

Article

Uptake of Potentially Toxic Elements by Four Plant Species Suitable for Phytoremediation of Turin Urban Soils

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Received: 7 May 2020; Accepted: 4 June 2020; Published: 6 June 2020



Abstract: This study investigated the concentrations of 22 elements in two Turin urban soils located in the city center (Campana Street garden (CA)) and in a peripheral area (Nobile Park (NOB)). The former was found contaminated by Pb, Zn, Ba, Cr and Ni and, to a lower degree, by As, Co, Cu and Cd, while the latter showed high concentrations of Co, Cr and Ni. The nature of Cr, Ni and Co in both sites is mainly geogenic, whereas the high content of Pb, Zn, Ba, As, Cu and Cd in the CA soil is probably due to exposure to atmospheric deposition linked to emissions from motor vehicles, domestic and industrial burning of fossil fuels and industrial emissions. We evaluated the uptake of potentially toxic elements (PTEs) by four plant species suitable for phytoremediation (*Brassica juncea*, *Helianthus annuus*, *Zea mays* and *Pteris vittata*) in controlled conditions in CA and NOB soils in order to assess their efficiency in the absorption of PTEs and suitability to restore the CA site. Results highlighted a different uptake ability of the plants according to the considered element; for example, *Brassica juncea* demonstrated a great capability in cadmium uptake. The effect of a soil improver, derived from the composting of green and organic waste, on absorption efficiency was also studied and it altered uptake preferences of specific elements by the tested plant species, thus suggesting that its use has to be evaluated according to the target.

Keywords: phytoremediation; potentially toxic elements; contaminated urban soil; soil improver

1. Introduction

Currently, about half of the world's population lives in cities, often in overcrowded conditions [1,2]. This, in addition to the growing industrialization, has led to massive overbuilding and to the reduction of available green areas. For this reason, the interest in the restoration of urban and peripheral areas, assigning their management to associations or private citizens for the cultivation of flowers, fruits and vegetables, has grown in the last few years [3].

However, the position of these gardens within the urban environment exposes the soil to widespread pollution coming from different sources, such as atmospheric deposition as a result of industrial emissions, vehicular traffic, energy production and waste treatment plants [4,5].

The nature of pollutants present in these soils ranges from inorganic compounds (such as potentially toxic elements (PTEs)) to recalcitrant organic compounds (e.g., polycyclic aromatic hydrocarbons) [6] at concentrations that, when exceeding legal limits, entail the application of soil reclamation strategies.

Various chemical–physical techniques for the remediation of contaminated soils are currently available, such as leaching, vitrification, isolation of the polluted soil and its removal and electrokinetic techniques [7]. Immobilization or extraction of metals from the contaminated site by physical techniques are generally chosen for areas where rapid or complete decontamination is required. Many of these methods, besides having high costs, can cause secondary pollution or negative effects on the biological activities, on the structure and on the fertility of the soil [8]. Furthermore, these reclamation processes often involve expensive environmental and civil engineering practices and are unattractive to citizens, making them inappropriate for urban spaces.

Therefore, phytoremediation, among the less expensive and impactful remediation techniques, is an effective approach to achieve the abatement of potentially toxic elements (PTEs). It basically involves the use of green plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environment by exploiting the great ability of plants to take up pollutants from soils and to detoxify them through various mechanisms [9,10].

Phytoremediation techniques can be classified as follows:

- Phytoextraction, that uses pollutant-accumulating plants to absorb contaminants from soil by concentrating them in the harvestable biomass;
- Phytodegradation, that is related to the use of plants and associated microorganisms to degrade organic pollutants;
- Rhizofiltration, that utilizes plant roots to absorb pollutants, mainly metals, from water and aqueous waste streams;
- Phytostabilization, that refers to the use of plants to reduce the bioavailability of pollutants in the environment; and
- Phytovolatilization, that uses plants to volatilize pollutants [11,12].

The choice of the kind of phytoremediation depends on the matrix to be treated, on the type and bioavailability of pollutants and on the characteristics of the plants used [13].

In particular, plants for phytoextraction should ideally respect some characteristics, namely rapid growth, large biomass production, huge accumulation of metals (or other pollutants of interest) in the parts of the plant developed above the soil surface (which can subsequently be used for non-food purposes or ashed, followed by disposal in a landfill or, in the case of valuable metals, recycling of the accumulated element [13]) and good tolerance to high concentrations of pollutants [14,15].

The aim of this study was to evaluate the efficiency of a phytoremediation approach for the recovery of contaminated soil located in Turin by applying a green and low-cost process. For this purpose, the capability of PTE accumulation of four plant species (*Brassica juncea*, *Helianthus annuus*, *Zea mays* and *Pteris vittata*) cultivated in two Turin urban soils characterized by different PTE contents was studied. These plants, in fact, are known to be capable of accumulating high concentrations of PTEs in aerial parts—*H. annuus* exhibits a strong accumulation potential for Pb and Zn especially in the aerial part, which makes it particularly effective for phytoextraction [16,17], *P. vittata* shows a great ability in the absorption of As [18], *B. juncea* is suitable for the phytoremediation of Cd and Pb [19,20], whereas *Z. mays* was studied for the absorption of various heavy metals and, in particular, of Cd and Pb [21,22].

The phytoextraction efficiency of the four plants was assessed through the following means:

- determination of the metal concentration in plant tissues;
- assessment of the best extracted elements and of the possible selectivity of plant species towards some of them; and

- identification of the main accumulation site in the plant, root or aerial parts, with the latter representing the best condition in the case of an experiment in the field as leaves and stems are the easiest part to collect and dispose of.

The effect of adding a soil improver, derived from the composting of green and organic waste, on the absorption efficiency of the plants selected for phytoremediation was also assessed.

2. Materials and Methods

In the present study, two different soils, located in Turin (Italy), were used—one came from a central area of Turin, i.e., Campana Street garden (CA, GPS coordinates: 45°3′14″ N, 7°40′57″ E), and the other came from a peripheral area, i.e., Nobile Park (NOB, GPS coordinates: 45°3′1″ N, E 7°42′52″ E) (Figure 1).

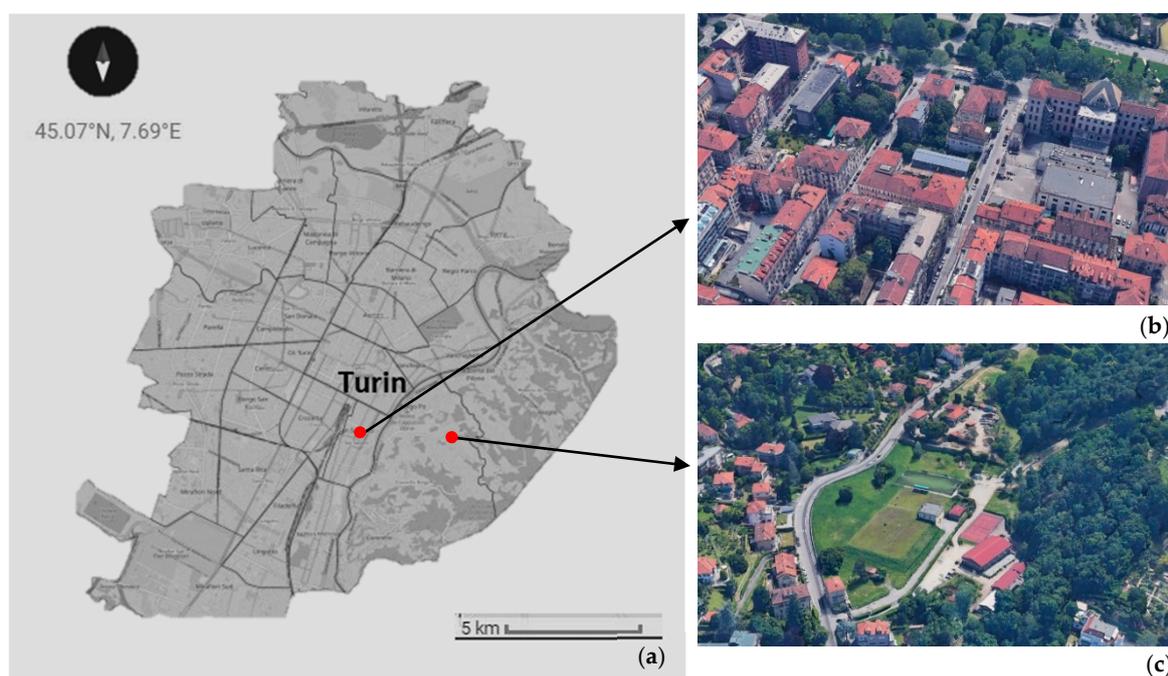


Figure 1. (a) Turin map and satellite (Google Earth) images of the considered sites: (b) Campana street garden (CA) and (c) Nobile Park (NOB).

Furthermore, the study was conducted to evaluate alterations in plant mineral accumulation caused by the addition of a soil improver, named Florawiva (FW), and supplied by Acea Pinerolese. This concentrated soil amendment comprises composted green waste and anaerobically digested organic materials from several sources [23].

The composting process consists of two phases, namely the bio-oxidation (active composting time) of the most easily degradable organic components and the maturation (curing phase) in which the product is stabilized and enriched with humic substances [24].

