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The Effects of Rabbit-Manure-Derived Biochar Co-Application with Compost on the Availability and Heavy Metal Uptake by Green Leafy Vegetables

Agnieszka Medyńska-Juraszek *, Katarzyna Marcinkowska, Dariusz Gruszka and Kamila Kluczek

Institute of Soil Science, Plant Nutrition and Environmental Protection, Wroclaw University of Environmental and Life Sciences, Grunwaldzka 53 St., 50-357 Wroclaw, Poland

* Correspondence: agnieszka.medynska-juraszek@upwr.edu.pl

Abstract: The use of organic amendments to enhance plant growth is increasing due to horticulture activities and vegetable cultivation in urban areas. Consequently, as organic amendments impact heavy metal solubility and plant uptake of unknown contaminants, the risk of human exposure to potentially toxic elements from contaminated soils and compost is increasing. Biochar co-application with compost may reduce the risk-related increased metal uptake by edible plants. To verify this thesis, a greenhouse experiment was established to examine the effects of rabbit-manure-derived biochar (RBC) on Cu, Cr, Cd and Pb uptake by five green leafy vegetables (lettuce—*Lactuca sativa* L., spinach—*Spinacia oleracea* L., corn salad—*Valerianella locusta* L., kale—*Brassica oleracea* L., mustard greens—*Brassica juncea* L.) cultivated in compost substrate and soil amended with a 30% (v/w) mix of compost and biochar. The results indicated that the addition of biochar decreased Cu, Cr, Cd and Pb availability in the tested substrates, reducing the uptake of Cd in spinach by 61% and Pb in mustard greens by 73%. The application of RBC also had some adverse effects, such as enhanced accumulation of Cr by kale, lettuce and mustard greens cultivated in compost. Compost co-application with biochar to soil decreased the availability of metals, reducing the content of Pb and Cd in tissues of the tested vegetables, while uptake of Cu and Cr was enhanced in spinach and lettuce by 20%. In conclusion, the application of compost and biochar can be beneficial in improving the quality of urban soil used for horticulture purposes. However, more attention by gardeners should be paid to soil and compost testing in terms of heavy metal contamination and possible adverse effects of organic amendments application for green leafy vegetable cultivation.

Keywords: biochar; compost; soil; rabbit manure; heavy metals; trace elements; vegetables

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1. Introduction

The accumulation of heavy metals and metalloids in edible crops is an increasing concern nowadays. Potentially toxic metals can be introduced to soil and growing medium from many different sources. Anthropogenic sources such as agricultural inputs, municipal solid and liquid waste deposition or fallout of industry and urban emissions are considered global threats and the main cause of soil chemical degradation. Soil acidification, together with excessive accumulation of heavy metals in agricultural and urban soils, has consequences such as deteriorating the quality of crops and food products. Urban horticulture activities are increasing globally, with at least 100 million people involved worldwide [1]. Growing fruits and vegetables in urban and peri-urban areas may improve food security and access, public health and dietary quality. However, the benefits of urban horticulture should be balanced with the potential risks of exposure to contaminants such as heavy metals and pesticides [2]. Air, water and soil pollution constrain urban horticulture, and more attention should be paid to the quality of the environment. The main sources of heavy metals in urban soils are atmospheric fallout from fuel combustion, vehicle emissions, fertilizer use, municipal solid waste disposal and industrial waste [3]. Dense levels of habitation, centralized vehicle emissions, increasing amounts of industrial wastes, domestic emissions and weathering of building and pavement surfaces have led to continuous and increased emissions of heavy metals [4]. Potentially toxic elements accumulated in soil, compost and other organic amendments can be easily introduced to the food chain via vegetable cultivation on contaminated soil or the use of poor-quality organic amendments. Food, especially vegetables, is an essential part of the human diet, but it is more often considered a major intake source of toxic compounds [5]. Microgreens and baby greens are commonly cultivated and harvested vegetables grown in gardens and soilless substrates. Baby greens are generally harvested without roots 15 to 40 days from seeding. The chemical composition of a microgreen differs considerably from that of the mature form, and the content of minerals, nutrients and vitamins is usually higher [6]. As most green leafy vegetables belong to the family of *Asteraceae* and *Brassicaceae*, they are tolerant to elevated concentrations of heavy metals in soil [7]; however, several have been known to be heavy metal accumulators and have been evaluated as potential phytoextraction plants [8]. In terms of *Asteraceae* and *Brassicaceae* grown as micro- and baby greens, heavy metal accumulation in plant tissues brings the risk of unintentional heavy metal consumption by humans and animals.

With the increasing interest in urban horticulture, the demand for organic fertilizers, e.g., green manures, animal manures or compost, is also increasing. Organic fertilization and biological plant protection ensure people that the quality of food products is high and safe. Recently, a lot of attention has been paid to compost use in horticulture, used as a conditioner to improve soil physical and biological properties or as an organic fertilizer and relevant source of nutrients. Compost can also be an excellent substrate for soilless vegetable production [9,10], containing large amounts of soluble organic matter, which can be transformed into humic substances, mainly humic and fulvic acids, having a positive effect on plant growth and plant health [11]. Increases in soil organic carbon and improved plant growth are commonly reported; however, compost quality and its actions in soil may differ significantly depending on initial feedstock [12] and composting conditions [13]. One of the concerns of using organic amendments of anthropogenic origin is the presence of inorganic and organic compounds. The application of compost brings the risk of dissemination of emerging contaminants having adverse effects on animal and human health [14]. Heavy metals, arsenic, pesticide residues, petroleum hydrocarbons, antibiotics and healthcare products have been reported to be found in compost [15–17]. To mitigate the problem of release of emerging compounds from compost, biochar has been proposed as a co-amendment during the composting process [18] and compost utilization in soil, as well as as a growing medium component [19]. Biochar is produced by the pyrolysis process of biomass under limited oxygen conditions [20]. Its abilities in improving soil fertility and plant growth have been reported in thousands of scientific papers. However, in terms of its use as an adsorbent of toxic compounds, its remarkable properties, such as high carbon content, large surface area and porosity, high cation exchange capacity, aromatic structure and the presence of oxygen functional groups able to bind metal cations, should be mentioned. Further incorporating biochar into compost or soil increases pH, reducing the mobility and bioavailability of toxic compounds [21–24]. The co-application of biochar and compost is beneficial, as increased soil fertility encourages plants to overcome the negative impact of soil contamination and acidification. Many different feedstock materials have been used to design a proper material for heavy metal adsorption. The efficiency of metal removal depends on biochar properties, and biochar derived from rabbit manure has not been recently studied in terms of heavy metal immobilization in the solid phase. Biochar derived from the waste of animal production such as animal feces is less often considered in terms of environmental use. The main obstacles related to animal feces gasification are high moisture and protein content with an unknown pathway of organic substance changes during thermal decomposition, which brings a higher risk of toxic compound formation in biochar and material exclusion from environmental uses [25,26]. In terms of biochar manure, some of the problems can be avoided, as rabbit feces contains less than 20% of water and the rabbit diet is based on fresh and dry plants. When animals are fed properly, the risk of contamination and transformation of potentially toxic compounds during gasification is low. Another advantage of using rabbit manure biochar is its application in the form of granules, avoiding dusting and keeping leaching of biochar from soil. A new approach is also the co-application of rabbit manure biochar with compost, and recently, no investigation on this topic has been published. In this study, two greenhouse experiments with the application of rabbit-manure-derived biochar to municipal green waste compost and a mix of compost with biochar to soil were conducted to investigate the effects of rabbit manure biochar and compost on heavy metal uptake by green leafy vegetables commonly grown in urban gardens.

