Bioassays for evaluation of sanitary risks from food crops cultivated in potentially contaminated sites

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Abstract

Environmental pollution in agricultural contexts cannot be neglected since food crops can be an important exposure pathway for soil pollutants. In Italy the Law on the Environment (Law Decree 152/2006) is unclear with regard to agricultural areas, since the specific regulation provided for in Art. 241 has not been enacted. Current risk analysis is based only on direct risk (*i.e.* via ingestion and inhalation of soil particles), while indirect risk via food consumption is not considered. For defining potential risks for consumers, assessment of exposure to potentially toxic elements (PTEs) can be made through the analysis of their content in food crops and the estimation of the related hazard quotient (HQ) based on the ratio between PTE intake through food and the health risks due to PTE doses during a lifetime. In order to obtain a precautionary estimate, the worst case approach was followed by selecting species well known for their PTE accumulation capacity, such as chicory (*Cichorium intybus* L.), lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.), radish (*Raphanus sativus* L.) and rocket salad (*Eruca vesicaria* L.), and by cultivating these plants in hotspots with the highest PTE concentrations. The PTE contents in such crops show wide variability, with values from 0.005 to 0.054 mg kg^{-1} for arsenic (As), from 0.004 to 3.9 mg kg^{-1} for cadmium (Cd), from 0.17 to 0.79 mg kg^{-1} for chromium (Cr), from 0.22 to 1.18 mg kg^{-1} for copper (Cu), from 0.03 to 0.43 mg kg^{-1} for lead (Pb) and from 1.43 to 25.06 mg kg^{-1} for zinc (Zn). Lead and Cd contents in vegetables exceeded thresholds established by EC Reg. 1881/2006 in 14% and 25% of samples, respectively. By contrast, from HQ analysis, any potential risk of dietary exposure due to ingestion of As, Cr, Cu, Pb or Zn was excluded. Only Cd content in lettuce, spinach and chicory resulted in a potential health risk due to intake of such foodstuffs. The proposed method could resolve the lacuna in current Italian legislation regarding the evaluation of risks for human health due to the intake of contaminants through the food chain.

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Key words: Agricultural soils; health risk; transfer factors; vegetables safety; dietary exposure.

Italian Journal of Agronomy 2018; 13(s1):45-52

Introduction

The content of potentially toxic elements (PTEs) in agricultural soils, their bioaccumulation in food crops and their dispersion in the environment are major exposure pathways for humans (Chen *et al*., 2018). However, in Italy current environmental legislation (Law Decree 152/2006) does not provide for assessing the suitability of a soil for agricultural use. Currently, the main risk analysis software programs, such as ReasOnable Maximum Exposure - R.O.M.E. (APAT), GIUDITTA (Milan province) and RBCA ToolKit (GSI), only allow calculation of direct health risks due to dermal contact, ingestion and inhalation of contaminants by people who frequent a site, while indirect risks, associated to PTE uptake by food crops and their consumption, are not taken into account.

In the rest of Europe, only Germany and Austria have guidelines for assessing the suitability of soil for crop production, reporting trigger values based on PTE bioavailable contents in soil (Carlon, 2007), thus requiring analysis of PTEs in crops whenever the trigger level is exceeded. The bioavailability of PTEs is an important aspect, because it can show the potential mobility of elements and the consequent potential uptake by plants as described by Adamo *et al.* (2014) and Rocco *et al.* (2018). Nevertheless, its assessment by chemical methods (*i.e.* by using extractants with different strength) allows indirect evaluation, while direct assessment can be made by analysing PTE accumulation in food crops.

Lead and Cd contents in foodstuffs can be evaluated by comparing them with the thresholds reported by EC Reg. 1881/2006 for some vegetables, while for As and mercury (Hg) the values could be compared with the thresholds reported in EC Dir. 32/2002 relative to forage. For other potentially toxic contaminants, health risks could be assessed by estimating the intake of contaminants through the most common food in normal diets, as described by national or international agencies (ISS, 2013; US EPA, 1989).

