HortScience 57(12):1548–1557. 2022. https://doi.org/10.21273/HORTSCI16791-22

Effect of the Growing Season, *Trichoderma*, and Clinoptilolite Application on Potentially Toxic Elements Uptake by *Cucumis melo* L.

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Keywords. clinoptilolite, food safety, heavy metals, plant uptake, *Trichoderma*

Abstract. **The extent to which different agricultural strategies may affect the uptake of potentially toxic elements (PTEs) by cropped plants is not entirely understood at a field scale. This study addresses the effect of seasonality, *Trichoderma* inoculation alone, or combined with different applications of commercial-grade clinoptilolite (i.e., foliar action, fertigation, and pellet) on the PTE content of early- and late-ripening cultivars of *Cucumis melo* L. Two similar field experiments were performed in spring and summer. For each cultivar/treatment combination, the input of PTEs [namely, chromium (Cr), copper (Cu), and lead (Pb)] into the soil-crop system through irrigation water, fertilizers, pesticides, and treatment products (i.e., *Trichoderma* and clinoptilolite products), as well as the PTE content of melon stem, leaves, and fruit, were measured through inductively coupled plasma-optic emission spectrometry (ICP-OES). Neither *Trichoderma* alone nor with clinoptilolite had a visible effect on PTE uptake by plants, whereas early season cultivation was strongly associated with reduced uptake of Cu and Pb. The high correlation of Cu and Pb content with stem and leaf calcium (Ca) content (used as a proxy for different transpiration rates under different growing seasons) indicated a possible uptake of these metals through Ca nonselective cation channels as a defense against drought stress. Reduced Cu and Pb concentrations were found in early-ripening fruit cultivated in spring. Concerning Cu and Pb risk management, in case of significant contamination in Mediterranean calcareous soils, early-ripening *Cucumis melo* L. cultivars are suggested instead of late-ripening ones.**

In cultivated soils, high PTE concentration of allochthonous origin are commonly attributed to anthropogenic industrial activities and the utilization of amendments, fertilizers, and poorly treated water (He et al. 2005; Thornton 1981). High levels of copper (Cu) and zinc (Zn) can accumulate in plants, as they are actively assimilated as essential micronutrients (Clemens et al. 2002). Similarly, nonessential elements, such as lead (Pb), arsenic (As), and cadmium (Cd), can be taken up by the crop root system and translocated into edible tissues (Lasat 2002; Sekara et al. 2005; Uchimiya et al. 2020).

In the European Union, excessive concentration of some PTEs (such as Cu and Pb) in food edible parts are considered a cause of food noncompliance, and regulation set a maximum amount tolerated within the inner market (European Commission 2006, 2008). Nevertheless, lower maximum PTEs concentration may be set by member states or within certain market segments (Buscaroli et al. 2021). The use of Cu salts as sulfates, commonly known as Bordeaux mixture, is permitted with some limitations on several crops (including melon) in integrated pest management (European Parliament 2009) and in organic crop production. Cu-based products have been massively used in the past century due to their fungicidal properties (Lamichhane et al. 2018), thus increasing the Cu pool in cultivated soils (La Torre et al. 2018). Agriculture intensification and reduction of crop diversification favored Cu accumulation in soils (Lamichhane et al. 2018). Moreover, the increasingly adopted practice of plowing crop residues (green manure) reduces Cu and other nutrition elements removal by harvest.

From an environmental point of view, the reduction of heavy metal content in polluted soils is generally achieved through phytoremediation techniques (Ali et al. 2013), soil washing (Buscaroli et al. 2019), and soil dilution (Dermont et al. 2008). Otherwise, agricultural strategies aimed at mitigating the risk of PTEs entering the food chain prevent their plant uptake and translocation through soil conditioning or pH corrections to reduce metal ion mobility and availability (Uchimiya et al. 2020).

Alternative solutions have been investigated by academia or proposed by professional horticulture companies. This is the case of all-purpose natural zeolite-based products, such as clinoptilolite, whose use in agriculture was proposed in past years owing to zeolite's high cation exchange capacity and nutrient content (Nakhli et al. 2017). More controversial is their use as PTE immobilizers, as studies on clinoptilolite's ability to stabilize PTEs are scarce and limited to particular applications (Leggo and Ledésert 2001; Leggo et al. 2006; Oste et al. 2002).

Another proposed strategy currently evaluated to prevent PTEs translocation to crops is soil treatment based on *Trichoderma* sp. fungi inoculation. *Trichoderma* sp. is a competitor of pathogenic fungi and can establish a mutualistic symbiotic endophytic association with certain crops (Berg 2009; Woo et al. 2006). Recent studies pointed out the capacity of symbiotic *Trichoderma* sp. to reduce micronutrient plant assimilation (de Santiago et al. 2011; Téllez Vargas et al. 2017), especially Cu. This mechanism is unclear, but it has been hypothesized that competition between *Trichoderma* and the inoculated crop for micronutrients is involved. If confirmed in multiple tests and fully understood, *Trichoderma* association with crops cultivated in Cu-rich substrates could be a valuable strategy to reduce excessive Cu accumulation.

Melon (*Cucumis melo* L.) is the third most largely cultivated horticultural crop in Italy [Italian Institute of Statistics (ISTAT) 2021]. The consumption of melon fruit peaked at 9.5 annual kilograms per capita in 2020 [Services Institute for Agri-food Market (ISMEA) 2020]. Italy is also the second largest European producer of this crop [Food and Agriculture Organization of United Nations (FAO) 2021]. Like other genera of the Cucurbitaceae family, melon may mobilize, uptake, and translocate organic and inorganic contaminants (Campbell et al. 2009; Mattina et al. 2004, 2006).

The aim of this study was to evaluate different agricultural strategies to elucidate the uptake and translocation of PTEs (i.e., Cu, Cr, and Pb) to three different melon cultivars of relevant commercial interest in the Italian market. Specifically, the effect of different applications of commercial-grade clinoptilolites, *Trichoderma* inoculation, and seasonality on PTE content of three different melon cultivars were evaluated. In parallel, a detailed mass

Received for publication 25 Jul 2022. Accepted for publication 14 Sep 2022.
Published online 15 Nov 2022.
All authors certify that they have no affiliations with or involvement in any organization or entity with any financial or nonfinancial interest in the subject matter or materials discussed in this manuscript.
This study was funded by Agricola Don Camillo S.C.a.R.L. and HM.Clause.
No experimentation on humans or animals has been performed.
Giampaolo Nitti (Department of Agricultural and Food Sciences and Technology, University of Bologna), Walter Benelli, Ilaria Orsi, Lorenzo Pezzali (Agricola Don Camillo), Sergio Guardigli, and Mauro Gallerani (HM. Clause Italia) are deeply thanked for their fundamental support to experimental design and field activities and for fruitful discussion.
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balance of PTEs introduced into the soil-crop system through irrigation water, fertilization, and pest management was assessed. Results indicated that *Trichoderma* alone or combined with clinoptilolite treatments did not affect PTE uptake, whereas early season cultivation was strongly associated with lower Cu and Pb uptake.

Materials and Methods

Experimental field design and bulk soil analysis. The experimental field was in the municipality of Viadana, MN, Italy (44°58′21″N, 10°35′11″E) within the production area of Agricola Don Camillo S.C.a.R.L. (Fig. 1), one of the largest melon producers in Italy. The plot used for the trials had an extension of 0.5 ha.