2.1. Apparatus and Reagents

Soil and plant sample mineralization for the determination of element content was performed with a Milestone MLS-1200 Mega (Milestone, Sorisole, Italy) microwave digestion system. Hydrogen peroxide (30% w/w), nitric acid ($\geq 65\%$) and hydrochloric acid ($\geq 37\%$) used for the digestion were provided by Merck (Darmstadt, Germany). Sample blanks were prepared using the same reagents.

Element quantification was conducted with an inductively coupled plasma atomic emission spectrometer (ICP-AES, Optima 7000 DV PerkinElmer, Waltham, MA, USA) or with a high-resolution inductively coupled plasma mass spectrometer (HR-ICP-MS, Element 2 Thermo Finnigan, Waltham,

MA, USA) or with a graphite furnace atomic absorption spectrometer (GF-AAS, Analyst 600 PerkinElmer, Waltham, MA, USA), alternatively chosen on the basis of the required sensitivity and of the matrix of samples.

Calibration curves were carried out using the external standard calibration method. Standard solutions were prepared by diluting single element concentrated (1000 mg L^{-1}) stock solutions (Sigma-Aldrich TraceCERT, St. Louis, MO, USA) with aliquots of sample blanks obtained by using the same acid mixtures of samples (matrix matching method) [25]. For the freeze-drying of the samples, a LaboGene ScanVac CoolSafe instrument (Lillerød, Denmark) was utilized. Water was purified in a Milli-Q system (Merck Millipore, Burlington, MA, USA), resulting in high purity water (HPW) with a resistivity of $18.2 \text{ M}\Omega \text{ cm}$.

2.2. Soil Sampling and Characterization

Before sampling, 15 mm of topsoil were removed to avoid collecting the turf. A composite sample of about 30 kg was taken through the mixing of 20 soil samples, each collected randomly with a plastic spade at depths of 0–10 cm in the areas of NOB and CA.

Plastic tools were used throughout to avoid contamination by metal abrasion, and the samples were stored in polyethylene bags. Before starting the experimentation ($t = 0$), representative soil samples were air-dried, sieved (2 mm), ground in a centrifugal ball mill, stored in polycarbonate jars and subsequently analyzed to determine the initial concentrations of elements, pH, organic carbon, organic matter content and particle size distribution. Furthermore, pH was determined by potentiometric analysis on a soil– CaCl_2 suspension, organic carbon and organic matter were measured with the Walkley–Black method according to Italian official soil chemical analysis methods described by the Ministerial Decree 13 September 1999 [26].

The remaining untreated soils were used for pot experiments. The concentration of 22 elements (transition metals, alkaline and alkaline earth metals, P, As and two lanthanides (La and Ce)) was measured on t_0 samples. The analysis required a pretreatment consisting of a microwave-assisted acid digestion. Soil lots of 50 mg were added to 5 mL of aqua regia in tetrafluoromethoxyl (TFM) vessels and digested following the heating program, i.e., first step of 2 min at 250 W, second step of 2 min at 0 W, third step of 6 min at 250 W, fourth step of 5 min at 400 W, fifth step of 5 min at 650 W and 20 min of ventilation. Three replicates were prepared for each sample. The obtained solutions were filtered on Whatman Grade 5 cellulose paper filters before being diluted to 50 mL with HPW in polyethylene containers. Most elements were analyzed by ICP-AES, except the cases where the observed concentrations below the detection limit (DL) of this technique made it necessary to employ the GF-AAS technique.

2.3. Plants, Growth Conditions and Phytoextraction Assessment

Studies on the accumulation of PTEs in plants were carried out in pots. Plants were grown in standard plastic nursery pots (diameter, 12 cm; capacity, 600 mL) arranged as follows: at the bottom of each pot, 60 mL of coarse-grained quartz sand (sterilized at $150 \text{ }^\circ\text{C}$ in a stove for 48 h) were placed in order to facilitate drainage. The pots were then filled with unaltered soil or soil mixed with the soil improver FW (70% soil and 30% soil improver (v/v)).

Brassica juncea (L.) Czern. ISC199, *Helianthus annuus* L. Nano semplice and *Zea mays* L. Panna plants were obtained from seeds (the first provided by Arcoiris, Modena, Italy; sunflower and corn by Dotto Sementi, Udine, Italy). Since preliminary experiments showed a reduction in germination induced by the presence of the soil improver, we sowed 100 seeds per species in trays containing the CA soil without amendment in order to get plants as uniform as possible. After germination, uniformly grown plants were transplanted, three per pot, in the CA soil, with or without amendment. About two weeks from transplanting, they were thinned to one per pot.

P. vittata plants were obtained from spores collected from plants growing at the Botanical Garden of Torino (Italy) and dispersed on the CA soil that was kept constantly humid. About three months

after spore germination, evenly developed sporophytes were transplanted into pots containing CA soil tests.

Five plants per treatment were cultivated under controlled conditions in a growth chamber under the following conditions: 16/8 h light/dark photoperiod, and 23 ± 1 °C/ 20 ± 1 °C light/dark thermoperiod. The light source consisted of four GreenPower LED production modules (Certhon, Poeldijk, The Netherlands) per shelf with a 2:1 red (626 nm peak) and blue (470 nm peak) emission. Plants were watered three times a week with deionized water, provided to limit leaking from the pots, and the experiment was carried out according to a randomized design, changing the position of each pot during the experiment in order to avoid the effect of position on plant growth.

Based on the results obtained from this first experimental trial, two more experiments were carried out on the four plants including, for *P. vittata* and *B. juncea*, the cultivation in the NOB soil, with and without amendment, following the same methodology described above.

The sampling took place at early flowering, eight weeks from sowing for *B. juncea* and *P. vittata* and fifteen weeks for *H. annuus* and *Z. mays*. For each plant, the fresh weight was determined.

Harvested plants were washed to eliminate soil residues and epigeal and hypogeal parts were separated. Then, they were weighed, freeze-dried and ground to obtain samples as homogeneous as possible.

Leaves and stems or roots were pretreated through a microwave-assisted acid digestion [27], and three replicates were prepared for each sample.

In all, 20 elements (V, Cr, Co, Ni, Cu, As, Cd, Pb, Zn, P, Mg, K, Ca, Al, Ti, Mn, Ba, Sr, Fe and Na) were determined by ICP-AES or HR-ICP-MS depending on the concentration range. Bioconcentration factor (BF) was calculated in order to assess the amount of PTEs that were transferred from the soil to the plant. It was calculated according to the following formula [28,29]:

$$BF = \frac{\text{PTE concentration in plant biomass (mgkg}^{-1} \text{ dry weight)}}{\text{PTE concentration in soil (mgkg}^{-1} \text{ dry weight)}} \quad (1)$$

Moreover, to evaluate in which part of the plant the PTEs were preferentially accumulated, the translocation factor (TF) was calculated using the following relationship [28,29]:

$$TF = \frac{\text{PTE in leaves and stems (mgkg}^{-1} \text{ dry weight)}}{\text{PTE concentration in roots (mgkg}^{-1} \text{ dry weight)}} \quad (2)$$

2.4. Data Analysis

Data about plants' fresh weights were processed with Excel 2013 calculating averages and standard deviation. The significance of differences found between treatments was assessed by the Tukey test or by the Mann–Whitney test for data normally distributed or not, respectively, using the PAST 3 program (PAleontological STatistics, Hammer & Harper, Oslo, Norway).

The experimental results obtained by chemical analyses were processed by chemometric treatment using the XlStat 2017 software package, an add-on of Microsoft Excel. Analysis of variance (ANOVA), Tukey test with a level of confidence of 95% and principal component analysis (PCA) were performed. Information on the principles of this technique can be found elsewhere [30,31]. The analytes whose concentrations were below the detection limits (DLs) in more than 30% of the samples were not included in the treatments [32]. For performing PCA, values below the DL were replaced by an imposed random value lower than the DL value; the whole dataset was then autoscaled.

3. Results and Discussion

3.1. Soil Characterization

The obtained values for organic carbon, organic matter, pH and particle size distribution are reported in Table 1.

Table 1. General characteristics of the soils.

	CA	NOB
Organic carbon (% w/w)	1.66	2.05
Organic matter (% w/w)	2.86	3.54
pH	7.10	7.30
Particle size distribution		
% sand	65.1	64.8
% silt	28.2	28.2
% clay	6.78	6.95

Organic matter content of a soil is of great importance since it can form soluble or insoluble complexes with metals, therefore influencing their tendency to be retained or to migrate throughout it [33].

Organic carbon, which makes up about 60% of the organic substance present in soils, has an essential positive impact on many soil properties. It promotes the aggregation and stability of soil particles, thereby reducing erosion, compaction, crevices and soil crust formation; it binds effectively with numerous substances improving soil fertility and its buffer capacity. Additionally, it improves microbial activity and the availability of nutrients for plants such as nitrogen and phosphorus [34]. pH can influence heavy metal adsorption capacity since these trace elements are very mobile in acidic conditions and their mobility and availability decreases as pH approaches neutrality [33]. Finally, soil texture also plays an important role in the mobility of metals; it reflects the particle size distribution of the soil and thus the content of fine particles like oxides and clay that are important adsorption media for heavy metals in soils [35].

The percentages of sand, silt and clay determined in the investigated soil samples allowed us to classify it as a sandy-loam soil. The texture, the medium-high percentages of organic matter and organic carbon and the pH close to neutrality suggested that the metals were not extremely mobile in the studied soils and were therefore less available.