These methods have already been used and validated in other surveys (Zheng *et al*., 2007; Huang *et al*., 2008; Beccaloni *et al*., 2013; Osaili *et al*., 2016; Salvo *et al*., 2018), in which estimation of contaminant intake allowed identification of the effective risks for human health that were often unrelated to major soil contaminants. Indeed, in an agricultural area near a Zn smelting plant, Zheng *et al.* (2007) excluded risks derived from accumulation in foodstuffs of the elements with higher concentrations (Zn, Cu and Hg), but highlighted potential risks due to Cd and Pb. On the other hand, in industrial areas, Huang *et al.* (2008) did not find health risks for each single element (Hg, As, Cr, Cu, Ni, Pb and Zn), but focused on calculating aggregate risk due to their cumulative effect. Beccaloni *et al.* (2013) found no health risks associated to consumption of local vegetable products in potentially contaminated croplands in Sardinia, while research conducted in Sicily (Salvo *et al*., 2018) revealed health risks due to Cd and As accumulation in vegetables cultivated in fields close to a petrochemical plant, correlating the contamination of soil and food crops with the use of contaminated groundwater for irrigation.

To evaluate the suitability of a field for agricultural use, a precautionary assessment can be made by using the worst case approach in which food crops well known for their PTE accumulation capacity, such as lettuce (Antoniadis *et al*., 2017; França *et al.*, 2017), spinach (Agrawal *et al.*, 2007), chicory (Aksoy, 2008), rocket salad (Mourato *et al.*, 2015) and radish (Liu *et al.*, 2006) are cultivated in potentially contaminated soils to evaluate the risk of PTE transfer to the food chain.

In light of the above, the aim of this work was to evaluate the suitability for agricultural use (*i.e.* the risks for human health due to contaminant accumulation in foodstuffs) of a site defined as potentially contaminated by Cr and Zn due to illegal tannery sludge disposal, in which a more detailed environmental characterization highlighted some hot spots potentially contaminated also by As, Cd, Cu and Pb (Adamo *et al.,* 2017).

Materials and methods

Description of the study area

The study area (60,000 m^2) is situated in the Campania plain in which fruit (*e.g.* strawberry, melon and peach) and vegetables (*e.g.* tomato, lettuce, broccoli) are mainly cultivated. In 2008 the site was sequestered due to illegal disposal of tannery sludge, and from 2015 onwards it was characterized and remediated by using the ECOREMED protocol (2017).

Experimental model

To evaluate the potential uptake of PTEs without uncertainties due to field conditions (*e.g.* leaching and weather adversity), some soil hot spots with higher PTE concentrations were collected from the study area and used in a pot experiment in a greenhouse. The *worst case* approach was used, which means growing selected crop species already known for their ability to accumulate PTEs in their edible portions in soils from such hot spots.

Soil and vegetable selection

Starting from a previous detailed characterization (Figure 1) four areas were selected with different contaminant levels (F4 with the highest concentration of Cd, F2 with the highest concentration of Cr and Zn, C13 with the highest concentration of Pb, and A7 with the lowest concentration of PTEs as control) (Table 1). Massive samples (1 m^2 x 0.2 m) of soils were collected and transported to an experimental greenhouse at the University of Naples.

The following five species commonly grown in the field area and known for their high PTE accumulation capacity were selected: i) Lettuce (*Lactuca sativa* L.): known to accumulate Pb, As, Hg (Antoniadis *et al.*, 2017) and Zn (França *et al.*, 2017); ii) Spinach (*Spinacia oleracea* L.): reported by Agrawal *et al.* (2007) as accumulating various PTEs (Pb, Cd, Cr and As); iii) Chicory (*Cichorium*