Before the beginning of the experimental trial, on 1 Jun 2020, a bulk soil sampling was performed. The soil was sampled according to nonsystematic "W" pattern sampling. Four soil core samples of 30 cm in depth were collected at positions shown in Fig. 1. The soil sampling was established at the expected melon roots development depth. Single soil core samples were homogenized, air-dried, manually ground, and sieved at 2 mm. Samples were analyzed for pH [International Organization for Standardization (ISO) 2021], electrical conductivity (EC) (ISO 1994), total organic carbon (TOC) (ISO 1995a), total nitrogen (TN) (ISO 1995b), and total carbonates (ISO 1995c). Particle size distribution was determined by the pipette method (Gee and Bauder 1986) using sodium hexametaphosphate as a dispersant.

Pseudo-total metal content of soil samples was determined through microwave acid digestion in *aqua regia* (ISO 2012). Digested extracts were filtered through Whatman no. 42 filter paper and analyzed by ICP-OES (ISO 2008a), using an ICP-OES Spectro Arcos (Ametek, Germany).

The fraction of potentially available-to-plant metal content was assessed by diethylentriaminopentaacetic acid (DTPA) extraction, adapting the method from Lindsay and Norvell (1978). The DTPA extractable metal fraction is considered a valuable proxy for the determination of element potential bioavailability for plants in alkaline soils (Lindsay and Norvell 1978). Briefly, soil samples were added to a DTPA solution (1.97 $g \cdot L^{-1}$ DTPA, 1.46 $g \cdot L^{-1}$ $CaCl_2 \cdot 2H_2O$, 14.92 $g \cdot L^{-1}$ triethanolamine) with 1:2 w:v ratio, at pH 7.3, shaken for 2 h, filtered through Whatman no. 42 paper, and analyzed by ICP-OES.

All analyses were performed in triplicate.

Field trials. Three commercial melon hybrids were selected for experimental cultivation: 1) Django F1, an early-ripening cultivar, 2) Costantino F1 and 3) EXPE504 F1, both late-ripening. All cultivars (from now on, called Django, Costantino, and 504, respectively, for the sake of brevity) were chosen for their commercial value, and provided by HM.Clause. No data were available beforehand on their specific capacity to mobilize or uptake PTEs differently. To even out the differences in PTEs uptake between early and late-ripening cultivars, Django, Costantino, and 504 were cultivated in both periods.

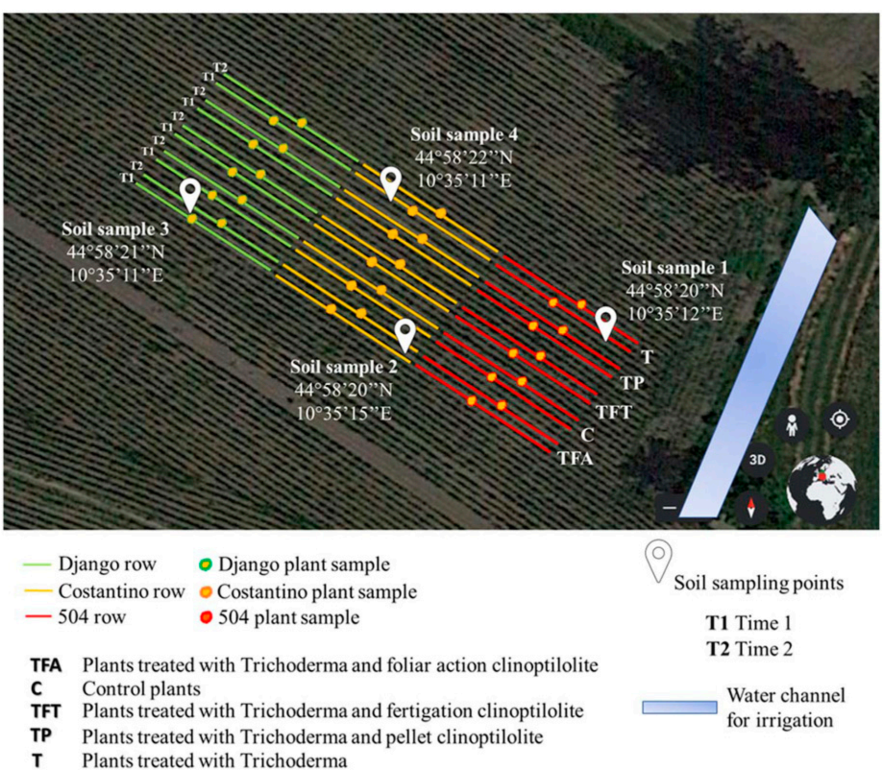

Fig. 1. Experimental field. Soil and plant sampling positions are indicated. Plants were sampled from the inner part of each cultivar row to avoid, as much as possible, side effects from the different vicinal varieties.

In 2020, two field trials with a duration of 84 d each were performed during spring (Time 1: planted on 2 Apr and harvested on 25 Jun) and summer (Time 2: planted on 1 Jun and harvested on 24 Aug). The planting layout provided for the rows to be laid in line on growing beds of 20 cm high from ground level. The distance between rows was 2 m and, along the row, the distance between plants was 1 m. The cultivars were planted according to the scheme indicated in Fig. 1. All three cultivars were fertilized and protected according to the integrated pest management protocol given as supporting information in Supplemental Table 1.

At both growing periods (Times 1 and 2) and on all three crop varieties, different treatments were evaluated: *Trichoderma* inoculation alone or combined with the most common clinoptilolite-based commercial products.

The acronyms of the different treatments are reported as follows:

- C: control not treated
- T: inoculation with *Trichoderma*
- TFA: inoculation with *Trichoderma* and foliar application of powdered clinoptilolite ("foliar action")
- TFT: inoculation with *Trichoderma* and application of powdered clinoptilolite via fertigation
- TP: inoculation with *Trichoderma* and soil application of clinoptilolite pellets

The products used, the dosage, and the application conditions for *Trichoderma* inoculation and clinoptilolite treatments are reported as supporting information in Supplemental Table 1.

A weather station ECO 4M (DigitEco Srl., Bologna, Italy) was installed at 20-m height from ground level close to the experimental plot (44°58′17″N, 10°35′03″E) for meteorological data collection. The average daily temperature, rainfall, and relative humidity of the period corresponding to the two experimental trials are reported as a supporting information in Supplemental Fig. 1. The average daily temperature showed a trend to increase from 13 °C on the first day of the experimentation (2 Apr) to 22.5 °C on the last one (20 Aug). During the first growing season, the total amount of rainfall was 126 mm spread over 31 d, whereas, during the second growing season, it was 197 mm spread over 26 d. The average relative humidity curve shows a variable trend with peaks of 90% corresponding to rainy days.

Trace elements analysis of fertilizers, pesticides, and products used for treatments. Pseudo-total metal content of the fertilizers, the pesticides, and the inoculation product used in the trials were determined through a wet acid attack on flame. Briefly, 5 g of each sample was placed in a 250-mL wide-neck flask. In general, 21 mL of hydrochloric acid (37% HCl for trace analysis; Honeywell Fluka, Muskegon, MI, USA) and 7 mL of nitric acid (65% HNO_3 for trace analysis;

Table 1. Physical and chemical characterization of 0 to 30-cm topsoil samples. Errors are expressed as a standard deviation.

