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

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Abstract

An accurate assessment of the absorption and accumulation of soil pollutants by plants is 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 a mixed compost (CP) and a vermicompost (VC) from digestate, at doses of 10 t ha^{-1} (CP_L and VC_L) and 30 t ha^{-1} (CP_H and VC_H), in sustaining the growth of cucumber (*Cucumis sativus* L.) plants and reducing the uptake of contaminats, such as the fungicide metalaxyl-m (MET-M) and the endocrine disruptors bisphenol A (BPA), 4-tert-octylphenol (OP) and nonylphenol (NP). Plant response to soil amendment with CP and VC was tested in parallel in both contaminated and uncontaminated soil. All treatments significantly promoted cucumber growth in both soil conditions. After 38 days of cucumber growth in contaminated soil, CP_L, CP_H, VC_L and VC_H increased dry biomass of roots and shoots by 42, 128, 118, 147%, and 46, 113, 271, 443%, respectively, compared to unamended soil (control). Root and shoot elongation and the number of leaves and their expansion were also significantly promoted by the application of CP and

VC at both doses. All treatments, in the order: $VC_H > VC_L > CP_H > CP_L$, considerably 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 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 that the use of CP and VC can be very effective and sustainable both from an economic 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 residues in food plants.

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

1. Introduction

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 intensive agricultural practices. However, even in more remote areas and in uncultivated soils, researchers have reported the presence of persistent organic contaminants that are very harmful to wildlife and humans. According to the United Nations, nearly 2 billion ha (22.5%) of agricultural land, pasture, forest, and woodland are affected by soil pollution (United Nations 2019). A clean soil is essential for the maintenance of the ecosystem biodiversity and soil functioning thus ensuring global food security and 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 loss of the self-depollution capacity of soil.

Currently, a major concern among the international scientific community is due to the so-called anthropogenic organic pollutants (AOPs), a wide class that include different compounds such as agrochemicals, pharmaceuticals, dyes, wood preservatives, industrial 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, the normal chemical and biological equilibria and processes in both undisturbed and cultivated soils. AOPs can reach the soil on purpose, as in the case of agrochemicals for controlling crop diseases, or inadvertently and sometimes illegally through the incorporation into soil of not completely decontaminated liquid and solid wastes, such as sewage sludges, wastewaters, biosolids and biowaste of agro-industrial origin (Geissena et al. 2015; Silva et al. 2019).

Agrochemicals, including herbicides, fungicides, insecticides, nematicides and so on, 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 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 to different tissues where they accumulate, which is particularly dangerous in the case of staple food crops because toxic residues can enter the food chain of animals and humans. Furtheremore, these chemicals, especially the more polar ones, can pose serious environmental problems due to their movement into soil and transport in surface- and groundwater (Loffredo et al. 2021).

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, MET-M 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).

Multiple contamination can be considered the norm for intensively cultivated soils, where agrochemicals can be simultaneously present in soil with other classes of AOPs 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 and metabolism of animals and humans (Corrales 2015; EC 2020). EDCs are used in many industrial products and, consequently, they are constantly released into terrestrial and aquatic environments where represent a serious threat to wildlife, especially aquatic, 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 and/or disposal of urban and industrial effluents, sludges, biowaste from various production activities, including agriculture, and biosolids application (Loffredo 2022).

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

Another aspect related to intensive agriculture is the general progressive reduction of 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, 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, improving soil fertility, stimulating plant growth and microbial activity, and promoting carbon sequestration (Diacono and Montemurro, 2010; Schimmelpfennig et al. 2014). CP 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 et al. 2015). This process allows to control pollutants bioavailability and limit their transport into natural waters (Gámiz et al. 2016; Parlavecchia et al. 2019). Furthermore, there is growing concern about the residues of pollutants and their metabolites that remain in harvested crops, particularly in the edible parts of plants, and can then be ingested by humans and animals via food or feed.

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 considered an important indicator of the general stress status of the plant. The plant root system has a certain variability (plasticity) that represents a major survival strategy to cope with a wide range of soil factors and external stresses. Various morphological parameters such as the length, surface area, volume and diameter are used as potential 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 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). All these morphological parameters are commonly used to evaluate plant responses to 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.

2. Materials and methods

2.1 Chemicals, soil, amendents and plant

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

A loamy calcareous agricultural soil sampled at 0–20 cm depth at an experimental station located at Valenzano, South Italy, was used. The soil was air-dried, sieved at <3-mm particle size to remove the coarser fraction and thoroughly homogenized. Soil properties were determined according to standard methods (Sparks et al. 1996). Briefly, soil moisture was measured after heating at 105 °C overnight; pH was potentiometrically measured in a 1:2.5 (w/v) soil-to-0.01 M $CaCl_2$ solution mixture (pH_{CaCl2}); electrical conductivity was measured at 25 °C in a 1:2 (w/v) soil-to-water ratio slurry ($EC_{1:2}$ 25 °C); total organic C and N were determined by an elemental analyzer LECO CN628 (LECO Corporation, MI, USA); total $CaCO_3$ was determined by the gas-volumetric method using a Dietrich–Fruhling calcimeter; cation exchange capacity (CEC) was measured by using

0.1 M $BaCl_2$ 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 (Calabra Maceri & Servizi S.p.a., Rende, Italy) after a 3-month accelerated composting process of mixed animal and plant waste. The VC sample was provided by C&F Energy, Società Agricola S.r.l. (Silentina, Italy) after 2-month vermicomposting with redworms (*Lumbricus rubellus*) of a digestate resulting from the anaerobic digestion process of a mixture of buffalo manure, chicken manure and olive oil mill wastewater. Major characteristics of CP and VC are reported in Table 2. Before use, CP and VC samples were air-dried, finely ground and 0.5-mm sieved.