2. Materials and Methods

2.1. Soil and Compost Collection

Soil for the pot experiment was collected from the top 30 cm layer from the cultivated area near Biedaszków Mały village in Poland (51°40′10.6″ N 17°10′57.3″ E). The whole agricultural area is part of the Dolina Baryczy nature region, and lands are used for organic and traditional farming due to low inputs of airborne emissions and limited use of mineral fertilization, as most parts of this area are protected by law. Compost used for the experiment was produced in the Gać Biological Treatment of Municipal Wastes Plan, located near Oława city (50°40′79.3″ N 17°36′54.4″ E) in the Lower Silesia region of Poland. The substrate used for the composting was mainly the biodegradable fraction of municipal wastes mixed with green wastes from urban parks and gardens and wastes from the methane fermentation process, composted for approx. 90 days in a composting reactor under controlled conditions of air and moisture. The plant has an appropriate permit required by Polish law, regulating the conditions of composting organic wastes.

2.2. Biochar Preparation

Rabbit manure was collected from household rabbits, and only solid fractions (rabbit droppings) were used for biochar production. Approximately 500 g of air-dried rabbit droppings was packed tightly in 0.5 L steel cans and closed before being placed in a muffle furnace to avoid external air flow (Figure 1). Samples were heated for 1 h at 550 °C and then left in the closed furnace for 12 h to cool down.

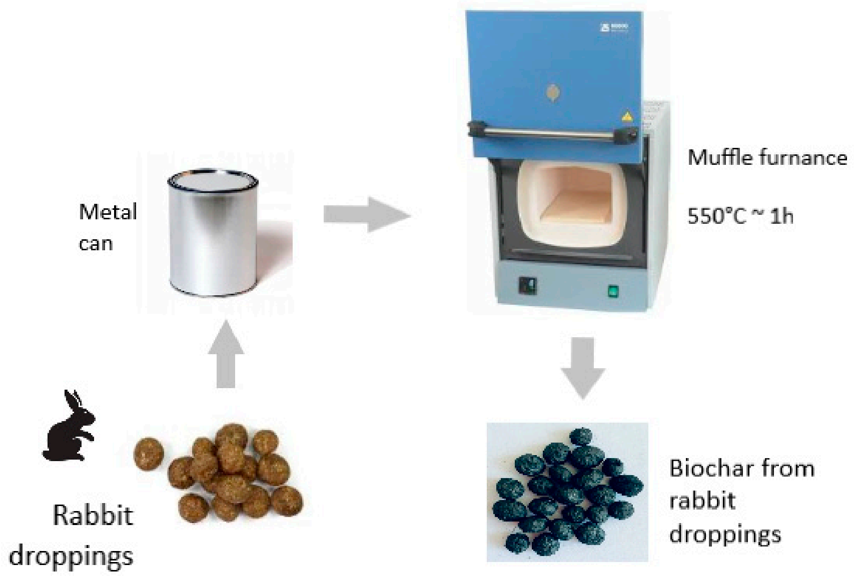

Figure 1. Scheme of rabbit-manure-derived biochar production.

2.3. Substrate Analysis

Standard properties of soil, biochar and compost before use in the experiment were determined at the Wroclaw University of Environmental and Life Sciences (WUELS) and the Wroclaw University of Science and Technology. Analyses for the characterization of substrates (air-dried soil, compost and biochar) were conducted as follows: the pH in water was measured in a 1:5 (v/v) ratio using a pH meter (Mettler-Toledo, Greifensee, Switzerland). Cation exchange capacity, determined as the sum of base cations, was measured on an MP-AES 4200 Spectrometer (Agilent Technologies, Santa Clara, CA, USA) at pH 7.0 after extraction with 1 M ammonium acetate. Total organic carbon (TOC) and total nitrogen (TN) content in soil and compost were measured using the EnviroTOCCube analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). The specific surface area of biochar (SSA) was measured using the TrisStar II 3020 (Micrometrics®, Norcross, GA, USA) surface area analyzer (N2-BET method) [21]. Soil texture was determined after dispersion with hexametaphosphate-bicarbonate with the hydrometer method for silt and clay fractions and sieved for sand [27]. The properties of substrates used for the experiment (soil, biochar and compost) are summarized in Table 1.

Table 1. Properties of soil, biochar and compost used in the experiment.