Parameter	Soil samples 1	2	3	4	Avg	
pH H_2O	8.80 ± 0.01	8.80 ± 0.01	8.80 ± 0.01	8.80 ± 0.01	8.80 ± 0.01	
pH $CaCl_2$	7.90 ± 0.01	8.00 ± 0.01	8.10 ± 0.01	8.10 ± 0.01	8.00 ± 0.01	
Electrical conductivity ($ds \cdot m^{-1}$)	0.151 ± 0.004	0.143 ± 0.003	0.145 ± 0.006	0.154 ± 0.001	0.148 ± 0.002	
Total organic carbon (%)	1.329 ± 0.008	1.429 ± 0.029	1.412 ± 0.117	1.535 ± 0.019	1.426 ± 0.018	
Total nitrogen (%)	0.193 ± 0.003	0.210 ± 0.008	0.205 ± 0.003	0.226 ± 0.004	0.229 ± 0.005	
Carbonates ($CaCO_3$%)	7.952 ± 0.420	7.251 ± 1.055	8.210 ± 1.087	7.798 ± 0.776	7.803 ± 0.834	
Textural class (USDA[i] class)	Silty Clay Loam	Silty Clay Loam	Silty Clay Loam	Silty Clay Loam		
PTEs content ($mg \cdot kg^{-1}$)						Italian threshold concentration in soils[ii]
Chromium[iii]	208.2 ± 2.9 (0.01%)	212.1 ± 2.7 (0.01%)	197.0 ± 4.2 (0.01%)	205.6 ± 2.0 (0.01%)	205.0 ± 2.9 (0.01%)	150
Copper[iii]	114.1 ± 1.4 (11.84%)	91.3 ± 0.5 (10.20%)	99.2 ± 3.9 (12.25%)	72.4 ± 0.8 (10.05%)	87.6 ± 1.8 (11.08%)	100
Lead[iii]	33.8 ± 4.6 (6.88%)	33.4 ± 6.2 (7.93%)	35.4 ± 1.8 (7.48%)	36.1 ± 0.8 (7.73%)	35.0 ± 3.0 (7.51%)	200

[i] U.S. Department of Agriculture (Soil Survey Staff 1999).
[ii] D.Lgs. N°46 01/03/2019.
[iii] Diethylentriaminopentaacetic acid extractable potentially toxic elements (PTEs) content expressed in brackets as a percentage of the total PTEs amount.

Honeywell, Fluka) were added to the sample, and the mixture was heated on a Bunsen flame and brought to boiling. Only for organic fertilizers (namely, Lieta Veg, Agriges and Examine L®, K&A), digestion was completed by adding a further volume of 65% nitric acid and a few milliliters of hydrogen peroxide (30% H_2O_2 for electronic use; Honeywell, Fluka) until complete dissolution of the solid matrix. Pseudo-total metal content of the clinoptilolite-based products were determined through microwave acid digestion by adding to 0.250 g of each sample, 6 mL HCl (37%), 2 mL HNO_3 (65%), 2 mL HF (40% for trace analysis; Honeywell, Fluka), and 0.5 mL H_2O_2 (30%).

The digestates obtained were filtered through Whatman no. 42 filter paper, diluted to 20 mL with milliQ® water and analyzed by ICP-OES.

To evaluate to what extent any product contained PTEs readily available to plants, the metal fraction potentially available to plants of fertilizers, pesticides, and products used for treatments was assessed on DTPA extracts as already described for soil.

All analyses were performed in triplicate.

Irrigation protocol and water analysis. The irrigation was performed with water from a freshwater canal that is adjacent to the experimental field (see Fig. 1) through a drop-by-drop system equipped with a unit control that allowed us to measure the water daily distributed to each experimental field rows. The total irrigation water used separately for crops during the first and second growing periods was 192 and 218 m^3, respectively.

The irrigation water was collected monthly and characterized. On 15 Apr, 15 May, 15 Jun, 15 Jul, and 15 Aug 2020, a sample (10 L) of water was collected from the suction pipe connected to the freshwater canal. Water samples were filtered by Whatman 0.45-μm pore size nylon membrane filter to separate suspended solids from the liquid phase (ISO 1997). Both liquid and solid fractions were analyzed.

Filtered water was characterized for pH (ISO 2008b), EC (ISO 1985), and trace elements content. Trace elements analysis was carried out by adding 0.15 mL of HNO_3 (1% v/v) to 15 mL of each filtered water sample and analyzed by ICP-OES.

Suspended solids were air-dried, ground, and analyzed for pseudo-total metals content through microwave acid digestion (ISO 2012). Digested samples were filtered through Whatman no. 42 filter paper and analyzed by ICP-OES (ISO 2008a).

All analyses were performed in triplicate.

Table 2. Physical and chemical characterization of irrigation water. Error expressed as a standard deviation. In brackets, the detection limit (DL) value of the analytical method.

Parameter	Sampling date	Water samples 15 Apr 2020	15 May 2020	15 Jun 2020	15 Jul 2020	15 Aug 2020
pH		9.5 ± 0.1	8.0 ± 0.1	8.2 ± 0.1	8.4 ± 0.1	8.2 ± 0.1
Electrical conductivity ($dS \cdot m^{-1}$)		0.479 ± 0.040	0.286 ± 0.008	0.279 ± 0.007	0.271 ± 0.010	0.258 ± 0.005
Suspended solids ($mg \cdot L^{-1}$)		81 ± 1.0	83 ± 1.0	83 ± 1.0	93 ± 1.0	90 ± 1.0
Chromium ($mg \cdot L^{-1}$)	Filtered water	<DL (0.001)	<DL (0.001)	<DL (0.001)	<DL (0.001)	<DL (0.001)
	Suspended solids	0.012 ± 0.015	0.049 ± 0.001	0.049 ± 0.001	0.030 ± 0.001	0.034 ± 0.001
	Total	0.012	0.049	0.049	0.03	0.034
Copper ($mg \cdot L^{-1}$)	Filtered water	0.006 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.016 ± 0.001	0.016 ± 0.001
	Suspended solids	0.008 ± 0.001	0.024 ± 0.001	0.024 ± 0.001	0.010 ± 0.001	0.010 ± 0.001
	Total	0.014	0.027	0.027	0.026	0.026
Lead ($mg \cdot L^{-1}$)	Filtered water	<DL (0.003)	<DL (0.003)	<DL (0.003)	<DL (0.003)	<DL (0.003)
	Suspended solids	0.003 ± 0.001	0.010 ± 0.001	0.010 ± 0.001	0.010 ± 0.001	0.016 ± 0.001
	Total	0.003	0.010	0.010	0.010	0.016
Calcium ($mg \cdot L^{-1}$)	Filtered water	12.72 ± 0.290	35.69 ± 0.17	35.69 ± 0.17	17.35 ± 0.001	16.19 ± 0.406
	Suspended solids	17.72 ± 1.000	18.79 ± 1.000	18.79 ± 1.000	31.10 ± 1.000	32.43 ± 1.000
	Total	30.44	54.48	54.48	48.45	48.62
Silicon ($mg \cdot L^{-1}$)	Filtered water	0.156 ± 0.010	0.643 ± 0.010	0.643 ± 0.010	<DL (0.001)	<DL (0.001)
	Suspended solids	0.471 ± 0.001	0.524 ± 0.001	0.524 ± 0.001	0.491 ± 0.001	0.481 ± 0.001
	Total	0.627	1.167	1.167	0.491	0.481

Table 3. Total calcium, silicon, and potentially toxic elements (PTEs) content of fertilizers, pesticides, and tested products used during both growing seasons, expressed in $mg \cdot kg^{-1}$. Errors are expressed as a standard deviation. Diethylentriaminopentaacetic acid (DTPA) extractable PTEs content expressed in brackets as a percentage of the total PTEs amount.