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

2.2 Experimental protocol

Cucumber seeds were germinated in Petri dishes (9-cm diameter) kept in the dark into a Phytotron growth chamber (F.lli Della Marca S.r.l., Roma, Italy, model 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, UN) or added into the upper soil layer (~ 3 cm), thus obtaining in the whole soil a concentration of 1 µg g^{-1} of each compound. After about 2 h, uniformly sized cucumber seedings were selected and not transplanted (bare soil) or transplanted (one seedling in each pot, planted soil) into the pots. Subsequently, a volume of 10 mL of distilled H_2O was added to each pot (with and without seedling). Thus, the following treatments were obtained: control (contaminated soil), CP_L, CP_H, VC_L, VC_H, NC (not contaminated soil), 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 H_2O per day. The pots were placed in the growth chamber according to a completely randomized design with 5 replications.

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 shoot fresh weights, shoot length, number of leaves per plant and length of the main leaf 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 Régent Inc., Canada) at a resolution of 600 dpi for morphological analyses. Scanned 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 shoot dry weights were determined by oven-drying at 70°C for 16 h. Total plant dry weight was obtained by summing root and shoot dry weight. Based on the measurements 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 of root dry weight, SRSA), specific root volume (root volume per unit of root dry weight, SRV) and root tissue density (root dry mass per unit root volume, RTD) which represent functional parameters.

2.3 Extraction and quantification of contaminant residues from pot mixtures and plant tissues

After cucumber plant removal, each pot mixture was thoroughly homogenized; then an aliquot of 20 g sample was collected, added with 50 mL of methanol and kept under 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 solution was evaporated to dryness at a temperature of 40 °C using a rotatory evaporator. The solid residue was dissolved in a volume of 1 mL of acetonitrile/water mixture (60:40 *v/v*), filtered through 0.45 µm Millipore™ cellulose acetate filters and analysed by reverse 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 µg g^{-1} were, respectively, 92.20 ± 1.61, 92.43 ± 0.80, 91.82 ± 2.08 and 94.6 ± 4.6% (*n=4*). The percentages of the compounds disappeared in pot mixtures during the trial were calculated as the difference between the initial amounts and those extracted after 38 d.

Absorbed contaminant were extracted from plant tissues according to the procedure of Ferrara et al. (2006). Briefly, 0.1 g of dried shoot and root mass, individually, was 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 6 mL was evaporated to dryness at a temperature of 40 °C using a rotary evaporator. The residue was dissolved in a volume of 1 mL of acetonitrile/water mixture (60:40 *v/v*), filtered through 0.45 µm Millipore™ cellulose acetate filters and analysed by UHPLC technique (section 2.4).

2.4 Analytical measurement

The UHPLC system (Dionex Ultimate 3000 RSLC, Waltham MA, USA) used was equipped with an HPG-3200 RS pump, a WPS-3000 autosampler and a TCC-3000 column compartment connected to a Supelco™ LC-18 column (250 mm × 4.6 mm × 5 μm). The mobile phase was a mixture of water (A) and acetonitrile (B). The elution gradient adopted was the following: 0-1 min 40% A, 1-6 min from 40 to 30% A, 6-8 min 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 and 13.5, respectively. MET-M was detected using a DAD-3000 RS diode array detector (Dionex Ultimate 3000 RSLC, Waltham MA, USA) at a wavelength of 220 nm, while BPA, OP and NP were detected using a FLD-3400 RS fluorescence detector (Dionex Ultimate 3000 RSLC, Waltham MA, USA) operating at wavelengths of 200-nm excitation and 290-nm emission.

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 0.05*P*, 0.01*P*, and 0.001*P* levels. Data of residual compounds extracted from soil were analysed by two-way ANOVA and the means were separated at 0.05*P* and 0.01*P* levels using the Duncan's multiple range test for the main factors and the LSD test for the interaction.

3. Results and discussion

3.1 Plant response to soil amendment

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 significantly higher in presence of both CP_L and VC_L, compared to the unamended control (NC), while VC_H positively affected only shoots (Fig. 1A and B). A similar trend was found for root dry weights (Fig. 1C), while shoot dry weights were increased only by the 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 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) and on the average length of the main leaf vein (Fig. 1G) which can be considered in indicator of leaf extension.

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).

Unfortunately, no results are present in the literature on toxic effects of NP on agricultural plants.

In the treatments with CP and VC, the toxic effects of the contaminants appeared noticeably mitigated by the presence of both amendments, with the only exception of CP_L on root fresh weight (Fig. 1A), and the stimulation was positively related to the 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 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 contaminated soil where shoot elongation was generally favoured by the amendments (Fig. 1E). Also in contaminated soil, CP and VC generally increased the number of leaves per plant (Fig. 1F) and the average length of the main leaf vein (Fig. 1G).

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 benefit from the antitoxic activity of these amendments. These positive effects may be 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 in soil pore water and their bioavailability for plants (Hurtado et al. 2017). In a recent study, the toxicity of BPA and OP on hemp plants was significantly attenuated by the addition of a CP which increased root and shoot dry weights by more than 50%, compared to unamended soil (Loffredo et al. 2021). Evident antitoxic effects on rocket plants grown on a soil spiked with a mixture of contaminants, including BPA and OP, were observed 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 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 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. 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 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 20% of VC with soil resulted in 98% average increase of *Dracocephalum moldavica* biomass, compared to soil only (Ose et al., 2021).

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 all morphological parameters compared to control plants (NC), particularly with VC_L 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 the control soil, but lesser than that in NC soil. The two-way ANOVA (*F*-ratios) 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 to indicate root functionality because it expresses the potential for solute and water uptake

(Ryser, 2007). In uncontaminated soil, root diameter increased significantly in the 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" 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 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 indicated that the amendment dose, rather than the presence of the contaminants, caused the highest variability in the morphological parameters (Table S2).

Specific root length (SRL), specific root area (SRA) and specific root volume (SRV) describe the potential of the root apparatus to develop and contact soil by investing a given amount of photosynthate. All these parameters are significantly increased on 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 aspect of the root system and is frequently used as an indicator of root fineness (Panuccio *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 contrasting responses could be in part explained by considering that SRL is a complex parameter that includes variations in length, diameter and root tissue density, which respond to environmental conditions differently. Root tissue density (RTD) is defined as 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 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 density are generally associated with a slow growth in infertile soil (Ryser 2007). On this basis, the significant increase of SRL on contaminated soil (Fig. S1) can be in part due to 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 S2). Two-way ANOVA showed that in general the concentration rather than the contamination determined the greatest variability in morphological ratios, particularly 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 advantageous in high-resource environments leading to a fast plant growth. Conversely, this acquisition strategy is disadvantageous when resources are scarce, due to excess tissue building costs. In fact, as reported by Fitter (1991), even if roots with a smaller diameter can contact a larger soil volume per unit surface area, the maintenance carbon cost of producing finer roots is higher as these will have to be replaced more frequently. 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 the exploration of a larger soil volume.