Characteristics		Value
Texture	Soil	Loamy sand (78% sand, 23% silt, 3% clay)
pH_{H_2O}		5.36 ± 0.16 *
Total organic carbon (TOC, %)		1.18 ± 0.06
Total nitrogen (TN, %)		0.081 ± 0.05
Cation exchange capacity (CEC, cmol(+)/kg)		1.71
Substrate	Compost	Urban green and municipal waste
Composting time		90 days
Composting method		Composting reactor
pH_{H_2O}		7.56 ± 0.12
Total organic carbon (TOC, %)		9.31 ± 0.08
Total nitrogen (TN, %)		0.71 ± 0.05
Cation exchange capacity (CEC, cmol(+)/kg)		181.2 ± 0.03
Substrate	Biochar	Rabbit droppings
Pyrolysis time		1 h
Pyrolysis temperature		550 °C
pH_{H_2O}		6.98 ± 0.04
Total organic carbon (TOC, %)		72.67 ± 0.12
Total nitrogen (TN, %)		2.28 ± 0.08
Cation exchange capacity (CEC, cmol(+)/kg)		23.1 ± 0.1
Specific surface area (BET) g/m^2		95.6

* Values are means ± standard deviation (n = 3).

The content of heavy metals in substrates was measured as the semi-total content after microwave digestion with 10 mL of HNO_3 by the EPA 3051A method [22]. Measurements were performed on MP-AES 4200 (Agilent Technologies, Santa Clara, CA, USA). For calibration, standard solutions were used (LGC Standards Ltd., Teddington, UK). Samples were measured in triplicate, and then the average was calculated by MP Expert software (Agilent Technologies, Santa Clara, CA, USA). Heavy metal contents are presented in Table 2.

Table 2. Total contents of trace elements in substrates.

Metal (Oid) (mg/kg)	Soil	Compost	Biochar
Pb_{total}	24.79 [a] ± 0.03 (100) [b]	45.51 ± 0.03 (140)	0.44 ± 0.012
Cd_{total}	5.20 ± 0.02 (2)	5.51 ± 0.03 (5)	<0.008 ± 0.01
Cu_{total}	39.63 ± 0.04 (100)	101.43 ± 0.12 (n.r.) [c]	9.5 ± 0.04
Cr_{total}	21.26 ± 0.03 (150)	36.84 ± 0.08 (100)	1.3 ± 0.03
Ni_{total}	3.5 ± 0.03 (100)	10.38 ± 0.02(60)	0.2 ± 0.05
Hg_{total}	0.062 ± 0.012	0.10 ± 0.012(2)	<0.002 ± 0.004
As_{total}	0.15 ± 0.08 (10)	1.39 ± 0.08 (n.r.)	0.03 ± 0.01

[a] Values are means _ standard deviation (n = 3); [b] values in brackets represent standard maximum values for soil and organic amendments according to Polish law regulation and recommendation for soil and compost when applied as a soil amendment for agriculture and horticulture purposes; [c] not regulated by Polish law.

2.4. Experimental Design

Two pot experiments were set up in square plastic pots of approximately 1 L volume. The first experiment (EX 1) was carried out only with compost and biochar. A 10% amount of RBC (v/w) was mixed with the entire volume of compost. In the second experiment (EX 2), a mix of 90% of compost and 10% of rabbit manure biochar (called MIX) in a dose of 30% (v/w) was applied to soil and well mixed with the whole volume of the pot (Table 3). Soil and compost were homogenized with added materials, moisturized with distilled water and left to stabilize for 1 week for equilibration.

Table 3. Summary of the treatments in greenhouse experiment.

Experiment Setup	Description	Abbreviation
Experiment 1 (EX 1)	Control compost without organic amendments	CC
Experiment 1 (EX 1)	Compost with 10% (v/w) of rabbit manure biochar	CBC
Experiment 2 (EX 2)	Control soil without organic amendments	CS
Experiment 2 (EX 2)	Soil with 30% (v/w) rabbit manure biochar and compost MIX *	SCBC

* Composition of the mix of 90% of compost and 10% of rabbit manure biochar.

Green leafy vegetables commonly grown by gardeners, also called "microgreens" (lettuce—*Lactuca sativa* L., spinach—*Spinacia oleracea* L., corn salad—*Valerianella locusta* L., kale—*Brassica oleracea* L. var. *sabellica* L., mustard greens—*Brassica juncea* L.) were planted in EX 1 and EX 2. Each variant was set up in six replicates to produce enough plant biomass for investigations. Five seeds were sown in each pot, and after germination, some of the plants (depending on plant species) were removed to keep the space biomass development (e.g., for lettuce). The pots were placed in a greenhouse under a controlled light regime (16 h of light/8 h of darkness), temperature (~23 °C) and humidity conditions and watered with distilled water to maintain the moisture (ca. 70%) of water-holding capacity (WHC). No additional mineral fertilization was applied during the experimental period. After 8 weeks of growth, when the edible parts of plants were fully developed (Figure 2), vegetable shoots and leaves were harvested, washed with tap water and deionized water, dried in a forced-air oven for 48 h at 60 °C, weighted and ground using a stainless steel mill.

Figure 2. Lettuce growth in 6th week of the EX 2 experiment on control soil without MIX (CS) (on the **left**) on soil with MIX (SCBC) (on the **right**).

2.5. Chemical Analysis of Samples after the Experiments

After 8 weeks of incubation, the soil was collected from pots to repeat the analyses of standard chemical properties. The material was air-dried, homogenized and passed through a 2 mm sieve, then stored in a dry place at room temperature. Analyses of pH, CEC, TOC and TN were repeated by the same methods as described in Section 2.3. To investigate the effect of biochar on heavy metal mobility in compost and soil, the sequential extraction procedure proposed by the European Community Bureau of Reference (BCR) was performed according to the procedure described by Medyńska et al. [28]. Four fractions of heavy metals with different mobilities were obtained: Fraction F1—easily soluble fraction and bond to carbonates, Fraction F2—reducible, bound to Fe and Mn oxides, Fraction F3—oxidizable, bound to organic matter, Fraction F4—residual. Obtained extracts representing four fractions were analyzed to determine heavy metal content on MP-AES 4200 (Agilent Technologies, Santa Clara, CA, USA). Freshly collected vegetable leaves were washed in distilled water to reduce the risk of external contamination of the material with soil and dried at 45 °C for 12 h in a laboratory dryer (POL-EKO, Wodzisław Śląski, Poland). Content of Pb, Cd, Cr and Cu in plant tissues was determined with MP-AES 4200 (Agilent Technologies, Santa Clara, CA, USA) after microwave digestion with a mixture of 36% H_2O_2 and 10 mL of HNO_3, following the United States Environmental Protection Agency (US-EPA) 3052A method on the STAR D digestion system (Milestone, Shelton, CT, USA). To avoid analytical errors, standard solutions (LGC Standards Ltd., Teddington, UK) for MP-AES 4200 and a certified reference material RTH 953 Heavy Clay Soil from LGC Promochem (LGC Standards Ltd., Teddington, UK) was used, and for plants, white clover BCR-402 and plankton BCR-414 from the European Commission Joint Research Center (Geel, Belgium) were used for calibration with each sample set. The recovery of Cu, Cr, Pb and Cd from the certified reference material (CRM) was 84–93%, and the maximum values of RSD were 2.3%. Detection limits were 0.01 mg/kg for Cu, 0.01 for Cr, 0.002 mg/kg for Pb and 0.008 mg/kg for Cd in the soil, compost and plant samples. The biological accumulation coefficient (BAC) was defined as the concentration of heavy metals in plant shoots divided by the heavy metal concentration in soil (BAC = (Metal) shoot/(Metal) soil) [29,30]. BAC indicates the ability of plants to tolerate and accumulate heavy metals.