Fertilizer commercial name	Element				
	Chromium	Copper	Lead	Calcium	Silicon
Lieta Veg	1.180 ± 0.271 (64.41%)	4.904 ± 1.263 (38.15%)	<DL	425.5 ± 32.45 (n.a.)	2.448 ± 0.052 (n.a.)
Examine L	1.470 ± 0.111 (67.81%)	2.079 ± 0.000 (10.08%)	0.510 ± 0.053 (0.00%)	8545 ± 361.2 (n.a.)	17.74 ± 0.122 (n.a.)
New Ferstim Idro	1.769 ± 0.266 (40.58%)	0.295 ± 0.107 (0.00%)	<DL	472.2 ± 20.53 (n.a.)	34.61 ± 1.660 (n.a.)
Idrocomplex New Blu	1.851 ± 0.012 (92.83%)	<DL	<DL	123.0 ± 19.85 (n.a.)	36.60 ± 0.212 (n.a.)
Pesticide commercial name					
Presidium One	5.45 ± 1.887 (2.31%)	2.639 ± 0.763 (48.16%)	<DL	314.7 ± 98.67 (n.a.)	10,750 ± 2.3000 (n.a.)
Volare	0.474 ± 0.002 (19.31%)	1.428 ± 0.084 (48.16%)	<DL	262.8 ± 86.82 (n.a.)	8351 ± 34.70 (n.a.)
Zoxium	7.57 ± 0.422 (0.86%)	1.343 ± 0.361 (100.0%)	<DL	2212 ± 128.2 (n.a.)	11,433 ± 203.2 (n.a.)
Thiopron	0.427 ± 0.142 (0.00%)	4.996 ± 1.597 (4.00%)	<DL	409.1 ± 2.183 (n.a.)	12,966 ± 800.4 (n.a.)
Poltiglia Disperss	9.33 ± 0.216 (2.39%)	19,099 ± 19.30 (38.66%)	4.283 ± 1.110 (0.00%)	74,722 ± 943.3 (n.a.)	1424 ± 660.4 (n.a.)
Clinoptilolite commercial name					
Clinogold (pellet)	6.879 ± 0.248 (1.72%)	3.846 ± 1.181 (1.76%)	38.62 ± 1.578 (0.00%)	1990 ± 68.05 (n.a.)	31,557 ± 825.8 (n.a.)
Clinogold (fertigation)	10.12 ± 0.432 (0.00%)	8.350 ± 1.209 (3.95%)	20.65 ± 1.520 (16.7%)	1877 ± 8.143 (n.a.)	30,968 ± 965.0 (n.a.)
Rock Powder (foliar action)	13.76 ± 0.000 (0.00%)	10.29 ± 0.000 (14.3%)	30.92 ± 1.050 (13.0%)	1825 ± 180.0 (n.a.)	31,363 ± 762.6 (n.a.)
Tusal (Inoculant)	4.318 ± 0.374 (0.00%)	7.603 ± 0.873 (19.93%)	47.62 ± 0.429 (0.00%)	843.6 ± 1.810 (n.a.)	1054 ± 67.71 (n.a.)
Detection limit	0.001	0.001	0.010	0.010	0.030

n.a. = not available (no official procedure to assess the potentially bioavailable-to-plant fraction is suitable).

Plant sample preparation and trace element analysis. At the end of each trial period, when most melon fruits were considered ripe for the market (i.e., 25 Jul and 24 Aug 2020, for Time 1 and Time 2, respectively), the epigeal portion of one single plant per variety per treatment per growing period (for a total of 30 plants) was collected at the positions shown in Fig. 1. The plants collected had a variation of the epigeal biomass within ± 10%. Nonripe fruit was discarded. Each plant was divided into three subsamples: stem, leaves, and fruit. Plant parts were thoroughly washed with tap water and then rinsed with deionized water. The parts were then dried in a ventilated oven at 60 °C for 72 h and finely ground with a food blender. The variation of the dry mass of all parts for each plant was < 10%. The water content of fruit samples was determined as a ponderal loss.

Trace element analysis was performed for each plant part (stem, leaves, and fruit). The metals content of the plant parts was determined through microwave acid digestion using 3:1 v/v ratio of 65% nitric acid and 30% hydrogen peroxide. After digestion, the solutions were filtered and analyzed by ICP-OES. Analyses were performed in triplicate.

Statistical analysis. Statistical analysis of the data on metals content in the epigeal plant's biomass (stem, leaves, and fruit) was conducted in the R environment (R Core Team 2020). The effects of variety, treatment, and time were assessed using a split-split plot analysis of variance (ANOVA) ($P < 0.05$), followed by an least significant difference (LSD) post hoc test ($P < 0.05$) with Bonferroni adjustment. The differences in metal content in the different epigeal fractions of plants (stem, leaves, fruit) for the two times of transplanting considered were tested with a two-way ANOVA ($P < 0.05$) followed by an LSD post hoc test ($P < 0.05$) with Bonferroni adjustment. The correlation matrix of the metal concentration in plant parts was computed using Pearson's correlation coefficient.

Results

Characterization of soil, irrigation water, fertilizers, pesticides, and treatment products. Characteristics of soil, irrigation water, and all products used for crop production are presented in Tables 1, 2, and 3, respectively.

The soil was silty clay loam, according to the U.S. Department of Agriculture classification system (Soil Survey Staff 1999), with an average alkaline pH ($pH_{H2O} = 8.8$; $pH_{CaCl2} = 8.0$) that was in line with its total carbonate content (7.8%) (Table 1).

Table 4. Calcium, silicon, and potentially toxic elements (PTEs) input ($g \cdot ha^{-1}$) to the soil/crop system due to irrigation water, fertilizers, pesticides, and trial products. Errors are expressed as a standard deviation.

Element	Chromium	Copper	Lead	Calcium	Silicon
Time 1					
Irrigation water	12.14 ± 3.552	6.964 ± 3.648	2.908 ± 1.228	16,018 ± 2298	247.4 ± 112.4
Fertilizers	0.226 ± 0.022	0.300 ± 0.036	0.040 ± 0.004	708.4 ± 30.62	3.044 ± 0.056
Pesticides	0.466 ± 0.022	802.4 ± 0.844	0.180 ± 0.044	3164 ± 39.04	704.0 ± 60.46
of which Poltiglia Disperss	*0.392 ± 0.010*	*802.2 ± 0.810*	*0.180 ± 0.046*	*3138 ± 39.60*	*59.80 ± 27.72*
Treatments					
Clinogold Clinoptilolite (pellet)	*1.030 ± 0.040*	*0.580 ± 0.180*	*5.800 ± 0.240*	*298.5 ± 10.20*	*4733 ± 123.8*
Clinogold Clinoptilolite (fertigation)	*0.100 ± 0.040*	*0.080 ± 0.010*	*0.210 ± 0.020*	*18.77 ± 0.080*	*309.6 ± 9.650*
Rock Powder Clinoptilolite (foliar action)	*0.100 ± 0.010*	*0.070 ± 0.010*	*0.220 ± 0.010*	*12.77 ± 1.260*	*219.5 ± 5.330*
Tusal (fertigation)	*0.005 ± 0.001*	*0.008 ± 0.001*	*0.045 ± 0.001*	*0.803 ± 0.003*	*1.003 ± 0.063*
Time 2					
Irrigation water	17.58 ± 1.206	11.63 ± 0.382	5.380 ± 0.956	22,234 ± 414.0	340.0 ± 15.19
Fertilizers	0.266 ± 0.030	0.550 ± 0.088	0.052 ± 0.003	886.8 ± 38.80	2.676 ± 0.010
Pesticides	0.466 ± 0.022	802.4 ± 0.844	0.180 ± 0.044	3164 ± 39.04	704.0 ± 60.46
of which Poltiglia Disperss	*0.392 ± 0.010*	*802.2 ± 0.810*	*0.180 ± 0.046*	*3138 ± 39.60*	*59.80 ± 27.72*
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Rock Powder Clinoptilolite (foliar action)	*0.100 ± 0.010*	*0.070 ± 0.010*	*0.220 ± 0.010*	*12.77 ± 1.260*	*219.5 ± 5.330*
Tusal (fertigation)	*0.005 ± 0.001*	*0.008 ± 0.001*	*0.045 ± 0.001*	*0.803 ± 0.003*	*1.003 ± 0.063*

Data in italics report the specific name of products used for the tests and the pesticide of major relevance.