3.2 Absorption and accumulation of contaminants in plant tissues

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 ($P \leq 0.01$) for MET-M, BPA and OP, and significant ($P \leq 0.05$) 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 mechanisms and forces of various type and strength which include ionic, hydrogen and covalent bonding, charge-transfer or electron donor-acceptor mechanisms, dipole–dipole 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 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 adsorption/immobiliation and, possibly, biological degradation, plant uptake contributed to the removal of contaminants, as found in planted pot soil. The distribution of contaminants between soil and pore water (adsorption) in combination with biodegradation controls the bioavailability of the compounds to plant uptake. Absorption of most organic chemicals by plant roots is a passive and diffusive process that depends 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 occurred with different intensity based on the presence or absence of the plant and the 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 rhizosphere during cucumber growth, being a source of nourishment for microorganisms, might have promoted the dissipation of contaminants (rhizodegradation or phytostimulation), or (ii) the rapid root uptake of contaminants might have reduced their time of exposure to soil microorganisms with consequent lower biodegradation. Unfortunately, the experimental conditions of this study do not allow to discriminate and quantify the two possible processes.

Another factor that influences the absorption of contaminants by the plant are the physicochemical properties of the compound, primarily its hydrophobicity. When the percentages of residual compounds extracted from bare soil or planted soil or their 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 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 absorbed by the plant, assuming that the difference between the amount of residual compound in bare soil and planted soil may be approximated to the amount of absorbed compound. Negative correlation between contaminant lipophilicity and their uptake by plants was previously demonstrated for the contaminants in this study and other plant species (Jayampathi et al. 2021; Gong et al. 2020; Loffredo et al. 2021). Despite the very low solubility of OP and NP, data obtained clearly evidenced the ability of cucumber to take up these molecules. Bokern and Harms (1997) found that plants incorporated NP 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 was minimal.

Among the compounds examined, MET-M showed the maximum disappearance in both bare and planted soil and, on average, in all treatments (Table 3). This can be attributed to the relatively high water solubility of MET-M which allows the molecule to easily reach the roots and enter the plant with the water flow. The addition to the soil of CP or VC, especially at the higher dose, significantly ($P \leq 0.01$) increased the percentage of MET-M residues found in both bare and planted soil (Table 3). The effects were particularly evident in planted pot soil where residual MET-M was about 44% in the control and much more in the treatments with a maximum of 91% in VC_H (Table 3). It is not easy to explain these results which may depend on several factors. One hypothesis is that the increased retention capacity of the amended soil may have reduced the availability of the contaminant for microbial degradation; another hypothesis is that the increased retention capacity of the amended soil may have involved also simple organic compounds, including root exudates, which constitute a nutrient source for microbial 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 largely demonstrated that MET-M adsorption occurs mainly on the organic fraction of soil and markedly increases after the addition of C-rich materials (Fernandes et al. 2003). 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 biomixture for MET-M was much higher than that of the soil only (Karanasios et al. 2010).

The general behaviour of BPA in both bare and planted soil was not very different 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 decreased the quantity of residual BPA, OP and NP in soil, compared to bare soil (Table 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 fact, averagely for soil treatments, the removal of BPA, OP and NP in amended soil was only about 25, 28 and 21%, respectively, of the amounts removed in the control. All the considerations done for MET-M can be reasonably extended also to these molecules. Parlavecchia et al. (2020) found higher removals of BPA and OP in soil planted with rocket salad, compared to unplanted soil. Brown et al. (2009) reported that NP degradation was enhanced by the presence of winter wheat plants, compared to unplanted soil, and concluded that a significant plant uptake of this molecule was unlikely.

3.3 Accumulation of contaminants in cucumber plants

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. (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 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 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. Based on the results obtained, it is possible to state that residual contaminants did not accumulate homogeneously in the plant but were generally found at higher concentrations in the green organs. Teixeira et al. (2011) reported that the higher accumulation of MET-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 vessels, especially via the transpiration stream. In unamended soil (control), all 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 were always more concentrated in the shoots than in the roots, while OP and NP were in general slightly more concentrated in roots than in shoots. That may depend on the higher hydrophobicity of the latter molecules that made plant translocation more difficult. Abril 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 this with the poor translocation to aerial organs due to the hydrophobicity of the compound and its rapid metabolization by plant cells.

A very interesting finding of this study is the relevant reduction of contaminant 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 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 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 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, considering the contamination as a whole, residues accumulation in roots followed the trend $VC_H < CP_H < VC_L < CP_L <$ control. In shoots, MET-M and BPA residues followed the trend $VC_H = VC_L < CP_H = CP_L <$ control, while OP and NP residues were similar in the treatments VC_H, VC_L and CP_H, significantly higher in CP_L and much higher in the control.

When the amounts of contaminants accumulated in the whole plant were compared 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 hydrophobicity of the contaminant (Table 5). Li et al. (2019) studied the distribution of a large number of contaminants in the soil-water-plant systems and found that they were metabolized in plant tissues via different dissipation patterns. Compared to the control, any treatment significantly reduced the quantity of residues accumulated by the plant, being CP and VC at both doses not statistically ($P \leq 0.05$) different for MET-M and BPA, while CP_H was slightly more efficient for OP and NP (Table 5).

In order to evaluate a possible influence of the amendments also on the transformation rate of the contaminants by the plant, the percentages of accumulated contaminants with respect to removed (degraded + absorbed) contaminants were calculated (Fig. 5). Results obtained suggest that soil treatment with CP and VC, in addition to influencing contaminant absorption, appear to be able to regulate the biological breakdown of all compounds, as the transformation of contaminants seems faster in the plants grown in amended soil (Fig. 5). However, further studies are needed to better clarify this aspect.