In Table 2, the concentrations of the 22 elements determined in the two soils are reported with the concentration limits imposed by Italian legislation [36], which are different according to the intended use of the land (public green area or industrial area).

High concentrations of Pb, Ni and Zn and Co, Cr and Ni were found in the CA and NOB sites, respectively; their values exceeded public green area limits but not industrial area limits.

The content of Co, Cr and Ni is high in both sites according to the geogenic nature of these elements in the Turin valley. In fact, Piedmont plains originated during past fluvio-glacial events and are therefore made of sediments, which partly derive from serpentinitic areas resulting in high background levels of Co, Cr and Ni in soils, as already documented by other researchers [37,38].

Cu and Zn, which are micronutrients and therefore necessary in low concentration in agricultural lands, are less concentrated in the NOB soil; this confirms that they partially derive from anthropogenic contamination in the CA site. The load of Ba and Pb represents the most significant data—lead concentration in the CA soil is an order of magnitude greater than that of NOB, whereas barium shows a three times greater concentration in CA. This can result from anthropogenic pollution sources such as urban vehicular traffic. In fact, they are typical markers of road dust, since the deposition of Pb on the urban soil usually derives from tetraethyl lead (TEL), used as an antiknock additive in gasoline [39], whereas Ba is added in lubricating oils to prevent smoke and diesel engine abrasion and,

as barite, is employed in both the brake pads and in the tires of motor vehicles [40]. The presence of barium, usually used in paints and glass [41], can also be traced back to the history of this site, which, according to our information, has been a public garden for decades, but is located in an area of Turin that underwent several bombings during the Second World War, with the consequent accumulation of debris.

Table 2. Element concentration (mg/kg) \pm standard deviation (SD) in NOB and CA sites and Italian legislation limits.

	NOB t_0	CA t_0	Public Green Area Limit	Industrial Area Limit
Al	22,298 \pm 3280	23,184 \pm 1140		
As	9.33 \pm 0.70	15.8 \pm 1.7	20	50
Ba	104 \pm 15	322 \pm 48		
Ca	11,487 \pm 1493	21,412 \pm 8483		
Cd	0.21 \pm 0.03	1.16 \pm 0.09	2	15
Ce	42.4 \pm 0.3	36.5 \pm 3.6		
Co	23.2 \pm 1.5	16.6 \pm 1.8	20	250
Cr	248 \pm 37	150 \pm 74	150	800
Cu	39.5 \pm 3.3	75.2 \pm 3.8	120	600
Fe	33,552 \pm 2204	32,312 \pm 2960		
K	4969 \pm 1207	3867 \pm 126		
La	18.8 \pm 0.3	19.7 \pm 5.6		
Mg	22,819 \pm 3971	16,458 \pm 5670		
Mn	870 \pm 13	958 \pm 136		
Na	330 \pm 138	445 \pm 17		
Ni	253 \pm 29	147 \pm 22	120	500
P	617 \pm 29	1169 \pm 79		
Pb	34.3 \pm 0.6	312 \pm 44	100	1000
Sr	33.1 \pm 2.7	49.4 \pm 20.8		
Ti	865 \pm 108	795 \pm 83		
V	43.5 \pm 5.0	46.1 \pm 3.1	90	250
Zn	96.7 \pm 7.4	221 \pm 21	150	1500

Table 3 reports the concentrations of the elements determined in the soil added with a soil improver before filling the pots for plant cultivation in the growth chamber. The composition of FW is also reported.

FW has low concentrations of most PTEs, while it is rich in macronutrients such as P, Ca, Mg and K and in micronutrients such as Na, Zn and Cu.

The high load of some elements present in FW, e.g., P, Na, K and Ca, determines an increased amount of these elements in the soils added with the improver. The concentration of Zn in FW is very high; however, the addition of the improver in a percentage of 30% does not alter the concentration of Zn, whereas it causes a slight increase in the NOB soil.

Considering the variability of analytical data for soil and FW element concentration, we can assess that the addition of FW does not significantly affect the content of most of the examined elements.

The Tukey test with a level of confidence of 95% was used for multiple pairwise comparison after the ANOVA test in order to check if the analytes' concentrations determined in the CA and NOB samples without and with improver addition were significantly different (Table S1, Supplementary Materials). Cu, Pb, Ba and Zn have statistically different concentrations in the two sites, regardless of the addition of FW. No significant difference was found for the concentration of Al, Ca, Ce, Cr, Fe, La, Mg, Mn, Na, Ni, Ti and V, so the probable geogenic origin of Cr and Ni is confirmed. The different concentration of P in each soil highlights its different amount in the pristine soils and the influence of FW on its concentration. K concentration differs mainly according to the addition of the soil improver, whereas for As, Cd and Co the differentiation derives from the combination of soil type and addition of FW.

Table 3. Element concentration (mg/kg) \pm SD in Campana and Nobile soils, after adding a 30% portion of soil improver (Florawiva (FW)), and in FW, before starting the experimentation (t_0).

	NOB with FW		CA with FW		FW	
Al	22,367	\pm 865	22,075	\pm 6571	16,534	\pm 1014
As	9.23	\pm 2.05	12.3	\pm 0.4	3.75	\pm 0.12
Ba	133	\pm 15	369	\pm 16	170	\pm 7
Ca	20,031	\pm 1481	31,311	\pm 8496	52,258	\pm 3084
Cd	0.24	\pm 0.02	0.55	\pm 0.04	0.48	\pm 0.02
Ce	43.0	\pm 1.8	47.6	\pm 13.9	17.3	\pm 0.1
Co	20.6	\pm 1.8	15.6	\pm 1.4	6.42	\pm 0.47
Cr	271	\pm 39	142	\pm 24	70.7	\pm 0.9
Cu	51.6	\pm 6.1	87.6	\pm 3.1	95.4	\pm 2.7
Fe	30,309	\pm 320	28,704	\pm 2326	16,211	\pm 995
K	8469	\pm 632	7935	\pm 2164	13,920	\pm 470
La	19.2	\pm 1.4	20.9	\pm 9.5	9.25	\pm 0.82
Mg	26,175	\pm 7400	13,220	\pm 2424	11,746	\pm 1624
Mn	814	\pm 20	837	\pm 64	506	\pm 15
Na	871	\pm 59	909	\pm 341	2900	\pm 82
Ni	235	\pm 55	138	\pm 22	53.0	\pm 3.1
P	2383	\pm 194	3275	\pm 82	9271	\pm 635
Pb	34.4	\pm 0.8	267	\pm 3	49.9	\pm 1.3
Sr	54.3	\pm 7.4	112	\pm 5	192	\pm 5
Ti	903	\pm 101	950	\pm 145	733	\pm 41
V	44.4	\pm 1.4	50.3	\pm 9.8	24.4	\pm 1.8
Zn	116	\pm 6	229	\pm 10	225	\pm 23

3.2. Pot Experiments

This section reports the results obtained from the analyses carried out on the plants used for phytoextraction and the soil in which they were grown. The effect of the soil improver FW was also assessed.

Statistically significant differences between fresh weights (reported in Table 4) of plants grown in soils treated with soil improver or without were always found, indicating a growth stimulation by the amendment due to its content in organic substances and macronutrients and probably to its protective action against microbial pathogens [23].

Table 4. Total fresh weight (aerial parts + roots) (g/plant) \pm SD of the plants.

	<i>Helianthus annuus</i>		<i>Zea mays</i>		<i>Brassica juncea</i>		<i>Pteris vittata</i>	
CA	22.2	\pm 2.6	34.4	\pm 3.5	7.1	\pm 1.4	10.6	\pm 1.5
CA + FW	52.8	\pm 6.8	68.3	\pm 8.6	62.2	\pm 6.4	12.0	\pm 1.4
NOB	-	-	-	-	13.6	\pm 1.7	7.0	\pm 1.6
NOB + FW	-	-	-	-	70.5	\pm 8.0	10.6	\pm 1.6

Reported data refer to results obtained from one of the experiments, established that they did not differ significantly from the others.

PTE concentration values (reported in Tables S2 and S3, Supplementary Materials, as an average of the data obtained from the three replications) were processed using a chemometric treatment aimed to evaluate the uptake efficiency of the plants towards the different elements. BF and TF were calculated as well to assess the plant mineral uptake and the transport from roots to the aerial part, respectively.

In Figure 2a,b, the score and loading biplots resulting from the principal component analysis (PCA) on the autoscaled data relating to the element concentrations in aerial parts (leaves and stems) and roots of the plants are reported. Figure 2a presents a close correlation between *P. vittata* and arsenic, i.e., epigeal levels of As in *P. vittata* are higher when plants are grown in the two soils added with FW.

Under these conditions, the same happens in roots. However, As lowers in roots growing in NOB soils without FW.