Table 5. Total and diethylentriaminopentaacetic acid (DTPA) extractable input of chromium (Cr), copper (Cu), lead (Pb), calcium (Ca), and silicon (Si) per single treatment (C = control not treated; T = *Trichoderma* inoculation; TFA = *Trichoderma* inoculation and clinoptilolite for foliar action; TFT = *Trichoderma* inoculation and clinoptilolite in fertigation; TP = *Trichoderma* inoculation and clinoptilolite pellet). Bioavailability of Cr, Cu, and Pb was assessed as a DTPA extractable fraction. Ca and Si bioavailability was assessed as a water-soluble fraction.

Treatment		Cr	Cu	Pb	Ca	Si
		Time 1				
C	Total input ($g{\cdot}ha^{-1}$)	12.57	810.1	2.863	24,154	1,031
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.168	312.0	0.000	10,368	142.30
	Bioavailable (green) fraction (%)					
T	Total input ($g{\cdot}ha^{-1}$)	12.57	810.1	2.911	24,155	1,032
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.546	312.1	0.000	10,369	142.39
	Bioavailable (green) fraction (%)					
TFA	Total input ($g{\cdot}ha^{-1}$)	12.67	810.2	3.127	24,168	1,252
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.546	312.1	0.028	10,369	142.4
	Bioavailable (green) fraction (%)					
TFT	Total input ($g{\cdot}ha^{-1}$)	12.68	810.2	3.117	24,174	1,342
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.546	312.1	0.030	10,369	142.4
	Bioavailable (green) fraction (%)					
TP	Total input ($g{\cdot}ha^{-1}$)	13.61	810.7	8.704	24,454	5,765.8
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.563	312.1	0.000	10,369	142.4
	Bioavailable (green) fraction (%)					
		Time 2				
C	Total input ($g{\cdot}ha^{-1}$)	17.88	814.6	5.176	26,286	1,046
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.633	315.04	0.000	10,876	120.7
	Bioavailable (green) fraction (%)					
T	Total input ($g{\cdot}ha^{-1}$)	17.88	814.6	5.224	26,287	1,047
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.633	315.0	0.000	10,876	120.7
	Bioavailable (green) fraction (%)					
TFA	Total input ($g{\cdot}ha^{-1}$)	17.88	814.6	5.440	26,299	1,267
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.633	315.0	0.030	10,876	120.7
	Bioavailable (green) fraction (%)					
TFT	Total input ($g{\cdot}ha^{-1}$)	17.88	814.6	5.430	26,305	1,357
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.633	315.0	0.030	10,876	120.7
	Bioavailable (green) fraction (%)					
TP	Total input ($g{\cdot}ha^{-1}$)	18.915	815.2	11.017	26,585	5,781
	Bioavailable content ($g{\cdot}ha^{-1}$)	0.651	315.1	0.000	10,876	120.7
	Bioavailable (green) fraction (%)					

The soil had low salinity (EC = 0.15 $ds{\cdot}m^{-1}$) and a well-endowment of TOC and TN (1.43 and 0.23%, respectively), according to quality criteria defined for surrounding soils (ARPAV 2007).

Among PTEs, the average total content of Cr (205.0 ± 2.9 $mg{\cdot}kg^{-1}$) was found to exceed the legal threshold of 150 $mg{\cdot}kg^{-1}$ [Legislative Decree (D. Lgs.) N°46 01/03/2019] (Table 1). Moreover, the total Cu content at sampling positions 1 (114.1 ± 1.4 $mg{\cdot}kg^{-1}$) and 3 (99.2 ± 3.9 $mg{\cdot}kg^{-1}$) slightly exceeded the threshold of 100 $mg{\cdot}kg^{-1}$. The average total content of Pb was safely far from the legal threshold. Nevertheless, Pb was reported in the table because of its peculiar accumulation profile in the melon crop, as is discussed further on.

The potential bioavailability-to-plant content, expressed as a percentage of the total content, was in the order: Cu > Pb >> Cr (11.1%, 7.5%, and 0.01% of the corresponding average total content, respectively, Table 1).

Regarding the characterization of irrigation water samples that were collected monthly from Apr to Aug 2020 (Table 2), the samples were similar for pH (8.0–8.4) and EC (from 0.258 to 0.271 $dS{\cdot}m^{-1}$), except for that collected on 15 Apr (pH = 9.5; EC = 0.479 $dS{\cdot}m^{-1}$). The total metal amount of irrigation water is given as a sum of the amount contained in the filtered water and the suspended solids contained within. Cr and Pb were found only in the suspended solids of the water samples with concentrations ≤ 0.049 and ≤ 0.016 $mg{\cdot}L^{-1}$, respectively. Cu was found in filtered water and suspended solids with total content of ≤ 0.027 $mg{\cdot}L^{-1}$. In general, all the water parameters were compliant with international water quality standards for irrigation water (Pescod 1992).

The total content and the bioavailable fraction of PTEs of all the products used for crop production (namely, fertilizers, pesticides, clinoptilolite, and *Trichoderma*-based products) are reported in Table 3. Among the products, Poltiglia Disperss contained the highest amount of Cu (19,099 $mg{\cdot}kg^{-1}$), 38.66% of which was assessed as potentially bioavailable-to-plant (DTPA extractable). Appreciable levels of Pb were found in the *Trichoderma*-based product Tusal (47.62 $mg{\cdot}kg^{-1}$) and in the three clinoptilolite-based products (20.65–38.62 $mg{\cdot}kg^{-1}$) with measurable bioavailable fractions in the formulates for fertigation and foliar action only (16.7% and 13.0% of the total, respectively). Low levels of Cr were found in pesticides (≤ 9.33 $mg{\cdot}kg^{-1}$) and in clinoptilolite samples (≤ 13.76 $mg{\cdot}kg^{-1}$) with bioavailable percentages low and highly variable among the products.

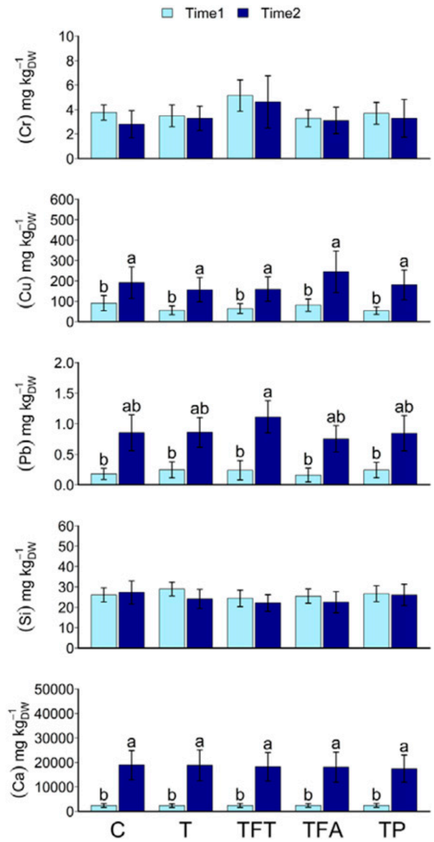

Fig. 2. Marginal means of chromium (Cr), copper (Cu), lead (Pb), silicon (Si), and calcium (Ca) content in melon plants (overall potentially toxic element concentration in aerial biomass), expressed as dry weight (DW) for the tested treatments. Error bars represent the standard errors and different lower-case letters indicate significant differences ($P < 0.05$) between treatment:time interaction as determined by the Fisher's least significant difference test. C = control not treated; T = *Trichoderma* inoculation; TFA = *Trichoderma* inoculation and clinoptilolite for foliar action; TFT = *Trichoderma* inoculation and clinoptilolite in fertigation; TP = *Trichoderma* inoculation and clinoptilolite pellet.

