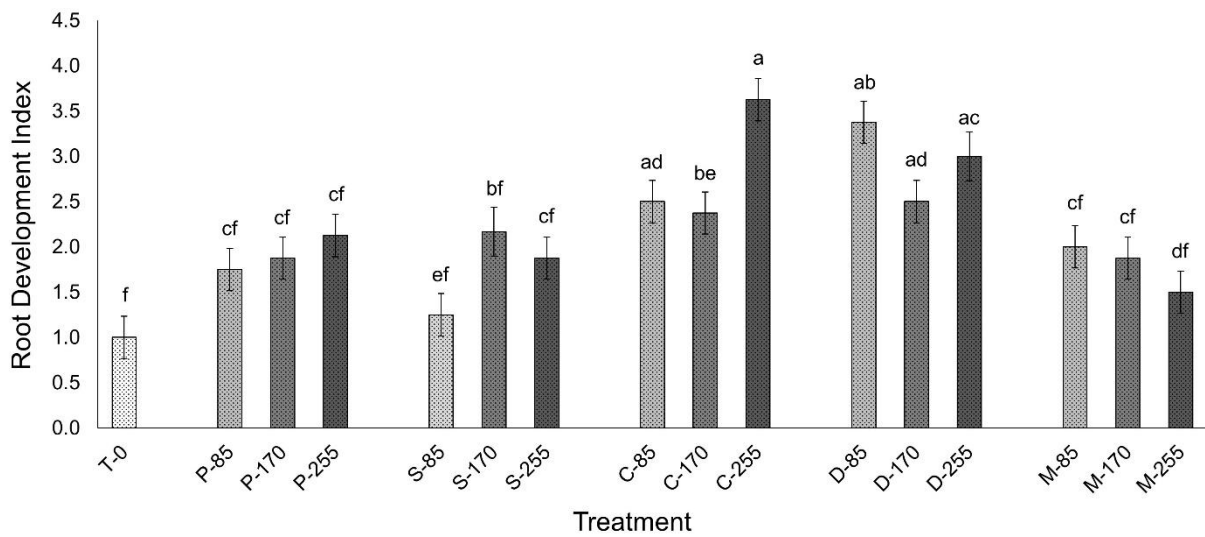
418 **Figure 6.** Mean Root Development Index of *C. sativus* grown on sandy soil.

419 Each data point represents mean of replicates to mean of control replicates ratio ± mean

420 standard error; different letters indicate differences between treatments and concentrations of N

421 that are significant at $P < 0.05$ (Tukey HSD).

422



423

424

425 Root apparatus was mostly developed in plants grown on C and D treatments on sandy soil
426 (**Figure 6**). Indeed, C255 (3.625), D85 (3.375), D255 (3.000), D170 (2.500), C85 (2.500) and
427 C170 (2.375) revealed an RDI significantly higher than control. Data on peat substrate did not
428 respect the homogeneity of variances (P-value = 0.0449) (data not shown).

429

430

431 **4. Discussion**

432

433 All digestates showed interesting contents in macronutrients (N > 5% and P > 4%) as well as in
434 meso- and micronutrients. Indeed, these values were even slightly higher than the mean ones
435 published in other works (N_{Tot} = 3.6%; P_{Tot} = 2.5%). In the case of nitrogen, dewatering probably
436 induced an immobilisation effect, remarked by the increasing levels of N_{Org}/N_{Tot} . On the
437 contrary, despite K levels were a little bit low if compared to other studies (e.g. mean values of
438 works in the references: K = 0.59 %), the applied dosage in this work was sufficient for the early
439 growth stages (Adjei and Rechcigl, 2002; Alvarenga et al., 2016; Antonkiewicz et al., 2018;
440 Asagi and Ueno, 2008; Belhaj et al., 2016; de Andrés et al., 2010; Singh and Agrawal, 2008;
441 Hussein, 2009; Ferreiro-Domínguez et al., 2011; Tarrasón et al., 2008; Wang et al., 2008).
442 However, this aspect can negatively affect the proper potassium supply when SSAD is applied
443 as fertilizer, especially in the phase of fruit maturation (Hawkesford et al., 2012). The main
444 disadvantage of these digestates was the presence of heavy metals. Despite all the analysed
445 ones complied with the limits imposed by the Italian Law on Sewage Sludge Land Application
446 (Italian Decree Law 99/1992), in some cases (i.e. Zn, Cu and Ni) the thresholds imposed by
447 Italian Discipline on Fertilizers (Italian Decree Law 75/2010) were overcome. Moreover, heavy
448 metals concentrations were generally lower than those published elsewhere (Cu: 413 mg/kg,
449 Zn: 922 mg/kg, Pb: 116 mg/kg, Cd: 3.9 mg/kg, As: 3.5 mg/kg), except for Cr and Ni (93 mg/kg
450 and 72 mg/kg, respectively) (Adjei and Rechcigl, 2002; Alvarenga et al., 2016; Antonkiewicz et
451 al., 2018; Asagi and Ueno, 2008; Belhaj et al., 2016; de Andrés et al., 2010; Singh and Agrawal,

452 2008; Hussein, 2009; Ferreiro-Domínguez et al., 2011; Tarrasón et al., 2008; Wang et al.,
453 2008).

454 The sandy soil was alkaline and carbonate-rich, with very low concentration of organic matter
455 and nutrients. Several reports have shown that SS application in soils with these peculiarities
456 can provide a good nutrient supply with a relatively small risk of pollution (Navas et al., 1998;
457 García-Gil et al., 2004; Antolín et al., 2005).

