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Phytoextraction of copper from a contaminated soil using arable and vegetable crops

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HIGHLIGHTS

- Arable and vegetable crops showed different response to copper concentration.
- Copper concentration was determined in different tissues of crops.
- Arable and vegetable crops had not stored copper above the safety standards.

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ABSTRACT

Copper (Cu) is among the main contaminant of agricultural soil. The reclamation of Cu polluted soils can be achieved with phytoextraction even if, in general, plants are Cu-excluders and uncommon are Cu-accumulators. The research objectives were to establish the Cu removal capacity by arable and vegetable crops and to investigate the distribution of Cu in their roots, stems and leaves, and fruits. Pot trials were conducted for two subsequent years in Tuscany (Italy). Cu was added into soil in four levels (0, 200, 400, 600 mg kg^{-1} of Cu). At harvesting, the crops roots, stems and leaves, and fruits or seeds were separately collected, oven dried, weighted, milled and separately analyzed.

The results show that the GDUs value to reach the physiological maturity for barley, common bean, Indian mustard, and ricinus was significantly positively correlated with Cu concentration in soil in contrast with observed in sorghum, spinach, and tomato. Leaves and stems of spinach and ricinus have a good storage capacity in contrast with common bean, tomato, Indian mustard sorghum and barley. Tomato storage Cu mainly in fruits and roots which show a remarkable concentration of Cu that increases progressively with the increase of Cu concentration in the soil. In addition, the roots of common bean and ricinus showed a very high concentration of Cu. All species can be considered Cu-excluders because of their low capacity to uptake high quantity of Cu. Indian mustard can be considered a plant able to translocate the metal from root to epigeal tissue.

1. Introduction

Copper (Cu) is an essential micronutrient for plant, playing an important role in biological and physiological process such as photosynthesis, protein synthesis, and respiratory processes (Fernandes and Henriques, 1991; Kabata-Pendias and Pendias, 2001; Yruela, 2005). Normally Cu concentration in plant tissue ranges from 5 to 30 mg kg^{-1} (Clarkson and Hanson, 1980; Ballabio et al., 2018). Both deficiency and excess of Cu affect plant growth with drastic effects on plant biomass production and yield. Cu deficiency inhibits photosynthesis process and causes a plant premature ageing with scarce biomass production (Rahimi and Bussler, 1973; Bowen, 1979; Fernandes and Henriques, 1991; Kabata-Pendias and Pendias, 2001). On the contrary, Cu excess can alter seed germination and plant growth and morphology (Fernandes and Henriques, 1991; Kabata-Pendias and Pendias, 2001; Yang et al., 2002; Yruela, 2005; Nagajyoti et al., 2010; Adrees et al., 2015). Moreover, when the concentration of this heavy metal in the soil exceeds 60–125 mg kg^{-1} it becomes toxic also for tolerant plants, negatively influencing their biological and physiological process (Kabata-Pendias and Pendias, 2001; Pugh et al., 2002; Yang et al., 2002).

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Cu is naturally present in the soil, ranging from 2 to 100 mg kg^{-1} (mean: 25 mg kg^{-1}) (Shacklette and Boerngen, 1984; Marschner, 1995; Baize, 1997; Adriano, 2001) but its continuous and prolonged use in agriculture, as antibacterial and antifungal, has led to strong soil pollution in specialized agricultural areas (Lado et al., 2008; Ballabio et al., 2018). In fact, as Cu concentration in agricultural soil generally varies from 5 to 30 mg kg^{-1}, it was found to reach even 500 mg kg^{-1} in some vineyards (Brun et al., 2003; Adrees et al., 2015). Heavy metals such as Cu are among the main contaminant of agricultural soil in Europe and globally by representing an immediate and serious threat to food safety, human health and environment (Järup, 2003; CEC, 2006; Duruibe et al., 2007; Khan et al., 2008; Peralta-Videa et al., 2009; Kong, 2014; Van Liedekerke et al., 2014; Tóth et al., 2016a; Tóth et al., 2016b; Khalid et al., 2017). The Italian directive establishes concentration levels of Cu contained in soil at 120 mg kg^{-1} in the areas destined to green public spaces and residential areas; while for the soil destined for industrial and commercial use, the limits are 600 mg kg^{-1} (D.Lgs 152/2006). For agricultural soil, the Italian law defines no limits of Cu concentration, but the D.Lgs 99/1992 establishes at 100 mg kg^{-1} the maximum level of the heavy metals in soil in which sewage sludge is distributed.

The reclamation of Cu polluted soils can be achieved with different techniques and technologies (i.e. by physical-mechanical, chemical and biological systems) depending on soil properties, the extent of polluted areas, the concentration of pollutant, and the economic costs. On wide areas, where the pollutant concentration on the soil surface does not exceed the limits of toxicity for plants, biological system by adapted plants able to adsorb and store Cu in their tissue must be preferred for its simplicity and its low cost. Phytoextraction is a subprocess of phytoremediation in which plants roots uptake metal contaminants from the soil and translocate them to their aboveground harvestable tissues (Baker, 1990; Salt et al., 1995, 1998; Chaney et al., 1997; Padmavathiamma and Li, 2007). There are two basic strategies of phytoextraction: induced phytoextraction and long term continuous phytoextraction (Salt et al., 1995; Raskin et al., 1997; Padmavathiamma and Li, 2007). The induced phytoextraction, which involves the addition of chelates agents, is used when metal concentration in soil exceeds the plant uptake capacity (Huang et al., 1997; Lasat, 2002; Wu et al., 1999; Lombi et al., 2001). On the contrary, continuous phytoextraction is a clean-up plant-based technology which depends on plant metal accumulating capacity and biomass production (Kumar et al., 1995; Cunningham and Ow, 1996; McGrath, 1998; McGrath and Zhao, 2003; Pilon-Smits, 2005).