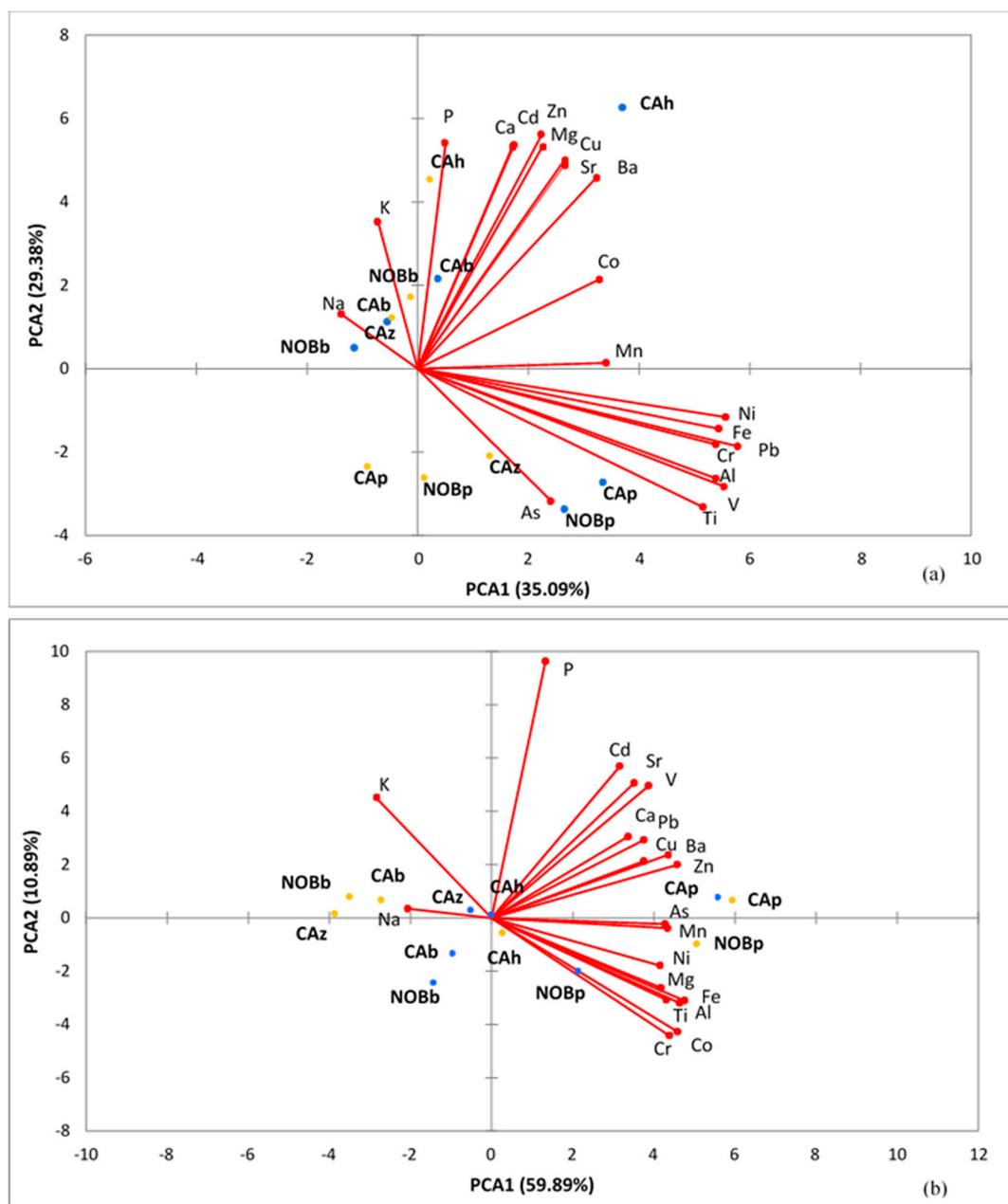


Figure 2. Score and loading plot from principal component analysis (PCA) (PC1 vs. PC2) on element concentration data of (a) epigeal and (b) hypogeeal parts. NOB = plants grown in Nobile soil, CA = plants grown in Campania soil, yellow spot = addition of FW to the soil, blue spot = no addition of FW, p = *Pteris vittata*, b = *Brassica juncea*, h = *Helianthus annuus*, z = *Zea mays*.

These results are not unexpected since this species was chosen on the basis of previous studies that assessed its capability to absorb both arsenate and arsenite [42]. Arsenate is an analogue of phosphate (P), so arsenate is taken up via the P transporters in higher plants including *P. vittata* [43], and it competes with P uptake. The uptake mechanism of arsenite by *P. vittata* is mainly controlled by a transporter-mediated active process. Since arsenite often occurs as a neutral species in the environment, it has been hypothesized that arsenite is taken up by plants via aquaporin channels [44]. With respect to Pb, one of the major pollutants, results suggest that the plants used for phytoextraction did not have a

strong uptake efficiency, although *B. juncea* was suggested for the phytoremediation of lead. However, the main obstacle to the Pb phytoextraction could be its low bioavailability. It was estimated that only 0.1% of total soil Pb is amenable for extraction [45] and, in most cases, the addition of chelating agents (e.g., ethylene diamine tetra-acetate (EDTA) anion) in aqueous solutions was commonly required to mobilize Pb and facilitate its extraction [19,46]. In the absence of a chelating agent, the mobility of lead remains very low and it cannot be effectively extracted.

When comparing the absorption of elements in the epigeal part of selected plants grown in the presence or absence of FW, *B. juncea* and *H. annuus* take up more Cd, Zn, Cu and Co without soil improver, although the difference is not marked. On the other hand, the behavior of *Z. mays* is very different and its effectiveness is strictly connected to the addition of the soil improver. This renders this plant species more efficient towards elements with negative loadings on the PC2, such as As, Pb, Ni and Cr.

Figure 3 shows the values of bioconcentration factors of plants grown in Campana (a, b) and Nobile (c, d) soils for PTEs, and the results obtained by the PCA are confirmed. *P. vittata* shows an excellent bioconcentration ability—its action is particularly marked for As and Pb, but it is also efficient for other PTEs to become one of the most capable species for lead uptake.

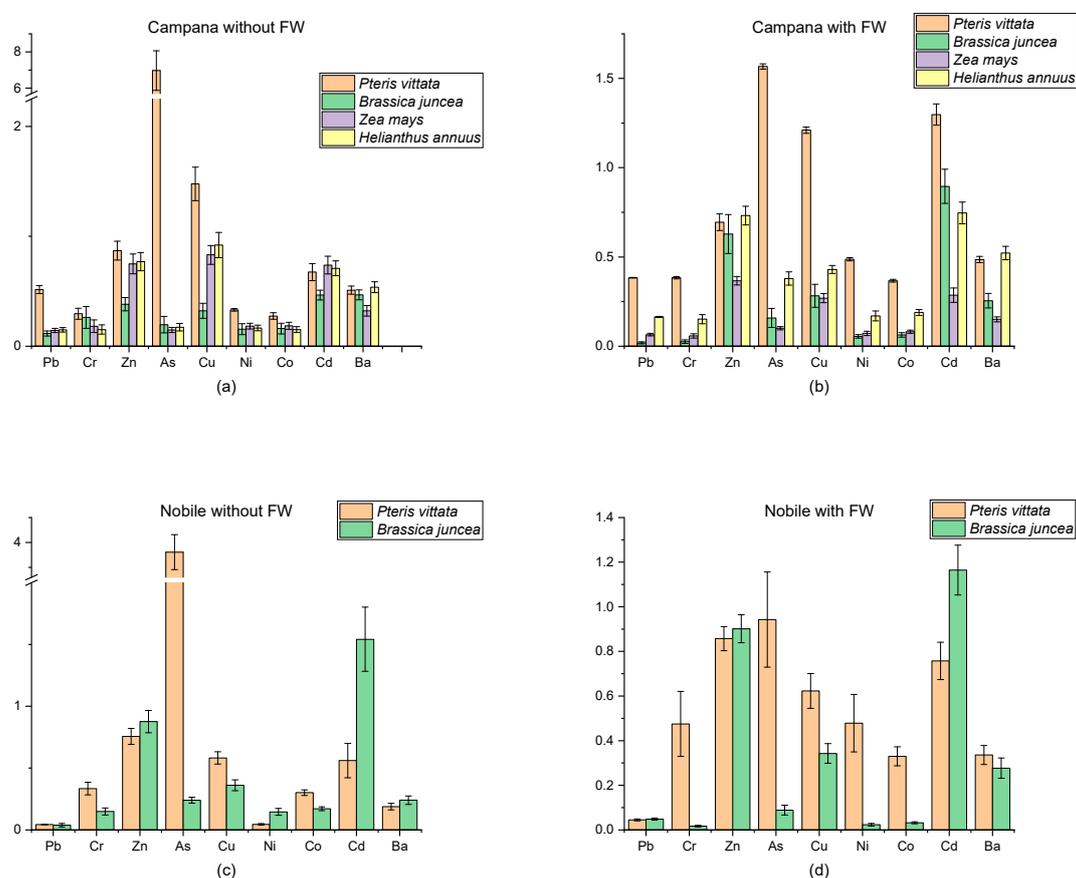


Figure 3. Bioconcentration factors (BFs) of plants chosen for phytoremediation and grown in (a) Campana without FW, (b) Campana with FW, (c) Nobile without FW and (d) Nobile with FW. Values are expressed as mean \pm SD.

The ANOVA and Tukey tests (Table S4, Supplementary Materials) confirm a significant difference between the BF values of *P. vittata* for As and those of all other plants.