2.6. Data Analysis

All experiments and measurements were performed in triplicates. Data are presented as the mean values with relative standard deviation (RSD). Student's *t*-tests were used to test for significant differences in the pH and content of metals in amended and control soil and compost ($p < 0.05$). Obtained data were compiled using Microsoft Excel 2016.

3. Results

3.1. Effect of Biochar Amendment on Compost and Soil Psychochemical Properties

After biochar application to compost (in EX 1), some of the compost properties were significantly improved. Compost pH increased by 0.2 units, as well as the content of TOC, while the content of TN and surprisingly CEC of compost was not affected by RBC. In terms of EX 2, all soil properties were significantly improved in the SCBC treatment. The most important properties for the process of sorption and desperation of metals such as soil pH by 0.8 units, organic carbon content by 0.39% and a tenfold increase in soil CEC from 1.71 c(mol)/kg to 17.38 c(mol)/kg, were recorded after the application of 30% (*v*/*w*) of biochar–compost mix to sandy soil (Table 4).

Table 4. Effects of biochar and compost mix application on soil chemical properties after 8 weeks of incubation.

Treatment	pH in H_2O	CEC [1] cmol(+)/kg	TOC [2] %	TN [3] (%)	C:N
CC	7.62 [a]	181.24 ± 0.12 [a]	9.31 ± 0.06 [a]	0.71 ± 0.04 [a]	12
CBC	7.82 [b]	182.94 ± 0.16 [a]	9.54 ± 0.04 [b]	0.69 ± 0.03 [a]	14
CS	5.36 ± 0.02 [a]	1.71 ± 0.12 [a]	1.18 ± 0.08 [a]	0.081 ± 0.04 [a]	22
SCBC	6.10 ± 0.03 [b]	17.38 ± 0.14 [b]	1.57 ± 0.09 [b]	0.090 ± 0.05 [b]	20

[1] CEC—cation exchange capacity [2] TOC—total organic carbon [3] TN—total nitrogen. Different lowercase letters ([a] and [b]) indicate significant differences between treatment type in EX 1 (CC and CBC) and EX 2 (CS and SCBC ($p < 0.05$)).

3.2. Sequential Extraction of Heavy Metals in Compost Amended with Rabbit Manure Biochar

The effect of rabbit manure biochar application on the speciation of the tested metals in compost varied between elements and, in terms of Pb and Cd, was more efficient in the reduction of easily soluble and bioavailable forms compared to Cu and Cr. Prioritizing the availability of metals in the studied compost, Cd > Cu > Cr > Pb. Biochar caused a decrease in Cu in Fraction F1 from 5.1% to 4.3%, also reducing the amounts of Cu in residual form and causing a shift of Cu to Fractions F2 and F3 (Figure 2). In biochar-amended compost, 33% of Cu was in F4 and, respectively, 31% in Fractions F2 and F3, compared to 54.6% of Cu in F4, 14.5% in F3 and 25.7% in F2 in pure compost without RBC. Similarly for Cr, a significant decrease in Fraction F1 from 1.41% to 0.44% and a shift to Fractions F2 and F3 was observed; however, more than 60% of Cr in the tested soil was in residual Fraction F4. The addition of RBC caused a significant decrease in Cd in F1 from 9.6% to 0.2% and an element shift to Fraction F3. The solubility and availability of Pb in the tested compost was low, only 1.3%; however, RBC-affected Pb speciation caused a decrease in F1 to 0.03% and an element shift to Fractions F2 and F3.

3.3. Sequential Extraction of Heavy Metals in Soil Amended with Rabbit Manure Biochar and Compost Mix

Prioritizing the availability of metals in the control soil, Cu > Cd > Cr > Pb. The effect of MIX application varied between metals; however, for all the tested metals, a significant decrease in exchangeable forms and shifts in metal species were observed after 8 weeks of soil incubation. The content of Cu in the Fraction F1 decreased from 22.5% to 4.7%, and Cu was shifted to Fractions F2 and F3, increasing the amounts of Cu bound with organic matter from 0.4% to 15.3% and bound to Fe and Mn oxides from 12.8% up to 29.9% (Figure 3). In terms of Cr, Fraction F1 was reduced from 6.6% to 1.9%, and chromium was shifted from Fractions F1 and F2 to F3, while no significant effect was observed for residual Fraction F4, and approx. 70% of Cr remained in residual forms. Significant changes were observed for Pb, and easily soluble and available forms were decreased from 2.6% to 0.03%, while MIX addition caused shifts from F1 and F2 to Fraction F3, and almost 44% of Pb remained in a form bound with organic matter. The content of Cd in easily soluble and available forms was also remarkably decreased from 19.1% to 0.7%, and metal was shifted to Fractions F3 and F4, increasing the amounts of residual forms of Cd from 53.6 to 69.5%.