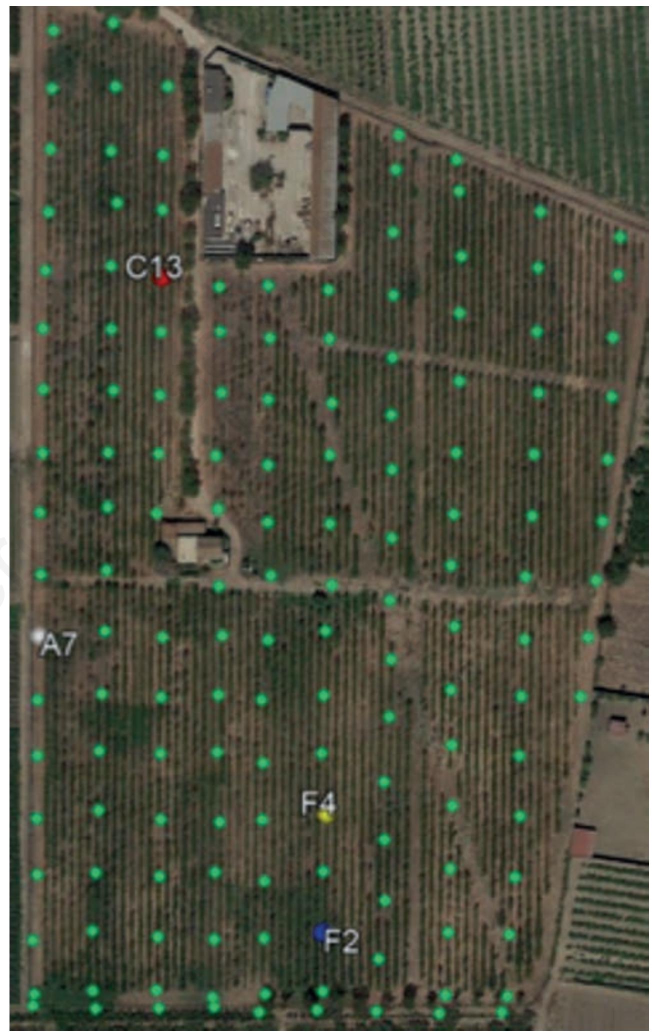

Figure 1. Hot spots in the experimental site (A7 = control; F2 = highest Cr and Zn concentration; F4 = highest Cd concentration; C13 = highest Pb concentration) (from Adamo *et al.*, 2017).

Table 1. Total and bioavailable content (mg kg^{-1}) of PTEs in the selected hot spots.

Hot spots	Copper		Lead		Zinc		Arsenic		Cadmium		Chromium	
	T	B	T	B	T	B	T	B	T	B	T	B
A7	29	0.11	45	0.06	122	0.14	13	---	0	0.05	112	0.30
C13	88	0.45	*147*	0.02	*684*	0.82	20	---	1	0.07	*650*	0.54
F2	49	0.17	63	0.01	*846*	1.78	16	---	1	0.01	*2399*	0.23
F4	64	0.19	70	0.01	*342*	0.49	20	---	*13*	0.18	*716*	0.50

T, total content; B, bioavailable content. Values in italics exceed the Italian screening values (DL 152/2006).

intybus L.): accumulating Pb, Cd and Zn (Bandiera *et al.*, 2016); iv) Rocket salad (*Eruca vesicaria* L.): accumulating Pb and Zn (Nikaido *et al.*, 2010); v) Radish (*Raphanus sativus*): known for its ability to accumulate Pb, Cd, As and Cr (Liu *et al.*, 2006).

Experimental activities: preparation, sampling and analysis

The selected soil was homogenized, sieved and used to fill 1.5-L pots in which the five plant species were grown, with five replicates per crop. During the first ten days of December 2016, all plants were seeded except the lettuce, which was transplanted, while baby leaf lettuce was seeded at the beginning of February 2017. For all plants the optimal growth conditions (temperature, water, *etc.*) were guaranteed.

The plants were cultivated until commercial maturity, harvested, weighed and washed with tap water and then with deionized water to remove any residual soil particles from the samples. Determination of PTE concentrations in fresh plant samples was made by using ICP-MS after acid digestion (HNO_3) in a microwave oven.