Plants Cu tolerance varies with species and cultivar, but in general plants are Cu-excluders and uncommon are Cu-accumulators plants. Until now about 34 species has been discovered to be hyperaccumulators for Cu, among which *Ipomea alpine*, *Aeolanthus biformifolius*, *Eleocharis acicularis*, *Haumaniastrum katangense*, *Commelina communis*, *Rumex acetosa* and *Artemisia argyi* but they produce few biomass and have slow growth (Baker and Brooks, 1989; Baker and Walker, 1989; Cunningham and Ow, 1996; Tang et al., 1999; Lasat, 2002; Li et al., 2003; Reeves, 2003; Ghosh and Singh, 2005; Sheoran et al., 2009; Chaney et al., 1997; Sakakibara et al., 2011; Shan et al., 2011). In this context, it could be interesting to know the capability of storing Cu by plants easy to harvest and evaluate their ability to produce considerable amount of biomass in a short time, even on very polluted soil. The research objectives were to evaluate the Cu removal capacity by seven arable and vegetable crops and to investigate the distribution of Cu in root, stem and leaves, and fruits. Further objectives were to determine the Cu removal per unit of above ground dry matter produced, and if the removal was influenced by the Cu concentration in the soil.

2. Material and methods

2.1. Experiment set-up

Pot trials were conducted for two subsequent years (January 2009–December 2010) at the University of Florence's Farm in Montepaldi, San Casciano Val di Pesa, Italy (WGS84; 43° 39′ to 43° 40′ N; 11° 8′ to 11° 9′ E; 256 m a.s.l.). The local climate was sub-Mediterranean, with a rainfall regime characterized by a summer minimum in July and two winter maxima in November and February (Napoli et al., 2017) (Fig. 1). The average annual rainfall was approximately 854 mm and the average annual temperature was 14.9 °C (Napoli et al., 2013). The site was instrumented with a meteorological station (SIAP comp. SM3830 meteorological station, Italy) for measuring air temperature (T) and rainfall amount (R) (Caracciolo et al., 2012). In 2009, the climate was characterized by hot and dry conditions. In particular, the annual R amount was about 14.3% below the long-term average, with the 49% of the total

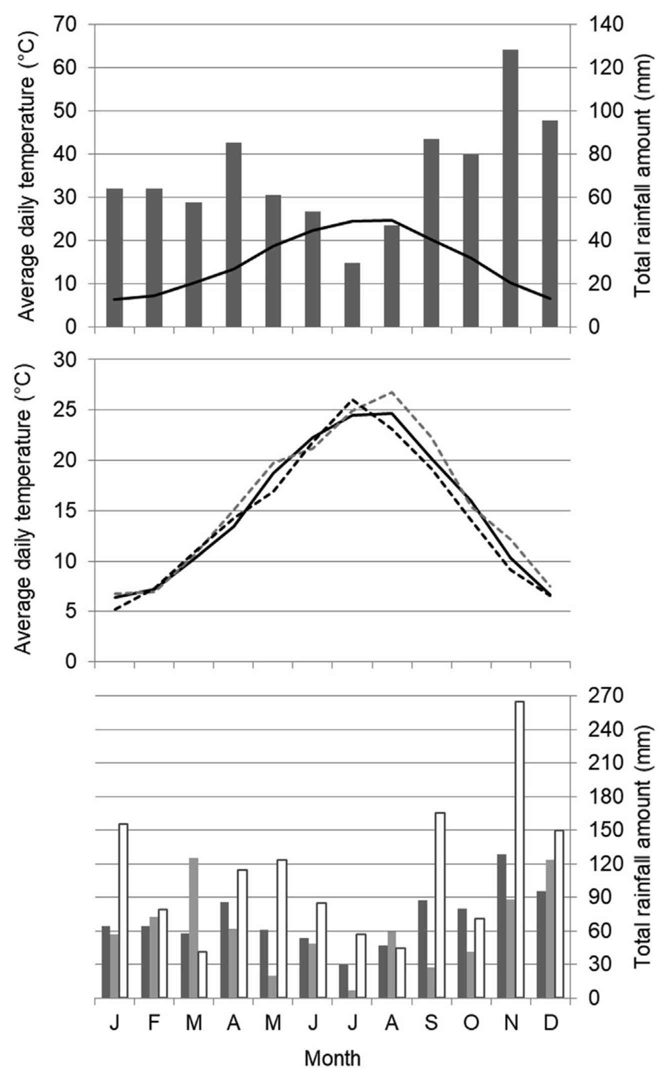

Fig. 1. Walter-Lieth climate diagram of the study site (data 1991–2010), with monthly daily average temperature (°C, black continous line) and montly average rainfall amount (mm, dark grey histograms). Center: Comparison of the monthly daily average temperature (mm) measured during 1991–2010 (black continous line), 2009 (black dashed line) and 2010 (grey dashed line); Bottom: Comparison of the monthly rainfall amount (mm) measured during 1991–2010 (dark grey histograms), 2009 (light grey histograms) and 2010 (white histograms).

annual R reached in May instead of July. Moreover, the average annual T exceeded the long-term average by about 0.7 °C, with the spring and autumn T exceeding the long-term average by about 1 and 1.2 °C, respectively. On the contrary, in 2010, the annual R exceeded the long-term average by about 58.1% and experienced annual average temperature by about 0.5 °C lower than the long-term average. The growing degree units (GDU) were used to relate the relative temperature to phenological development of crop (Parthasarathi et al., 2013). GDU values were computed for each crop separately, starting from the seeding or transplanting date, as the accumulation of daily average temperature exceeding a base temperature of 0 °C.