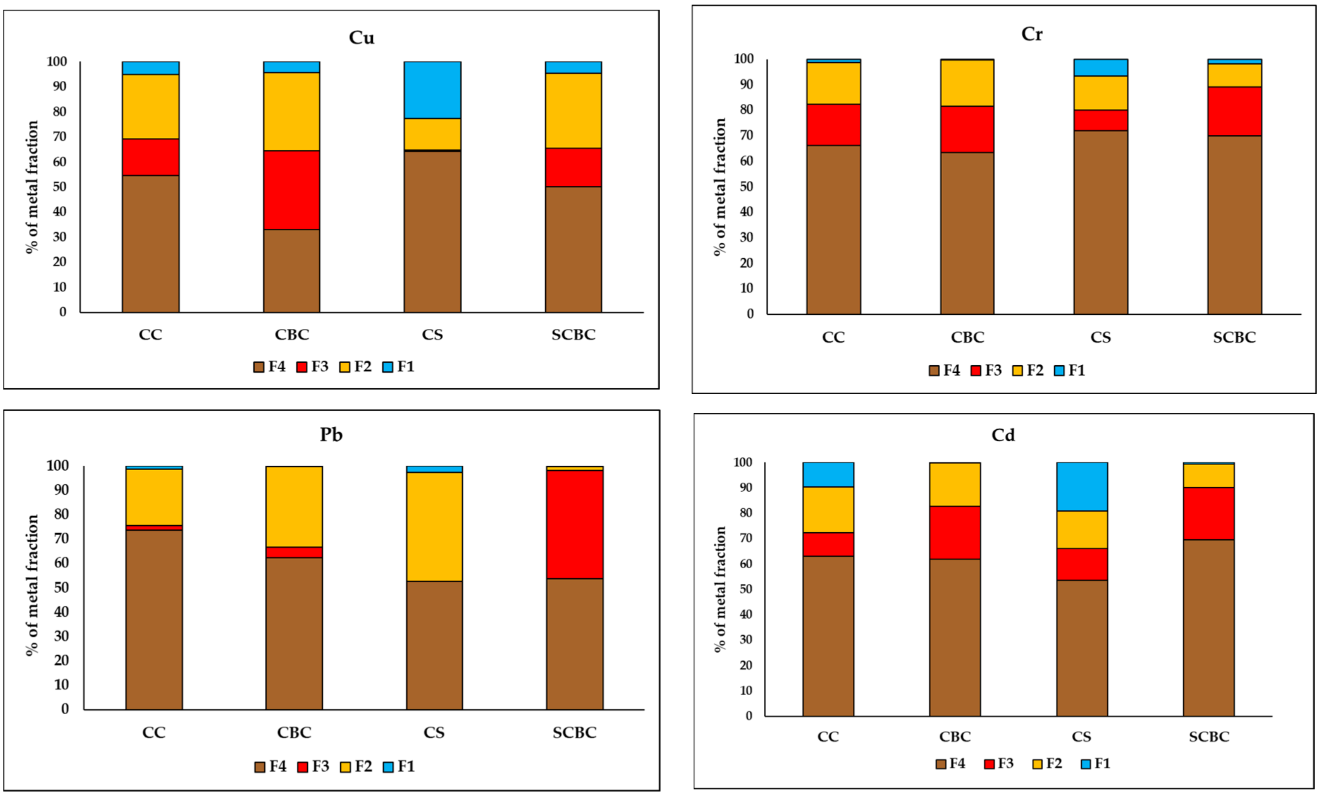

Figure 3. Effects of the application of rabbit manure biochar and co-application of rabbit manure biochar with compost on the speciation of heavy metals (Cu, Cr, Cd and Pb) in compost (CBC) and soil (SCBC) compared with untreated compost (CC) and soil (CS).

3.4. Heavy Metals Accumulation in Leafy Vegetables Grown on Compost with Biochar Amendment

Response to the application of rabbit manure biochar to compost in heavy metal content in plant leaves varied between the tested elements and plant species. The highest contents of Cu, Cr, Pb and Cd were determined in spinach, followed by lettuce and mustard greens, with the lowest in kale and corn salad (Figure 4). The application of RBC to compost significantly ($p > 0.05$) reduced the uptake of Cd and Cr by spinach by 61% and 40%, respectively, and Pb and Cd in mustard greens by 73% and 45%. However, some negative effects of biochar presence were also observed for some of the tested species. The application of RBC enhanced the accumulation of Cr by kale, lettuce and mustard greens, respectively, by 30%, 12% and 17% and of Cu in lettuce by 16% (Figure 4). From all the tested species, corn salad had a tendency to accumulate Cd and Pb and, respectively, up to 40% and up to 63% more of this metal were accumulated in plants grown on compost with RBC amendment; however, corn salad should not be considered a good metal accumulator compared with typical accumulators such as lettuce and spinach.