Total PTE contents in soil samples were determined by *aqua regia* extraction, while the readily soluble and bioavailable content of PTEs was extracted by 1 mol L^{-1} ammonium nitrate (DIN 19730, 1997). The metal contents in the extracts were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Health risk assessment

To evaluate the potential non-cancer risk of PTEs, the hazard quotient (HQ) equation was used for each PTE (US EPA, 1986; 2000), while the hazard index (HI) equation (US EPA, 1989; Huang *et al.*, 2008) was used to assess the cumulative potential effects of PTEs. The calculation formulas are shown below:

$$HQ = ADD/RfD \qquad (1)$$

where:
HQ is the hazard quotient for a single PTE (adimensional);
ADD is the average daily dose of the element (mg kg^{-1} day^{-1});
RfD is the reference dose of the element (mg kg^{-1} day^{-1}) that represents an estimate of daily oral exposure of the population which is likely to be without an appreciable risk of detrimental effects during a lifetime. If the ratio exceeds unity (HQ>1) there could be a potential risk to human health.

Exposure to contaminants is estimated by calculating the average daily dose (ADD) as follows:

$$ADD = [(C \times IR) \times EF \times ED]/(BW \times AT_{ADD}) \qquad (2)$$

where:
ADD is the average daily dose of the element (mg kg^{-1} day^{-1});
C is the concentration of an element in a vegetable (mg g^{-1});
IR is the per capita food consumption rate differentiated by age group (g day^{-1});
EF is the exposure frequency (day $year^{-1}$), indicating the number of days in one year in which an individual comes into contact with the contaminant (it is a variable parameter which may depend on seasonality and the availability of the food in question). The highest degree of caution is to consider 365;
ED is the exposure duration (years), indicating the number of years during which the population is exposed to intake of contaminated food. If the real exposure time is unknown, a precautionary value can be considered 70 years. If the risk assessment is for children, the maximum value of the age group is considered (*e.g.* for the 0-10 years age group, ED is equal to 10);
BW is the body weight (kg). Internationally a value of 60 (for adults) is generally considered;
AT_{ADD} is the time over which exposure is averaged (days), AT_{ADD} = ED x 365.

As mentioned above, it is important to calculate also the potential cumulative risk due to the addition of single risks:

$$HI = \Sigma HQ = ADD_1/RfD_1 + ADD_2/RfD_2 + ADD_n/RfD_n \qquad (3)$$

where:
HI is the hazard index as the sum of single hazard quotients (adimensional);
$ADD_{[1,2\ldots n]}$ are the average daily doses of the single PTEs (mg kg^{-1} day^{-1});
$RfD_{[1,2\ldots n]}$ are the reference doses of the single PTEs (mg kg^{-1} day^{-1}).

When HI exceeds unity, there could be a potential health risk. The single parameters used for calculating HQ values from national and international agencies (INAIL, 2015; EFSA, 2006, 2015) are reported in Table 2.

Statistical analysis

One-way ANOVA was performed by using SPSS (version 21). Mean separation was carried out by using the LSD test.

Results and discussion

As shown in Table 3, there were no phytotoxic effects depending on soil PTE contents, since plant growth was not significantly affected by the extent of soil contamination (Table 4). From Table 5, it may be noted that 14% and 25% of samples exceeded the European thresholds (EC Reg. 1881/06) for Pb and Cd, respectively. With regard to As, only in two plots were soil concentrations (Table 4) close to Italian screening values for potential contamination in residential/urban soils (20 mg kg^{-1}), but the bioavailability

Table 2. Parameters used for calculating the hazard quotient.