A total of 560 pots (30 cm diameter; 30 cm depth) were filled with 18 kg of soil collected from a vineyard of the University of Florence's Farm (Napoli et al., 2015) in the layer 0–0.15 m. After air-drying, the soil was crushed, sieved to 5 mm and then homogenized with a concrete mixer. The main physical and chemical properties of the used soil are reported in Table 1. The soil analyses were executed using the official methodology indicated by the Italian Ministry of Agriculture and Forestry (MiPAF, 1994).

The experimental plan included 28 treatments, comprising seven field crops and four levels of Cu (Cu), replicated 20 times. The crops used in this experiment were: barley (*Hordeum vulgare* L.), common bean (*Phaseolus vulgaris* L.), Indian mustard (*Brassica juncea* Czern), ricinus (*Ricinus communis* L.), sorghum (*Sorghum vulgare* L.), spinach (*Spinacia oleracea* L.), tomato (*Solanum lycopersicum* L.). Four level of Cu were realized by treating and mixing homogeneously the soil of each pot with a solution containing 0, 14.1, 28.2, 42.3 g of copper sulphate corresponding to 0 (control), 200, 400, 600 mg kg^{-1} of Cu, respectively. The above mentioned crops were sown or transplanted into the pots on the dates shown in Table 2.

To avoid water stress the crops were automatically irrigated with a dripping system, depending on soil moisture. For each treatment, three pots were randomly chosen and equipped with sensors for measuring the soil moisture and connected to an automatic irrigation scheduling system.

The experimental data of Cu concentration and dry matter production were analyzed for all crops and separately for each part of them (stems and leaves, fruits or seeds, roots) for evaluating their Cu storage capacity and for assessing the effects of different Cu soil treatment on plant dry matter.

2.2. Reagents

Superpure nitric acid (HNO_3) for trace metal analysis (Carlo Erba, Rodano, Milan, Italy) was diluted to 10% (v/v) with deionized water. According to De Leonardis et al. (2000), containers and test tubes, were in poly-propylene and were previously cleaned with a solution of hydrochloridric acid (HCl) for analytic analysis (Carlo Erba, Rodano, Milan, Italy) diluted to 5% (v/v) with deionized water. The purity of plasma torch argon was greater than 99.99%.

Table 1
Physical and chemical properties of the soil used in the experiment.

Properties	Measure unit	Value
Sand	%	18.7
Silt	%	40.3
Clay	%	41
Organic carbon	%	1.17
Total nitrogen	%	0.07
Available phosphorous	mg kg^{-1}	2.8
Total calcium carbonate	%	18.9
pH		7.9
Cation Exchange Capacity	cmol kg^{-1}	15.2
Copper	mg kg^{-1}	55.5
Nickel	mg kg^{-1}	62.2
Lead	mg kg^{-1}	32.6
Zinc	mg kg^{-1}	87.2

Table 2
Seeding (or transplanting) date for each crops and year.

Crop	Seeding or Transplanting date	
	2009	2010
Barley	02-03-2009	25-02-2010
Common bean	02-03-2009	25-02-2010
Indian mustard	02-03-2009	25-02-2010
Ricinus	02-03-2009	25-02-2010
Sorghum	20-05-2009	24-05-2010
Spinach	24-03-2009	30-03-2010
Tomato	20-05-2009	24-05-2010

2.3. Sample preparation and spettrophotometric determination

At the end of the biological cycle of the crops, roots, stems and leaves, and fruits or seeds were separately collected, oven dried at 105 °C, weighted and milled. Dried samples (1 g) for each different components of the plants for each crop were ignited at 550 °C and kept at that temperature until a white ash was obtained. The resulting ashes were treated with 5 mL of the nitric acid solution and the mixture was slowly heated to dissolve the residues. The solution was filtered by using a Whatman filter paper, transferred to a 10 ml volumetric flask and then made up to volume with distilled water. The blank consisted of the 10% dilute nitric acid used for extraction.

The Cu determination was performed by using PerkinElmer Optima 7300 DV ICP-OES (PerkinElmer, Inc. Shelton, CT, USA) with the following instrumental conditions: forward power 1300 W; argon plasma flow rate 15 L min^{-1}; argon nebulizer flow rate 0.7 L min^{-1}; auxiliary argon flow rate 1.5 L min^{-1}; wavelengths 324.747 nm.

2.4. Data analysis

The experimental data of Cu concentration and dry matter production have been analyzed for all crops and separately for each part of them (stems and leaves, fruits or seeds, roots) for evaluating their Cu storage capacity and for assessing the effects of different Cu soil treatment on plant dry matter. The uptake of heavy metal (mg kg^{-1} DW) was based on the DW of the harvested plant material.

In order to assess the Cu absorption and/or phytoremediation potential of investigated crops the following indicators were calculated:

Bioaccumulation factor (BAF) (Equation (1)) indicates the ability of plant to uptake the metals from soil to plant tissues.

$$BAF = \frac{CCu_{plant}}{CCu_{soil}} \tag{1}$$

where CCu_{plant} and CCu_{soil} were the Cu concentration in plant and soil, respectively. With a BAF lower than 1 plant is considered an excluder, with BAF value ranging between 1 and 10 plant is considered an accumulator, and with a BAF value higher than 10 plant is considered an hyperaccumulator (Ghavri and Singh, 2010; Lam et al., 2017). Plants characterized by a BAF value higher than 1 can be considered appropriate for phytoextraction.

Translocation factor (TF) (Equation (2)) indicates the ability of plant to translocate heavy metal from roots to aerial tissues and then the potential of plants to accumulate heavy metal in aerial organs (Lam et al., 2017).

$$TF = \frac{CCu_{aereal\ tissues}}{CCu_{roots}} \quad (2)$$

where $CCu_{aereal\ tissues}$ and CCu_{roots} were the Cu concentration in aereal tissues and roots, respectively. High value of TF ($TF > 1$) indicates a great capacity of plant to translocate heavy metals from roots to aerial tissues. At the opposite, a low value ($TF < 1$) indicates a limited capacity of plant to translocate the metal to aerial tissues (Lam et al., 2017).