CONCLUSIONS

When present in soil, the fungicide MET-M and the endocrine disruptors BPA, OP and NP can be absorbed by the root system of horticultural plants, like cucumber, and translocated to various organs. This is more likely to occur at low organic matter content of the soil, and poses a serious threat to animal and human health. Soil amendment with 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 potential to support the growth of cucumber plants in both uncontaminated and multi-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 and VC improved all biometric parameters of cucumber plants, especially fresh and dry biomass. Soil amendment appeared to reduce the availability of each contaminating molecule and was very effective in preventing the uptake and accumulation of the contaminants by cucumber plants. At the end of the experiments, residues of all contaminants, especially the less hydrophobic ones, were found both in roots and in shoots of all plants at much lower concentrations in amended soil than in not amended soil. Our findings suggest that beside this key role in managing the soil fertility and increasing plant productivity (Chen et al., 2018; Blouin et al., 2019), soil addition of composted materials may help cultivated soils to mitigate toxic pressure from environmental contamination. Finally, the overall results obtained indicated that both amendments, in addition to influencing plant uptake and accumulation of organic contaminants, may be able to regulate their metabolic fate in plant tissues.

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Figure Captions

Figure 1. Biometric data of cucumber plants grown on uncontaminated soil (NC) and contaminated soil only (control) or amended with CP and VC at the lower dose (CP_L and VC_L) and the higher dose (CP_H and VC_H). The vertical line on each bar indicates the standard error (n = 3). Data were statistically treated with one-way ANOVA and the means of the treatments were compared to the corresponding soil only by the LSD test. $*,^{+}$ *$P \leq 0.05$*; $**,^{++}$ *$P \leq 0.01$*; $***,^{+++}$ *$P \leq 0.001$*

Figure 2. Root (RMR) and shoot (SMR) dry mass ratios and total dry weights of cucumber plants.

Figure 3. Growth 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, *$P \leq 0.05$; **$P< 0.01$; *** $P< 0.001$).

Figure 4. Relationships between residual compounds extracted from bare or planted soil or their difference and corresponding log Kow of the compounds.

Figure 5. Ratio between the amounts of residual compounds accumulated in the whole plant and the amounts removed (degraded + absorbed) from planted soil in a period of 38 days.

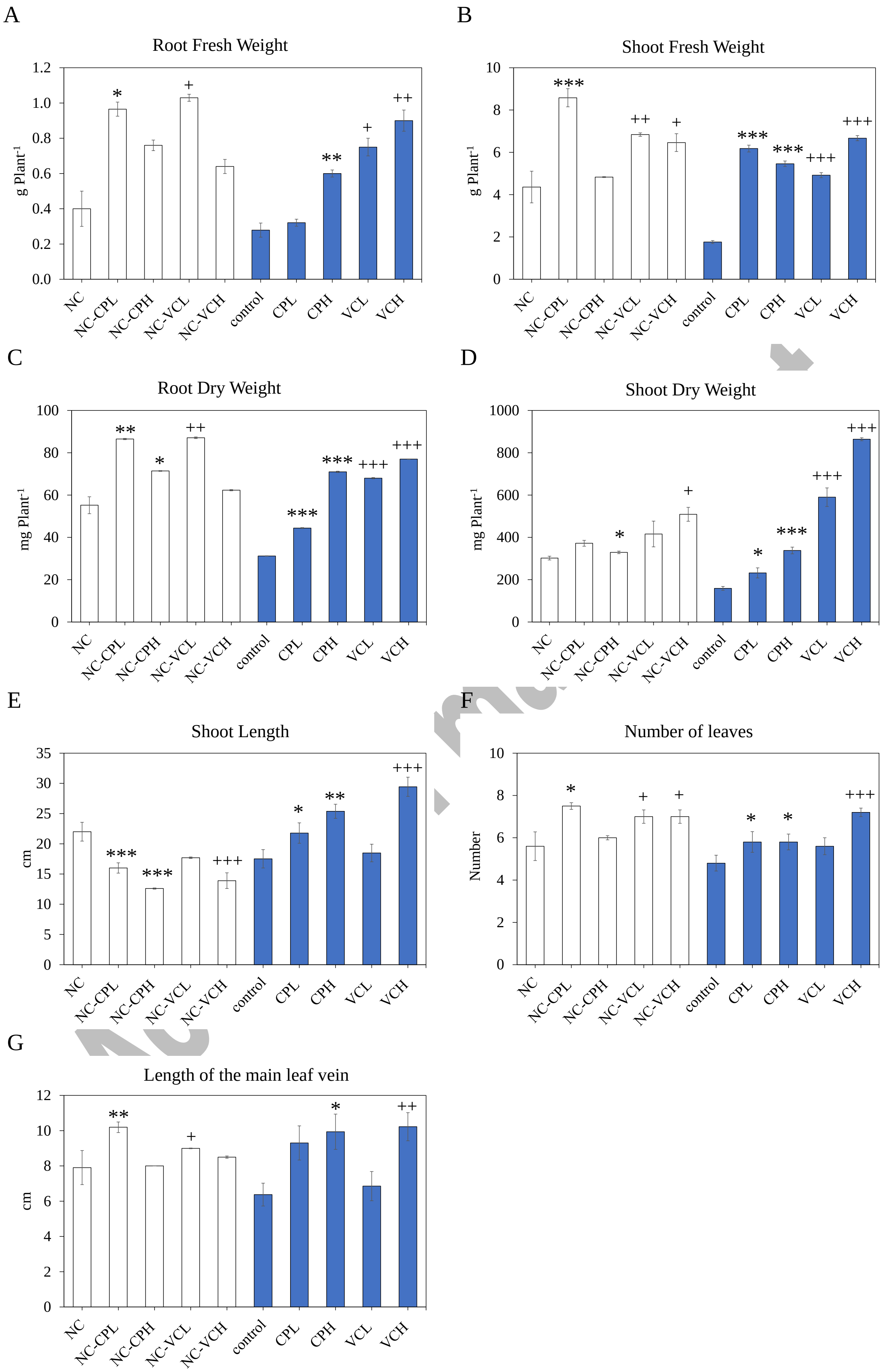
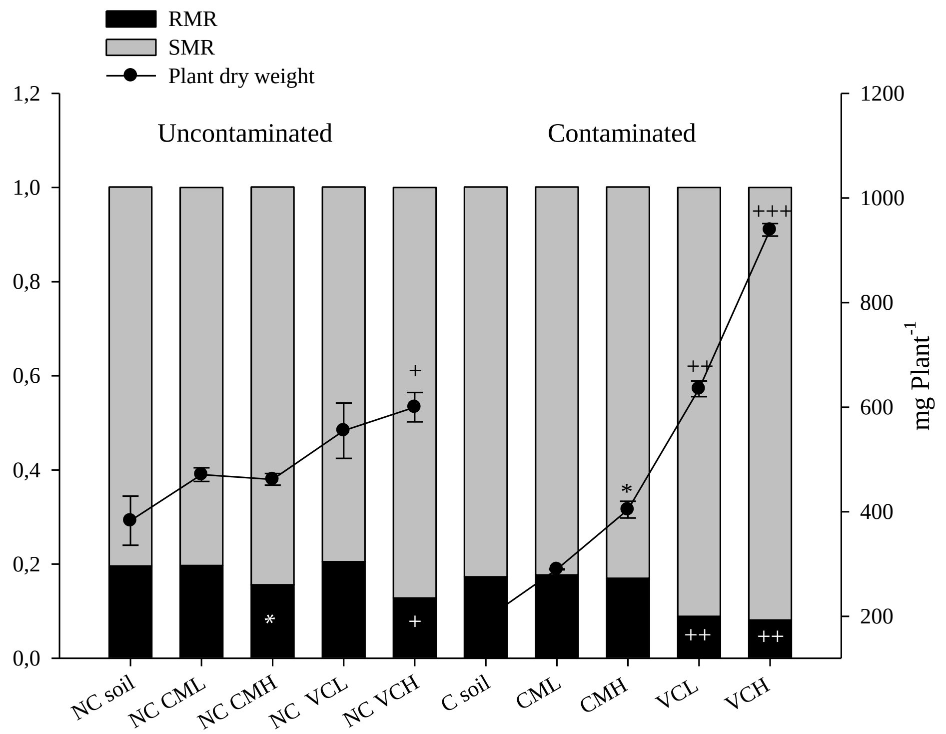

