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16 Compost and vermicompost in cucumber rhizosphere promote plant growth and

17 prevent the entry of anthropogenic organic pollutants

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26 Abstract

An accurate assessment of the absorption and accumulation of soil pollutants by plants is 27 28 essential to avoid the entry of toxic compounds into the human and animal food chain. Therefore, this study evaluated the effectiveness of the amendment of a loamy soil with 29 a mixed compost (CP) and a vermicompost (VC) from digestate, at doses of 10 t ha⁻¹ (CP_L 30 and VC_L) and 30 t ha⁻¹ (CP_H and VC_H), in sustaining the growth of cucumber (*Cucumis* 31 sativus L.) plants and reducing the uptake of contaminats, such as the fungicide 32 metalaxyl-m (MET-M) and the endocrine disruptors bisphenol A (BPA), 4-tert-33 octvlphenol (OP) and nonvlphenol (NP). Plant response to soil amendment with CP and 34 VC was tested in parallel in both contaminated and uncontaminated soil. All treatments 35 significantly promoted cucumber growth in both soil conditions. After 38 days of 36 cucumber growth in contaminated soil, CPL, CPH, VCL and VCH increased dry biomass 37 of roots and shoots by 42, 128, 118, 147%, and 46, 113, 271, 443%, respectively, 38 compared to unamended soil (control). Root and shoot elongation and the number of 39 leaves and their expansion were also significantly promoted by the application of CP and 40

VC at both doses. All treatments, in the order: $VC_H > VC_L > CP_H > CP_L$, considerably 41 42 reduced the absorption of all compounds by cucumber. Although small quantities of residues of each pollutant were found both in the roots and in the aerial organs of all 43 44 plants, their overall accumulation in plants grown in amended soil was significantly lower than that found in the control plants. Thus, on the basis of the results obtained, it is evident 45 46 that the use of CP and VC can be very effective and sustainable both from an economic 47 and environmental point of view, since, in addition to stimulate plant growth and increase soil fertility, it can represent a successful strategy to mitigate the presence of toxic 48 49 residues in food plants.

50

51 Keywords: soil contamination, root system, plant uptake, contaminant accumulation,
52 endocrine disrupting chemical, metalaxyl-m, contaminant mitigation

53

54 **1. Introduction**

55 The presence of contaminants in soil and food products is an alarming emergency in countries with a high concentration of industries, significant urbanization and prevailing 56 intensive agricultural practices. However, even in more remote areas and in uncultivated 57 soils, researchers have reported the presence of persistent organic contaminants that are 58 very harmful to wildlife and humans. According to the United Nations, nearly 2 billion 59 ha (22.5%) of agricultural land, pasture, forest, and woodland are affected by soil 60 pollution (United Nations 2019). A clean soil is essential for the maintenance of the 61 ecosystem biodiversity and soil functioning thus ensuring global food security and 62 63 mitigation of climate change. It is also true that continuate decline of stable organic matter in many soils has caused progressive alteration of biological equilibria and the consequent 64 loss of the self-depollution capacity of soil. 65

Currently, a major concern among the international scientific community is due to 66 67 the so-called anthropogenic organic pollutants (AOPs), a wide class that include different compounds such as agrochemicals, pharmaceuticals, dyes, wood preservatives, industrial 68 69 products and byproducts (FAO 2017). AOPs may be defined as organic chemicals that are foreign to natural ecosystems and may adversely affect, either directly or indirectly, 70 the normal chemical and biological equilibria and processes in both undisturbed and 71 cultivated soils. AOPs can reach the soil on purpose, as in the case of agrochemicals for 72 73 controlling crop diseases, or inadvertently and sometimes illegally through the incorporation into soil of not completely decontaminated liquid and solid wastes, such as 74 sewage sludges, wastewaters, biosolids and biowaste of agro-industrial origin (Geissena 75 et al. 2015; Silva et al. 2019). 76

Agrochemicals, including herbicides, fungicides, insecticides, nematicides and so on, 77 78 are widely used in conventional agriculture for plant protection, and their repeated applications to soil and/or plants over the years or incorrect dosage can generate residue 79 80 levels in soil that compromise soil fertility and food safety. Due to their prevailing low hydrophobicity, most agrochemicals can be taken up by plant roots and then traslocated 81 to different tissues where they accumulate, which is particularly dangerous in the case of 82 staple food crops because toxic residues can enter the food chain of animals and humans. 83 Furtheremore, these chemicals, especially the more polar ones, can pose serious 84 environmental problems due to their movement into soil and transport in surface- and 85 groundwater (Loffredo et al. 2021). 86

Metalaxyl-M [methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-D-alaninate, MET-M] is the bioactive R-enantiomer of the acylanilide chiral fungicide metalaxyl which is widely used for the control of phytopathogenic fungi of several crops and urban green areas turfgrasses (Leadbeater 2014). Besides target organisms, the non-selective

MET-M could adversely affect non-target organisms. For its systemic behaviour, METM is absorbed by plant roots and translocated into various organs where it is partly
metabolized and partly accumulated. Due to its moderate dissipation in soil (half-life of
about 39 d in field conditions), MET-M can accumulate and reach concentrations up to 1
mg kg⁻¹ (Kurek 2017).

96 Multiple contamination can be considered the norm for intensively cultivated soils, where agrochemicals can be simultaneously present in soil with other classes of AOPs 97 98 such as the so-called endocrine disrupting chemicals (EDCs). They are a group of compounds known for their capacity to severely disturb the normal hormonal functions 99 and metabolism of animals and humans (Corrales 2015; EC 2020). EDCs are used in 100 many industrial products and, consequently, they are constantly released into terrestrial 101 and aquatic environments where represent a serious threat to wildlife, especially aquatic, 102 103 farm animals and humans (de Bruin et al. 2019; Kim et al. 2019). These compounds are often found in cultivated soils where they may enter through the application, discharge 104 105 and/or disposal of urban and industrial effluents, sludges, biowaste from various 106 production activities, including agriculture, and biosolids application (Loffredo 2022).