Data for the dependent variables (cumulated GDU for each phenological stage; DW; Cu concentration in plant tissues) were subjected to analysis of variances (ANOVA) utilizing the R statistical software package (R Core Team, 2017). ANOVA was performed for each crop separately. A split plot design was set up, where the main factor was the year of trial and the second factor was the Cu level. The year of trial was considered as random effect factor, while the four Cu levels were considered as fixed effect factors. The multiple mean comparison comparisons were performed by means of Tuckey honest significant difference (Tuckey HSD) test at $P \leq 0.05$ probability level (R Core Team, 2017).

3. Results and discussion

For none of the tested species there was a significant inter-annual difference between the GDUs accumulated between a phenological phase and the next (Table 3). In contrast, GDUs differences were observed within the same crop for different Cu level. The GDUs value to reach the physiological maturity for barley, common bean, Indian mustard, and ricinus was significant positive correlated ($p < 0.05$) with Cu concentration in soil. On the contrary, significant negative correlations ($p < 0.05$) were found for sorghum, whilst no relationships were found for spinach and tomato. Brun et al. (2003) and Jin et al. (2015) found that high concentrations of the metal in the soil resulted in phenological maturity delay for herbaceous plants. At the same time, the weight values of the dry-biomass at the physiological maturity for barley, common bean, Indian mustard, and ricinus were significant positive correlated ($p < 0.05$) with Cu concentration in soil. Whilst, negative correlations were found for sorghum, and no relationships were found for spinach and tomato. These results were consistent with Stapper and Harris (1989) who found that the durations of the vegetative growth period was positively correlated with the dry-biomass accumulation.

For all crops, no significant differences were found in terms of dry matter production (Table 4) and Cu concentration (Table 5) in different part of plant between the two years of trials. Data did not allow carrying out a proper assessment of the inter-annual variability and of the impacts of meteorological variables on Cu absorption by plants.

In barley, grains and roots of plants grown in treated soil contained a higher Cu concentration than that grown in no treated soil. In both years, roots were the first organ of accumulation of Cu. In grain, the higher Cu concentration was observed at 200 mg kg^{-1} of metal added to soil, followed by 600 and then 400 mg kg^{-1}, however without significant differences. Consistent with previous studies (Sekara et al., 2005), barley roots contained from 3 to 6.7 times more Cu in comparison to leaves and stems. For what concerns Cu accumulation in stems and leaves, results showed an increasing trend with the increase of its concentration level in the soil, as was also reported by Ali et al. (2004). A significant difference ($p < 0.05$) was noticed between grains DW at the lowest and at the highest soil Cu concentration. In contrast to what measured by

Table 3
Average cumulate growing degree unit from Seeding (or transplanting) date measured for different part of plant using different amounts of Cu in the soil. Lowercase letters indicate different means ($p < 0.05$) according to the post hoc Tuckey test.