A
Root Fresh Weight
g Plant-1
B
Shoot Fresh Weight
g Plant-1
C
Root Dry Weight
mg Plant-1
D
Shoot Dry Weight
mg Plant-1
E
Shoot Length
cm
F
Number of leaves
Number
G
Length of the main leaf vein
cm
NC
NC-CPL
NC-CPH
NC-VCL
NC-VCH
control
CPL
CPH
VCL
VCH

Fig. 1



RMR
SMR
Plant dry weight
Uncontaminated
Contaminated
1,2
1,0
0,8
0,6
0,4
0,2
0,0
1200
1000
800
600
400
200
mg Plant-1
NC soil
NC CML
NC CMH
NC VCL
NC VCH
C soil
CML
CMH
VCL
VCH

Fig. 2

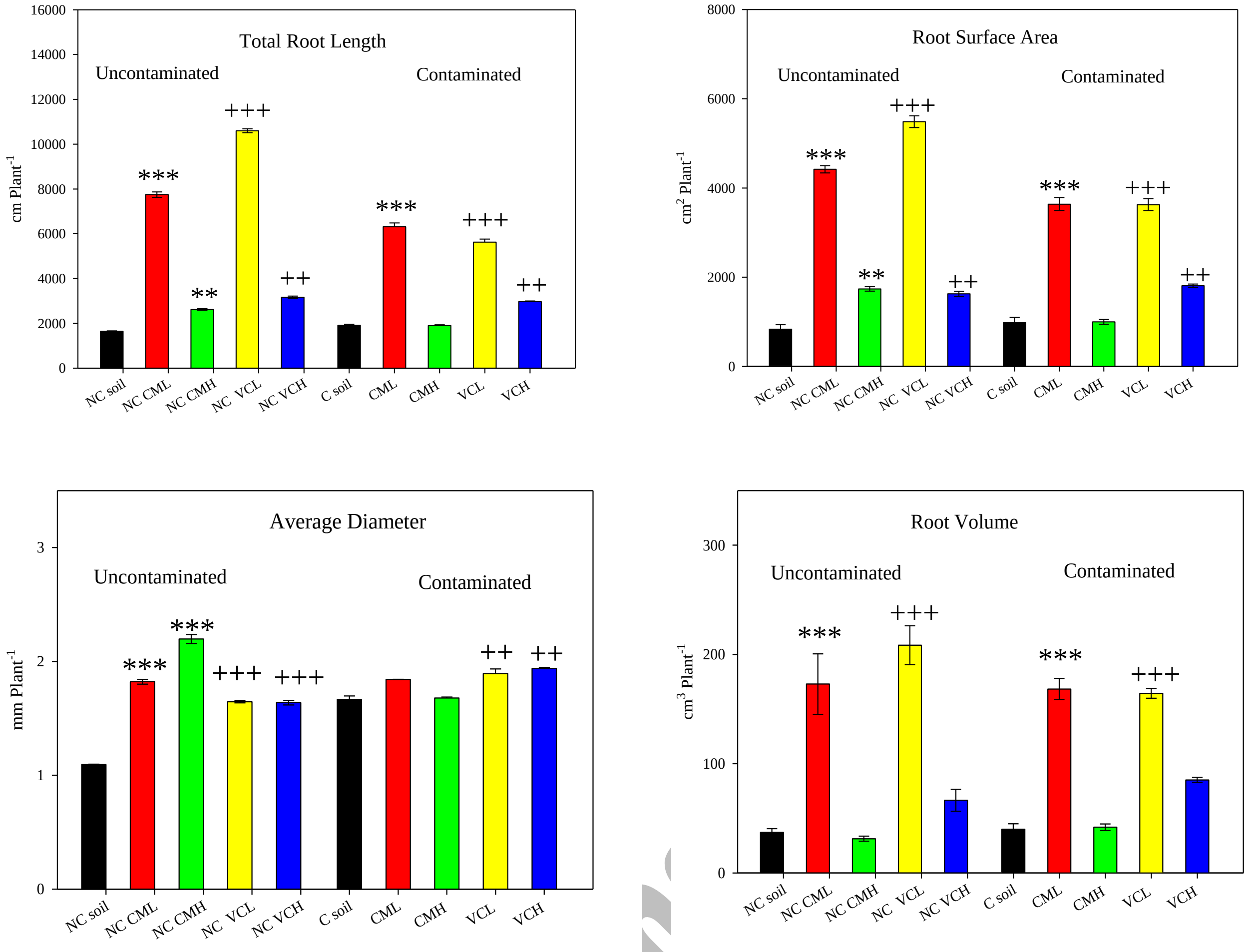

Total Root Length
Uncontaminated
Contaminated
cm Plant-1
Root Surface Area
cm2 Plant-1