458 Germination of cress increased with dilution and this trend is confirmed elsewhere (Abdullahi et
459 al., 2008). GI of P, at 2.5% concentration, showed a significative improvement compared to 0%.
460 In order to determine a more precise GI trend in this range, the approach proposed by Lencioni
461 and colleagues (2016) should be applied, exploring the interval 0% - 10%, with steps of 1%.
462 Differently, results of GI of S did not show any significative variation from not treated samples
463 until the 7.5% concentration. At higher concentration rates, GI presents a sudden decrease. For
464 sure, concentration of 15% for P and 10% for S were the highest ones with a germination index
465 of at least of 60%, which is considered the GI threshold to support the absence of phytotoxic
466 effects (Zucconi et al., 1985). Compared to other sewage sludges, the two digestates used in
467 this study revealed higher GI values. For instance, in the work of Mañas and De las Heras
468 (2018) a not-digested sewage sludge was utilized: at 10% concentration revealed a lower GI
469 (1.4%) than the one obtained in the present work (81% on primary digestate and 64% on
470 secondary digestate). Albuquerque and colleagues (2012) used twelve different kinds of
471 anaerobic digestates from animal origin (obtained from co-digestion of different organic matrix)
472 and only two of them showed a GI higher than 50% at concentration of 10%. Interesting results
473 derived from the comparison of the GI of an anaerobic digestate from microalgae, and
474 digestates from a co-digestion of microalgae with primary sewage sludges: while the former
475 showed a GI comparable to the one obtained with P of the present work (at concentration of
476 10%), the latter displayed a GI of nearly 100% for the same concentration (Solé-Bundò et al.,
477 2017). Thus, the minor toxicity of primary co-digested sludge could be justified by the synergic
478 effect of co-digestion, which has been demonstrated to be more advantageous than mono-
479 digestion ones due to a dilution effect of inhibitory compounds, among other factors (Tritt, 1992).

480 Hence, SS co-digestion could be a nice suggestion to elevate GI at higher digestate
481 concentrations.

482 The germination of cucumber seedlings grown on peat substrate was higher compared to sandy
483 soil, which may be due to pH values of the growing substrate. Optimal pH conditions for
484 cucumber germination are between 5.5 and 6.5 (Nersisyan et al., 2017), that are values roughly
485 similar to peat substrate, but far away from sandy soil ones (pH 8.7). Moreover, D255 induced a
486 significantly low germination within all treatments applied on sandy soil. This effect could be
487 explained by the high E.C. of D, which is 300% and 50 % higher than liquid and centrifuged
488 digestates, respectively. Indeed, other authors (Sánchez-Monedero et al., 2004 and Eklind et
489 al., 2001) demonstrated a clear correlation between the E.C. increase in soil and germination
490 decrease.

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

509 Moving to a broader perspective, other works designed with a pot experiment approach
510 assessed the fertilizing effect of sewage sludge on different species. Asagi and Ueno (2008)
511 and Shaheen and co-workers (2014) reported examples of komatsuna (*Brassica rapa* L. var.
512 perviridis) grown on sandy soil, and rocket (*Eruca sativa* Mill.), grown on calcareous soil, which
513 quintupled and doubled their dry biomass yield, respectively. Furthermore, relevant outcomes
514 have been described on sunflower (*Heliantus annuus* L.) (Belhaj et al., 2016) and kenaf
515 (*Hibiscus cannabinus* L.; de Andrés Parlorio et al., 2010) grown in presence of dewatered
516 anaerobic digestates similar to C and D treatments, providing well comparable results with this
517 study. Qasim and colleagues (2001) and Alvarenga and co-workers (2016) provided examples
518 of cereal crops (maize and sorghum, respectively) fertilized with an unstabilized sewage sludge
519 and a yield increase of 40% and 400%, respectively, over untreated control was reported. Even
520 if it's difficult to compare the behaviour of different plants exposed to diversely treated sludges, it
521 is conceivable that weaker performances of digestates of this study may be due not only to
522 lower application rates, but also to the nitrogen fractionation. In fact, in the present work, this is
523 skewed in favour of organic nitrogen (N_{Org} / N_{Tot} ranging from 79% to 94%), with lower
524 concentrations of "readily-available" nitrogen (i.e. NH_4^+ and NO_3^-).

525 Nevertheless, the main drawbacks of sewage sludge land application are the phytotoxic effects
526 occurring at higher application rates, preventing the optimal growth of the plant. Indeed, this
527 aspect has been deeply investigated as regards the presence of organic and inorganic
528 pollutants, such as heavy metals. These ones can interfere with the biomass yield as widely
529 reported in literature (Singh and Agrawal, 2007; Nagajyoti et al., 2010). In the present work, the
530 decrease of dry weight with higher application rates was observed only in few cases (e.g. P255
531 and S255 on sandy soil, and P255, C255 and D255 on peat substrate). These reductions can
532 be justified in part with the metal-derived toxicity, especially in the case of peat substrate. Its
533 slightly acidic conditions maybe allowed a more sustained metal bioavailability, which was
534 instead down modulated by high pH in sandy soil (Sukreeyapongse et al., 2002; Belhaj et al.,
535 2016). On the other hand, another conceivable hypothesis is the ammonia-connected toxicity
536 occurring in alkaline conditions: increasing soil pH induces higher NH_3 percentage of total
537 ammoniacal nitrogen (Masoni and Ercoli, 2010), according to the NH_4^+/NH_3 acid-base

538 equilibrium (Gay and Knowlton, 2005). Thus, at the pH of sandy soil exploited in this work (8.7),
539 around 20-25% of ammoniacal nitrogen is represented by NH_3 , which can negatively affect the
540 plant growth under different aspects as described by van der Eerden (1982). This aspect has
541 been observed mainly on plants exposed to liquid digestates, which revealed ammonia-nitrogen
542 concentrations up to six times higher than dewatered ones. On the contrary, dehydration of
543 SSAD might have had a positive effect on the ammonia abatement, which resulted in an overall
544 slighter phytotoxicity exhibited by solid SSADs (C and D, in this study). In this respect, this
545 aspect is confirmed by Alvarenga et al. (2016) and de Andrés Parlorio et al. (2010). Moreover,
546 the last one devoted particular attention to the treatment formulation (pelletization, in this case),
547 which can be an aspect to take into account even for future work.