Crop	Phenological stage (BBCH)	Average Cumulate GDU from Seeding/Transplanting date							
		Year 2009				Year 2010			
		0	200	400	600	0	200	400	600
Barley	1	121.6 ± 6.9	122.6 ± 6.2	119 ± 6.4	123.1 ± 6.5	121.6 ± 5.4	122.3 ± 6.6	119.6 ± 5.8	122.6 ± 6.1
	6	831.8 ± 8.7c	859.7 ± 6.1b	858.7 ± 4.5b	877.7 ± 0a	832.4 ± 8.1ac	861.3 ± 8.3b	858.9 ± 7.4b	882.3 ± 6.5a
	8	1340 ± 9.5d	1403.1 ± 7.2c	1487.9 ± 8.8b	1571.5 ± 9.4a	1341.8 ± 8.1d	1400.7 ± 5.8c	1485.4 ± 0b	1572.5 ± 10a
	9.9	1513.7 ± 0d	1555.6 ± 0c	1618.1 ± 0b	1680.7 ± 0a	1508.4 ± 0d	1555.6 ± 0c	1600.3 ± 0b	1669 ± 0a
Common bean	1	210.7 ± 6.7	212.2 ± 5.2	211.3 ± 6.2	210.7 ± 5.6	211 ± 5.3	210.4 ± 6.4	211.5 ± 6.4	210.5 ± 5.3
	6	757.9 ± 8.9d	834.6 ± 3.5c	874.3 ± 8.9b	946.5 ± 10a	758.2 ± 6.8d	835.3 ± 7.7c	873.4 ± 7.3b	947.9 ± 6.3a
	7	1072.1 ± 10.6d	1184.6 ± 5.1c	1234.4 ± 0b	1345.9 ± 6.3a	1078.4 ± 8.4d	1184.8 ± 10.4c	1238.1 ± 6.8b	1347.7 ± 9.9a
	8	1378.6 ± 8.9d	1517.1 ± 4.2c	1590.6 ± 10.2b	1722.7 ± 9.9a	1380.7 ± 9d	1517.2 ± 12.5c	1583.9 ± 8.8b	1727.2 ± 8.9a
	9.9	1578.5 ± 0d	1742 ± 0c	1829.1 ± 0b	1969.6 ± 0a	1580.3 ± 0d	1750.7 ± 0c	1823.7 ± 0b	1989.4 ± 0a
Indian mustard	1	117.5 ± 6.4	118.1 ± 6.2	116.5 ± 5.4	117.5 ± 5.3	119.9 ± 6.3	120.7 ± 7.9	118.6 ± 7	118.6 ± 5.8
	6	519.8 ± 7.8b	545.9 ± 8.7a	542.5 ± 7.5a	550.2 ± 8a	524.1 ± 5.7b	546.3 ± 6.7a	544.1 ± 7.9a	553.3 ± 6.7a
	7	902.7 ± 5.5b	913.5 ± 8.5 ab	925.4 ± 9.2a	922.6 ± 7.7a	906.1 ± 6.9b	921.2 ± 8.1a	923.9 ± 6.9a	925.2 ± 6.5a
	8	1234.4 ± 0c	1293.1 ± 10.9 ab	1287.6 ± 6.8b	1309.5 ± 6.1a	1240.9 ± 8.7b	1288.2 ± 9.4a	1287.2 ± 9.8a	1310.6 ± 8.4a
	9.9	1538.3 ± 0d	1620.9 ± 0c	1644.4 ± 0b	1693.6 ± 0a	1554.3 ± 0d	1626 ± 0c	1648.8 ± 0b	1692.1 ± 0a
Ricinus	1	195.8 ± 9.3	197.2 ± 6.3	196.6 ± 8.1	196 ± 7.9	194.5 ± 7.9	195.7 ± 5.9	194.3 ± 7.1	195.3 ± 6.4
	6	1090.4 ± 10.7d	1115 ± 9c	1136.1 ± 9.5b	1165.1 ± 7.1a	1093.7 ± 8.1d	1115.4 ± 6c	1132.4 ± 6.3b	1168.6 ± 9.5a
	7	1391.9 ± 7.7d	1417.5 ± 0c	1449.2 ± 10.4b	1487.7 ± 10.7a	1388.9 ± 7.5d	1414.6 ± 0c	1447 ± 11.5b	1485.2 ± 9.5a
	8	1686.1 ± 11.8d	1717.9 ± 0c	1759.3 ± 11.6b	1808.2 ± 6.5a	1688.1 ± 8.2d	1727.2 ± 8.9c	1761.3 ± 8.8b	1808.2 ± 9.2a
	9.9	2280.1 ± 0d	2326.4 ± 0c	2379.9 ± 0b	2456.2 ± 0a	2291.8 ± 0d	2320.7 ± 0c	2376 ± 0b	2457.6 ± 0a
Sorghum	1	81.3 ± 11.7 ab	88.5 ± 5.4a	65.8 ± 0c	70.5 ± 9.8bc	83.3 ± 8.4 ab	89.4 ± 10.4a	71.8 ± 9.3c	75.5 ± 7.6bc
	6	1038.4 ± 12.5b	1093.7 ± 5a	878.6 ± 10.1d	932.4 ± 12.3c	1034.4 ± 10.1b	1087.3 ± 0a	881.7 ± 14.1d	930 ± 11.6c
	8	1225 ± 12.5b	1287 ± 0a	1042.2 ± 11d	1102.6 ± 10.9c	1228.6 ± 0b	1282.9 ± 0a	1035.8 ± 11.3d	1097.7 ± 14.6c
	9.9	1645.1 ± 0b	1723.3 ± 0a	1405 ± 0d	1487.4 ± 0c	1643.5 ± 0b	1736.4 ± 0a	1391.1 ± 0d	1471 ± 0c
Spinach	1	60.7 ± 7.5	63.2 ± 2.9	61.2 ± 5.4	62.7 ± 6.2	65.1 ± 7.2	65.7 ± 6.3	65.4 ± 8.1	64.6 ± 7.1
	9.9	407 ± 7.8	410.2 ± 5.8	408.6 ± 7.1	409.4 ± 6.5	408.7 ± 9.4	408.7 ± 9.4	409.6 ± 9.3	408.7 ± 9.4
Tomato	6	290.7 ± 9.5	293.6 ± 8	292.6 ± 8.6	291.7 ± 9.1	290 ± 9.4	288.9 ± 8.7	288.9 ± 8.7	288.9 ± 8.7
	7	390.5 ± 9.5	397.1 ± 4.2	393.3 ± 8.4	398.1 ± 0	386.1 ± 11.6	400.4 ± 10.7	395.2 ± 13.1	397.8 ± 12.2
	8	790.6 ± 9	805.8 ± 0	800.7 ± 9	805.8 ± 0	788.9 ± 0	808.2 ± 11.4	792.8 ± 9.4	805.6 ± 12.6
	9.9	1287 ± 0	1314.8 ± 0	1303.7 ± 13.9	1314.8 ± 0	1284.2 ± 6	1309.6 ± 0	1302.9 ± 11.8	1310.9 ± 5.9

Table 4
Average dry weight (DW) measured for different part of plant using different amounts of Cu in the soil. Lowercase letters indicate different means ($p < 0.05$) according to the post hoc Tuckey test.