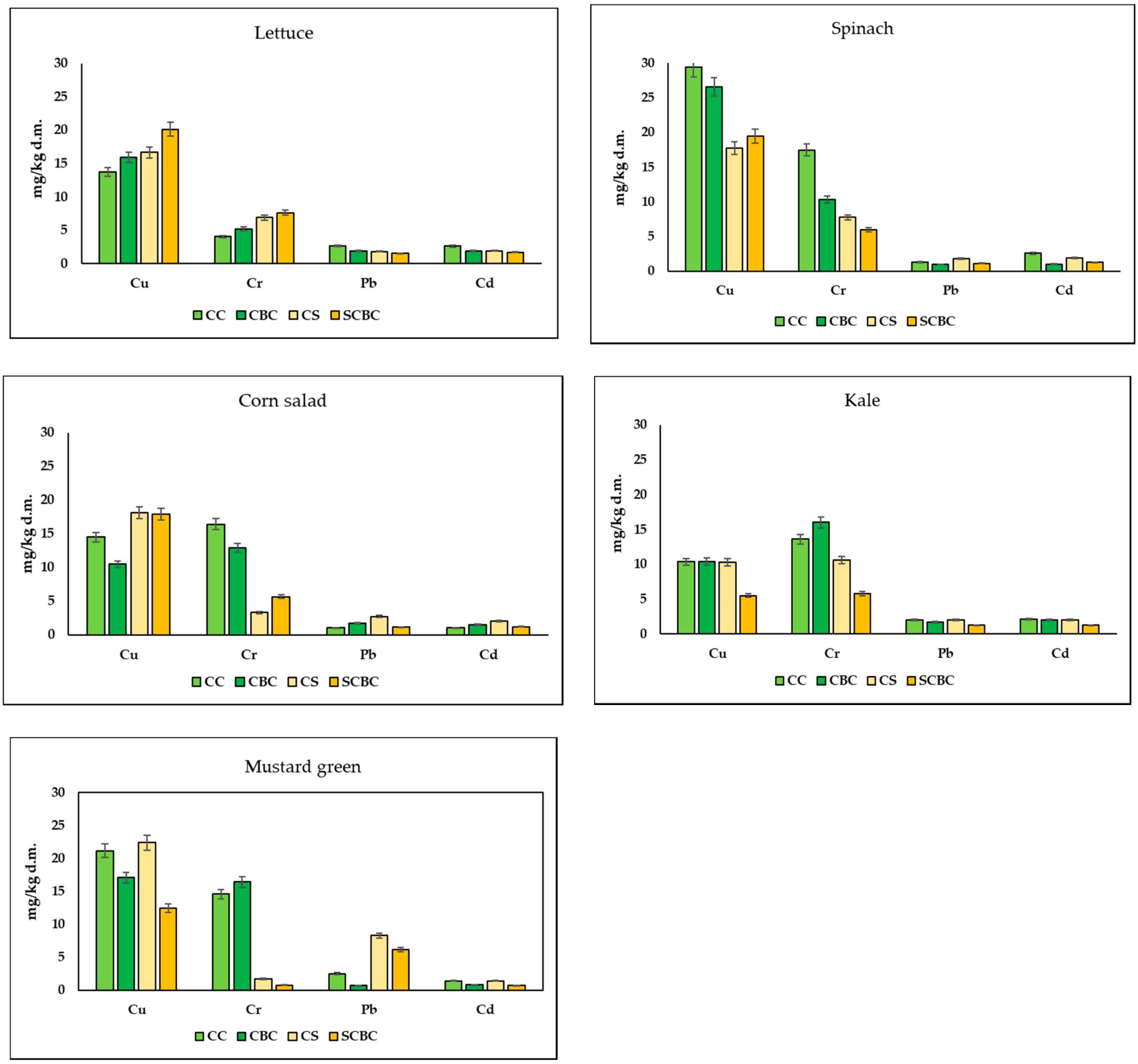

Figure 4. Concentration of Cu, Cr, Pb and Cd in plants grown without (CC—control compost and CS—control soil) and with (CBC—compost with rabbit manure biochar, SCBC—soil with mix of biochar and compost) organic amendments.

3.5. Heavy Metals Accumulation in Leafy Vegetables Grown in Soil with Biochar–Compost Amendment

The application of 30% MIX reduced the uptake of the tested metals by two species: mustard greens and kale. The accumulation of Cu, Cr, Cd and Pb in leaves of both species decreased by 56% for Cr and by 52% for Cd, but also, compared to other species, the content of Cu was reduced by 46% in plants grown with soil amendment. In EX 2, corn salad also exhibited a high tendency for Cr accumulation in the presence of MIX in soil; however, the accumulation of Pb and Cd was reduced by 41% and 58%, respectively, which is an opposite result compared with EX 1. In the other tested vegetables, the application of MIX caused a reduction in the amounts of Pb and Cd accumulated in edible plant parts, respectively, by 37% and 34% in spinach and by 16% and 12% in lettuce, while the content of Cu in both species increased by 20%. For Cr, the content was reduced by 23% in spinach and enhanced by 10% in lettuce, showing no similar tendencies.

3.6. Heavy Metal Accumulation Coefficient

Calculated bioaccumulation coefficients (BAC) differed between plant species and types of metal. Spinach, kale and mustard greens had a tendency to accumulate Cr and Cu in leaf tissues (BAC > 1) when cultivated on pure compost amendment (Table 5). The application of 10% (v/w) RBC reduced the risk of metal accumulation, similarly to the co-application of RBC and compost in 30% (v/w) to soil. All calculated BAC values were <1. BAC calculations confirmed that green leafy vegetables have a tendency to accumulate Cr when present in bioavailable forms in substrates. Mustard green also had a tendency to accumulate Cu, while Pb and Cd were not easily transferred to the edible parts of the tested vegetables.

Table 5. Bioaccumulation coefficient (BAC) of Cu, Cr, Pb and Cd in five species of green leafy vegetables grown on compost without/with biochar and soil without/with biochar–compost mix.

Treatment	Cu	Cr	Pb	Cd	Treatment	Cu	Cr	Pb	Cd
		Lettuce					Lettuce		
CS	0.81	0.94	0.09	0.38	CC	0.50	0.11	0.08	0.74
SBCB	0.73	0.72	0.06	0.38	CBC	0.49	0.17	0.05	0.41
		Spinach					Spinach		
CS	0.86	1.06	0.09	0.38	CS	0.77	0.49	0.04	0.73
SBCB	0.71	0.56	0.05	0.28	SBCB	0.70	0.29	0.03	0.28
		Corn salad					Corn salad		
CS	0.88	0.46	0.14	0.40	CS	0.38	0.46	0.03	0.30
SBCB	0.65	0.53	0.05	0.26	SBCB	0.32	0.54	0.05	0.33
		Kale					Kale		
CS	0.50	1.45	0.10	0.40	CS	0.27	0.38	0.06	0.60
SBCB	0.20	0.54	0.05	0.27	SBCB	0.32	0.52	0.05	0.44
		Mustard greens					Mustard greens		
CS	1.09	0.24	0.41	0.28	CS	0.55	0.41	0.08	0.40
SBCB	0.45	0.07	0.25	0.15	SBCB	0.52	0.54	0.02	0.17

Colors indicate the intensity of metal bioaccumulation: dark and light green = low bioaccumulation, dark and light yellow = medium, dark and light orange = high, red = very high bioaccumulation of given metal. Abbreviations of the treatments are defined in Table 3.