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

567 The results of gas analysis measurements were not directly comparable to other values in
568 literature because these are strictly depending on environmental conditions (light, temperature,
569 irrigation and phenological phase). On peat substrate, almost no difference was appreciable;
570 just in C case, A_n and g_s values were higher than T; anyway, these differences reflect values
571 obtained in biomasses and CCI measurements. To the best of our knowledge, no
572 measurements of physiologic parameters and gas exchange have been performed on
573 cucumber exposed to sewage sludge treatments with pot experiments. However, some
574 comparisons can be done with studies on physiologic parameters of plants exposed to sewage
575 sludge and studies on physiologic parameters of cucumber. Antolín et al. (2010) and Bouriou
576 et al. (2015) carried out pot experiments with alfalfa (*Medicago sativa* L.) and European larch
577 (*Larix decidua* L.), applying both sewage sludge rates like the ones of this study. The
578 significative differences reported in the case of cucumber grown on sandy soil are in good
579 agreement with A_n and g_s values of the first work, while in the second study only with A_n ones.
580 Furthermore, similar results of A_n and g_s have been assessed using two different dosages of
581 sewage sludge in field on rice crop (*Oryza sativa* L.) (Singh and Agrawal, 2010). On the other
582 hand, studies with sewage sludge on beet (*Beta vulgaris* L.) (Singh and Agrawal, 2007) and
583 okra (*Abelmoschus esculentus* L.) (Singh and Agrawal, 2009) showed lower results in terms of
584 A_n and g_s , probably due to the higher SS doses, provoking phytotoxic effects.

585 Physiologic parameters of cucumber plants were studied mainly as regards metals stress, such
586 as toxicity derived from copper (Alaoui-Sossé et al., 2004) and sodium (Chartzoulakis, 1994):
587 their increasing concentration caused the decrease of the physiologic parameters. Anyway, in
588 the present study, concentrations of copper and sodium were lower and, consequently, A_n and
589 g_s values were higher. Moreover, an increase of stomatal conductance in presence of heavy
590 metals was explained by Singh and Agrawal (2010), claiming that it may be due to high nutrient
591 availability through SS amendment which nullified the heavy metal toxicity.

592 The trend of biomass production did not match always with a sustained root development
593 (RDI). This mismatch between shoot and roots biomass in cucumber has been already reported
594 in literature (Xu et al., 2012). Root development results clearly revealed that C and D gave best
595 outcomes, with an RDI similar between them and higher than liquid digestates and M.

596 Furthermore, these findings demonstrate that the kind of treatment had a greater effect on roots
597 growth than the nitrogen amount (except for the case of C255). This observation is in contrast to
598 the study of Gulyás and co-workers (2012), which described a root reduction in ryegrass (*Lolium*
599 *perenne* L.) treated with same dosages of SSAD, probably due to excessive ammonium
600 content. Despite comparable nitrogen application rate, root development was not inferior than
601 control presumably because of a lower $\text{NH}_4^+/\text{N}_{\text{Tot}}$ ratio of the SSADs used in the present work.
602 Lastly, this work contributed to deepen the knowledge about the agronomic recycling of SSAD.
603 As far as we know, this was the first study conducting a systematic comparison of the fertilizing
604 and phytotoxic effects of anaerobic digestates from primary, secondary, centrifuged and dried
605 sludges. The significative differences between the SSADs likely indicated that the ways in which
606 the digestate is treated at WWTP level had an effect not only on its chemical peculiarities but
607 also on its agronomic potential. Thus, these findings can pave the way for a wiser recycling of
608 this waste, through its usage for the improvement of nutrient-deficient soils.

609

610 **5. Conclusions**

611

612 Four different SSADs (two liquid and two dewatered) coming from WWTP were characterized
613 and exploited as soil improver for promoting cucumber growth in pot experiments. Application of
614 SSADs improved plant growth according to the exploitation of nitrogen dosages commonly used
615 in field operations. In general, an intermediate nitrogen dosage (170 kg N/ha) showed the best
616 results in terms of biomass, chlorophyll content, net photosynthesis, stomatal conductance and
617 root development. All these results were much more evident for cucumber plants grown on an
618 alkaline, sandy and poor (concerning organic matter and nutrients) soil than an acid and rich
619 cultivation substrate, such as peat substrate. However, in some cases phytotoxicity effects
620 occurred probably due to an excessive addition of ammonia nitrogen or heavy metals. Hence,
621 considering these observations, SSADs share many peculiarities with improvers for nutrient-
622 poor soils. Future work should include the study of long-term effects and of repeated
623 applications consequences deriving from SSAD land application; on the other hand, strategies

624 for contaminants abatement or recovery of valuable substances should be investigated, within a
625 circular economy approach.