Plant	Copper added to the soil (mg kg^{-1})	Dry weight of different part of the plant (D.W.) (g)					
		2009			2010		
		Leaves and stems	Fruits	Roots	Leaves and stems	Fruits	Roots
Barley	600	8.2 ± 1.1 b	1.3 ± 0.2 c	1.2 ± 0.2 a	7.8 ± 1 b	1.2 ± 0.2 c	0.9 ± 0.1 b
	400	9.3 ± 1.4 a	2.4 ± 0.4 b	1.2 ± 0.2 a	8.8 ± 1.4 a	2.5 ± 0.3 b	1.1 ± 0.1 ab
	200	6.3 ± 0.9 d	3.9 ± 0.6 a	1.3 ± 0.2 a	5.2 ± 0.9 d	3.4 ± 0.4 a	1.2 ± 0.1 a
	0	6.8 ± 1 c	2.3 ± 0.3 b	1.3 ± 0.2 a	6.8 ± 0.7 c	2.2 ± 0.3 b	1.3 ± 0.2 a
Common bean	600	6.2 ± 0.7 a	7.4 ± 1.3 a	0.5 ± 0.1 a	5.9 ± 0.8 a	7.5 ± 0.9 a	0.4 ± 0.1 a
	400	5.7 ± 0.9 b	8.4 ± 1.3 a	0.5 ± 0.1 a	4.9 ± 0.5 b	7.7 ± 1.1 a	0.4 ± 0.1 a
	200	5.1 ± 0.7 c	8.4 ± 1 a	0.4 ± 0.1 a	5.3 ± 0.7 b	7.6 ± 1.1 a	0.4 ± 0.1 a
	0	4.6 ± 0.5 d	7.9 ± 1.1 a	0.4 ± 0.1 a	3.3 ± 0.4 c	7.4 ± 1.1 a	0.5 ± 0.1 a
Indian mustard	600	7.5 ± 0.9 a	–	1.1 ± 0.1 a	7.4 ± 1 a	–	1 ± 0.1 a
	400	7 ± 1 ab	–	0.7 ± 0.1 a	6.7 ± 0.8 ab	–	0.7 ± 0.1 a
	200	5.3 ± 0.7 c	–	0.5 ± 0.1 a	4.6 ± 0.6 c	–	0.4 ± 0.1 a
	0	6.1 ± 0.9 bc	–	0.5 ± 0.1 a	5.7 ± 0.8 bc	–	0.5 ± 0.1 a
Ricinus	600	30.8 ± 3.6 a	4.2 ± 0.6 b	2.7 ± 0.4 a	23.7 ± 3.2 a	4.2 ± 0.6 b	2.2 ± 0.3 a
	400	24 ± 3.1 b	5.9 ± 1.1 a	2.1 ± 0.2 a	21.7 ± 2.8 a	6.2 ± 0.9 a	1.6 ± 0.2 b
	200	22.3 ± 4.6 b	5.5 ± 0.7 a	1.9 ± 0.3 a	21.5 ± 3 a	5.5 ± 0.7 ab	1.9 ± 0.3 ab
	0	17.1 ± 2.7 c	4.1 ± 0.7 b	1.9 ± 0.3 a	14.5 ± 2.1 b	4.3 ± 0.5 b	1.7 ± 0.2 ab
Sorghum	600	18.6 ± 2.8 a	17.7 ± 1.8 a	7.1 ± 1.1 a	18.4 ± 2.6 a	16.1 ± 2.1 a	6.7 ± 1 b
	400	18.9 ± 2.3 a	18.9 ± 2.2 a	6.5 ± 0.9 a	17.3 ± 2.5 a	15.9 ± 2.5 a	5.8 ± 0.8 a
	200	23.5 ± 2.1 a	19.7 ± 2.1 a	8.1 ± 1.1 a	20.3 ± 2.2 a	17.6 ± 2.4 a	7.9 ± 1.1 a
	0	22.8 ± 3.1 a	20.9 ± 2.7 a	4.5 ± 0.4 b	21.1 ± 2.7 a	16.5 ± 1.9 a	4.1 ± 0.6 b
Spinach	600	2.3 ± 0.3 a	–	–	2.2 ± 0.3 a	–	–
	400	2.7 ± 0.4 a	–	–	2.2 ± 0.3 a	–	–
	200	2.3 ± 0.3 a	–	–	2.2 ± 0.2 a	–	–
	0	2.5 ± 0.3 a	–	–	1.8 ± 0.2 a	–	–
Tomato	600	31.2 ± 4.7 a	7 ± 1 d	4.4 ± 0.6 a	27.2 ± 3.7 ab	6.3 ± 1.2 a	4.0 ± 0.5 a
	400	34.3 ± 4 a	6.2 ± 0.9 b	4.8 ± 0.4 a	27.7 ± 4.6 ab	5.9 ± 0.6 a	4.5 ± 0.5 a
	200	31.4 ± 4.1 a	7.4 ± 1 a	4.7 ± 0.6 a	30.3 ± 3.7 a	6.5 ± 0.7 a	4.4 ± 0.6 a
	0	28.2 ± 4.3 a	5.6 ± 0.8 c	4.0 ± 0.5 a	22.3 ± 3.2 b	4.5 ± 0.5 b	3.7 ± 0.5 a

Table 5
Average Cu concentration measured for different part of plant using different amounts of Cu in the soil. Lowercase letters indicate different means ($p < 0.05$) according to the post hoc Tuckey test.