The xenoestrogen bisphenol A [2,2-(4,4 dihydroxydiphenyl) propane, BPA] is the 107 building block of epoxy resins and polycarbonates and is adopted as a stabilizer for 108 polyvinyl chloride. BPA may severely affect the human endocrine system and act as a 109 prominent EDC (Michałowicz, 2014). Octylphenol (OP) and nonylphenol (NP) originates 110 111 from the breakdown of alkylphenol polyethoxylates, which are the largest group of nonionic surfactants used in cleaning products, cosmetics and pesticides (Chokwe et al. 2017; 112 Olaniyan et al. 2018). All these three EDCs, in addition to being recalcitrant to 113 biodegradation, are being constantly released into the environment where they represent 114 an alarming risk for terrestrial and aquatic organisms (Metcalfe et al. 2022). 115

Another aspect related to intensive agriculture is the general progressive reduction of 116 117 soil organic matter that exposes soil to degradation, alters the biological equilibria and determines inadequate levels of water and nutrients for plants. To counter this situation, 118 119 unexpensive C-rich materials, such as compost (CP) and vermicompost (VC), can be incorporated into soil with the multiple benefits of supplying stable organic matter, 120 improving soil fertility, stimulating plant growth and microbial activity, and promoting 121 carbon sequestration (Diacono and Montemurro, 2010; Schimmelpfennig et al. 2014). CP 122 123 and VC have shown excellent capacity in the retention of organic and inorganic pollutants through various mechanisms that involve their numerous functional sorption sites (Senesi 124 et al. 2015). This process allows to control pollutants bioavailability and limit their 125 transport into natural waters (Gámiz et al. 2016; Parlavecchia et al. 2019). Furthermore, 126 there is growing concern about the residues of pollutants and their metabolites that remain 127 128 in harvested crops, particularly in the edible parts of plants, and can then be ingested by humans and animals via food or feed. 129

130 The root system is the main interface between plants and their environment, therefore under heavy soil contamination it represents the most exposed plant organ and may be 131 considered an important indicator of the general stress status of the plant. The plant root 132 system has a certain variability (plasticity) that represents a major survival strategy to 133 cope with a wide range of soil factors and external stresses. Various morphological 134 parameters such as the length, surface area, volume and diameter are used as potential 135 136 indicator of root plasticity. Further morphological traits derived from the formers and having a functional significance are: specific root length (root length per unit of root dry 137 138 weight, SRL), root fineness (root length per unit root volume, RF), tissue density (root dry mass per unit root volume, RTD), root surface area (root length per unit of diameter). 139 All these morphological parameters are commonly used to evaluate plant responses to 140

interfering agents, such as compost (Lazcano et al. 2009; Gelsomino et al. 2014; Busato
et al. 2018), organic (Wei et al. 2021) or inorganic (Ryser and Emerson 2007; Panuccio
et al. 2014) pollutants.

Considering all this, the aim of this study was to evaluate the potential of two doses of CP and VC to promote the growth of cucumber (*Cucumis sativus L.*) plants in both uncontaminated and contaminated soil and limit the entry and accumulation in plants of the contaminants MET-M, BPA, OP and NP.

148

149 **2. Materials and methods**

150 2.1 Chemicals, soil, amendents and plant

151 MET-M with purity \geq 98%, BPA at 99.0% purity and OP at 99.5% purity were 152 purchased from Sigma-Aldrich S.r.l., Milano, Italy, while NP at 99.5% purity was 153 provided by Dr Ehrenstorfer GmbH, Augsburg, Germany. Some chemical properties of 154 these AOPs are shown in Table 1. All other chemicals of extra pure grade were obtained 155 from commercial sources and used without further purification.

A loamy calcareous agricultural soil sampled at 0–20 cm depth at an experimental 156 station located at Valenzano, South Italy, was used. The soil was air-dried, sieved at <3-157 mm particle size to remove the coarser fraction and thoroughly homogenized. Soil 158 properties were determined according to standard methods (Sparks et al. 1996). Briefly, 159 soil moisture was measured after heating at 105 °C overnight; pH was potentiometrically 160 measured in a 1:2.5 (w/v) soil-to-0.01 M CaCl₂ solution mixture (pH_{CaCl2}); electrical 161 conductivity was measured at 25 °C in a 1:2 (w/v) soil-to-water ratio slurry (EC_{1:2} 25 °C); 162 total organic C and N were determined by an elemental analyzer LECO CN628 (LECO 163 Corporation, MI, USA); total CaCO₃ was determined by the gas-volumetric method using 164 a Dietrich–Fruhling calcimeter; cation exchange capacity (CEC) was measured by using 165

0.1 M BaCl₂ buffered to pH 8.2 with triethanolamine (2.25%, v/v). Soil characteristics
are shown in Table 2.

The CP sample was obtained from a local municipal solid waste processing plant 168 (Calabra Maceri & Servizi S.p.a., Rende, Italy) after a 3-month accelerated composting 169 process of mixed animal and plant waste. The VC sample was provided by C&F Energy, 170 Società Agricola S.r.l. (Silentina, Italy) after 2-month vermicomposting with redworms 171 (Lumbricus rubellus) of a digestate resulting from the anaerobic digestion process of a 172 mixture of buffalo manure, chicken manure and olive oil mill wastewater. Major 173 characteristics of CP and VC are reported in Table 2. Before use, CP and VC samples 174 175 were air-dried, finely ground and 0.5-mm sieved.

176 Cucumber (*Cucumis sativus* L.) seeds were purchased from L'Ortolano S.r.l., Cesena,
177 Italy.

178

179 2.2 Experimental protocol

180 Cucumber seeds were germinated in Petri dishes (9-cm diameter) kept in the dark
181 into a Phytotron growth chamber (F.lli Della Marca S.r.l., Roma, Italy, model
182 60043/THTL) at a temperature of 22 ± 1 °C for 3 days.

Plastic pots (13-cm diameter and 13-cm height) were filled to a height of about 10 cm with 800 g of air-dried soil only (control), or with 800 g of mixtures of soil and 1.12% (w/w) of amendment (CP_L and VC_L), or soil and 3.36% (w/w) of amendment (CP_H and VC_H). The lowest and the highest dose of CP and VC correspond, respectively, to a soil application of about 10 and 30 t ha⁻¹. Then, pot mixtures were bought to 60% field capacity by adding distilled water.