Average Diameter
mm Plant-1
Root Volume
cm3 Plant-1
NC soil
NC CML
NC CMH
NC VCL
NC VCH
C soil
CML
CMH
VCL
VCH

Fig. 3

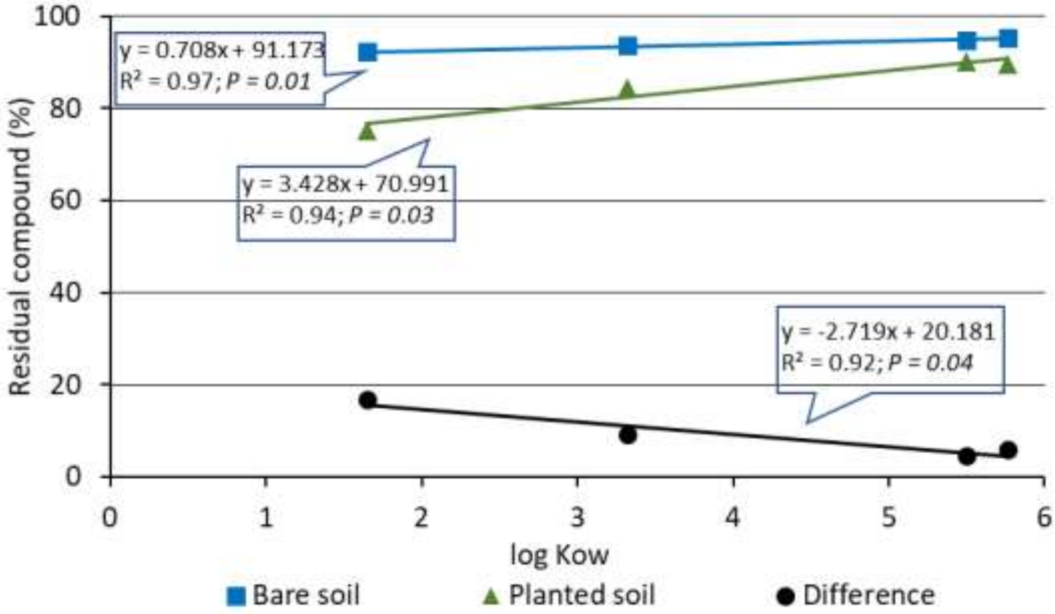

100
80
60
40
20
0
0
1
2
3
4
5
6
Residual compound (%)
log Kow
y = 0.708x + 91.173
R² = 0.97; P = 0.01
y = 3.428x + 70.991
R² = 0.94; P = 0.03
y = -2.719x + 20.181
R² = 0.92; P = 0.04
Bare soil
Planted soil
Difference

Fig. 4

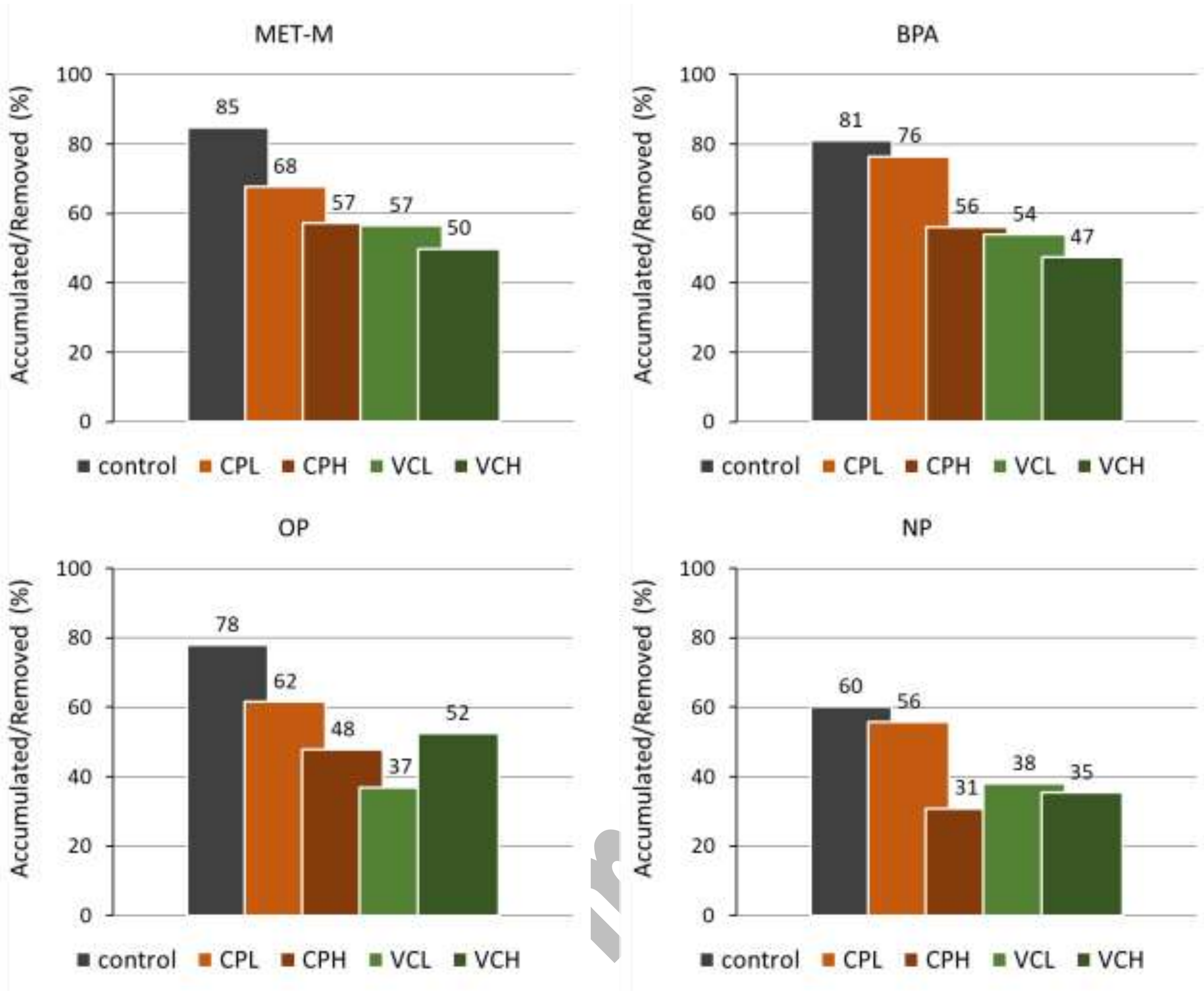
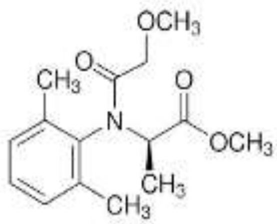
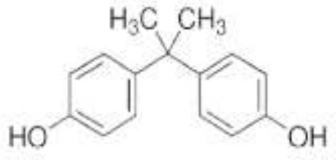
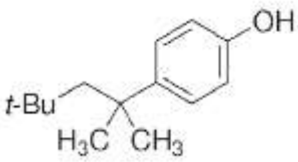
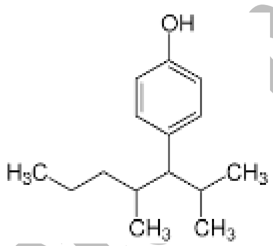