4. Discussion

Rabbit farming on a large scale raises several problems related to the disposal of feces (solid wastes) produced in large quantities [31]. The reuse of animal residues can generate added value and provide benefits to soil, including a high amount of nutrients, organic matter and microbial biomass, as well as increased plant yields [32]. Transformation of rabbit manure to biochar and its utilization as a soil or growing medium amendment is very promising and can bring benefits related to better growth of plants and plant protection against metal stress and unintentional uptake from substrates. In addition, it eliminates the problem of odor pollution, contamination of soil and water bodies, and potential microbial contamination of soil and vegetables [33]. The application of rabbit-manure-derived biochar (RBC) for heavy metal (HM) problem mitigation is a novel application of this material. Only limited scientific papers have been published describing the effects of rabbit-manure-derived biochar on soil health. The results of this study showed that the application of labile organic matter with RBC significantly improved heavy-metal-polluted soil enzymatic activity, soil aeration and pH [34]; however, RBC significantly affected Zn availability, enhancing metal uptake by *Brassica napus* [35]. More can be found about RBC use for the removal of pharmaceuticals and antibiotics (e.g., ciprofloxacin) from aquatic solution, and the results show high adsorption capacity for the removal of potentially toxic compounds [36]. Animal-manure-derived biochar has a higher heavy metal sorption capacity compared with plant biomass feedstock, which is attributed to high polarity and ash content [37,38], and such properties can be used for designing soil conditioners having both good characteristics: substrates for plant growth due to its fertilizing properties and contaminant sorbents.

4.1. Heavy Metal Immobilization by Rabbit Manure Biochar

Biochar actions in soil or compost are related to direct and indirect changes in substrate properties important for the heavy metal sorption/desorption process [21]. Metal cation binding onto biochar depends on several factors, such as affinity between element and biochar, specific surface area of BC, concentration of metal in soil solution or initial soil pH [39]. In EX 1, the possible mechanism of Cu, Cr, Cd and Pb immobilization can be related to biochar properties, and the most probable scenario is the direct effect of the addition of stable forms of carbon in the form of well-charred biochar able to actively bind metals on its surface and metal precipitation with minerals (e.g., Fe and Mn oxides and carbonates) present in biochar. As evidence, significant changes in metal speciation were determined in RBC-amended compost where metals were shifted from Fractions F1 (exchangeable and soluble metal forms) and F4 (residual) to Fractions F2 (bound to Fe and Mn oxides) and F3 (bound with organic matter). This phenomenon was previously observed in another study with Co speciation in alkaline soil amended with wheat straw biochar [28]. The direct sorption of metals on biochar active sites provided by functional groups (e.g., carboxyl, hydroxyl and carbonyl groups) has been recently suggested as the main mechanism of metal precipitation under alkaline soil conditions [40,41]. This mechanism has been recently called "char-assisted metal retention" [42], as cations can be absorbed by mineral (ash) components of biochar. In EX 2, soil mechanisms related to the decrease in available heavy metals after biochar–compost MIX application can be related to the significant improvement of sandy soil pH and CEC. Biochar is commonly alkaline and thus can be used as a soil amendment to neutralize soil acidity and increase soil pH [43]. The adsorption of heavy metal(loid)s strongly depends on soil pH and increases as a function of pH increases [44]. The enhancement of cation exchange capacity increases the soil retention capacity of metals, e.g., Cu and Cd [45]. In EX 2, the co-application of compost and biochar added to soil caused an increase in species binding with organic matter and Fe–Mn oxides, while no effect on residual Fraction F4 was observed. The application of organic amendments in the form of compost and biochar increases the soil sorption capacity for divalent cations [19,46]; in addition, biochar contains a substantial amount of free Al^{3+} and Fe^{3+} oxides, which can also serve as additional metal sorption sites [47].

4.2. Effect of Biochar Co-Application with Compost

The most desired effect of biochar application to compost and soil is reduced content of potentially toxic elements (PTEs) in the edible parts of plants, as their presence in vegetables has potential adverse effects on human health. Unfortunately, it is more common that organic materials such as manure and manure-derived biochar are recognized as a major source of metal input to soils, with repeated applications having resulted in elevated concentrations of metal(loid)s in soil [44,48]. It is probably due to this problem that animal-manure-derived biochar is less studied in terms of its utilization in soil protection and remediation of inorganic contaminants [49]. The application of compost to soils with elevated concentrations of pollutants can be beneficial; however, some studies have indicated that compost can increase the mobilization of As [50], Zn [19] or Cu [51]. The co-application of compost and biochar can be beneficial in reducing the risk related to the presence of heavy metals in both materials due to the dilution effect and potential of biochar for adsorbing metals released during compost decomposition [52]. The long-term benefits of compost and biochar co-application to soil include greater stabilization of organic matter, slower nutrient release from added organic matter and improved retention of cations due to enhanced CEC [53]. RBC produced for this study was not an important source of potentially toxic elements, probably due to the more controlled diet of domestic rabbits compared with large-scale farming. However, this implication is important for managing the risk related to heavy metal transfer from feed to manure in animal farming.

4.3. Heavy Metal Accumulation in Green Leafy Vegetables Grown with Organic Amendments

The results of both experiments showed that rabbit manure biochar (RBC) and the mix of RBC with compost can have a profound effect on heavy metal uptake by different green vegetable species, able to accumulate excessive amounts of potentially toxic elements (PTEs) when grown on contaminated urban soils. *Brassicaceae* and *Asteraceae* species can accumulate significant amounts of Cd [54], Pb [55], Cu [56] and Cr [57,58]; however, they are a common choice of vegetables cultivated by gardeners. The application of RBC to compost decreased the uptake of Pb and Cd by most of the tested species. A similar finding was observed in a previous study by Medyńska-Juraszek et al [19]. Puga et al. [59] showed that biochar application caused a significant decrease in Pb and Cd availability in soil cultivated with jack beans and *Mucuna aterrima*, and this decrease corresponded to a 54% and 50% reduction of Cd and Pb uptake by the tested species. Van Poucke et al. [60] confirmed in their study that biochar is much more efficient in Cd immobilization, and the concentrations of Cd in soil solution after application of 4% of biochar were reduced by 67% compared to peat and compost, which caused a significant increase in Cd in available forms (by 231%). Similarly, Cui et al. [46] described that with the increase in soil pH with increasing rates of biochar application, the content of Cd in rice was reduced by 57%. Netherway et al. [61] showed that poultry-derived biochar can effectively bind Pb due to precipitation with phosphates. Similarly, precipitation is a dominant mechanism for Cu removal [62]; however, in our study, the effect of RBC and MIX application on Cu uptake was less significant compared to Pb and Cd. Opinions in the literature about Cu sorption by compost and biochar are divided, showing more relevant dependence on initial soil properties, e.g., soil pH or organic matter content, than substrate direct performance. Vila-Rodriguez et al. [63] demonstrated that Cu uptake into leafy greens is significantly reduced in alkaline soil, which confirms our finding showing enhanced uptake of Cu by plants grown in control, more acidic soil.