After about 2 h, aliquots of 0.8 mL of individual methanol solutions of MET-M,
BPA, OP and NP at a concentration of 1000 µg mL⁻¹ were not added (uncontaminated,

191 UN) or added into the upper soil layer (~ 3 cm), thus obtaining in the whole soil a 192 concentration of 1 μ g g⁻¹ of each compound. After about 2 h, uniformly sized cucumber 193 seedings were selected and not transplanted (bare soil) or transplanted (one seedling in 194 each pot, planted soil) into the pots. Subsequently, a volume of 10 mL of distilled H₂O 195 was added to each pot (with and without seedling). Thus, the following treatments were 196 obtained: control (contaminated soil), CP_L, CP_H, VC_L, VC_H, NC (not contaminated soil), 197 NC-CP_L, NC-CP_H, NC-VC_L, NC-VC_H.

Plants were grown in the chamber for 38 d using a 11-h daylight photoperiod. Relative humidity and air temperature were, respectively, 50% and 24 ± 1 °C during light hours, and 60% and 17 ± 1 °C during dark hours. Each pot (with and without plants) was watered with 20 mL of distilled H₂O per day. The pots were placed in the growth chamber according to a completely randomized design with 5 replications.

203 At the end of the experiments, plants were gently removed from pot mixtures, roots were rinsed with distilled water and separated from shoots. Immediately after, root and 204 205 shoot fresh weights, shoot length, number of leaves per plant and length of the main leaf 206 vein were measured. The root system was stained with 0.1% (w/v) toluidine blue O for 5 min, thoroughly washed with water and then scanned (WinRhizo STD 1600, Instruments 207 Régent Inc., Canada) at a resolution of 600 dpi for morphological analyses. Scanned 208 209 images were processed using the WinRhizo[®] root analysis software (Régent Instruments) to measure total root length, volume, surface area and average diameter. Then, root and 210 shoot dry weights were determined by oven-drying at 70°C for 16 h. Total plant dry 211 212 weight was obtained by summing root and shoot dry weight. Based on the measurements 213 above, the following morphological ratios were calculated: specific root length (root length per unit of root dry weight, SRL), specific root surface area (surface area per unit 214 of root dry weight, SRSA), specific root volume (root volume per unit of root dry weight, 215

SRV) and root tissue density (root dry mass per unit root volume, RTD) which representfunctional parameters.

218

2.3 Extraction and quantification of contaminant residues from pot mixtures and planttissues

After cucumber plant removal, each pot mixture was thoroughly homogenized; then 221 an aliquot of 20 g sample was collected, added with 50 mL of methanol and kept under 222 223 mechanical shaking overnight (16 h). Then, the suspension was filtered and an aliquot of 15 mL was centrifuged at 10,000 g for 10 min. Subsequently, 10 mL of supernatant 224 solution was evaporated to dryness at a temperature of 40 °C using a rotatory evaporator. 225 The solid residue was dissolved in a volume of 1 mL of acetonitrile/water mixture (60:40 226 v/v), filtered through 0.45 µm MilliporeTM cellulose acetate filters and analysed by reverse 227 228 phase ultra-high performance liquid chromatography (UHPLC) technique (section 2.4). Recoveries from soil of MET-M, BPA, OP and NP each at an initial concentration of 1 229 μ g g⁻¹ were, respectively, 92.20 ± 1.61, 92.43 ± 0.80, 91.82 ± 2.08 and 94.6 ± 4.6% (*n*=4). 230 The percentages of the compounds disappeared in pot mixtures during the trial were 231 calculated as the difference between the initial amounts and those extracted after 38 d. 232

Absorbed contaminant were extracted from plant tissues according to the procedure 233 of Ferrara et al. (2006). Briefly, 0.1 g of dried shoot and root mass, individually, was 234 235 added with 10 mL of pure methanol and kept under mechanical shaking for 4 h. Successively, the suspension was centrifugated for 10 min at 10,000 g and an aliquot of 236 6 mL was evaporated to dryness at a temperature of 40 °C using a rotary evaporator. The 237 residue was dissolved in a volume of 1 mL of acetonitrile/water mixture (60:40 v/v), 238 filtered through 0.45 µm MilliporeTM cellulose acetate filters and analysed by UHPLC 239 technique (section 2.4). 240

242 2.4 Analytical measurement

The UHPLC system (Dionex Ultimate 3000 RSLC, Waltham MA, USA) used was 243 equipped with an HPG-3200 RS pump, a WPS-3000 autosampler and a TCC-3000 244 column compartment connected to a SupelcoTM LC-18 column (250 mm \times 4.6 mm \times 5 245 um). The mobile phase was a mixture of water (A) and acetonitrile (B). The elution 246 gradient adopted was the following: 0-1 min 40% A, 1-6 min from 40 to 30% A, 6-8 min 247 248 from 30% to 20% A, 8-13 min from 20 to 10% A, 13-15 min 10% A. The flow rate was 1 mL min⁻¹ and the retention times of MET-M, BPA, OP and NP were about, 3.8, 4.2, 8.0 249 and 13.5, respectively. MET-M was detected using a DAD-3000 RS diode array detector 250 (Dionex Ultimate 3000 RSLC, Waltham MA, USA) at a wavelength of 220 nm, while 251 BPA, OP and NP were detected using a FLD-3400 RS fluorescence detector (Dionex 252 253 Ultimate 3000 RSLC, Waltham MA, USA) operating at wavelengths of 200-nm excitation and 290-nm emission. 254

255

256 2.5 Statistical analysis

Biometric data of plants and data of residual compounds extracted from plants were statistically analysed by one-way analysis of variance (ANOVA) and the means of the treatments were compared to the control by the least significant difference (LSD) test at 0.05P, 0.01P, and 0.001P levels. Data of residual compounds extracted from soil were analysed by two-way ANOVA and the means were separated at 0.05P and 0.01P levels using the Duncan's multiple range test for the main factors and the LSD test for the interaction.

264

265 **3. Results and discussion**

266 3.1 Plant response to soil amendment

267 3.1.1 Effects on the root system and aerial plant organs

Although the main objective of this study was to evaluate the effects of the two soil amendments in soil contaminated conditions, the response of plants to the application of CP and VC in uncontaminated soil was also evaluated, which allowed for a more comprehensive discussion of the role of these materials in the rhizosphere and on cucumber growth. For this purpose, various biometric parameters of both root system and aerial tissues of the plant were measured (Fig. 1).