Plant	Copper added to the soi (mg kg^{-1})	Copper concentration in different part of the plant (mg kg^{-1} of D.W.)					
		2009			2010		
		Leaves and stems	Fruits	Roots	Leaves and stems	Fruits	Roots
Barley	600	30.2 ± 3.6 a	24.4 ± 3.7 a	127.4 ± 17.4 a	27.8 ± 3.3 a	20.4 ± 2.8 ab	96.2 ± 11.1 a
	400	23.2 ± 3.3 b	20.1 ± 2.3 a	108.1 ± 16.2 ab	25.4 ± 2.9 a	16.7 ± 2.4 b	83.7 ± 13.3 a
	200	13.7 ± 2 c	25.5 ± 4.1 a	85.9 ± 11.3 b	11.1 ± 1.6 b	23.3 ± 3.5 a	73.5 ± 12.9 a
	0	10.7 ± 1.1 c	10.4 ± 1.1 b	41.4 ± 3.5 c	9.1 ± 1.2 b	8.4 ± 1.1 c	42 ± 6.3 b
Common bean	600	68.1 ± 7.1 a	19.5 ± 2.4 a	6.1 ± 0.8 d	50.5 ± 6.5 a	14.9 ± 2.4 a	4.2 ± 1.3 d
	400	55.1 ± 8.2 a	13.8 ± 1.7 b	47.1 ± 4.8 b	45.7 ± 7.1 a	14.4 ± 1.3 a	38.9 ± 5.9 b
	200	35.4 ± 4 b	17.6 ± 2.5 a	105.2 ± 10.6 a	28.6 ± 4 b	15.2 ± 1.7 a	83.6 ± 12.5 a
	0	18.5 ± 2 c	9.4 ± 1.4 c	22.2 ± 3.4 c	15.5 ± 2.1 c	10.4 ± 1.5 b	20.8 ± 2.1 c
Indian mustard	600	43.5 ± 7.2 a	–	43.4 ± 6.5 a	39.5 ± 6.6 a	–	39.6 ± 4.9 a
	400	27.7 ± 3.9 b	–	28.7 ± 3.2 b	28.3 ± 3.3 b	–	25.7 ± 3.8 b
	200	26.5 ± 3.2 b	–	24.6 ± 3.5 b	26.1 ± 3 b	–	22.6 ± 3.6 b
	0	14.8 ± 2.2 c	–	18 ± 2.3 c	13 ± 1.8 c	–	16.4 ± 2 c
Ricinus	600	63.5 ± 8.8 a	15.2 ± 2 ab	99.6 ± 16 a	58.9 ± 8 a	15.6 ± 2 ab	84 ± 13.4 a
	400	58.1 ± 7.9 a	12.2 ± 1.4 b	74.2 ± 11 a	55.3 ± 7.5 a	12.4 ± 1.6 b	73 ± 9.1 a
	200	39.3 ± 5.9 b	17.6 ± 2 a	85.7 ± 13.5 a	33.5 ± 4 b	16.2 ± 2.1 a	69.3 ± 9.8 a
	0	23.7 ± 3.2 c	11.7 ± 1.1 b	44.7 ± 5.9 b	21.7 ± 2.5 c	12.9 ± 1.7 ab	38.7 ± 5.8 b
Sorghum	600	35.8 ± 5 a	9.1 ± 1.1 a	86.4 ± 12.3 a	31.2 ± 3.9 a	7.7 ± 0.9 a	78.4 ± 9.1 a
	400	22.2 ± 2.8 b	7.2 ± 0.8 b	80.6 ± 11.3 a	20 ± 2.7 b	7.4 ± 1 a	70.6 ± 9.2 ab
	200	20.9 ± 2.9 b	8.7 ± 1.1 ab	57 ± 6.7 b	19.3 ± 2.9 b	6.9 ± 0.9 a	59.6 ± 9.4 b
	0	15.6 ± 1.8 c	8.8 ± 1.5 ab	38 ± 5.2 c	14.4 ± 1.6 c	7.8 ± 1.2 a	35.2 ± 4.5 c
Spinach	600	101.4 ± 13.6 a	–	–	95.4 ± 14.2 a	–	–
	400	99.1 ± 10.5 a	–	–	86.1 ± 13.8 a	–	–
	200	91.2 ± 14.9 a	–	–	91.6 ± 13.9 a	–	–
	0	74.9 ± 11.8 b	–	–	63.9 ± 9.1 b	–	–
Tomato	600	48.7 ± 7.4 a	40.3 ± 6.5 a	121.7 ± 18.5 a	44.7 ± 6.9 a	33.3 ± 4 a	102.3 ± 14.6 a
	400	29.3 ± 3.9 b	30.7 ± 3.9 b	81.7 ± 14.5 b	28.9 ± 4.2 b	25.9 ± 2.9 b	86.3 ± 12.2 a
	200	32.4 ± 4.9 b	25.5 ± 4 bc	51.6 ± 7.9 c	29 ± 3.9 b	23.7 ± 3.7 b	46 ± 6.4 b
	0	21.2 ± 2.6 c	7.9 ± 1.1 c	31.2 ± 4 d	17.2 ± 2.7 c	7.1 ± 0.9 c	28.8 ± 3.6 c

Žaltauskaitė and Šliumpaitė (2013) under hydroponic condition, no significant differences were noticed in leaves and stems, and roots DW at the various soil metal concentrations.

In common bean, data showed a progressive increase of Cu concentration in stems and leaves with the increase of its level in the soil. In fruits, Cu accumulation in plant grown on Cu treated soil was similar between the treatments, but higher in comparison with that of control. Data showed that roots have a good Cu storage capacity, even if its efficiency must be further verified. As measured by Sanchez et al. (1999) and Stingu et al. (2009), roots Cu concentration was higher than that measured in stems or leaves for control and 200 mg kg^{-1} of Cu treatment. On the contrary, Cu concentration was found higher in stems or leaves than in roots for the 400 and 600 mg kg^{-1} of Cu treatment. Consistent with Stingu et al. (2009), no significant differences were noticed between the various level of treatments DW either on fruits or roots or stems and leaves, even if in these latter the average DW was higher in treated soil in comparison with the no-treated one.

In Indian mustard, a significant ($p < 0.05$) increasing trend of Cu concentration in leaves and roots with the increase of soil Cu concentration was observed. Moreover, significant differences ($p < 0.05$) were observed in leaves and roots DW at different levels of Cu added to soil. On the opposite, Chigbo et al. (2013) observed a reduction in root and shoot DW, with respect to the control treatments, by adding 50 and 100 mg kg^{-1} of Cu to the soil. In addition, contrary to what reported by Feigl et al. (2013), the Cu concentration in the roots was not significantly different from that measured in the shoot.

Consistent with Andreazza et al. (2013), Cu contaminated soil enhanced the phytomass production of ricinus plants. In fact, while no significant differences were noticed in the roots and fruits at the increasing levels of Cu added to soil, leaves and stems DW showed a significant positive relationship. Metal concentration in leaves and stems, and roots of ricinus showed a decreasing trend with the decrease of soil Cu concentration. In fruits, the results did not show a trend of translocation trend as a function of soil Cu concentration. Andreazza et al. (2013), after 57 days from seeding in the greenhouse, found a tissue Cu concentration in the root 90–100 times higher in roots than in leaves and stems. On the contrary, our results showed that at the end of the growing season (172 days from seeding), ricinus seemed to be a good root stored and, to a lesser extent, a leaves and stems stored. However, considering biomass, the amount of Cu in leaves and stems was higher than in roots.