626

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628

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632

633 **Bibliography**

634 Abdullahi, Y.A., Akunna, J.C., White, N.A., Hallett, P.D., Wheatley, R., 2008. Investigating the
635 effects of anaerobic and aerobic post-treatment on quality and stability of organic fraction of
636 municipal solid waste as soil amendment. *Bioresource Technology* 99, 8631–8636.
637 <https://doi.org/10.1016/j.biortech.2008.04.027>

638 Adjei, M.B., Rechcigl, J.E., 2002. Bahiagrass production and nutritive value as affected by
639 domestic wastewater residuals. *Agronomy Journal* 94, 1400–1410.
640 <https://doi:10.2134/agronj2002.1400>

641 Alaoui-Sossé, B., Genet, P., Vinit-Dunand, F., Toussaint, M.L., Epron, D., Badot, P.M., 2004.
642 Effect of copper on growth in cucumber plants (*Cucumis sativus*) and its relationships with
643 carbohydrate accumulation and changes in ion contents. *Plant Science* 166, 1213–1218.
644 <https://doi.org/10.1016/j.plantsci.2003.12.032>

645 Albuquerque, J.A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M.,
646 Bernal, M.P., 2012. Assessment of the fertiliser potential of digestates from farm and
647 agroindustrial residues. *Biomass and Bioenergy* 40, 181–189.
648 <https://doi.org/10.1016/j.biombioe.2012.02.018>

649 Alvarenga, P., Mourinha, C., Farto, M., Santos, T., Palma, P., Sengo, J., Morais, M.C., Cunha-
650 Queda, C., 2015. Sewage sludge, compost and other representative organic wastes as
651 agricultural soil amendments: Benefits versus limiting factors. *Waste Management* 40, 44–52.
652 <https://doi.org/10.1016/j.wasman.2015.01.027>

653 Alvarenga, P., Farto, M., Mourinha, C., Palma, P., 2016. Beneficial Use of Dewatered and
654 Composted Sewage Sludge as Soil Amendments: Behaviour of Metals in Soils and Their
655 Uptake by Plants. Waste and Biomass Valorization. <https://doi.org/10.1007/s12649-016-9519-z>

656 Andrés, P., Mateos, E., Tarrasón, D., Cabrera, C., Figuerola, B., 2011. Effects of digested,
657 composted, and thermally dried sewage sludge on soil microbiota and mesofauna. Applied Soil
658 Ecology. <https://doi.org/10.1016/j.apsoil.2011.03.001>

659 Antolín, M.C., Pascual, I., García, C., Polo, A., Sánchez-Díaz, M., 2005. Growth, yield and
660 solute content of barley in soils treated with sewage sludge under semiarid Mediterranean
661 conditions. Field Crops Research. <https://doi.org/10.1016/j.fcr.2005.01.009>

662 Antolín, M.C., Muro, I., Sánchez-Díaz, M., 2010. Application of sewage sludge improves growth,
663 photosynthesis and antioxidant activities of nodulated alfalfa plants under drought conditions.
664 Environmental and Experimental Botany. <https://doi.org/10.1016/j.envexpbot.2009.11.001>

665 Antonkiewicz, J., Kolodziej, B., Bielinska, E.J., Glen-Karolczyk, K., 2018. The use of
666 macroelements from municipal sewage sludge by the multiflora rose and the Virginia fanpetals.
667 Journal of Ecological Engineering. <https://doi.org/10.12911/22998993/92889>

668 Asagi, N., Ueno, H., 2008. Determination of application effects of sewage sludge on growth, soil
669 properties, and N uptake in Komatsuna by using the indirect ¹⁵N isotope method.
670 Communications in Soil Science and Plant Analysis.
671 <https://doi.org/10.1080/00103620802134685>

672 Bae, J., Mercier, G., Watson, A.K., Benoit, D.L., 2014. Seed germination test for heavy metal
673 phytotoxicity assessment. Canadian Journal of Plant Science. [https://doi.org/10.4141/cjps-2014-](https://doi.org/10.4141/cjps-2014-018)
674 [018](https://doi.org/10.4141/cjps-2014-018)

675 Belhaj, D., Elloumi, N., Jerbi, B., Zouari, M., Abdallah, F. Ben, Ayadi, H., Kallel, M., 2016.
676 Effects of sewage sludge fertilizer on heavy metal accumulation and consequent responses of
677 sunflower (*Helianthus annuus*). Environmental Science and Pollution Research.
678 <https://doi.org/10.1007/s11356-016-7193-0>

679 Bianchini, A., Bonfiglioli, L., Pellegrini, M., Sacconi, C., 2016. Sewage sludge management in
680 Europe: a critical analysis of data quality. International Journal of Environment and Waste
681 Management. <https://doi.org/10.1504/ijewm.2016.080795>

682 Bouriou, M., Alaoui-Sehmer, L., Laffray, X., Benbrahim, M., Aleya, L., Alaoui-Sossé, B., 2015.
683 Sewage sludge fertilization in larch seedlings: Effects on trace metal accumulation and growth
684 performance. *Ecological Engineering*. <https://doi.org/10.1016/j.ecoleng.2015.01.031>

685 Camargo, F.P., Sérgio Tonello, P., dos Santos, A.C.A., Duarte, I.C.S., 2016. Removal of Toxic
686 Metals from Sewage Sludge Through Chemical, Physical, and Biological Treatments—a
687 Review. *Water, Air, and Soil Pollution*. <https://doi.org/10.1007/s11270-016-3141-3>

688 Chartzoulakis, K.S., 1994. Photosynthesis, water relations and leaf growth of cucumber
689 exposed to salt stress. *Scientia Horticulturae*. [https://doi.org/10.1016/0304-4238\(94\)90088-4](https://doi.org/10.1016/0304-4238(94)90088-4)

690 de Andrés Parlorio, E.F., Tenorio, J.L., Walter, I., 2010. Biomass production and nutrient
691 concentration of kenaf grown on sewage sludge-amended soil. *Spanish Journal of Agricultural*
692 *Research*. <https://doi.org/10.5424/sjar/2010082-1202>

693 DIVAPRA, IPLA, ARPA, 1998. *Metodi di analisi del compost: determinazioni chimiche, fisiche,*
694 *biologiche e microbiologiche; analisi merceologica dei rifiuti.* Collana Ambiente, Regione
695 Piemonte, Assessorato all'Ambiente.