Root and shoot fresh weights of cucumber plants grown on uncontaminated soil were 274 significantly higher in presence of both CP_L and VC_L, compared to the unamended control 275 (NC), while VC_H positively affected only shoots (Fig. 1A and B). A similar trend was 276 found for root dry weights (Fig. 1C), while shoot dry weights were increased only by the 277 278 higher dose of CP and VC (Fig. 1D). A different trend was observed for shoot elongation in NC soils where both doses of CP and the higher dose of VC appeared to depress this 279 280 parameter compared to unamended soil (Fig. 1E). Furthermore, positive effects of CP and VC were also generally observed in NC soils on the number of leaves per plant (Fig. 1F) 281 and on the average length of the main leaf vein (Fig. 1G) which can be considered in 282 indicator of leaf extension. 283

As expected, the multi-contamination of soil (control) exerted phytotoxic effects on cucumber plants producing an evident decrease of root and shoot biomass, as well as shoot length and leaf parameters, compared to NC (Fig. 1). Similarly, Patama et al. (2019) found a significant inhibition of both root and shoot elongation of *Gypsophila elegans* treated with OP. In a recent study, an evident phytotoxicity due to soil treatments with BPA and OP on rocket salad plants was reported (Parlavecchia et al. 2020). 290 Unfortunately, no results are present in the literature on toxic effects of NP on agricultural291 plants.

In the treatments with CP and VC, the toxic effects of the contaminants appeared 292 293 noticeably mitigated by the presence of both amendments, with the only exception of CP_{1} . on root fresh weight (Fig. 1A), and the stimulation was positively related to the 294 295 amendment dose (Fig. 1). This was particularly evident in VC_H treatment, where the fresh weights of root and shoot increased by 223 and 279%, respectively (Fig. 1A and B), and 296 297 dry weights by 147 and 443%, respectively (Fig. 1C and D), compared to the control. The apparent stress observed on the aerial organs in amended NC soils was not confirmed in 298 contaminated soil where shoot elongation was generally favoured by the amendments 299 (Fig. 1E). Also in contaminated soil, CP and VC generally increased the number of leaves 300 per plant (Fig. 1F) and the average length of the main leaf vein (Fig. 1G). 301

302 These results indicate that the stimulation of cucumber plants by CP and VC observed in NC soil is even enhanced under multi-contamination conditions where plants greatly 303 304 benefit from the antitoxic activity of these amendments. These positive effects may be 305 attributed, at least in part, to the ability of C-rich materials like CP and VC to adsorb contaminants through various physicochemical mechanisms, thus reducing their presence 306 in soil pore water and their bioavailability for plants (Hurtado et al. 2017). In a recent 307 study, the toxicity of BPA and OP on hemp plants was significantly attenuated by the 308 309 addition of a CP which increased root and shoot dry weights by more than 50%, compared 310 to unamended soil (Loffredo et al. 2021). Evident antitoxic effects on rocket plants grown 311 on a soil spiked with a mixture of contaminants, including BPA and OP, were observed 312 following the application of a green CP (Parlavecchia et al. 2020).

The presence of VC_H in cucumber rhizosphere produced the maximum plant biomass (Fig. 2). Furthermore, results showed that plants grown on contaminated soil enriched

with VC relocated carbon from belowground production to aboveground production, as 315 316 pointed out by the root (RMR) and shoot dry mass ratios (SMR) that reflect the proportion of resources distributed between the root and shoot apparatus (Fig. 2). The two-way 317 318 ANOVA (Tab. S1) confirmed that in soil amended with CP, regardless of the dose applied, plant growth was mostly affected by the presence of the contaminants. 319 320 Conversely, it was the amount of added VC rather than the contamination itself that affected the biomass production of cucumber plants (Tab. S1). These results confirm what 321 322 already observed by Liu et al. (2021) who demonstrated that the addition of CP to soil significantly increases the height and shoot fresh and dry weights of roselle plants. Mixing 323 20% of VC with soil resulted in 98% average increase of Dracocephalum moldavica 324 biomass, compared to soil only (Ose et al., 2021). 325

326

327 3.1.2 Root morphological analysis

The root system of cucumber plants was evaluated for a number of morphological parameters such as total root length, root surface area, mean root diameter and root volume (Fig. 3). Geldner and Salt (2014) emphasized the importance of roots and their architecture for a good ability of plants to absorb water and nutrients from the soil.

In plants grown on soil without contaminants, the presence of CP and VC increased 332 all morphological parameters compared to control plants (NC), particularly with VC_L 333 334 addition (Fig. 3). A similar trend was observed in contaminated soil, where the increase of all root parameters of plants grown in amended soil was still significant compared to 335 the control soil, but lesser than that in NC soil. The two-way ANOVA (F-ratios) 336 337 evidenced that among the parameters examined the most affected by the treatments was the total root length (Tab. S2). Root length is considered more important than root weight 338 to indicate root functionality because it expresses the potential for solute and water uptake 339

(Ryser, 2007). In uncontaminated soil, root diameter increased significantly in the 340 341 presence of CP and VC, while in contaminated soil only VC significantly enhanced this parameter (Fig. 3). Root diameter distribution is usually expressed as the "mean diameter" 342 343 and sometimes does not characterize a response of the root system structure adequately, as fine and coarse roots should be considered separately having different responses in 344 345 terms of functionality. In both uncontaminated and contaminated soil, root volume was greatly increased by the lower dose of both amendments (Fig. 3). The ANOVA results 346 347 indicated that the amendment dose, rather than the presence of the contaminants, caused the highest variability in the morphological parameters (Table S2). 348

Specific root length (SRL), specific root area (SRA) and specific root volume (SRV) 349 describe the potential of the root apparatus to develop and contact soil by investing a 350 given amount of photosynthate. All these parameters are significantly increased on 351 352 contaminated soil in plants treated with the lower concentration of CP and VC (Fig. S1). SRL is the root length per unit of root dry mass; it is believed to characterize economic 353 354 aspect of the root system and is frequently used as an indicator of root fineness (Panuccio 355 et al., 2014). SRL has been shown to increase, decrease, or remain constant in response to local heterogeneity of the soil and nutrient limitation (Eissenstat et al., 2000). These 356 contrasting responses could be in part explained by considering that SRL is a complex 357 parameter that includes variations in length, diameter and root tissue density, which 358 respond to environmental conditions differently. Root tissue density (RTD) is defined as 359 360 the amount of structural material invested by unit of volume (ratio between dry mass and volume) and is commonly associated with critical aspects of plant growth in unproductive 361 362 environments. Low-density tissues enable a fast relative growth rate and a rapid resource acquisition with a low investment on dry matter. Conversely, roots with high tissue 363 density are generally associated with a slow growth in infertile soil (Ryser 2007). On this 364