4.4. Negative Impact of Organic Amendments Application on Heavy Metal Uptake by Vegetables

This study also indicated some negative effects of RBC and RBC with compost co-application in terms of Cr accumulation in the tested species. Kale, lettuce and mustard greens accumulated up to 30% more Cr compared to untreated compost. Enhanced uptake of chromium by plants can be related to its speciation and form in soil. Chromium (Cr) is a potentially toxic heavy metal that does not have any essential metabolic function in plants. Chromium occurs in different chemical forms (primarily as chromite (Cr(III)) and chromate (Cr(VI)) in soil), which vary markedly in terms of their biogeochemical behavior [64]. The high oxidizing potential and solubility make Cr (VI) more mobile and toxic than Cr (III); however, the oxidation status and toxicity of Cr in soil can vary depending on the moisture content and redox potential of soil [57]. Chromium behavior in soil, its soil–plant transfer and accumulation in different plant parts vary with its chemical form, plant type and soil physicochemical properties [64]. Soil acidification affects the solubility of Cr through its effect on adsorption/precipitation and oxidation/reduction reactions. While the adsorption of Cr(VI) in soil increases with decreasing pH, the adsorption of Cr(III) decreases [65]. Some plant species, including the *Brassicaceae* family, have been reported to be good accumulators of chromium. For example, *Brassica juncea* (Indian mustard) has been widely used in phytoremediation because of its remarkable capacity to accumulate high levels of heavy metals, including Cr [58]. The effectiveness of chromium uptake by plants depends on Cr bioavailability in soil and plant tolerance to this metal; however, *Brassicaceae* species are able to uptake chromium and accumulate it in roots and shoots, even if the amounts of Cr present in soil solution are low [57]. High ability to uptake and tolerate chromium, as well as a detoxification mechanism leading to the transition of potentially toxic Cr (VI) into less toxic Cr (III) in roots, can explain the increased uptake of chromium by the tested species in mix-amended and nonamended soil. For spinach, the findings are partly in agreement with the study of Sehrish et al. [66], describing that poultry-derived biochar decreased the content of bioavailable forms of Cr and uptake of metal by spinach. Zakir et al. [67] showed that spinach grown in farm and industrial contaminated soils accumulates Cr in excessive amounts, and this can pose potential health concerns for consumers, which is in agreement with the findings of our study. Vegetable species differ widely in their ability to take up and accumulate heavy metals, even among cultivars and varieties within the same species [68]. BAC calculations confirmed that some species such as spinach, kale and mustard green accumulate Cr in leaves (BAC > 1), while the application of RBC and MIX to soil reduced the uptake of Cr by all tested species. Our results are in agreement with the results of a previous study by Medyńska-Juraszek et al. [19], showing that the application of compost alone increased the uptake and accumulation of Cr by spinach. All tested species showed a tendency to accumulate Cu, Cr and Cd, and the concentration of these elements exceeded permissible heavy metal limits indicated by the World Health Organization and the Food and Agriculture Organization of the United Nations (FAO) for edible plants. WHO indicated the permissible limits as follows: 0.02 mg/kg cadmium (Cd), 1.3 mg/kg chromium (Cr), 10 mg/kg copper (Cu) and 2 mg/kg lead (Pb) [69]. However, these limits are more reliable for plants cultivated in unpolluted soils or soils with a low anthropogenic impact. Guerra et al. [70] indicated that the average content of Pb, Cd and Cr consumed by citizens of Sao Paulo, Brazil, with lettuce is, respectively, 0.41, 0.08 and 0.25 mg/kg, while spinach is able to accumulate more, respectively, 1.05, 0.13 and 0.27 mg/kg, showing that even under controlled greenhouse growing conditions, the content of heavy metals can exceed safe levels. Gupta et al. [69] showed that growing vegetables in urban areas bring the risk of vegetable contamination with different heavy metals, reporting that leafy vegetables exhibit a tendency to accumulate metals in the following pattern: Zn > Cu > Pb > Ni > Cd, which is in agreement with the findings of our study. The concentration of different elements in vegetables depends upon the relative level of exposure of plants to the contaminated soil [71]; however, the application of organic amendments, e.g., rabbit-manure-derived biochar, can reduce the risk of metal transfer to plants significantly and can be recommended as a method for health risk mitigation related to food production in urban gardening. The application of single compost may increase the concentration of heavy metals in some vegetable species, bringing the potential risk of adverse health effects and increase in daily intake of contaminants in the diet. The co-application of compost with biochar seems to be beneficial in minimizing the risk related to urban horticulture on contaminated soil, but also in terms of compost use as a growing medium, which is a popular choice for gardeners. Rabbit manure seems to be a good feedstock for biochar production. As a nutrient-rich material, rabbit droppings can be converted into macro- and micronutrient soil conditioner and improve plant growth. Another advantage of rabbit-manure-derived biochar is its easy application in the form of slowly solubilizing granulates, limiting the problem of biochar dusting and runoff.

5. Conclusions

- The results of the study showed that rabbit-manure-derived biochar and its co-application with compost can reduce the uptake of Cd and Pb by leafy vegetables, reducing the risk related to food contamination with potentially toxic elements
- The application of compost and biochar to soil significantly improved physicochemical properties, e.g., pH, cation exchange capacity and carbon content and plant growth, decreasing the availability of Cu, Cr, Cd and Pb and toxicity of metals for plants
- The application of rabbit-manure-derived biochar to compost and co-application of organic amendments to soil may also have some adverse effects, and increased uptake of chromium and copper for some of the tested vegetable species was observed. This finding can pose a potential health concern related to organic amendment application in urban horticulture.

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