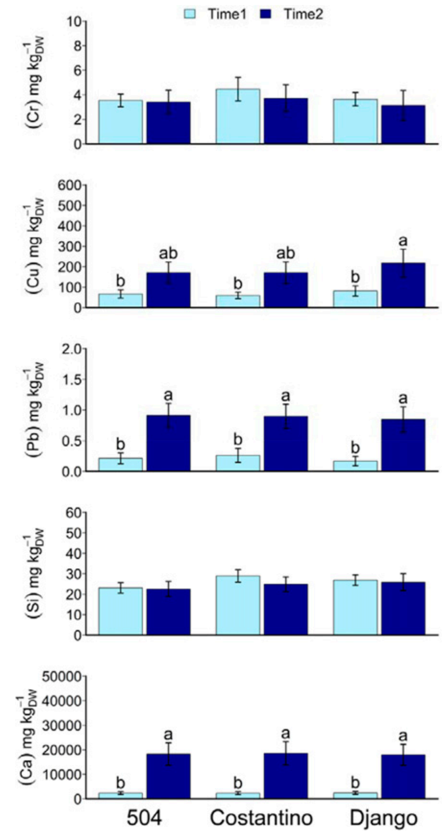

Fig. 3. Marginal means of chromium (Cr), copper (Cu), lead (Pb), silicon (Si), and calcium (Ca) content in melon plants (overall potentially toxic element concentration in aerial biomass), expressed as dry weight (DW), for variety (namely, '504', 'Costantino', and 'Django'). Error bars represent standard errors. Different lower-case letters indicate significant differences ($P < 0.05$) between crop variety:time interaction as determined by the Fisher's LSD test.

Total and bioavailable PTEs input. The metal input from irrigation water to each crop-soil system corresponding to a given treatment was obtained by multiplying the total metal content of the water collected on the 15th day of every month by the volume of irrigation water distributed to the corresponding field plot during that month.

As detailed in Tables 4 and 5, Cr input at both growing periods was mainly due to irrigation water (12.14–17.58 g·ha^{-1}) and, to a minor extent, to clinoptilolite pellet in TP treatment (1.03 g·ha^{-1}). Total Cr amounts received by different treatments (i.e., irrigation, fertilization, application of pesticides, and treatment products) were limited and in the range of 12.57 to 13.61 g·ha^{-1} at Time 1 and of 17.88 to 18.91 g·ha^{-1} at Time 2. Nevertheless, the bioavailable fraction that entered the soil-crop system was negligible in all treatments with respect to the total amount. Similar observations could be extended to the total input of Pb that was low and in the range of 2.86 to 8.70 g·ha^{-1} at Time 1 and of 5.18 to 11.02 at Time 2 (Table 5). The primary Pb sources were clinoptilolite pellet (TP treatment) and irrigation water (5.80 g·ha^{-1} and in the range 2.91–5.38 g·ha^{-1}, respectively) (Table 4) with no effect on the bioavailable portion (Table 5). On the contrary, for all treatments, the total input of Cu was high (≥ 810.1 g·ha^{-1}) and mainly due to Poltiglia Disperss (802.2 g·ha^{-1}, Supplemental Table 1) with a consistent bioavailable fraction (38.7%, Table 3).

Effect of variety, treatments, and seasonality on PTEs uptake by crop. Figure 2 shows the average Cr, Cu, and Pb contents in the aerial part of plant biomass (stem, leaves, and fruit) for each treatment in both growing periods. Plant Ca content was reported as a proxy for verifying a differential transpiration rate (Marschner 2011; McLaughlin and Wimmer 1999; White and Broadley 2003) under different growing seasons. Si content was reported as well due to the claim reported in De Smedt et al. (2015) that some clinoptilolite-based products, foliar action especially, can create a silica-film coating to protect the leaves from external pathogens.

Cr levels in plants did not show statistically different concentrations either among treatments or between growing periods (Fig. 2). For Cu, Pb, and Ca, no differences among treatments were observed for plants within the same growing period. In contrast, statistically significant differences resulted between Time 1 and Time 2. Cu, Pb, and Ca uptake resulted, on average, approximately three (69.25–187.0 mg·kg^{-1}), four (0.214–0.885 mg·kg^{-1}), and seven (2384–18,254 mg·kg^{-1}), respectively, times higher at Time 2 with respect to Time 1 (Fig. 2).

In Fig. 3, the marginal means of the metal uptake of each cultivar (namely, 504, Costantino, and Django) in the two growing periods were reported. The three cultivars did not uptake statistically different amounts of Cr and Si, neither within the same growing period nor between the two growing periods. Moreover, the Cu, Pb, and Ca uptake of the cultivars was not statistically different within the same growing period.

The trend in metals uptake by melon plants shown in Figs. 2 and 3 highlighted the lack of significant variability among both treatments and melon cultivars.

Figure 4 shows the marginal means of metal content in the stem, leaves, and fruit of plants grown at Time 1 and Time 2. Statistically significant differences in Cr, Cu, and Pb accumulation among the plant parts were observed. The higher plant uptake of Pb and Cu at Time 2 with respect to Time 1 seems to affect leaves and stem (Pb) mostly or leaves only (Cu). The fruit was the least accumulating plant part for Cu and Pb. In fruit, Cu content did not seem to be affected by the seasonality of the growing period, whereas Pb content seemed to be only partially affected (Fig. 4). On a dry weight (DW) basis, the increase of Cu average concentration in fruit at Time 2 with respect to Time 1 resulted moderate (15.83 and 13.68 mg·kg^{-1} DW, respectively) whereas the increase of Pb, on average, was more consistent (0.224 and 0.064 mg·kg^{-1} DW, respectively).

As big differences in Cu, Pb, and Ca concentration were observed from Time 1 and Time 2 in plant parts, the Pearson correlation (r) matrix for the metals in the plant parts was calculated (Table 6). As a result, positive correlations were observed both between Ca and Cu or Pb in leaves (r = 0.84 and 0.76, respectively) and stem (r = 0.89 and 0.74, respectively), thus supporting the figure that absorption and translocation of Pb and Cu are correlated with Ca uptake.

As far as the quality of melon fruit was concerned, the effect of variety and growing seasonality on Cu and Pb content of fruit was reconsidered on a fresh weight basis. Cu concentration in fresh fruit ranged from 1.88 mg·kg^{-1} in 'Django' to 2.22 mg·kg^{-1} in 'Costantino'. Despite a slight tendency, no significant effect of variety on Cu concentration was assessed. Concerning Pb, its concentration in fresh fruit spaced from a value of 0.01 mg·kg^{-1} in 'Costantino' to 0.03 mg·kg^{-1} in 'Django'.

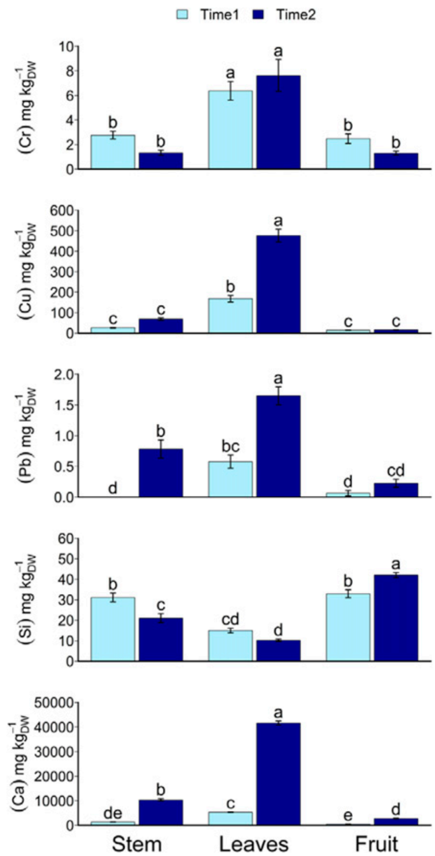

Fig. 4. Marginal means of chromium (Cr), copper (Cu), lead (Pb), silicon (Si), and calcium (Ca) content of plant fractions (stem, leaves, and fruit) expressed as dry weight (DW). Error bars represent standard errors. Different lower-case letters indicate significant differences ($P < 0.05$) between plant fractions:time interaction as determined by the Fisher's least significant difference test.