696 EEA, 2016. *European water policies and human health - Combining reported environmental*
697 *information.* European Environment Agency, Report n° 32 of 2016. ISBN 978-92-9213-834-9.
698 European Commission Web Sources: <http://ec.europa.eu/environment/waste/sludge/index.htm>
699 (verified in November 2018).

700 European Commission, 2017. *Ninth Report on the implementation status and the programmes*
701 *for implementation (as required by Article 17) of Council Directive 91/271/EEC concerning urban*
702 *waste-water treatment.*

703 European Council Directive, 1986. Council Directive 86/278/EEC of 12 June 1986 on the
704 Protection of the Environment, and in Particular of the Soil, When Sewage Sludge is Used in
705 Agriculture. *Official Journal of the European Communities*, L 181, 4 July 1986. [https://eur-](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A31986L0278)
706 [lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A31986L0278](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A31986L0278) (Verified in October 2018).

707 European Council Directive, 1991a. Council Directive 91/271/EEC of 21 May 1991 concerning
708 urban waste-water treatment, *Official Journal of the European Communities*. *Official Journal of*
709 *the European Communities*, L 135, 30 May 1991. [https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31991L0271)
710 [content/EN/TXT/?uri=celex%3A31991L0271](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31991L0271) (Verified in October 2018).

711 European Council Directive, 1991b. Council Directive 91/676/EEC of 12 December 1991
712 concerning the protection of waters against pollution caused by nitrates from agricultural
713 sources. Official Journal L 375, 31 December 1991. [https://eur-lex.europa.eu/legal-](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31991L0676)
714 [content/EN/TXT/?uri=CELEX:31991L0676](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31991L0676) (verified in July 2019).

715 European Council Directive, 1999. Council Directive 1999/31/EC of 26 April 1999 on the landfill
716 of waste. Official Journal of the European Communities, L 182, 16 July 1999. [https://eur-](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31999L0031)
717 [lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31999L0031](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31999L0031) (Verified in October 2018).

718 Eurostat, 2017. Water statistics from Eurostat - Statistic Explained. Data extracted in August
719 2017. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Water_statistics
720 (Verified in November 2018).

721 Eklind, Y., Rämert, B., Wivstad, M., 2001. Evaluation of growing media containing farmyard
722 manure compost, household waste compost or chicken manure for the propagation of lettuce
723 (*lactuca sativa* L.) transplants. Biological Agriculture and Horticulture.
724 <https://doi.org/10.1080/01448765.2001.9754919>

725 Erhardt, W., Prueß, A. 2001. Organic contaminants in sewage sludge for agricultural use. EC
726 JRC Institute for Environment and Sustainability Soil and Waste Unit.
727 http://ec.europa.eu/environment/archives/waste/sludge/pdf/organics_in_sludge.pdf (Verified in
728 November 2018).

729 Ferreiro-Domínguez, N., Rigueiro-Rodríguez, A., Mosquera-Losada, M.R., 2011. Response to
730 sewage sludge fertilisation in a *Quercus rubra* L. silvopastoral system: Soil, plant biodiversity
731 and tree and pasture production. Agriculture, Ecosystems and Environment.
732 <https://doi.org/10.1016/j.agee.2011.02.009>

733 García-Gil, J.C., Plaza, C., Senesi, N., Brunetti, G., Polo, A., 2004. Effects of sewage sludge
734 amendment on humic acids and microbiological properties of a semiarid Mediterranean soil.
735 Biology and Fertility of Soils. <https://doi.org/10.1007/s00374-003-0709-z>

736 Gay, S. W., Knowlton, K. F. 2005. Ammonia emissions and animal agriculture. Publication 442-
737 110. Virginia Cooperative Extension/Biological Systems Engineering.
738 <https://vtechworks.lib.vt.edu/bitstream/handle/10919/50720/442-110.pdf?sequence=1>

739 Güler, S., Büyük, G., 2007. Relationships among chlorophyll-meter reading value, leaf n and
740 yield of cucumber and tomatoes, in: Acta Horticulturae.
741 <https://doi.org/10.17660/ActaHortic.2007.729.50>

742 Gulyás, M., Tomocsik, A., Orosz, V., Makádi, M., Füleky, G., 2012. Risk of agricultural use of
743 sewage sludge compost and anaerobic digestate. Acta Phytopathologica et Entomologica
744 Hungarica. <https://doi.org/10.1556/aphyt.47.2012.2.4>

745 Han, S.H., Lee, J.C., Jang, S.S., Kim, P.G., 2004. Composted sewage sludge can improve the
746 physiological properties of *Betula schmidtii* grown in tailings. Journal of Plant Biology.
747 <https://doi.org/10.1007/BF03030638>

748 Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Møller, I.S., White, P.,
749 2012. Functions of Macronutrients, in: Marschner's Mineral Nutrition of Higher Plants: Third
750 Edition. <https://doi.org/10.1016/B978-0-12-384905-2.00006-6>

751 Hussein, A.H.A., 2009. Impact of sewage sludge as organic manure on some soil properties,
752 growth, yield and nutrient contents of cucumber crop. Journal of Applied Sciences.
753 <https://doi.org/10.3923/jas.2009.1401.1411>

754 IRSA-CNR, 1985. Metodi analitici per i fanghi – Parametri chimico-fisici. Quaderno 64 vol. 3.
755 Istituto di ricerca sulle acque, Consiglio Nazionale delle Ricerche (in Italian).

756 Italian Law 130/2018. Legge 16 novembre 2018, n. 130. "Disposizioni urgenti per la città di
757 Genova, la sicurezza della rete nazionale delle infrastrutture e dei trasporti, gli eventi sismici del
758 2016 e 2017, il lavoro e le altre emergenze." Published on: Gazzetta Ufficiale della Repubblica
759 Italiana, Serie Generale n.269 of 19-11-2018 – Supplemento Ordinario n. 55 (in Italian).
760 <http://www.gazzettaufficiale.it/eli/id/2018/11/19/18G00157/sg> (Verified in February 2019).