Overall, *H. annuus* and *Z. mays* are more efficient than *B. juncea* in the uptake of Zn and Cu and *B. juncea* shows a good capability in the cadmium absorption. The FW addition determines a change in the bioconcentration capacity of plants for phytoremediation, probably due to the input of organic carbon that decreases the mobility and bioavailability of PTEs [35]. Indeed, although *P. vittata* always

shows a greater ability to absorb all elements, the absolute value of As is much greater in the absence of FW, *Z. mays* is less efficient in the uptake of all the elements, whereas *H. annuus* shows approximately the same efficiency. Cd is adsorbed in slightly higher concentration by *B. juncea* in Campana soil; on the contrary, the addition of FW to Nobile soil inhibits the process, as also highlighted by ANOVA and Tukey tests.

Figure 4 reports translocation factors. These values indicate to what extent the elements absorbed by the plant are transferred to the aerial part or remain fixed in roots (higher values correspond to a greater transfer).

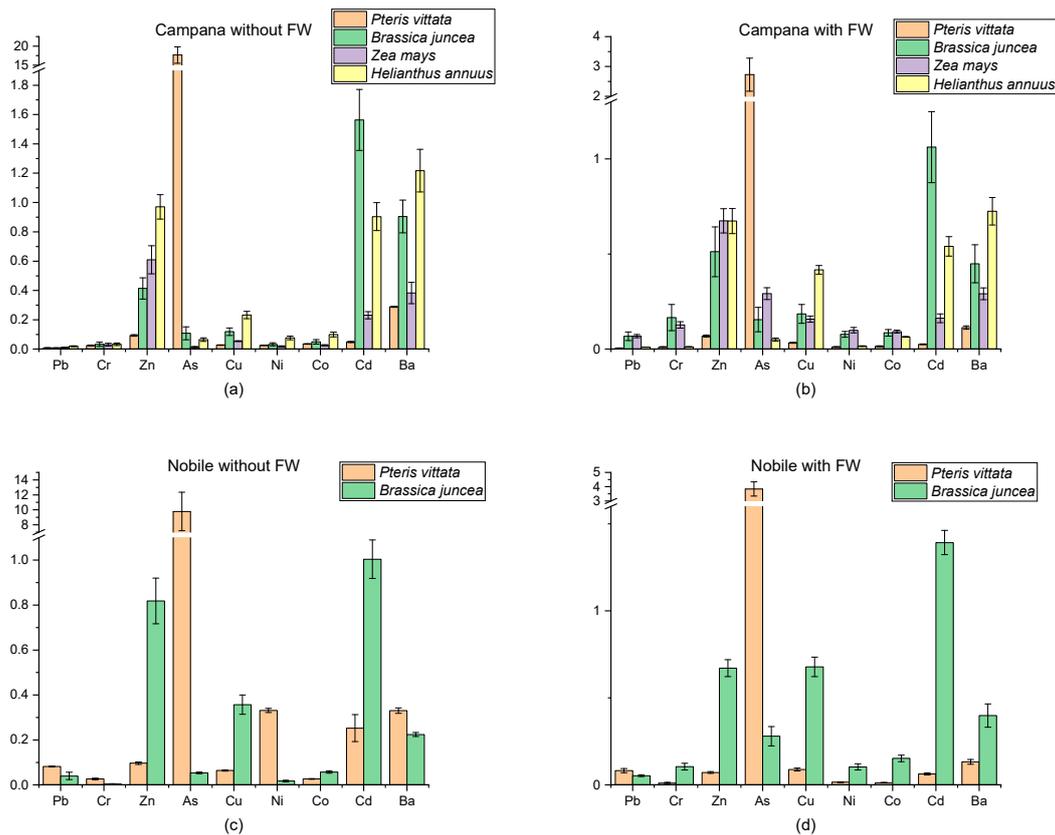


Figure 4. Translocation factors (TFs) of plants chosen for phytoremediation and grown in (a) Campana without FW, (b) Campana with FW, (c) Nobile without FW and (d) Nobile with FW. Values are expressed as mean \pm SD.

P. vittata shows a very high TF for As in any soil at any treatment, even if this capability was much higher in soils without FW. However, this is the only exception because the other elements are retained in the roots.

B. juncea has bioconcentration factors that are not particularly high; on the contrary, it shows good translocation factors, especially with respect to Cd in both soils. The ANOVA analysis (Table S5, Supplementary Materials) reveals a significant difference between the TF values of *P. vittata* for As and the TF values of *B. juncea* and *H. annuus* for Cd and those of all the other plants.

H. annuus and *Z. mays* exhibit a good translocation efficiency for the metals that were mainly absorbed, namely Zn, Cu, Cd and Ba.

The use of the soil improver provokes an increase of translocation factors of *B. juncea* towards Ba, Cd and Cr in the Nobile soil, whereas if we consider Campana soil the translocation of Cd and Ba in *B. juncea* is enhanced when FW is not added to the soil. With respect to arsenic, the transfer to aerial parts of *P. vittata* is more significant in the absence of the improver in both soils.

Despite these considerations, a plant is considered suitable for phytoextraction when it has TF and especially BF values greater than one, whereas when TF is less than one but BF is greater than one it can be a good candidate for phytostabilization [28]. *P. vittata* shows values of BF greater than one for arsenic in both soils, for copper in the soil of the CA site and for cadmium for the CA soil in the presence of FW. Furthermore, it has $TF \geq 1$ for arsenic (in the CA soil without FW, it reaches a value of 17). *B. juncea*, on the other hand, has $BF \geq 1$ for Cd in NOB soils and shows $TF \geq 1$ for Cd in both soils (Tables S6 and S7, Supplementary Materials).

In an attempt to estimate the feasibility of this treatment, the maximum percentage reductions for major anthropogenic contaminants were calculated for the most efficient plant, i.e., *P. vittata*.

These values take into account the ratio between the amount of PTEs detected in the soil volume of each pot and the amount of metal present in the plant related to its total biomass.

For the fern grown in the soil without FW, the maximum abatement percentages of 16%, 1.2%, 1.6%, 0.6%, 0.7%, 3.5%, 0.8%, 1.2% and 2% were estimated for As, Ba, Cd, Co, Cr, Cu, Ni, Pb and Zn, respectively, whereas for the same elements the maximum abatement percentages of 4%, 1.3%, 3.5%, 0.9%, 1%, 3.2%, 1.3%, 1% and 1.8% were obtained for fern grown in the presence of the soil improver.

4. Conclusions

Two Turin sites, exploitable as urban gardens, were compared for PTE content. The urban soil located in the city center, the CA site, was found contaminated by Pb, Ba and Zn and, to a lower degree, by As, Cu and Cd, due to exposure to urban pollution.

The study on the phytoextraction efficiency of four plant species cultivated in controlled conditions in both soils highlighted a great ability of *P. vittata* to abate As, even if it was not one of the major pollutants. Considering this kind of fern, it is possible to assume that, in the adopted experimental conditions, a 15% reduction of As after a life cycle could be obtained, whereas four vegetative cycles would be necessary to achieve the same result for Cu and, for Pb, the treatment must be further prolonged.

B. juncea is promising for the bioconcentration and translocation of cadmium, whereas *H. annuus* showed interesting TF values for Zn, Cd and Ba. *Z. mays*, on the contrary, did not demonstrate high performances in the examined experimental conditions.

The effect of the addition of a soil improver supplied by Acea Pinerolese is controversial. In the case of *B. juncea*, it seems to favor the absorption of PTEs by carrying out a positive activity on the growth of the plant and on the development of the root system. Moreover, it inhibits PTE extraction by *P. vittata* and *Z. mays* competing with them by the complexing and withholding of the PTEs, whereas the influence on *H. annuus* is negligible. Therefore, its use must be assessed according to individual cases and the desired effect.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2076-3417/10/11/3948/s1>, Table S1: Summary of multiple pairwise comparisons on element concentrations in soils obtained with ANOVA and Tukey tests. B identifies soil samples whose mean concentrations in the respective elements are significantly lower than the ones obtained for soil samples identified by A; the same concept applies to C with respect to B and D with respect to C, Table S2: Element concentration (mg/kg) \pm SD in the plants' aerial parts. NOB = plants grown in Nobile soil, CA = plants grown in Campana soil, FW = addition of amendment to the soil, *p* = *Pteris vittata*, *b* = *Brassica juncea*, *h* = *Helianthus annuus*, *z* = *Zea mays*, Table S3: Element concentration (mg/kg) \pm SD in the plants' roots. NOB = plants grown in Nobile soil, CA = plants grown in Campana soil, FW = addition of amendment to the soil, *p* = *Pteris vittata*, *b* = *Brassica juncea*, *h* = *Helianthus annuus*, *z* = *Zea mays*, Table S4: Summary of multiple comparisons for pairs of the plants' BF with Tukey test. NOB = plants grown in Nobile soil, CA = plants grown in Campana soil, FW = addition of amendment to the soil, *p* = *Pteris vittata*, *b* = *Brassica juncea*, *h* = *Helianthus annuus*, *z* = *Zea mays*. B identifies BF values whose means are significantly lower than the ones obtained for BF values identified by A; the same concept applies to C with respect to B, D with respect to C, E with respect to D and E with respect to F, Table S5: Summary of multiple comparisons for pairs of the plants' TF with Tukey test. NOB = plants grown in Nobile soil, CA = plants grown in Campana soil, FW = addition of amendment to the soil, *p* = *Pteris vittata*, *b* = *Brassica juncea*, *h* = *Helianthus annuus*, *z* = *Zea mays*. B identifies BF values whose means are significantly lower than the ones obtained for BF values identified by A; the same concept applies to C with respect to B, D with respect to C, E with respect to D, E with respect to F and G with respect to F, Table S6:

Bioconcentration factors (BFs) \pm SD. Values > 1 are reported in bold, Table S7: Translocation factors (TFs) \pm SD. Values > 1 are reported in bold.