MET-M
85
68
57
57
50
BPA
81
76
56
54
47
OP
78
62
48
37
52
NP
60
56
31
38
35
Accumulated/Removed (%)
0
20
40
60
80
100
control
CPL
CPH
VCL
VCH

Fig. 5

Table 1. Major properties of tested contaminants.

Compound	Chemical structure	Molecular weight (g mol^{-1})	Water solubility (mg L^{-1})	log K_{ow}
Metalaxyl-M		279.33	8.4	1.65
Bisphenol A		228.29	300	3.32
4-tert-Octylphenol		206.32	3.1	5.50
4-Nonylphenol		220.35	0.1	5.76

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

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

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 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%).

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
CP_L	91.59	71.23	81.41 Cd
CP_H	96.98	81.70	89.34 Bc
VC_L	92.09	88.37	90.23 Bb
VC_H	97.07	91.37	94.22 Aa
Average	92.22 Aa	75.31 Bb	
	BPA; 0.05P= 6.53; 0.01P=8.79		
control	85.19	60.95	73.07 Cc
CP_L	94.84	88.43	91.63 Bb
CP_H	97.42	88.24	92.83 ABb
VC_L	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= 2.38; 0.01P=3.20		
control	87.99	75.81	81.90 Cc
CP_L	95.20	91.29	93.25 Bb
CP_H	97.79	95.74	96.77 Aa
VC_L	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= 6.66; 0.01P=8.97		
control	89.19	69.88	79.53 Bc
CP_L	96.67	91.16	93.91 Ab
CP_H	99.06	96.36	97.71 Aa
VC_L	95.21	94.40	94.80 Aab
VC_H	96.99	95.85	96.42 Aab
Average	95.42 Aa	89.53 Ab	

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 $P \leq 0.05$ and $P \leq 0.01$.

[a]LSD for the interaction treatment × soil (bare or planted) at $P \leq 0.05$ and $P \leq 0.01$ (n = 3).

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

Compound	control	CP_L	CP_H	VC_L	VC_H
			Roots		
MET-M	105.47 ± 17.38[a] a	58.28 ± 11.51 b	16.16 ± 1.26 c	21.79 ± 2.95 c	10.04 ± 0.86 c
BPA	76.01 ± 7.47 a	29.73 ± 7.15 b	8.17 ± 0.01 c	18.25 ± 0.94 bc	7.40 ± 0.12 c
OP	51.33 ± 1.78 a	22.42 ± 1.64 b	7.38 ± 0.13 d	14.97 ± 1.79 c	6.30 ± 0.86 d
NP	46.69 ± 1.95 a	21.66 ± 2.05 b	4.62 ± 0.02 d	9.87 ± 1.21 c	6.12 ± 0.32 c
			Shoots		
MET-M	274.82 ± 28.96 a	97.47 ± 3.27 b	67.48 ± 2.51 b	30.78 ± 0.15 c	24.38 ± 3.63 c
BPA	177.03 ± 12.21 a	41.02 ± 1.19 b	44.64 ± 0.52 b	19.93 ± 0.56 c	14.73 ± 0.83 c
OP	99.56 ± 0.49 a	20.51 ± 0.58 b	8.91 ± 0.40 d	7.48 ± 0,12 d	12.04 ± 0.23 c
NP	98.16 ± 1.37 a	17.72 ± 0.57 b	4.38 ± 0.20 d	7.16 ± 0.04 c	5.64 ± 0.08 cd

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 $P \leq 0.05$.
[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	CP_L	CP_H	VC_L	VC_H
MET-M	6.30 ± 0.73[a] a	3.39 ± 0.29 b	3.11 ± 0.15 b	2.37 ± 0.12 b	2.20 ± 0.39 b
BPA	4.20 ± 0.87 a	1.46 ± 0.14 b	2.04 ± 0,09 b	1.57 ± 0.13 b	1.40 ± 0.29 b
OP	2.36 ± 0.37 a	0.77 ± 0.10 bc	0.52 ± 0.01 c	0.64 ± 0.06 c	1.14 ± 0.21 b
NP	1.99 ± 0.04 a	0.67 ± 0.07 b	0.28 ± 0.02 c	0.59 ± 0,05 b	0.56 ± 0.08 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 $P \leq 0.05$.
[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 (*$P \leq 0.05$; **$P < 0.01$; *** $P < 0.001$; ns, not significant) estimated by two-way ANOVA (contamination x organic dosage).