761 Italian Decree Law 152/1999. Decreto Legislativo 11 maggio 1999, n. 152.

762 "Disposizioni sulla tutela delle acque dall'inquinamento e recepimento della direttiva
763 91/271/CEE concernente il trattamento delle acque reflue urbane e della direttiva 91/676/CEE
764 relativa alla protezione delle acque dall'inquinamento provocato dai nitrati provenienti da fonti
765 agricole". Published on: Gazzetta Ufficiale della Repubblica Italiana, Serie Generale n.124 of
766 29-05-1999 – Supplemento Ordinario n. 101 (in Italian).

767 http://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=1999-07-30&atto.codiceRedazionale=099A6464&elenco30giorni=false
768
769 (Verified in October 2018).

770 Italian Decree Law 75/2010. Decreto Legislativo 29 aprile 2010, n. 75. "Riordino e revisione
771 della disciplina in materia di fertilizzanti, a norma dell'articolo 13 della legge 7 luglio 2009, n.
772 88". Published on: Gazzetta Ufficiale della Repubblica Italiana, Serie Generale n.88 25-06-2010
773 – Supplemento ordinario n. 106 (in Italian).

774 <http://www.gazzettaufficiale.it/eli/id/2010/05/26/010G0096/sg> (Verified in February 2019).

775 Italian Decree Law 99/1992. Decreto Legislativo 27 gennaio 1992, n. 99. "Attuazione della
776 direttiva n. 86/278/CEE concernente la protezione dell'ambiente, in particolare del suolo,
777 nell'utilizzazione dei fanghi di depurazione in agricoltura." Published on: Gazzetta Ufficiale della
778 Repubblica Italiana, Serie Generale n.38 15-02-1992 – Supplemento Ordinario n. 28 (in Italian).

779 <http://www.gazzettaufficiale.it/eli/id/1992/02/15/092G0139/sg> (Verified in February 2019).

780 Italian Decree Law 152/2006: Decreto Legislativo 3 aprile 2006, n. 152.

781 "Norme in materia ambientale". Published on: Gazzetta Ufficiale della Repubblica Italiana, Serie
782 Generale n.88 of 14-04-2006 – Supplemento Ordinario n. 96 (in Italian).

783 http://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2006-04-14&atto.codiceRedazionale=006G0171 (Verified in October 2018).

784
785 Italian Ministerial Decree, 1999. Decreto Ministeriale 13 settembre 1999.

786 "Approvazione dei "Metodi ufficiali di analisi chimica del suolo"". Published on: Gazzetta
787 Ufficiale della Repubblica Italiana, Serie Generale n.248 of 21-10-1999 – Supplemento
788 Ordinario n. 185 (in Italian). <http://www.gazzettaufficiale.it/eli/id/1999/10/21/099A8497/sg>
789 (Verified in October 2018).

790 Jahromi, M.G., Aboutalebi, A., Farahi, M.H., 2012. Influence of different levels of garden
791 compost (garden wastes and cow manure) on growth and stand establishment of tomato and
792 cucumber in greenhouse condition. African Journal Of Biotechnology.

793 <https://doi.org/10.5897/ajb11.4139>

794 Koutroubas, S.D., Antoniadis, V., Fotiadis, S., Damalas, C.A., 2014. Growth, grain yield and
795 nitrogen use efficiency of Mediterranean wheat in soils amended with municipal sewage sludge.
796 Nutrient Cycling in Agroecosystems. <https://doi.org/10.1007/s10705-014-9641-x>

797 Latare, A.M., Kumar, O., Singh, S.K., Gupta, A., 2014. Direct and residual effect of sewage
798 sludge on yield, heavy metals content and soil fertility under rice-wheat system. Ecological
799 Engineering. <https://doi.org/10.1016/j.ecoleng.2014.03.066>

800 Lemaire, G., Gastal, F., 1997. N Uptake and Distribution in Plant Canopies, in: Diagnosis of the
801 Nitrogen Status in Crops. https://doi.org/10.1007/978-3-642-60684-7_1

802 Lencioni, G., Imperiale, D., Cavirani, N., Marmiroli, N., Marmiroli, M., 2016. Environmental
803 application and phytotoxicity of anaerobic digestate from pig farming by in vitro and in vivo trials.
804 International Journal of Environmental Science and Technology. [https://doi.org/10.1007/s13762-](https://doi.org/10.1007/s13762-016-1088-y)
805 [016-1088-y](https://doi.org/10.1007/s13762-016-1088-y)

806 Mañas, P., De las Heras, J., 2018. Phytotoxicity test applied to sewage sludge using *Lactuca*
807 *sativa* L. and *Lepidium sativum* L. seeds. International Journal of Environmental Science and
808 Technology. <https://doi.org/10.1007/s13762-017-1386-z>

809 Masoni, A., Ercoli, L., 2010. Azoto nel terreno. Riduzione dell'inquinamento delle acque dai
810 nitrati provenienti dall'agricoltura, 211–241 (in Italian).
811 <https://www.iris.sssup.it/retrieve/handle/11382/306758/1104/7-Azoto%20nel%20terreno.pdf>
812 (Verified in February 2019).

813 McGrath, S.P., Chaudri, A.M., Giller, K.E., 1995. Long-term effects of metals in sewage sludge
814 on soils, microorganisms and plants. Journal of Industrial Microbiology.
815 <https://doi.org/10.1007/BF01569890>

816 Milieu Ltd., 2008. Report to European Commission's DG Environment: Environmental,
817 economic and social impacts of the use of sewage sludge on land. Part II: Report on Options
818 and Impacts (DG ENV.G.4/ETU/2008/0076r) 158.