basis, the significant increase of SRL on contaminated soil (Fig. S1) can be in part due to 365 366 the increase in total root length and the concomitant decrease in root tissue density found in cucumber plants grown at the lower concentration of both amendments (Figs. S1 and 367 368 S2). Two-way ANOVA showed that in general the concentration rather than the contamination determined the greatest variability in morphological ratios, particularly 369 370 with organic amendments (Tab. S3). Higher SRL values indicate longer roots per unit of root mass. This root apparatus is more effective in water and nutrient uptake and is 371 372 advantageous in high-resource environments leading to a fast plant growth. Conversely, this acquisition strategy is disadvantageous when resources are scarce, due to excess 373 tissue building costs. In fact, as reported by Fitter (1991), even if roots with a smaller 374 diameter can contact a larger soil volume per unit surface area, the maintenance carbon 375 cost of producing finer roots is higher as these will have to be replaced more frequently. 376 377 However, SRL can increase when resources are getting limited, as it is equally logical that species of stressed environments may need higher investment in root length to ensure 378 379 the exploration of a larger soil volume.

380

381 3.2 Absorption and accumulation of contaminants in plant tissues

382 3.2.1 Residual contaminants in soil

A decreasing amount of all organic contaminants was recovered after 38 d from bare and planted pot soil either not amended (control) or amended with CP and VC at the two doses (Table 3). Averagely for soil treatments, residue reduction in planted soil, compared to bare soil, was highly significant ($P \le 0.01$) for MET-M, BPA and OP, and significant ($P \le 0.05$) for NP (Table 3). During the experimental period, in bare soil, contaminants underwent adsorption on the solid fraction thus reducing their mobility and activity degradation. It is well known that soil pollutants can be adsorbed onto soil organic

fraction or soil amendment, like CP and VC, via specific physical and chemical binding 390 mechanisms and forces of various type and strength which include ionic, hydrogen and 391 covalent bonding, charge-transfer or electron donor-acceptor mechanisms, dipole-dipole 392 393 and van der Waals forces (Senesi et al. 2015). In particular, adsorption of low-polar pollutants can also occur through non-specific hydrophobic or partitioning processes 394 395 between the water phase and the hydrophobic active sites of organic matter, such as aliphatic side chains and aromatic structures (Senesi et al. 2015). Besides surface 396 397 adsorption/immobiliation and, possibly, biological degradation, plant uptake contributed to the removal of contaminants, as found in planted pot soil. The distribution of 398 contaminants between soil and pore water (adsorption) in combination with 399 biodegradation controls the bioavailability of the compounds to plant uptake. Absorption 400 of most organic chemicals by plant roots is a passive and diffusive process that depends 401 402 on the concentration of the compounds in soil pore water (Cheng et al. 2017).

It is reasonable to assume that both adsorption and, especially, biological degradation 403 404 occurred with different intensity based on the presence or absence of the plant and the 405 properties of the contaminant. The plant could have played different and contrasting roles on microbial degradation of the molecules, that are: (i) root exudates released in the 406 rhizosphere during cucumber growth, being a source of nourishment for microorganisms, 407 might have promoted the dissipation of contaminants (rhizodegradation or 408 phytostimulation), or (ii) the rapid root uptake of contaminants might have reduced their 409 time of exposure to soil microorganisms with consequent lower biodegradation. 410 411 Unfortunately, the experimental conditions of this study do not allow to discriminate and 412 quantify the two possible processes.

413 Another factor that influences the absorption of contaminants by the plant are the 414 physicochemical properties of the compound, primarily its hydrophobicity. When the

percentages of residual compounds extracted from bare soil or planted soil or their 415 416 differences, averagely for soil treatments, was related to the corresponding log Kow of the contaminants, significant correlations were found in all cases (Fig. 4). These results 417 418 indicate that, regardless of soil treatment, the least hydrophobic compounds are: (i) the most degraded in bare soil; (ii) the most removed in planted soil; and (iii) the most 419 absorbed by the plant, assuming that the difference between the amount of residual 420 compound in bare soil and planted soil may be approximated to the amount of absorbed 421 422 compound. Negative correlation between contaminant lipophilicity and their uptake by plants was previously demonstrated for the contaminants in this study and other plant 423 species (Jayampathi et al. 2021; Gong et al. 2020; Loffredo et al. 2021). Despite the very 424 low solubility of OP and NP, data obtained clearly evidenced the ability of cucumber to 425 take up these molecules. Bokern and Harms (1997) found that plants incorporated NP 426 427 into cell walls as a mechanism to reduce the phytotoxicity of this compound. Brown et al. (2009) reported that NP uptake by plants was very low and its persistence within the plant 428 429 was minimal.

Among the compounds examined, MET-M showed the maximum disappearance in 430 both bare and planted soil and, on average, in all treatments (Table 3). This can be 431 attributed to the relatively high water solubility of MET-M which allows the molecule to 432 easily reach the roots and enter the plant with the water flow. The addition to the soil of 433 CP or VC, especially at the higher dose, significantly ($P \le 0.01$) increased the percentage 434 of MET-M residues found in both bare and planted soil (Table 3). The effects were 435 particularly evident in planted pot soil where residual MET-M was about 44% in the 436 control and much more in the treatments with a maximum of 91% in VC_H (Table 3). It is 437 not easy to explain these results which may depend on several factors. One hypothesis is 438 that the increased retention capacity of the amended soil may have reduced the availability 439

of the contaminant for microbial degradation; another hypothesis is that the increased 440 retention capacity of the amended soil may have involved also simple organic 441 compounds, including root exudates, which constitute a nutrient source for microbial 442 443 degraders, with a consequent reduction in microbial activity and a longer permanence of the contaminant in the soil. Of course, other explanations are also possible. It has been 444 largely demonstrated that MET-M adsorption occurs mainly on the organic fraction of 445 soil and markedly increases after the addition of C-rich materials (Fernandes et al. 2003). 446 447 Parlavecchia et al. (2019) found that the addition of different types of VC to soil noticeably increased MET-M adsorption. The adsorption capacity of a CP-based 448 biomixture for MET-M was much higher than that of the soil only (Karanasios et al. 449 2010). 450