Author Contributions: Conceptualization, A.F. and P.C.; methodology, A.F. and M.M. (Mery Malandrino); formal analysis, E.G.; investigation, D.F. and G.B.; resources, M.M. (Mery Malandrino), D.F. and A.F.; data curation, G.B.; writing—original draft preparation, E.G. and M.M. (Mery Malandrino); writing—review and editing, D.F., M.M. (Marco Mucciarelli) and P.I.; visualization, E.G.; supervision, M.M. (Mery Malandrino), A.F. and P.C.; project administration, M.M. (Mery Malandrino) and P.C.; funding acquisition, P.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work received funding from Compagnia di San Paolo (project CSTO168877-ReHorti).

Acknowledgments: The authors kindly acknowledge ACEA Pinerolese for providing the soil improver.

Conflicts of Interest: The authors declare no conflict of interest.

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Supplementary Materials: Uptake of Potentially Toxic Elements by Four Plant Species Suitable for Phytoremediation of Turin Urban Soils

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Table S1. Summary of multiple pairwise comparisons on element concentrations in soils obtained with ANOVA and Tukey tests. B identifies soil samples whose mean concentrations in the respective elements are significantly lower than the ones obtained for soil samples identified by A; the same concept applies to C with respect to B and D with respect to C.

Soil Concentration	Group	Elements
CA, CA + FW, NOB, NOB + FW	A	Al, Ca, Ce, Cr, Fe, La, Mg, Mn, Na, Ni, Ti, V
CA, CA+FW NOB, NOB+FW	A B	Ba, Cu, Pb, Zn
CA CA + FW NOB, NOB + FW	A A B B	As
CA NOB CA+FW, NOB + FW	A A B B	K
NOB NOB + FW CA, CA + FW	A A B B	Co
CA CA + FW NOB, NOB + FW	A B C	Cd
CA CA + FW NOB NOB + FW	A B C D	P

Table S2. Element concentration (mg/kg) \pm SD in the plants' aerial parts. NOB = plants grown in Nobile soil, CA = plants grown in Campana soil, FW = addition of amendment to the soil, *p* = *Pteris vittata*, *b* = *Brassica juncea*, *h* = *Helianthus annuus*, *z* = *Zea mays*.

	<i>CAp</i>		<i>CApFW</i>		<i>CAb</i>		<i>CAbFW</i>		<i>CAz</i>		<i>CAzFW</i>		<i>CAh</i>		<i>CAhFW</i>									
Al	158	±	6	69	±	14	45	±	8	97	±	12	34	±	3	162	±	6	57	±	2	24,949	±	0.004
As	104	±	13	14	±	1	0.30	±	0.02	0.26	±	0.04	0.03	±	0.01	0.280	±	0.006	0.17	±	0.01	0.22	±	0.03
Ba	39.4	±	0.1	18.2	±	0.9	77	±	3	29.1	±	0.5	31	±	2	12.5	±	0.4	102	±	6	80.9	±	0.7
Ca	9005	±	121	4294	±	42	34,181	±	858	13949	±	302	8099	±	200	3955	±	154	37,029	±	1924	25,686	±	1338
Cd	0.036	±	0.001	0.017	±	0.001	0.330	±	0.003	0.252	±	0.003	0.161	±	0.008	0.0218	±	0.0004	0.392	±	0.004	0.14	±	0.01
Co	0.160	±	0.005	0.08	±	0.01	0.13	±	0.01	0.078	±	0.006	0.0816	±	0.0002	0.106	±	0.003	0.236	±	0.008	0.178	±	0.004
Cr	0.86	±	0.09	0.58	±	0.08	1.0	±	0.2	0.51	±	0.04	0.65	±	0.05	0.922	±	0.006	0.59	±	0.04	0.24	±	0.02
Cu	3.15	±	0.02	3.4	±	0.2	2.72	±	0.09	3.86	±	0.07	3.39	±	0.01	3.21	±	0.04	13.8	±	0.2	11.1	±	0.2
Fe	149	±	8	78	±	9	75	±	10	127	±	8	52	±	7	174	±	7	101	±	5	75	±	2
K	13,186	±	130	13,770	±	533	17,698	±	476	44,353	±	907	18,387	±	1509	31,616	±	241	36,924	±	1087	54,981	±	3436
Mg	2929	±	6	2021	±	114	2182	±	96	3795	±	28	3712	±	201	2639	±	120	6585	±	201	4538	±	261
Mn	79.7	±	0.5	26	±	2	22	±	1	35.1	±	0.3	56	±	1	24.0	±	0.6	44	±	3	32.7	±	0.9
Na	54	±	2	119	±	15	<9	±		6720	±	178	114	±	6	1230	±	63	72	±	3	1572	±	233
Ni	1.12	±	0.02	0.7	±	0.1	0.66	±	0.02	0.5	±	0.1	0.44	±	0.06	0.9	±	0.1	1.59	±	0.02	0.36	±	0.03
P	1970	±	27	1104	±	47	4030	±	27	4673	±	165	5217	±	34	2597	±	162	4888	±	169	6378	±	414
Pb	1.47	±	0.07	0.54	±	0.05	0.31	±	0.01	0.32	±	0.02	0.55	±	0.02	1.10	±	0.02	0.99	±	0.05	0.4552	±	0.0002
Sr	22.0	±	0.5	10.8	±	0.6	69	±	2	0.661	±	0.004	21.6	±	0.1	8.5	±	0.2	83	±	4	56	±	3
Ti	5	±	1	2.8	±	0.6	0.79	±	0.06	1.5	±	0.1	0.9	±	0.1	4.4	±	0.1	1.8	±	0.2	0.85	±	0.01
V	0.333	±	0.003	0.16	±	0.04	0.15	±	0.01	0.163	±	0.003	0.078	±	0.003	0.33	±	0.02	0.126	±	0.005	0.088	±	0.008
Zn	17	±	1	10.2	±	0.5	25.5	±	0.6	49	±	2	65	±	9	34	±	2	86	±	2	67	±	2
	<i>NOBp</i>				<i>NOBpFW</i>				<i>NOBb</i>				<i>NOBbFW</i>											
Al	155	±		12	±		97	±		5	±		40.3	±		2.4	±		116	±		10	±	
As	61	±		3	±		13.3	±		0.6	±		0.114	±		0.003	±		0.18	±		0.03	±	
Ba	14.8	±		0.5	±		12	±		1	±		4.60	±		0.03	±		10.5	±		0.1	±	
Ca	6907	±		171	±		5781	±		479	±		21,214	±		367	±		20,940	±		301	±	
Cd	0.061	±		0.003	±		0.024237	±		0.002	±		0.16	±		0.01	±		0.164	±		0.005	±	
Co	0.137	±		0.005	±		0.07313	±		0.00008	±		0.22	±		0.01	±		0.087	±		0.005	±	
Cr	1.2	±		0.2	±		0.7	±		0.2	±		0.167	±		0.005	±		0.43	±		0.03	±	
Cu	2.8	±		0.1	±		4.0	±		0.3	±		3.76	±		0.05	±		7.14	±		0.05	±	
Fe	165	±		8	±		112	±		13	±		63	±		2	±		172	±		19	±	
K	10,641	±		489	±		10,626	±		196	±		14,552	±		278	±		40,608	±		575	±	
Mg	2428	±		85	±		2150	±		215	±		3138	±		51	±		4233	±		108	±	
Mn	44	±		2	±		20	±		2	±		37	±		1	±		38.7	±		0.5	±	
Na	41	±		1	±		90	±		2	±		94	±		2	±		4891	±		28	±	
Ni	1.51	±		0.04	±		1.0	±		0.1	±		0.6	±		0.1	±		0.51	±		0.07	±	
P	986	±		21	±		1287	±		48	±		1462	±		11	±		5170	±		31	±	
Pb	1.03	±		0.01	±		1.1	±		0.1	±		0.050	±		0.006	±		0.084	±		0.002	±	
Sr	10.4	±		0.3	±		10.7	±		0.9	±		22.7	±		0.4	±		29.9	±		0.4	±	
Ti	4.5	±		0.8	±		3.4	±		0.5	±		0.8	±		0.1	±		1.54	±		0.05	±	
V	0.38	±		0.03	±		0.21	±		0.02	±		0.0560	±		0.0001	±		0.11	±		0.01	±	
Zn	15.1	±		0.6	±		12.9	±		0.8	±		38.1	±		0.2	±		41.9	±		0.8	±	

Table S3. Element concentration (mg/kg) ± SD in the plants' roots. NOB = plants grown in Nobile soil, CA = plants grown in Campana soil, FW = addition of amendment to the soil, *p* = *Pteris vittata*, *b* = *Brassica juncea*, *h* = *Helianthus annuus*, *z* = *Zea mays*.