819 Monfet, E., Aubry, G., Ramirez, A.A., 2018. Nutrient removal and recovery from digestate: a
820 review of the technology. Biofuels. <https://doi.org/10.1080/17597269.2017.1336348>

821 M.P.A.A.F. ed. 2006. Trinchera, A., Leita, L., Sequi, P. 2006. Metodi di analisi per i fertilizzanti.
822 Analytical methods for fertilizers. Ministero delle Politiche Agricole Alimentari e Forestali.
823 Osservatorio Nazionale Pedologico e per la Qualità del Suolo Agricolo e Forestale (in Italian).
824 Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M., 2010. Heavy metals, occurrence and toxicity for
825 plants: A review. Environmental Chemistry Letters. <https://doi.org/10.1007/s10311-010-0297-8>
826 Navas, A., Bermúdez, F., MacHín, J., 1998. Influence of sewage sludge application on physical
827 and chemical properties of Gypsisols. Geoderma. [https://doi.org/10.1016/S0016-](https://doi.org/10.1016/S0016-7061(98)00072-X)
828 [7061\(98\)00072-X](https://doi.org/10.1016/S0016-7061(98)00072-X)
829 Nersisyan, A., Baudoin W., Shamilov, A., Hodder, A., Gutierrez, D., Pascale S, D., Nicola, S.,
830 Urban, L., Tanny, J. 2017. Good Agricultural Practices for greenhouse vegetable production in
831 the South East European countries-Principles for sustainable intensification of smallholder
832 farms. FAO Plant Production and Protection Papers. <http://www.fao.org/3/a-i6787e.pdf> (Verified
833 in February 2019).
834 Nesme, T., Withers, P.J.A., 2016. Sustainable strategies towards a phosphorus circular
835 economy. Nutrient Cycling in Agroecosystems. <https://doi.org/10.1007/s10705-016-9774-1>
836 Ohtake, H., Tsuneda, S., 2019. Phosphorus Recovery and Recycling, Phosphorus Recovery
837 and Recycling. <https://doi.org/10.1007/978-981-10-8031-9>
838 Padilla, F.M., Peña-Fleitas, M.T., Gallardo, M., Giménez, C., Thompson, R.B., 2017. Derivation
839 of sufficiency values of a chlorophyll meter to estimate cucumber nitrogen status and yield.
840 Computers and Electronics in Agriculture. <https://doi.org/10.1016/j.compag.2017.07.005>
841 Parry, C., Blonquist, J.M., Bugbee, B., 2014. In situ measurement of leaf chlorophyll
842 concentration: Analysis of the optical/absolute relationship. Plant, Cell and Environment.
843 <https://doi.org/10.1111/pce.12324>
844 Pavel, V.L., Sobariu, D.L., Diaconu, M., Stătescu, F., Gavrilăscu, M., 2013. Effects of heavy
845 metals on *Lepidium sativum* germination and growth. Environmental Engineering and
846 Management Journal.
847 Perez-Murcia, M.D., Moral, R., Moreno-Caselles, J., Perez-Espinosa, A., Paredes, C., 2006.
848 Use of composted sewage sludge in growth media for broccoli. Bioresource Technology.
849 <https://doi.org/10.1016/j.biortech.2005.02.005>

850 Qasim, M., Javed, N., Subhan, M., 2001. Effect of Sewage Sludge on the Growth of Maize
851 Crop. Journal of Biological Sciences. <https://doi.org/10.3923/jbs.2001.52.53>

852 Rigueiro-Rodríguez, A.A., Ferreiro-Domínguez, N., Mosquera-Losada, M.R., 2010. The effects
853 of fertilization with anaerobic, composted and pelletized sewage sludge on soil, tree growth,
854 pasture production and biodiversity in a silvopastoral system under ash (*Fraxinus excelsior* L.).
855 Grass and Forage Science. <https://doi.org/10.1111/j.1365-2494.2010.00743.x>

856 Sánchez-Monedero, M.A., Roig, A., Cegarra, J., Bernal, M.P., Noguera, P., Abad, M., Antón, A.,
857 2004. Composts as media constituents for vegetable transplant production. Compost Science
858 and Utilization. <https://doi.org/10.1080/1065657X.2004.10702175>

859 Shaaban, M. M., El-Bendary, A. A., 1999. Evaluation of nitrogen status for snap bean, potatoes
860 and cucumber under field conditions using a portable chlorophyll meter. Alexandria Journal of
861 Agricultural Research.

862 Shaheen, S.M., Shams, M.S., Ibrahim, S.M., Elbehiry, F.A., Antoniadis, V., Hooda, P.S., 2014.
863 Stabilization of sewage sludge by using various by-products: Effects on soil properties, biomass
864 production, and bioavailability of copper and zinc. Water, Air, and Soil Pollution.
865 <https://doi.org/10.1007/s11270-014-2014-x>

866 Schulz, H., Glaser, B., 2012. Effects of biochar compared to organic and inorganic fertilizers on
867 soil quality and plant growth in a greenhouse experiment. Journal of Plant Nutrition and Soil
868 Science. <https://doi.org/10.1002/jpln.201100143>

869 Siciliano, A., Limonti, C., Stillitano, M. A., Panico, S., Garuti, G., 2017. Trattamento dei digestati
870 per il recupero dell'azoto ammoniacale sottoforma di struvite. Ingegneria dell'Ambiente (in
871 Italian). <https://doi.org/10.14672/ida.v4i2.1198>

872 Singh, R.P., Agrawal, M., 2007. Effects of sewage sludge amendment on heavy metal
873 accumulation and consequent responses of *Beta vulgaris* plants. Chemosphere.
874 <https://doi.org/10.1016/j.chemosphere.2006.12.019>

875 Singh, R.P., Agrawal, M., 2008. Potential benefits and risks of land application of sewage
876 sludge. Waste Management. <https://doi.org/10.1016/j.wasman.2006.12.010>