The general behaviour of BPA in both bare and planted soil was not very different 451 452 from that of MET-M and quite similar to that of the other two endocrine disruptors OP and NP. Averagely for soil treatments, the presence of cucumber plants significantly 453 454 decreased the quantity of residual BPA, OP and NP in soil, compared to bare soil (Table 455 3). Similarly to what observed for MET-M, the presence of CP and VC at both doses reduced the removal of each of the other three contaminants, compared to bare soil. In 456 fact, averagely for soil treatments, the removal of BPA, OP and NP in amended soil was 457 only about 25, 28 and 21%, respectively, of the amounts removed in the control. All the 458 considerations done for MET-M can be reasonably extended also to these molecules. 459 Parlavecchia et al. (2020) found higher removals of BPA and OP in soil planted with 460 rocket salad, compared to unplanted soil. Brown et al. (2009) reported that NP 461 degradation was enhanced by the presence of winter wheat plants, compared to unplanted 462 soil, and concluded that a significant plant uptake of this molecule was unlikely. 463

465 3.3 Accumulation of contaminants in cucumber plants

466 Results obtained showed that cucumber plants were able not only to absorb all contaminants from the soil but also to accumulate them in their tissues. Kubicki et al. 467 468 (2019), studying the dynamic of MET-M in tomato, reported that the compound was readily taken up by the roots with the normal water flow and uniformly translocated to 469 470 the aerial organs through the xylem vessels. The amounts of residual contaminants found in both roots and shoots of 38-day grown plants are reported in Table 4. The presence of 471 472 all compounds in the aerial parts indicates that the plant is able to translocate the molecules evidencing the risks of the possible contamination of the edible plant parts. 473 Based on the results obtained, it is possible to state that residual contaminants did not 474 accumulate homogeneously in the plant but were generally found at higher concentrations 475 in the green organs. Teixeira et al. (2011) reported that the higher accumulation of MET-476 477 M in shoots of Solanum nigrum, compared to roots, could be explained by the low log Kow of this molecule which is easily transferred upward through both phloem and xylem 478 479 vessels, especially via the transpiration stream. In unamended soil (control), all 480 compounds were more concentrated in shoots than in roots and according to their solubility. A different situation was observed in amended soil where MET-M and BPA 481 were always more concentrated in the shoots than in the roots, while OP and NP were in 482 general slightly more concentrated in roots than in shoots. That may depend on the higher 483 hydrophobicity of the latter molecules that made plant translocation more difficult. Abril 484 485 et al. (2021), studying the bioconcentration and translocation of different types of contaminants in radish, reported that BPA was only detected in radish bulb, and explained 486 487 this with the poor translocation to aerial organs due to the hydrophobicity of the compound and its rapid metabolization by plant cells. 488

A very interesting finding of this study is the relevant reduction of contaminant 489 490 residues in all plants grown on amended soil, compared to the control, clearly indicating the important role of CP and VC in counteracting the uptake and accumulation of 491 492 contaminants in plant tissues. The abatement of residual compounds in plants cultivated in amended soil occurred to varying degrees based on the type and dose of the amendment 493 494 applied and the nature of the contaminant. In almost all treatments and for all molecules, the higher dose was more effective in reducing the accumulation of the contaminants both 495 496 in roots and in shoots, indicating once again the importance of the organic fraction of soil in reducing the absorption and accumulation of contaminants in plants. Furthermore, 497 considering the contamination as a whole, residues accumulation in roots followed the 498 trend $VC_H < CP_H < VC_L < CP_L < control$. In shoots, MET-M and BPA residues followed 499 the trend $VC_H = VC_L < CP_H = CP_L < control, while OP and NP residues were similar in$ 500 501 the treatments VC_H, VC_L and CP_H, significantly higher in CP_L and much higher in the 502 control.

503 When the amounts of contaminants accumulated in the whole plant were compared 504 to the amounts initially added to the soil, it was evident that, even in the control, they were small percentages, ranging between 2 and 6%, and inversely related to the 505 hydrophobicity of the contaminant (Table 5). Li et al. (2019) studied the distribution of a 506 507 large number of contaminants in the soil-water-plant systems and found that they were 508 metabolized in plant tissues via different dissipation patterns. Compared to the control, 509 any treatment significantly reduced the quantity of residues accumulated by the plant, 510 being CP and VC at both doses not statistically ($P \le 0.05$) different for MET-M and BPA, 511 while CP_H was slightly more efficient for OP and NP (Table 5).

512 In order to evaluate a possible influence of the amendments also on the 513 transformation rate of the contaminants by the plant, the percentages of accumulated

514 contaminants with respect to removed (degraded + absorbed) contaminants were 515 calculated (Fig. 5). Results obtained suggest that soil treatment with CP and VC, in 516 addition to influencing contaminant absorption, appear to be able to regulate the 517 biological breakdown of all compounds, as the transformation of contaminants seems 518 faster in the plants grown in amended soil (Fig. 5). However, further studies are needed 519 to better clarify this aspect.

520

521 CONCLUSIONS

When present in soil, the fungicide MET-M and the endocrine disruptors BPA, OP 522 and NP can be absorbed by the root system of horticultural plants, like cucumber, and 523 translocated to various organs. This is more likely to occur at low organic matter content 524 of the soil, and poses a serious threat to animal and human health. Soil amendment with 525 526 carbon-rich materials, such as CP and VC, can effectively hinder the entry of pollutants into plants. Both CP and VC at the doses used in this study demonstrated significant 527 528 potential to support the growth of cucumber plants in both uncontaminated and multi-529 contaminated soils. In the latter condition, both amendments exerted a crucial antitoxic activity that helped the plant to tolerate the stress condition. Each soil treatment with CP 530 and VC improved all biometric parameters of cucumber plants, especially fresh and dry 531 biomass. Soil amendment appeared to reduce the availability of each contaminating 532 molecule and was very effective in preventing the uptake and accumulation of the 533 contaminants by cucumber plants. At the end of the experiments, residues of all 534 contaminants, especially the less hydrophobic ones, were found both in roots and in 535 shoots of all plants at much lower concentrations in amended soil than in not amended 536 soil. Our findings suggest that beside this key role in managing the soil fertility and 537 increasing plant productivity (Chen et al., 2018; Blouin et al., 2019), soil addition of 538

539 composted materials may help cultivated soils to mitigate toxic pressure from 540 environmental contamination. Finally, the overall results obtained indicated that both 541 amendments, in addition to influencing plant uptake and accumulation of organic 542 contaminants, may be able to regulate their metabolic fate in plant tissues.