Intake rate (grams day^{-1})	Adult	Child
Radish	32.1	16.7
Spinach	135.0	77.8
Lettuce*	100.8	43.3
Rocket	18.5	3.4
Chicory	150.0	75.9
BW (kg)	60	26
EF (day $year^{-1}$)	350	350
ED (year)	70	10
ATADD (days)	25550	3650
Reference dose (mg kg^{-1} day^{-1})		
As	0.0003	
Cd	0.0005	
Cr	1.5	
Pb	0.0035	
Cu	0.04	
Zn	0.3	

*Lettuce and baby leaf lettuce.

of all samples was almost zero. Therefore As content in all foodstuffs (Table 5) was always very low (maximum value = 0.05 mg kg^{-1} f.w. in the 4th harvest of rocket), as also shown by Corradini *et al.* (2017) in their survey. As a consequence, HQ for adults (Table 6) and children (Table 7) were low, with a maximum value of 0.2 in chicory. It should be pointed out that As concentrations in vegetables that could represent health risks (*i.e.* HQ=1) are very low both for adults (0.14 for spinach) and children (0.10 for spinach), due to the high toxicity of this element (Table 7).

Copper content in soils was low (Table 4) and normal for this kind of volcanic soil (De Vivo *et al*., 2012). Levels of bioavailable Cu (Table 5), as assessed by NH_4NO_3, were below the trigger value of 1 mg kg^{-1} defined by German DIN (1997) on agricultural areas, in agreement with the moderate accumulation in vegetables (maximum value was 1.2 mg kg^{-1} f.w. in the 1st harvest of rocket), thus confirming the tolerance to Cu of rocket salad (Zhi *et al*., 2015) and its low storage capacity as demonstrated by Ali *et al.* (2012). No significant relation was found between soil content and vegetable accumulation (Table 8). HQ values were very low both for adults (0.06 for chicory and spinach, Table 6) and children (0.08 for spinach), thanks to the low toxicity of this element (HQ=1 for children can be reached with 14 mg kg^{-1} of Cu in spinach and chicory, Table 7). The levels of Cr in the soil were very high (up to 2399 mg kg^{-1}) due to tannery sludge disposal. For this reason the site was classified as potentially contaminated (Table 4). However, the bioavailable values were low (up to 0.54 mg kg^{-1}), as well as accumulation in vegetables (maximum value was 0.79 mg kg^{-1} f.w. in lettuce and rocket, Table 5). Similar values were also found by

Table 3. Vegetable yields (g pot^{-1} f.w.).

Hot spots	Chicory	Lettuce	B.L. Lettuce	Radish	Rocket 1st	Rocket 4th	Spinach
A7	37.32^{b}	95.07^{a}	32.99	16.67^{c}	16.25^{b}	20.05	20.79^{c}
C13	47.72^{ab}	51.07^{b}	24.01	60.21^{a}	26.28^{a}	34.89	33.28^{a}
F2	53.50^{a}	93.88^{a}	39.17	32.77^{b}	15.39^{b}	40.99	28.59^{ab}
F4	42.07^{ab}	112.96^{a}	31.46	39.35^{b}	19.02^{b}	32.08	25.60^{bc}

Table 4. Soil physical and chemical properties (0-30 cm).

Hot spots	NO_3-N ppm	NH_4-N ppm	C %	N %	C/N	Clay %	Loam %	Sand %	USDA Class.
A7	13	2	1.77	0.17	10.5	16.0	29.5	54.5	Sandy loam
C13	42	4	2.84	0.45	6.4	14.5	25.5	60.0	Sandy loam
F2	19	4	2.04	0.25	8.3	16.0	25.5	58.5	Sandy loam
F4	19	4	1.66	0.21	7.9	15.0	25.5	59.5	Sandy loam

Table 5. PTE concentrations in soils (mg kg^{-1} dw) and in vegetable edible parts (mg kg^{-1} f.w.).