In sorghum, the results showed an increasing trend, even if not linear, of Cu concentration in leaves and stems, as well as in the roots, with the increase of Cu concentration in the soil. Copper concentration measured in leaves and stems was consistent with that measured by Wei et al. (2008). In roots, high levels of Cu were observed for each treatment and no difference was observed in grains concentration. No statistical difference in DW between treatment in neither leaves and stems, grain or roots were observed.

The leaves and stems of spinach showed a high Cu storage capacity even if the values measured in our research were higher than that found by Singh et al. (2012) for spinach and by Sekara et al. (2005) for chicory. No significant differences in DW were observed between treatments.

Generally, the Cu concentration in tomato leaves and stems, fruits and roots was found increasing progressively with the increase of Cu concentration in the soil, as was also measured by Martins and Mourato (2006). In accordance with findings by Liao et al. (2000) and Martins and Mourato (2006), the higher Cu content was noticed in roots. Plant height of tomato plant decreased with the increasing of soil Cu concentration, however, no statistical differences were observed in DW of roots, steam and leaves, and fruits. Thus, in contrast with Liao et al. (2000) and Sonmez et al. (2006) which showed that the DW of the roots and shoots of tomato were not sensitive to external Cu concentrations.

Considering the above ground dry biomass produced, at the highest Cu concentration in soil, ricinus and tomato removed about 1.7 and 1.6 mg of Cu per plant, respectively. Sorghum, common bean, Indian mustard, barley, and spinach showed a gradually decreasing Cu removal capacity, at the higher concentration in the soil, corresponding to 0.77, 0.48, 0.31, 0.26, and 0.23 mg of Cu per plant, respectively.

How suggested by Giordani et al. (2005), it is interesting to know the capacity of metal removal per unit of above ground dry matter produced, and if the removal was influenced by the Cu concentration in the soil. As an average of two years of trial, spinach was the most efficient in removal Cu, and such removal increase with the increase of metal concentration in the soil; at the highest Cu concentration in the soil, the removal resulted of 98.4 mg kg^{-1} of above ground dry biomass produced. On the contrary, ricinus, tomato, Indian mustard, common bean, barley, and sorghum evidenced a progressively decreasing efficiency in removal of Cu that was 55.1, 44.9, 41.5, 35.7, 28.1, and 21.7 mg kg^{-1} of above ground dry biomass, respectively.

To conclude, it is interesting to observe that none of the considered crops showed a BAF higher than 1 (Table 6). This result indicates a low capacity to uptake high quantity of Cu, so all species can be considered as excluders of Cu. The species considered, showed a TF lower than 1 with the exception of indian mustard. This latter exhibited a TF higher than 1 at level 200 and 600 mg kg^{-1}, and at 600 mg kg^{-1} of Cu in the soil in 2009 and 2010, respectively. The value of TF, even if lower than 1, was near to 1 at level 400 mg kg^{-1} of Cu and at 200 and 400 mg kg^{-1} of Cu added to soil in 2009 and 2010, respectively. Indian mustard can be

Table 6
Values of the translocation factor (TF) and bioaccumulation factor (BAF) calculated for different part of plant using different amounts of Cu in the soil.

Plant	Copper added to the soil (mg kg^{-1})	BAF		TF	
		2009	2010	2009	2010
Barley	600	0.07	0.06	0.23	0.28
	400	0.08	0.07	0.21	0.28
	200	0.13	0.11	0.21	0.22
	0	–	–	–	–
Common bean	600	–	–	–	–
	400	0.08	0.07	0.65	0.68
	200	0.13	0.11	0.23	0.25
	0	–	–	–	–
Indian mustard	600	0.07	–	1	1
	400	0.07	0.1	0.97	0.91
	200	0.13	0.42	1.08	0.87
	0	–	–	–	–
Ricinus	600	0.1	0.09	0.58	0.62
	400	0.13	0.12	0.66	0.63
	200	0.19	0.16	0.41	0.43
	0	–	–	–	–
Sorghum	600	0.06	0.05	0.26	0.26
	400	0.06	0.06	0.18	0.2
	200	0.11	0.11	0.27	0.23
	0	–	–	–	–
Spinach	600	–	–	–	–
	400	–	–	–	–
	200	–	–	–	–
	0	–	–	–	–
Tomato	600	0.09	0.08	0.39	0.42
	400	0.09	0.09	0.36	0.33
	200	0.17	0.15	0.6	0.61
	0	–	–	–	–

considered a plant able to translocate the metal from root to areal tissue.

4. Conclusions

The widely use of copper-based pesticides in conventional and organic farming, especially in vineyards, is one of the main causes of agricultural soil pollution worldwide. The remediation of Cu contaminated soil by plant is a low cost and environmentally friendly strategy, therefore knowing the response of plant to Cu is a considerable issue. This study evaluates the Cu removal and storage capacity in roots, stems and leaves, and fruits of seven arable and vegetable crops. The results evidenced the higher Cu storage capacity in the epigeal part of spinach, followed, in decreasing order, by ricinus, tomato, Indian mustard, common bean, barley and sorghum. The spinach appeared to be the most efficient in the removal of Cu referred to unit of above ground dry matter produced. At the higher Cu concentration in the soil the removal reached 98.4 mg for each kg of dry biomass produced. On the other hand, considering the total uptake by plants, ricinus removes the highest amount of Cu. Among the species considered, tomato results the most efficient to accumulate Cu in fruits and besides their roots show a remarkable storage capacity. In addition, Indian mustard can be considered a plant able to translocate Cu from root to areal tissue. The study showed that the phytoremediation capability of crops depends on soil Cu concentration. At very high Cu concentration, even if appropriate, phytoremediation needs considerable time for lowering the Cu at acceptable levels. However, it has to be considered that the investigated arable and vegetable crops could be used, for example as cover crops, to remediate Cu polluted soil, as costs are represented by the normal cultivation costs.

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