543

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706

708 Figure Captions

Figure 1. Biometric data of cucumber plants grown on uncontaminated soil (NC) and 709 contaminated soil only (control) or amended with CP and VC at the lower dose (CPL 710 711 and VC_L) and the higher dose (CP_H and VC_H). The vertical line on each bar indicates 712 the standard error (n = 3). Data were statistically treated with one-way ANOVA and 713 the means of the treatments were compared to the corresponding soil only by the LSD test. *, $P \le 0.05$; **, $P \le 0.01$; ***, $P \le 0.01$; ***, $P \le 0.001$ 714 715 Figure 2. Root (RMR) and shoot (SMR) dry mass ratios and total dry weights of cucumber 716 plants. Figure 3. Growth parameters of cucumber plants grown on uncontaminated (NC) or 717 contaminated (C) soils compared to the respective control plants: non contaminated 718 (NC soil) and contaminated (C soil). Significant differences were calculated between 719 720 control and compost (*) or vermicompost (+) treatments in uncontaminated or contaminated conditions (one-way ANOVA, $*P \le 0.05$; **P < 0.01; ***P < 0.001). 721 722 Figure 4. Relationships between residual compounds extracted from bare or planted soil 723 or their difference and corresponding log Kow of the compounds. Figure 5. Ratio between the amounts of residual compounds accumulated in the whole 724 plant and the amounts removed (degraded + absorbed) from planted soil in a period of 725 38 days. 726



Fig. 1



Fig. 2









Fig. 3





Compound	Chemical structure	Molecular weight (g mol ⁻¹)	Water solubility (mg L ⁻¹)	log K _{ow}
Metalaxyl-M	CH ₃ CH ₃ CH ₃ CH ₃ OCH ₃ OCH ₃ OCH ₃	279.33	8.4	1.65
Bisphenol A	H ₃ C CH ₃ HO OH	228.29	300	3.32
4-tert-Octylphenol	<i>t</i> -Bu H ₃ C CH ₃	206.32	3.1	5.50
4-Nonylphenol	H ₃ C CH ₃ CH ₃	220.35	0.1	5.76

Table 1. Major properties of tested contaminants.

Data from PubChem open chemistry database at the National Institutes of Health (2022)

Variable	Soil	Compost	Vermicompost
Sand (%)	37	-	-
Silt (%)	50	-	-
Clay (%)	13	-	-
pH ^a	8.0 ± 0.2	8.4 ± 0.3	6.9 ± 0.1
EC^{b} (dS m ⁻¹)	0.06 ± 0.01	5.42 ± 0.58	7.69 ± 0.37
Moisture (% fw)	4 ± 0.1	16 ± 0.1	19 ± 0.3
Ash (% dw)	-	-	26.7
TOC (% dw)	3.3 ± 0.1	27.0 ± 4.0	31.6 ± 3.1
C/N	18 ± 2	17	20
TN (% dw)	0.19 ± 0.02	1.60 ± 0.20	1.58 ± 0.10
Organic N (% dw)	-	1.4 ± 0.2	1.4 ± 0.1
Humic and fulvic C (% dw)	-	9.9 ± 1.5	13.4 ± 1.4
Total CaCO ₃ (% dw)	15.4 ± 0.06	-	-
CEC (cmol ₊ kg ⁻¹)	28.8 ± 3.8	-	-

Table 2. Major properties of the soil and the organic amendments

Treatment	Bare soil	Planted soil	Average			
MET-M; $0.05P = 1.76^{a}$; $0.01P = 2.37^{a}$						
control	83.36	43.88	63.62 De			
CPL	91.59	71.23	81.41 Cd			
CP _H	96.98	81.70	89.34 Bc			
VCL	92.09	88.37	90.23 Bb			
VC _H	97.07	91.37	94.22 Aa			
Average	92.22 Aa	75.31 Bb				
	BPA; 0.05 <i>P</i> =	6.53; 0.01 <i>P</i> =8.79				
control	85.19	60.95	73.07 Cc			
CPL	94.84	88.43	91.63 Bb			
CP _H	97.42	88.24	92.83 ABb			
VCL	93.92	91.15	92.53 ABb			
VC _H	97.45	94.16	95.80 Aa			
Average	93.76 Aa	84.59 Bb				
	OP; $0.05P = 2$					
control	87.99	75.81	81.90 Cc			
CPL	95.20	91.29	93.25 Bb			
CP _H	97.79	95.74	96.77 Aa			
VCL	94.27	92.40	93.34 Bb			
VC _H	98.68	95.63	97.16 Aa			
Average	94.79 Aa	90.17 Bb				
	NP; $0.05P = 6$	5.66; 0.01 <i>P</i> =8.97				
control	89.19	69.88	79.53 Bc			
CPL	96.67	91.16	93.91 Ab			
СРн	99.06	96.36	97.71 Aa			
VCL	95.21	94.40	94.80 Aab			
VC _H	96.99	95.85	96.42 Aab			
Average	95.42 Aa	89.53 Ab				

Table 3. Effects of plant, soil treatment and their interaction on the percentage of residual contaminant recovered from the soil after 38 days, compared to the initial amount added (100%).

Note: Data were statistically treated with two-way ANOVA. Significant differences between means are indicated by different letters according to the Duncan's multiple range test at $P \le 0.05$ and $P \le 0.01$.

^aLSD for the interaction treatment × soil (bare or planted) at $P \le 0.05$ and $P \le 0.01$ (n = 3).

Compound	control	CPL	CP _H	VCL	VC _H
		R	oots		
MET-M	$105.47 \pm 17.38^{a} a$	$58.28 \pm 11.51 \text{ b}$	$16.16 \pm 1.26 \text{ c}$	$21.79\pm2.95~c$	$10.04\pm0.86~c$
BPA	76.01 ± 7.47 a	$29.73\pm7.15~\text{b}$	$8.17\pm0.01\ c$	$18.25\pm0.94~bc$	$7.40\pm0.12~c$
OP	51.33 ± 1.78 a	22.42 ± 1.64 b	$7.38\pm0.13~\text{d}$	14.97 ± 1.79 c	$6.30\pm0.86~d$
NP	46.69 ± 1.95 a	21.66 ± 2.05 b	$4.62\pm0.02\;d$	9.87 ± 1.21 c	6.12 ± 0.32 c
		St	noots		
MET-M	274.82 ± 28.96 a	$97.47\pm3.27~b$	$67.48\pm2.51~b$	30.78 ± 0.15 c	24.38 ± 3.63 c
BPA	177.03 ± 12.21 a	$41.02\pm1.19~\text{b}$	$44.64\pm0.52~b$	19.93 ± 0.56 c	14.73 ± 0.83 c
OP	99.56 ± 0.49 a	$20.51\pm0.58~\text{b}$	$8.91 \pm 0.40 \; d$	$7.48 \pm 0.12 \text{ d}$	12.04 ± 0.23 c
NP	98.16 ± 1.37 a	$17.72\pm0.57~b$	$4.38\pm0.20~d$	7.16 ± 0.04 c	$5.64\pm0.08\ cd$

Table 4. Amounts (µg per g of dry plant mass) of residual compounds in 38-d grown cucumber plants.