Again, no significant effect of the variety, as well as of variety:time interaction, was found under the two-way ANOVA. The only significant factor was the growing period. Cu and Pb concentration in fresh fruit cultivated at Time 2 increased by 25% ($P < 0.001$) and 312% ($P = 0.044$), respectively, with respect to Time 1.

In Table 7, the marginal means of Cu and Pb concentration in the fresh fruit are reported. In all cases, the fruit was safely edible for the EU market according to the current maximum concentration of Pb and Cu permitted in melon fruit of 0.1 and 5.0 mg·kg^{-1} fresh weight, respectively (European Commission, 2006, 2008).

Discussion

PTEs pool in soil. In the studied plot, soil Cr and Cu measured content was slightly higher than legal thresholds (Table 1). Nevertheless, this farmland is still suitable for food production, as Italian regulation allows cultivation of areas whose natural background PTE levels are slightly higher than the thresholds. In fact, Cr and Cu background values for Viadana municipality, as reported in ARPAE (2020) (151 mg·kg^{-1} < Cr < 225 mg·kg^{-1}; 61 mg·kg^{-1} < Cu < 120 mg·kg^{-1}), exceed the legal thresholds. Such Cr and Cu levels are rather typical in Po alluvial valley, especially in the immediate vicinity of Po river, as in this case. Indeed, contamination levels were mild, and of no serious concern from an environmental safety standpoint.

When potentially available-to-plants soil PTEs fraction was considered, Cu and Pb showed a considerably higher availability with respect to Cr (Table 1). These figures were expected in that, generally, in alkaline soils the bioavailability of Cu and Pb is mainly due to their forms as carbonates or complexed by the negatively charged moieties of soil organic matter (Alloway 2012; Shahid et al. 2012). In these forms, PTEs are suitable to be complexed by plant-derived carboxylates and then to be absorbed by roots. On the contrary, the unavailability of Cr(III) for plants is mainly due to its ability to form stable and insoluble species in soils at pH values > 5.5 (Alloway 2012; Shahid et al. 2017).

Incidence of PTEs input due to irrigation, fertilization, and pest control. The physical and chemical characteristics of the monthly sampled irrigation water was rather homogeneous, with the sole exception of the sample collected on 15 Apr (Table 2). The sample showed the highest pH value and EC with respect to the others. Such a high pH could be reasonably explained by larger input to the water canal of ammonia coming from surrounding cropped fields owing to the use of animal-based fertilizers/amendments locally produced by intensive animal farming, which is very extended in the area. The interruption of animal-based fertilizer distribution in later periods, and in summer, brought the pH to more neutral values in the water samples that were subsequently collected (pH ≤ 8.4). Moreover, the high value of suspended solids in water lastly sampled (15 Jul and 15 Aug) could be reasonably explained by the drier conditions of the summer period. Despite the larger amount (+56%) of rainfall at Time 2 with respect to Time 1, in the last 2 months of Time 2 only a few rainy days and four heavy rainfalls occurred (Supplemental Fig. 1). The drier summer conditions imposed an irrigation volume for the crop at Time 2 larger (+14%) than that used at Time 1 (218 and 192 m^3, respectively).

Considering the overall PTEs total input to the soil-plant system due to all the agricultural practices, Cu input resulted homogeneously distributed among treatments because of the same primary input (copper salts). On the other hand, Cr and Pb input slightly varied among treatments within the same growing periods and increased from the first to the second growing period mainly because of the different volumes and composition of irrigation water.

Nevertheless, each element input was negligible compared with the soil pool. Therefore, it is reasonable to assume that such slight variations of PTEs input unlikely influenced metal assimilation.

Effect of seasonality on PTEs translocation to plants. Likely, Cr uptake by plants produced under different treatments and growing seasons (Fig. 2) was flattened by its unavailability either in soil (Table 1) and/or in its input to the soil-crop system through irrigation water and products for crop production (Table 5). Similarly, plant Si content did not statistically differ among treatments and between the growing periods, thus ruling out any detectable accumulation in plant trials treated with clinoptilolite-based products (namely, pellet, fertigation product, and foliar action product in TP, TFT, and TFA treatments, respectively).

For Ca, Cu, and Pb, their considerably higher average uptake assessed during Time 2 with respect to Time 1 was not supported by a proportional increase of their total and potentially bioavailable input to the soil-crop system through irrigation water and products for crop production at Time 2 (Table 5). More likely, the higher uptake was due to a higher nutrient flux through the soil-root-shoot system following the climate variation between the two growing periods (Supplemental Fig. 1), that is, a higher transpiration rate. In the plants, Ca content was within the

Table 6. Pearson's correlation coefficients matrix of copper (Cu), lead (Pb), and calcium (Ca) content of fruit, leaves, and stem.

	Cu in fruit	Cu in leaves	Cu in stem	Cr in fruit	Cr in leaves	Cr in stem	Pb in fruit	Pb in leaves	Pb in stem	Ca in fruit	Ca in leaves	Ca in stem
Cu in fruit	1											
Cu in leaves	0.46[i]	1										
Cu in stem	0.38[i]	0.84[ii]	1									
Cr in fruit	0.07	−0.31	−0.35	1								
Cr in leaves	0.3	−0.03	−0.07	−0.09	1							
Cr in stem	−0.04	−0.59[ii]	−0.53[ii]	0.63[ii]	0.29	1						
Pb in fruit	0.25	0.43[i]	0.42[i]	0	0.32	0.03	1					
Pb in leaves	0.34	0.47[ii]	0.47[ii]	−0.3	0.59[ii]	−0.23	0.26	1				
Pb in stem	0.5[ii]	0.53[ii]	0.58[ii]	−0.28	0.45[i]	−0.24	0.25	0.63[ii]	1			
Ca in fruit	0.46[ii]	0.84[ii]	0.76[ii]	−0.44[i]	0.13	−0.59[ii]	0.28	0.72[ii]	0.7[ii]	1		
Ca in leaves	0.45[i]	0.84[ii]	0.78[ii]	−0.46[i]	0.21	−0.55[ii]	0.34	0.76[ii]	0.75[ii]	0.97[ii]	1	
Ca in stem	0.4[i]	0.83[ii]	0.89[ii]	−0.43[i]	0.14	−0.52[ii]	0.44[i]	0.7[ii]	0.74[ii]	0.92[ii]	0.95[ii]	1

[i] Significant at $P < 0.05$.

[ii] Significant at $P < 0.01$.

Table 7. Marginal means of copper (Cu) and lead (Pb) content in the fresh fruit ($mg \cdot kg^{-1}$ fresh weight). Different lower-case letters indicate significant differences ($P < 0.05$) as determined by the Fisher's least significant difference test.

	Cu	Pb
Variety		
Costantino	2.22	0.010
Django	1.88	0.027
504	1.91	0.025
P	0.044	0.462
Time		
Time 1	1.77 b	0.008 b
Time 2	2.23 a	0.033 a
P	<0.001	0.044
Variety × Time		
P	0.738	0.959

typical range of 1 to 50 $mg \cdot g^{-1}$ DW (Kirkby and Pilbeam 1984) for higher vascular plants.