877 Singh, R.P., Agrawal, M., 2009. Use of sewage sludge as fertiliser supplement for *Abelmoschus*
878 *esculentus* plants: physiological, biochemical and growth responses. *International Journal of*
879 *Environment and Waste Management*. <https://doi.org/10.1504/ijewm.2009.024702>

880 Singh, R.P., Agrawal, M., 2010. Biochemical and physiological responses of Rice (*Oryza sativa*
881 L.) grown on different sewage sludge amendments rates. *Bulletin of Environmental*
882 *Contamination and Toxicology*. <https://doi.org/10.1007/s00128-010-0007-z>

883 Solé-Bundó, M., Cucina, M., Folch, M., Tàpias, J., Gigliotti, G., Garfí, M., Ferrer, I., 2017.
884 Assessing the agricultural reuse of the digestate from microalgae anaerobic digestion and co-
885 digestion with sewage sludge. *Science of the Total Environment*.
886 <https://doi.org/10.1016/j.scitotenv.2017.02.006>

887 Sonneveld, C., Voogt, W., Sonneveld, C., Voogt, W., 2009. Substrates: Chemical
888 Characteristics and Preparation, in: *Plant Nutrition of Greenhouse Crops*.
889 https://doi.org/10.1007/978-90-481-2532-6_11

890 Sonneveld, C., van den Ende, J., 1971. Soil analysis by means of a 1:2 volume extract. *Plant*
891 *and Soil*. <https://doi.org/10.1007/BF01372683>

892 Sukreeyapongse, O., Holm, P.E., Strobel, B.W., Panichsakpatana, S., Magid, J., Hansen,
893 H.C.B., 2002. pH-Dependent Release of Cadmium, Copper, and Lead from Natural and Sludge-
894 Amended Soils. *Journal of Environment Quality*. <https://doi.org/10.2134/jeq2002.1901>

895 Tarrasón, D., Ojeda, G., Ortiz, O., Alcañiz, J.M., 2008. Differences on nitrogen availability in a
896 soil amended with fresh, composted and thermally-dried sewage sludge. *Bioresource*
897 *Technology*. <https://doi.org/10.1016/j.biortech.2006.12.023>

898 Tritt, W.P., 1992. The anaerobic treatment of slaughterhouse wastewater in fixed-bed reactors.
899 *Bioresource Technology*. [https://doi.org/10.1016/0960-8524\(92\)90002-F](https://doi.org/10.1016/0960-8524(92)90002-F)

900 USEPA, 2006. Method 6010: Inductively coupled plasma-atomic emission spectrometry. *Test*
901 *Methods for Evaluating Solid Waste, Physical/Chemical Methods*. US Environmental Protection
902 Agency (EPA). <https://www.epa.gov/sites/production/files/2015-12/documents/6010d.pdf>
903 (Verified in October 2018).

904 USEPA, 2007a. Method 3051: Microwave assisted acid digestion of sediments, sludges, soils,
905 and oils, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*. US

906 Environmental Protection Agency (EPA). [https://www.epa.gov/sites/production/files/2015-](https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf)
907 [12/documents/3051a.pdf](https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf) (Verified in October 2018).

908 USEPA, 2007b. Method 7010: Graphite furnace atomic absorption spectrophotometry. Test
909 Methods for Evaluating Solid Waste, Physical/Chemical Methods. US Environmental Protection
910 Agency (EPA). <https://www.epa.gov/sites/production/files/2015-12/documents/7010.pdf> (Verified
911 in October 2018).

912 van der Eerden, L.J.M., 1982. Toxicity of ammonia to plants. Agriculture and Environment.
913 [https://doi.org/10.1016/0304-1131\(82\)90015-7](https://doi.org/10.1016/0304-1131(82)90015-7)

914 Waqas, M., Khan, S., Qing, H., Reid, B.J., Chao, C., 2014. The effects of sewage sludge and
915 sewage sludge biochar on PAHs and potentially toxic element bioaccumulation in *Cucumis*
916 *sativa* L. Chemosphere. <https://doi.org/10.1016/j.chemosphere.2013.11.064>

917 Wong, J.W.C., Li, G.X., Wong, M.H., 1996. The growth of *Brassica chinensis* in heavy-metal-
918 contaminated sewage sludge compost from Hong Kong. Bioresource Technology.
919 [https://doi.org/10.1016/S0960-8524\(96\)00121-6](https://doi.org/10.1016/S0960-8524(96)00121-6)

920 Wang, X., Chen, T., Ge, Y., Jia, Y., 2008. Studies on land application of sewage sludge and its
921 limiting factors. Journal of Hazardous Materials. <https://doi.org/10.1016/j.jhazmat.2008.03.046>

922 Wyrwicka, A., Steffani, S., Urbaniak, M., 2014. The effect of PCB-contaminated sewage sludge
923 and sediment on metabolism of cucumber plants (*Cucumis sativus* L.). Ecohydrology and
924 Hydrobiology. <https://doi.org/10.1016/j.ecohyd.2014.01.003>

925 Xu, D.B., Wang, Q.J., Wu, Y.C., Yu, G.H., Shen, Q.R., Huang, Q.W., 2012. Humic-Like
926 Substances from Different Compost Extracts Could Significantly Promote Cucumber Growth.
927 Pedosphere. [https://doi.org/10.1016/S1002-0160\(12\)60067-8](https://doi.org/10.1016/S1002-0160(12)60067-8)

928 Zucconi, F., Forte, M., Monaco, A., De Bertoldi, M., 1981. Biological evaluation of compost
929 maturity. BioCycle.

930 Zucconi, F., Monaco, A., Forte, M., 1985. Phytotoxins during the stabilization of organic matter,
931 in: Composting of Agricultural and Other Wastes.