Note: Data were statistically analysed by one-way ANOVA and significant differences between means of each row are indicated by different letters according to the Duncan's multiple range test at $P \le 0.05$. ^a Standard error of the mean (n = 3)

Table 5. Percentage of residual compounds accumulated in total plant mass compared to the initial quantity added to the soil.

Compound	control	CPL	CP _H	VCL	VC _H
MET-M	$6.30\pm0.73^{a}a$	$3.39\pm0.29~b$	3.11 ± 0.15 b	$2.37\pm0.12~b$	$2.20\pm0.39~b$
BPA	4.20 ± 0.87 a	$1.46\pm0.14~\text{b}$	$2.04 \pm 0{,}09 \text{ b}$	$1.57\pm0.13~b$	$1.40\pm0.29~b$
OP	2.36 ± 0.37 a	$0.77\pm0.10~\text{bc}$	$0.52\pm0.01~c$	$0.64\pm0.06~c$	$1.14\pm0.21~b$
NP	1.99 ± 0.04 a	$0.67\pm0.07~b$	$0.28\pm0.02\;c$	$0.59\pm0{,}05~b$	$0.56\pm0.08\ b$

Note: Data were statistically analysed by one-way ANOVA and significant differences between means of each row are indicated by different letters according to the Duncan's multiple range test at $P \le 0.05$. ^a Standard error of the mean (n = 3)

Supplementary Material

Compost and vermicompost in cucumber rhizosphere promote plant growth and prevent the entry of anthropogenic organic pollutants

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Table S1. Significant effects of the soil contamination treatment and organic amendment dosage (compost or vermicompost) on fresh and dry shoot and root weight of cucumber plants presented as *F*-values and level of significance (* $P \le 0.05$; **P < 0.01; *** P < 0.001; ns, not significant) estimated by two-way ANOVA (contamination x organic dosage).

		COMPOST	<u>[</u>		
	SHO	ТОС	R	ТОС	ТОТ
	F.W.	D.W.	F.W.	D.W.	D.W.
Organic dosage (D)	132.05***	41.47***	27.52**	169.51***	21.80**
Contamination (C)	44.80***	111.34***	55.52***	289.04***	62.32***
D imes C	23.10***	9.01**	16.22**	85.92***	5.55*
\mathbb{R}^2	0.967	0.946	0.960	0.993	0.951
r	0.984	0.973	0.980	0.996	0.975
		VERMICOMP	OST		
	SHO	ТОС	R	ТОС	TOT
	F.W.	D.W.	F.W.	D.W.	D.W.
Organic dosage (D)	103.08***	103.12***	52.10***	248.17***	113.54***
Contamination (C)	46.12***	23.03**	n.s.	52.65***	8.41*
D imes C	16.01***	35.12**	11.86**	86.03***	33.34**
\mathbb{R}^2	0.959	0.961	0.956	0.992	0.981
	0.080	0 981	0.978	0.996	0.990

Table S2. Significant effects of the soil contamination treatment and organic amendment dosage (compost or vermicompost) on growth parameters in cucumber plants presented as *F*-values and level of significance (* $P \le 0.05$; **P < 0.01; *** P < 0.001; ns, not significant) estimated by two-way ANOVA (contamination x organic dosage).

		COMPOST					
	ROOT LENGTH	SURFACE AREA	ROOT VOLUME	DIAMETER			
Organic dosage (D)	2661.64***	658.95***	102.49***	239.64***			
Contamination (C)	89.47***	28.44**	n.s.	n.s.			
D imes C	54.63***	15.99**	n.s.	216.49***			
\mathbb{R}^2	0.999	0.996	0.972	0.993			
r	0.999	0.998	0.986	0.997			
VERMICOMPOST							
	ROOT LENGTH	SURFACE AREA	ROOT VOLUME	DIAMETER			
Organic dosage (D)	4038.10***	838.78***	151.57***	188.28***			
Contamination (C)	721.33***	46.79***	n.s.	399.03***			
D imes C	757.65***	81.58***	7.20*	32.84**			
\mathbb{R}^2	0.999	0.997	0.982	0.993			
r	1.000	0.998	0.991	0.996			
r	1.000	0.998	0.991				



Figure S1. Morphological parameters of cucumber plants grown on uncontaminated (NC) or contaminated (C) soils compared to the respective control plants: non contaminated (NC soil) and contaminated (C soil). Significant differences were calculated between control and compost (*) or vermicompost (+) treatments in uncontaminated or contaminated conditions (one-way ANOVA, $*P \le 0.05$; **P < 0.01; ***P < 0.001).

Table S3. Significant effects of the soil contamination treatment and organic amendment dosage (compost or vermicompost) on morphological ratios in cucumber plants presented as *F*-values and level of significance (* $P \le 0.05$; **P < 0.01; *** P < 0.001; ns, not significant) estimated by two-way ANOVA (contamination x organic dosage).

		COMPOST		
	SRL	SRSA	SRV	RTD
Organic dosage (D)	133.82***	87.16***	56.84***	83.05**
Contamination (C)	17.13**	8.83*	n.s.	17.46**
D imes C	22.78**	18.31**	14.57**	32.20**
\mathbf{R}^2	0.982	0.973	0.961	0.976
r	0.991	0.987	0.980	0.988
	VI	ERMICOMPOST		
	SRL	SRSA	SRV	RTD
Organic dosage (D)	21.23**	118.47***	73.95***	280.09***
Contamination (C)	n.s.	16.57**	n.s.	6.62*
D imes C	n.s.	n.s.	n.s.	20.04**
\mathbb{R}^2	0.889	0.978	0.964	0.990
r	0.943	0.989	0.982	0.995