	CA _p		CA _p FW		CA _b		CA _b FW		CA _z		CA _z FW		CA _h		CA _h FW	
Al	7901	± 40	6279	± 572	4647	± 1561	1279	± 712	4270	± 830	5837	± 673	4826	± 1168	2633	± 314
As	5.9	± 0.1	5	± 1	3	± 1	1.7	± 0.6	2.6	± 0.5	4.4	± 0.4	2.3	± 0.3	1.0	± 0.1
Ba	137	± 1	161	± 9	85	± 10	65	± 14	84	± 8	112	± 11	80	± 14	43	± 4
Ca	26,366	± 1861	27,207	± 1352	5174	± 706	15,965	± 4799	9625	± 823	12,208	± 960	7525	± 372	6415	± 289
Cd	0.75	± 0.07	0.69	± 0.01	0.21	± 0.03	0.24	± 0.04	0.43	± 0.05	0.26	± 0.02	0.7	± 0.1	0.13	± 0.02
Co	4.6	± 0.1	5.7	± 0.5	2.6	± 0.8	0.9	± 0.2	2.38	± 0.38	2.78	± 0.03	3.1	± 0.5	1.16	± 0.09
Cr	35	± 3	54	± 10	31	± 11	3	± 1	18	± 4	21.3	± 0.4	21	± 6	7	± 1
Cu	114	± 6	103	± 5	23	± 5	21	± 6	59	± 6	27	± 1	63	± 3	20	± 2
Fe	9396	± 560	9628	± 806	5055	± 2265	984	± 388	4242	± 997	6349	± 46	4416	± 871	2113	± 39
K	9408	± 292	11,194	± 1586	11,041	± 588	9578	± 978	13,442	± 502	13,518	± 1836	12,570	± 350	18,834	± 4236
Mg	10,237	± 1103	8497	± 414	2994	± 1124	1822	± 386	3025	± 675	3479	± 113	2915	± 544	2104	± 69
Mn	206	± 2	275	± 12	145	± 38	51	± 23	147	± 23	205	± 7	177	± 34	69	± 6
Na	353.8	± 0.6	65	± 1	291	± 60	991	± 118	2880	± 299	7298	± 1275	595	± 61	9902	± 1154
Ni	44	± 1	66.7	± 0.9	20	± 7	7.0	± 0.7	21	± 3	23	± 1	24	± 4	9.0	± 0.7
P	2975	± 69	3291	± 211	2027	± 108	2731	± 447	2748	± 126	2006	± 75	2922	± 179	3063	± 421
Pb	176	± 9	102	± 8	40	± 8	5	± 2	50	± 6	43.4	± 0.8	49	± 6	16	± 2
Sr	54.7	± 0.3	65	± 1	19	± 2	43	± 8	31	± 2	42	± 9	21	± 2	19	± 7
Ti	123	± 3	149	± 18	73	± 23	13	± 10	79	± 16	122	± 10	86	± 22	49	± 8
V	16.8	± 0.1	26	± 3	11	± 3	3.1	± 0.1	12	± 2	11.4	± 0.8	8	± 2	4	± 0.8
Zn	181	± 1	149	± 10	62	± 11	95	± 24	89	± 7	100	± 9	106	± 7	50	± 3
	NOB _p				NOB _p FW				NOB _b				NOB _b FW			
Al	6422		±	62	7822		±	139	3776		±	943	1141		±	184
As	6		±	2	3.5		±	0.4	2.1		±	0.1	0.64		±	0.08
Ba	44.9		±	0.8	94		±	6	20.5		±	0.8	26		±	4
Ca	15,952		±	73	25,911		±	1534	10,447		±	1646	23,911		±	3962
Cd	0.24		±	0.06	0.38		±	0.02	0.160		±	0.005	0.118		±	0.005
Co	5.1		±	0.2	5.7		±	0.5	3.8		±	0.2	0.57		±	0.06
Cr	47		±	2	68		±	18	37		±	4	4.1		±	0.7
Cu	43.4		±	0.7	45		±	2	11		±	1	10.5		±	0.9
Fe	7488		±	432	9086		±	666	4433		±	976	1199		±	152
K	8066		±	75	9364		±	220	9256		±	320	13,397		±	531
Mg	5020		±	240	10,237		±	1103	4804		±	1691	2010		±	200
Mn	163		±	5	205		±	7	111		±	3	36		±	4
Na	230		±	6	370		±	22	146		±	5	1331		±	17
Ni	4.57		±	0.03	63		±	9	36		±	6	5.0		±	0.5
P	1243		±	25	3837		±	190	1245		±	127	3302		±	164
Pb	12.6		±	0.3	13		±	1	1.2		±	0.5	1.6		±	0.2
Sr	45.3		±	0.8	60.4		±	0.5	19		±	3	35		±	5

Ti	200	±	5	250	±	14	28	±	14	16.630	±	0.002
V	18.2	±	0.4	23.5	±	0.9	7	±	1	1.52	±	0.08
Zn	156	±	6	181	±	6	47	±	6	63	±	4

Table S4. Summary of multiple comparisons for pairs of the plants' BF with Tukey test. NOB = plants grown in Nobile soil, CA = plants grown in Campana soil, FW = addition of amendment to the soil, *p* = *Pteris vittata*, *b* = *Brassica juncea*, *h* = *Helianthus annuus*, *z* = *Zea mays*. B identifies BF values whose means are significantly lower than the ones obtained for BF values identified by A; the same concept applies to C with respect to B, D with respect to C, E with respect to D and E with respect to F.

BF	Group	Elements
<i>CAp</i>	A	
NOB <i>p</i>	B	
<i>CApFW</i>	C	As
NOB <i>pFW</i>	C D	
<i>CAh, CAhFW, CAb, CAbFW, CAz, CAzFW, NOBb, NOBbFW</i>	D	
<i>CAh, CAhFW, CAp, CApFW, CAb</i>	A	
<i>CAz, NOBpFW</i>	B	
NOB <i>bFW</i>	B C	
<i>CAbFW, NOBb</i>	B C D	Ba
NOB <i>p</i>	C D	
<i>CAzFW</i>	D	
NOB <i>b</i>	A	
<i>CApFW</i>	A B	
NOB <i>bFW</i>	B C	
<i>CAbFW</i>	C D	Cd
<i>CAh, CAhFW, CAz, CAp, NOBpFW</i>	D E	
<i>CAb, NOBp</i>	E F	
<i>CAzFW</i>	F	
<i>CApFW</i>	A	
NOB <i>p, NOBpFW</i>	A B	
<i>CAp</i>	B	
<i>CAhFW, CAz, CAb, NOBb</i>	C	Co
<i>CAh</i>	C D	
<i>CAzFW</i>	D E	
<i>CAbFW, NOBbFW</i>	E	
NOB <i>pFW</i>	A	
<i>CApFW</i>	A B	
NOB <i>p</i>	A B C	
<i>CAp, CAb</i>	B C D	Cr
<i>CAz</i>	C D E	
<i>CAh, CAhFW, NOBb</i>	D E	
<i>CAzFW, CAbFW, NOBbFW</i>	E	
<i>CAp</i>	A	
<i>CApFW</i>	B	
<i>CAh</i>	C	
<i>CAz</i>	C D	
NOB <i>pFW</i>	D E	Cu
NOB <i>p</i>	E	
<i>CAhFW</i>	E F	
<i>CAb, CAbFW, CAzFW, NOBb, NOBbFW</i>	F	
<i>CApFW, NOBpFW</i>	A	
<i>CAp</i>	B	
<i>CAz</i>	C	
<i>CAh, CAhFW, CAb</i>	C D	Ni
<i>CAzFW, NOBb</i>	C D E	
<i>CAbFW, NOBp</i>	D E	
NOB <i>bFW</i>	E	
<i>CAp</i>	A	Pb

CApFW	B	
CAhFW	C	
CAh, CAz	C D	
CAb	D	
CAzFW, CAbFW NOBb, NOBbFW, NOBp, NOBpFW	E	
CAp, NOBb, NOBbFW, NOBpFW	A	
CAh, CAhFW, CAz, CApFW, NOBp	A B	Zn
CAbFW	B	
CAb, CAzFW	C	

Table S5. Summary of multiple comparisons for pairs of the plants' TF with Tukey test. NOB = plants grown in Nobile soil, CA = plants grown in Campana soil, FW = addition of amendment to the soil, *p* = *Pteris vittata*, *b* = *Brassica juncea*, *h* = *Helianthus annuus*, *z* = *Zea mays*. B identifies BF values whose means are significantly lower than the ones obtained for BF values identified by A; the same concept applies to C with respect to B, D with respect to C, E with respect to D, E with respect to F and G with respect to F.