	COMPOST				
	SHOOT		ROOT		TOT
	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 × C	23.10***	9.01**	16.22**	85.92***	5.55*
R^2	0.967	0.946	0.960	0.993	0.951
r	0.984	0.973	0.980	0.996	0.975
	VERMICOMPOST				
	SHOOT		ROOT		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 × C	16.01***	35.12**	11.86**	86.03***	33.34**
R^2	0.959	0.961	0.956	0.992	0.981
r	0.980	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* ≤ 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 × C	54.63***	15.99**	n.s.	216.49***
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 × C	757.65***	81.58***	7.20*	32.84**
R^2	0.999	0.997	0.982	0.993
r	1.000	0.998	0.991	0.996

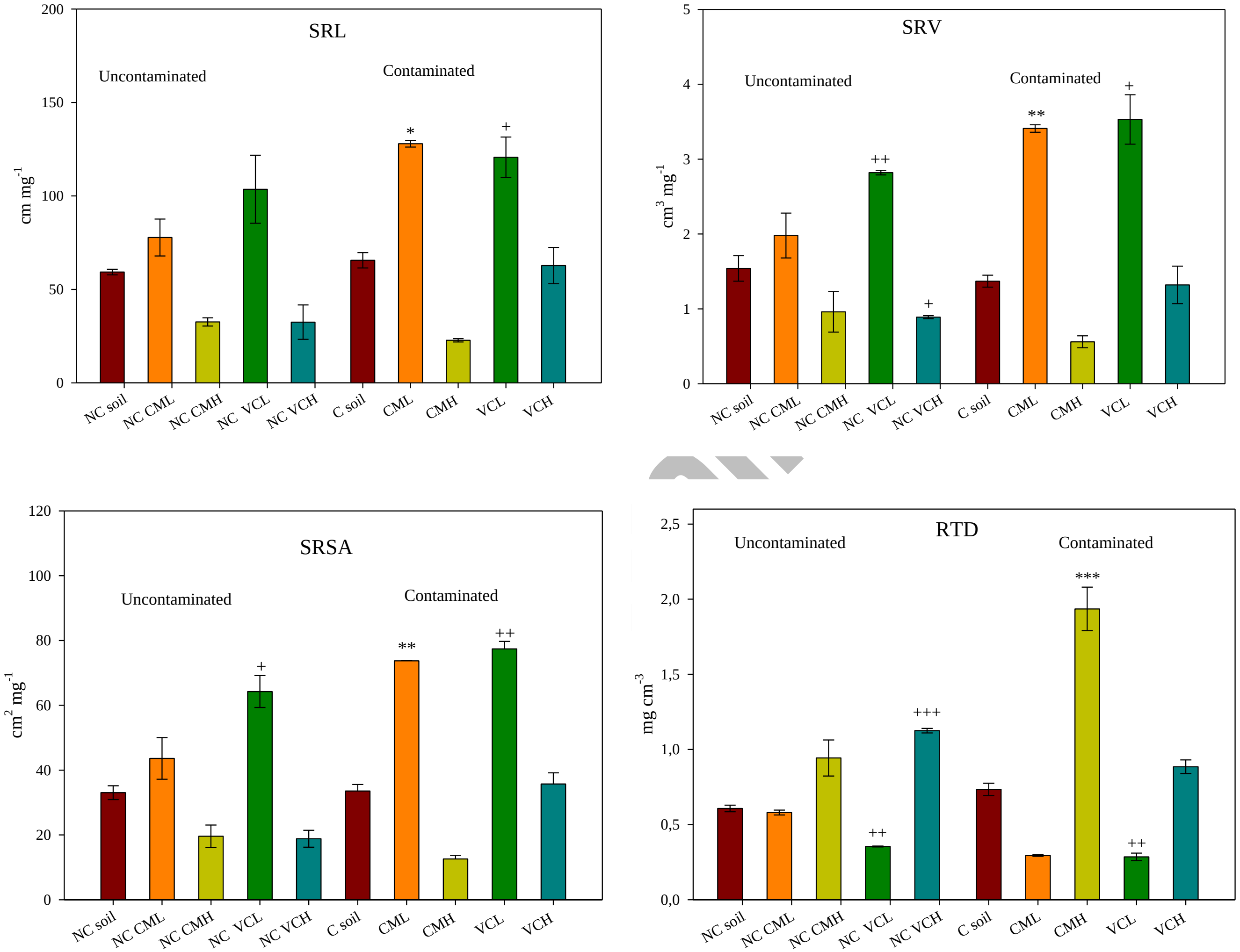

Figure S1. Morphological parameters of cucumber plants grown on uncontaminated (NC) or contaminated (C) soils compared to the respective control plants: non contaminated (NC soil) and contaminated (C soil). Significant differences were calculated between control and compost (*) or vermicompost (+) treatments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

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* ≤ 0.05; ***P* < 0.01; *** *P* < 0.001; ns, not significant) estimated by two-way ANOVA (contamination x organic dosage).

	SRL	SRSA	SRV	RTD
COMPOST				
Organic dosage (D)	133.82***	87.16***	56.84***	83.05**
Contamination (C)	17.13**	8.83*	n.s.	17.46**
D × C	22.78**	18.31**	14.57**	32.20**
R^2	0.982	0.973	0.961	0.976
r	0.991	0.987	0.980	0.988
VERMICOMPOST				
	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 × C	n.s.	n.s.	n.s.	20.04**
R^2	0.889	0.978	0.964	0.990
r	0.943	0.989	0.982	0.995