Largely, Ca is transported within roots through water mass flux (Kirkby and Pilbeam 1984). Absorption is mediated by Ca nonselective cation channels (Ca-NSCCs), specialized transmembrane pores permeable to different cations, which are activated/deactivated by ion flux or different stimuli (e.g., environmental stress) (Demidchik et al. 2002). These channels are involved in several defense processes against heat stresses (Naeem et al. 2020) and act as a heat sensor that causes hyperaccumulation of Ca and other compatible solutes. Moreover, Ca signal transduction has a role in leaf surface temperature regulation by regulating stomatal conductance (Demidchik and Maathuis 2007). Several studies indicated Ca-NSCCSs as a possible pathway for heavy metals uptake (Clemens 2006; Demidchik et al. 2018; Gallego et al. 2012; Pourrut et al. 2008; Robinson et al. 2009; Sanz et al. 2019; Wang et al. 2007) owing to the significant permeability for both mono and divalent cations (Demidchik and Maathuis 2007). Moreover, positive correlation between transpiration flux increase and Cu and Zn uptake is commonly found (Tani and Barrington 2005a, 2005b).

In our plants, most of the considered metals accumulated in leaves, except for Si. As defined by several studies on plant metal uptake and translocation mechanisms, the vacuole of leaf cells represents the main storage site for both excess nutrients and PTEs (Krämer 2010; Pilon-Smits 2005; Sharma et al. 2016). The metals translocation to above-ground tissues plays an ecological role as a detoxification and a herbivores defense strategy (Boyd 2007; Martinoia et al. 2012; Rascio and Navari-Izzo 2011; Sharma et al. 2016).

Because of these observations, it was reasonable to suppose that, in our study, the increase in Cu, Pb, and Ca uptake during the second growing season could depend mainly on the signal activation of Ca channels in response to the higher evapotranspiration rate typical of the summer growing period and the higher (+14%) irrigation volume used.

Conclusions

The field study was conducted on calcareous agricultural soil whose Cr and Cu contents were lower than the background levels but higher than legal threshold limits: a typical figure of intensively cropped soils that have been regularly fertilized, amended with composts, and treated with copper salts.

Under integrated pest management, Cr uptake by *Cucumis melo* L. was unaffected by seasonality, early- or late-ripening varieties, and *Trichoderma* inoculation alone or combined with pellet, foliar action, and fertigation clinoptilolite-based treatments, thus confirming its well-known low availability to plants.

On the contrary, the leaf translocation of Cu was significantly limited by the early cultivation period and, thus, by early-ripening varieties. A similar figure was observed with Pb, although its soil content was abundantly lower than the Italian threshold concentration. This result is of particular interest in reducing the overall uptake of Cu and Pb, whose presence in food is regulated within the European Union, in case the melon crop is cultivated in soils with sensible levels of these PTEs. The strong correlation between Ca uptake, used as a proxy of transpiration rate, and Cu and Pb accumulation, that affected leaves mainly, may indicate the involvement of Ca nonselective cation channels as a possible main entry for the PTEs in the epigeal biomass.

Concerning the market quality of fresh fruit, the lowest Cu concentration was found in the early-ripening 'Django'. Tendentially, lower average Pb concentration in fruit was found in the early cultivation season, but no significant effects of variety and growing period were observed.

These results indicate that a possible strategy to mitigate Cu and Pb uptake by melon plants, as well as Cu and Pb concentration of fresh fruit, can be achieved by anticipating the growing period of melons. Under field conditions, the cultivation of early-ripening cultivars had a significant impact on reducing the metal translocation and the Cu concentration in the fruit.

To the scope of PTEs risk management, the results of the present study support the strategies aimed to use early-ripening cultivars in place of late-ripening ones in case a significant PTEs contamination is expected (i.e., soils with critical PTEs levels). Moreover, as most Cu and Pb are accumulated in leave tissues, plowing of crop residues should be limited as much as possible. More generally, manage Cu-based pesticides to avoid long-term accumulation in soil.

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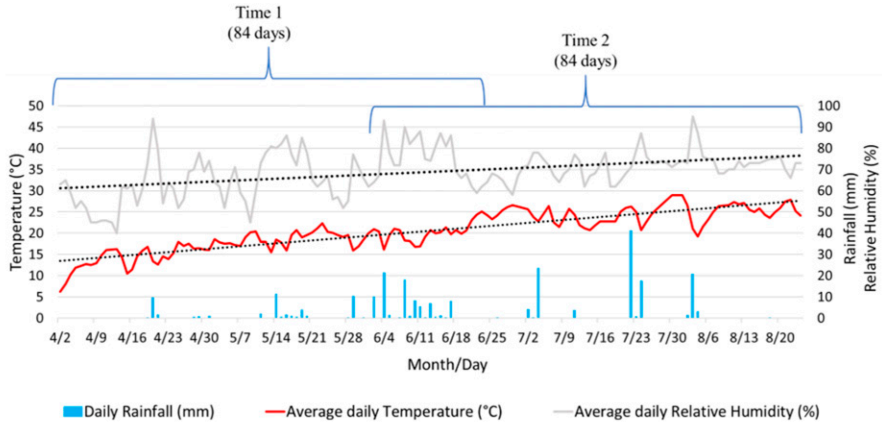

Supplemental Fig. 1. Graph of average temperature, average relative humidity, and daily rainfall from 2 Apr to 24 Aug 2020. The duration of the two growing periods (Time 1 and 2) is indicated.

Supplemental Table 1. Fertilizers, pesticides, and products used for treatments.

Products used in all five treatments			
		Dosage (kg·ha^{-1} or L·ha^{-1})	
Trade name	Composition or active ingredient	Time 2	Time 1
Fertilizers			
Lieta Veg, Agriges	N = 2.5%	20	70
Examine L®, K&A	N = 3%	80	100
New Ferstim Idro, Scam	NPK = 6–12–0	24	—
Idrocomplex New Blu, Scam	NPK = 20–20–20	20	20
Pesticides			
Volare® Bayer	fluopicolide + propamocarb (62.5 + 625 g·L^{-1})	3.2	3.2
Presidium One®, Gowan Italia	zoxamide + dimethomorph (180 + 180 g·L^{-1})	6	6
Zoxium® 240 SC, Gowan Italia	zoxamide (240 g·L^{-1})	3	3
Poltiglia Disperss®, UPL Italia	copper sulfate (Cu = 20%)	42	42
Thiopron®, UPL Italia	sulfur (825 g·L^{-1})	40	0

Treatment specific products			
Product name (description) *treatment*	Composition	Dosage (kg·ha^{-1}or L·ha^{-1})	
		Time 1	Time 1
Tusal® (Liquid for fertigation), *T, TFA, TFT, TP*	*Trichoderma atroviride* T11 + *Trichoderma asperellum* T25 (0.5 + 0.5%)	1	1
Clinogold, BHYP® (pellet - soil conditioner) *TP*	Clinoptilolite (95%)	150	150
Clinogold, BHYP® (powder for fertigation) *TFT*	Clinoptilolite (95%)	10	10
Rock powder, Midori® (powder for foliar action) *TFA*	Clinoptilolite (83% to 94%)	8	8

T = inoculation with *Trichoderma;* TFA = inoculation with *Trichoderma* and foliar application of powdered clinoptilolite (“foliar action”); TFT = inoculation with *Trichoderma* and application of powdered clinoptilolite via fertigation; TP = inoculation with *Trichoderma* and soil application of clinoptilolite pellets.