TF	Group	Elements
CAp	A	
NOBp	B	
NOBpFW	C	As
CApFW	C D	
CAb, CAbFW, CAh, CAhFW, CAz, CAzFW NOBb, NOBbFW	D	
CAh	A	
CAb, CAhFW	B	
CAbFW	C	
CAz, NOBbFW	C D	
NOBp	C D E	Ba
CAzFW, CAp	C D E F	
NOBb	D E F	
NOBpFW	E F	
CApFW	F	
CAb, NOBbFW	A	
CAbFW, CAh NOBb	B	Cd
CAhFW	C	
CAz, CAzFW, CAp, CApFW, NOBp, NOBpFW	D	
NOBbFW	A	
CAh	B	
CAzFW	B C	
CAbFW	B C D	
CAhFW	B C D E	Co
NOBb	D E	
CAb	E F	
CAp	E F G	
CAz, NOBp	FG	
CApFW, NOBpFW	G	
CAbFW, CAzFW, NOBbFW	A	Cr
CAh, CAhFW, CAb, CAz, CAp, CApFW, NOBb, NOBp, NOBpFW	B	
NOBbFW	A	
CAhFW, NOBb	B	
CAh	C	
CAbFW	C D	Cu
CAzFW	C D E	
CAb	D E F	
NOBpFW	E F G	
CAz, NOBp	F G	

CA _p , CA _p FW	G	
NOB _p	A	
CA _z FW, CA _b FW, CA _h , NOB _b FW	B	Ni
CA _b , CA _p , CA _p FW, CA _z , CA _h FW, NOB _b , NOB _p FW	C	
NOB _p , NOB _p FW	A	
CA _z FW, CA _b FW	A B	
NOB _b FW	B C	Pb
NOB _b	C D	
CA _h	D E	
CA _z , CA _h FW, CA _b , CA _p , CA _p FW	E	
CA _h	A	
NOB _b	A B	
CA _z FW, CA _h FW, NOB _b FW	B C	Zn
CA _z , CA _b FW	C D	
CA _b	D	
CA _p , CA _p FW, NOB _p , NOB _p FW	E	

Table S6. Bioconcentration factors (BFs) \pm SD. Values > 1 are reported in bold.

	CA no FW								CA FW															
	<i>P. vittata</i>		<i>B. juncea</i>		<i>Z. mays</i>		<i>H. annuus</i>		<i>P. vittata</i>		<i>B. juncea</i>		<i>Z. mays</i>		<i>H. annuus</i>									
As	7	\pm	1	0.20	\pm	0.08	0.15	\pm	0.02	0.17	\pm	0.03	1.57	\pm	0.01	0.16	\pm	0.05	0.10	\pm	0.01	0.38	\pm	0.04
Ba	0.51	\pm	0.04	0.47	\pm	0.05	0.32	\pm	0.05	0.54	\pm	0.05	0.49	\pm	0.02	0.26	\pm	0.04	0.15	\pm	0.01	0.52	\pm	0.04
Cd	0.67	\pm	0.08	0.47	\pm	0.04	0.74	\pm	0.08	0.71	\pm	0.07	1.30	\pm	0.06	0.9	\pm	0.1	0.29	\pm	0.04	0.75	\pm	0.06
Co	0.27	\pm	0.03	0.16	\pm	0.05	0.19	\pm	0.03	0.15	\pm	0.03	0.37	\pm	0.01	0.06	\pm	0.01	0.08	\pm	0.01	0.19	\pm	0.02
Cr	0.3	\pm	0.1	0.3	\pm	0.1	0.18	\pm	0.06	0.15	\pm	0.04	0.38	\pm	0.01	0.03	\pm	0.01	0.06	\pm	0.01	0.15	\pm	0.03
Cu	1.5	\pm	0.2	0.32	\pm	0.07	0.83	\pm	0.09	0.9	\pm	0.1	1.21	\pm	0.02	0.28	\pm	0.06	0.27	\pm	0.03	0.43	\pm	0.02
Ni	0.33	\pm	0.01	0.16	\pm	0.05	0.18	\pm	0.03	0.17	\pm	0.03	0.49	\pm	0.01	0.05	\pm	0.01	0.07	\pm	0.01	0.17	\pm	0.03
Pb	0.51	\pm	0.04	0.12	\pm	0.02	0.14	\pm	0.02	0.15	\pm	0.02	0.383	\pm	0.001	0.02	\pm	0.01	0.06	\pm	0.01	0.164	\pm	0.003
Zn	0.87	\pm	0.09	0.38	\pm	0.06	0.75	\pm	0.09	0.77	\pm	0.08	0.70	\pm	0.05	0.6	\pm	0.1	0.37	\pm	0.02	0.73	\pm	0.05
	NOB no FW								NOB FW															
	<i>P. vittata</i>				<i>B. juncea</i>				<i>P. vittata</i>				<i>B. juncea</i>											
As	3.8	\pm	0.4	0.24	\pm	0.02	0.9	\pm	0.2	0.09	\pm	0.02	0.09	\pm	0.02									
Ba	0.19	\pm	0.03	0.24	\pm	0.03	0.34	\pm	0.04	0.28	\pm	0.05	0.28	\pm	0.05									
Cd	0.56	\pm	0.14	1.5	\pm	0.3	0.76	\pm	0.08	1.2	\pm	0.1	1.2	\pm	0.1									
Co	0.30	\pm	0.02	0.17	\pm	0.02	0.33	\pm	0.04	0.032	\pm	0.004	0.032	\pm	0.004									
Cr	0.33	\pm	0.05	0.15	\pm	0.03	0.5	\pm	0.1	0.017	\pm	0.004	0.017	\pm	0.004									
Cu	0.58	\pm	0.05	0.36	\pm	0.04	0.6	\pm	0.1	0.34	\pm	0.04	0.34	\pm	0.04									
Ni	0.05	\pm	0.01	0.15	\pm	0.03	0.5	\pm	0.1	0.02	\pm	0.01	0.02	\pm	0.01									
Pb	0.043	\pm	0.001	0.04	\pm	0.01	0.045	\pm	0.004	0.049	\pm	0.005	0.049	\pm	0.005									
Zn	0.76	\pm	0.06	0.88	\pm	0.09	0.86	\pm	0.05	0.90	\pm	0.06	0.90	\pm	0.06									

Table S7. Translocation factors (TFs) \pm SD. Values > 1 are reported in bold.

	CA												CA+ FW											
	<i>P. vittata</i>			<i>B. juncea</i>			<i>Z. mays</i>			<i>H. annuus</i>			<i>P. vittata</i>		<i>B. juncea</i>		<i>Z. mays</i>		<i>H. annuus</i>					
As	18	\pm	2	0.11	\pm	0.04	0.013	\pm	0.004	0.06	\pm	0.01	2.7	\pm	0.6	0.16	\pm	0.06	0.29	\pm	0.03	0.05	\pm	0.01
Ba	0.289	\pm	0.003	0.9	\pm	0.1	0.38	\pm	0.07	1.2	\pm	0.1	0.11	\pm	0.01	0.4	\pm	0.1	0.29	\pm	0.03	0.72	\pm	0.07
Cd	0.049	\pm	0.005	1.6	\pm	0.2	0.23	\pm	0.02	0.9	\pm	0.1	0.025	\pm	0.002	1.1	\pm	0.2	0.16	\pm	0.02	0.54	\pm	0.05
Co	0.035	\pm	0.001	0.05	\pm	0.01	0.026	\pm	0.004	0.10	\pm	0.02	0.014	\pm	0.003	0.09	\pm	0.02	0.09	\pm	0.01	0.064	\pm	0.002
Cr	0.024	\pm	0.003	0.03	\pm	0.01	0.03	\pm	0.01	0.03	\pm	0.01	0.011	\pm	0.002	0.17	\pm	0.07	0.13	\pm	0.02	0.011	\pm	0.001
Cu	0.028	\pm	0.002	0.12	\pm	0.03	0.054	\pm	0.003	0.23	\pm	0.03	0.033	\pm	0.003	0.18	\pm	0.05	0.16	\pm	0.02	0.42	\pm	0.02
Ni	0.025	\pm	0.001	0.03	\pm	0.01	0.018	\pm	0.004	0.08	\pm	0.01	0.011	\pm	0.002	0.08	\pm	0.02	0.10	\pm	0.01	0.015	\pm	0.001
Pb	0.008	\pm	0.001	0.008	\pm	0.002	0.011	\pm	0.001	0.020	\pm	0.003	0.005	\pm	0.001	0.07	\pm	0.02	0.07	\pm	0.01	0.0105	\pm	0.0002
Zn	0.093	\pm	0.006	0.41	\pm	0.07	0.6	\pm	0.1	0.97	\pm	0.08	0.07	\pm	0.01	0.5	\pm	0.1	0.67	\pm	0.06	0.67	\pm	0.07
	NOB												NOB + FW											
	<i>P. vittata</i>			<i>B. juncea</i>			<i>P. vittata</i>			<i>B. juncea</i>														
As	10	\pm	3	0.054	\pm	0.004	3.8	\pm	0.5	0.28	\pm	0.06												
Ba	0.33	\pm	0.01	0.22	\pm	0.01	0.13	\pm	0.01	0.40	\pm	0.07												
Cd	0.25	\pm	0.06	1.00	\pm	0.09	0.06	\pm	0.01	1.39	\pm	0.07												
Co	0.027	\pm	0.001	0.06	\pm	0.01	0.013	\pm	0.001	0.15	\pm	0.02												
Cr	0.026	\pm	0.003	0.005	\pm	0.001	0.011	\pm	0.004	0.10	\pm	0.02												
Cu	0.064	\pm	0.003	0.36	\pm	0.04	0.09	\pm	0.01	0.68	\pm	0.06												
Ni	0.33	\pm	0.01	0.017	\pm	0.004	0.016	\pm	0.002	0.10	\pm	0.02												
Pb	0.082	\pm	0.002	0.04	\pm	0.02	0.08	\pm	0.01	0.05	\pm	0.01												
Zn	0.10	\pm	0.01	0.8	\pm	0.1	0.07	\pm	0.01	0.67	\pm	0.05												