PTEs	Hot spots	Soil T	Soil B	Chicory	Lettuce	B.L. Lettuce	Radish	Rocket 1st	Rocket 4th	Spinach
Arsenic	A7	13	0.01	0.01^{Acd}	0.01^{Ad}	0.04^{Aab}	0.02^{ABbc}	0.02^{bd}	0.04^{ABa}	0.00^{Ad}
	C13	20	0.01	0.03^{Aa}	0.01^{Aa}	0.02^{Aa}	0.01^{Ba}	0.02^{a}	0.02^{Aa}	0.02^{Aa}
	F2	16	0.01	0.03^{Aab}	0.01^{Abc}	0.02^{Abc}	0.03^{Aab}	0.02^{bc}	0.04^{ABa}	0.01^{Ac}
	F4	20	0.01	0.02^{Abd}	0.01^{Acd}	0.03^{Ab}	0.03^{ABbc}	0.01^{d}	0.05^{Ba}	0.02^{Abd}
Copper	A7	29	0.11	1.05^{Aa}	0.45^{Ab}	0.48^{Ab}	0.38^{Ab}	0.58^{Cb}	0.60^{Bb}	0.89^{Ba}
	C13	88	0.45	0.87^{Ab}	0.40^{Acd}	0.52^{Ac}	0.22^{Ad}	1.18^{Aa}	0.83^{Ab}	1.02^{ABab}
	F2	49	0.17	1.02^{Aa}	0.49^{Ab}	0.48^{Ab}	0.31^{Ab}	0.96^{ABa}	0.91^{Aa}	0.80^{Ba}
	F4	64	0.19	0.90^{Ab}	0.54^{Ac}	0.56^{Ac}	0.30^{Ad}	0.90^{Bb}	0.80^{ABb}	1.13^{Aa}
Chromium	A7	112	0.30	0.26^{Aab}	0.22^{Ab}	0.46^{Ba}	0.17^{Bb}	0.29^{ab}	0.36^{Bab}	0.26^{Aab}
	C13	650	0.54	0.34^{Abc}	0.39^{Ab}	0.79^{Aa}	0.18^{Bc}	0.39^{b}	0.53^{ABb}	0.46^{Ab}
	F2	2399	0.23	0.40^{Abd}	0.27^{Ad}	0.53^{Bbc}	0.41^{Abd}	0.58^{d}	0.79^{Aa}	0.33^{Acd}
	F4	716	0.50	0.36^{Aab}	0.22^{Ab}	0.47^{Ba}	0.18^{Bb}	0.37^{ab}	0.53^{ABa}	0.37^{Aab}
Zinc	A7	122	0.14	5.34^{Cbc}	3.55^{Bc}	5.53^{Abc}	1.43^{Ac}	6.03^{Bbc}	9.07^{Bb}	18.42^{Ba}
	C13	684	0.82	13.61^{Bb}	6.30^{Bc}	7.28^{Ac}	2.71^{Ac}	16.04^{Aab}	20.51^{Aa}	20.63^{ABa}
	F2	846	1.78	21.14^{Aab}	11.67^{Ac}	7.78^{Acd}	3.63^{Ad}	17.37^{Ab}	24.13^{Aa}	25.06^{Aa}
	F4	342	0.49	9.14^{BCbc}	7.51^{ABc}	6.69^{Acd}	2.11^{Ad}	10.37^{Bbc}	13.11^{Bb}	21.94^{ABa}
Cadmium	A7	0	0.05	0.04^{Ba}	0.03^{Ba}	0.03^{Ba}	0.01^{Aa}	0.01^{a}	0.05^{Ba}	0.03^{Ba}
	C13	1	0.07	0.07^{Ba}	0.02^{Ba}	0.02^{Ba}	0.00^{Aa}	0.01^{a}	0.03^{Ba}	0.01^{Ba}
	F2	1	0.01	0.12^{Ba}	0.10^{Ba}	0.02^{Ba}	0.01^{Aa}	0.01^{a}	0.04^{Ba}	0.03^{Ba}
	F4	13	0.18	*3.98*^{Aa}	*2.23*^{Ab}	*1.76*^{Ac}	*0.14*^{Ad}	*0.47*^{d}	*1.36*^{Ac}	*2.61*^{Ab}
Lead	A7	45	0.06	0.04^{Ab}	0.03^{Ab}	0.08^{Ab}	0.08^{Cb}	0.06^{b}	*0.34*^{Aa}	0.05^{Ab}
	C13	147	0.02	0.07^{Ac}	0.06^{Ac}	0.12^{Abc}	*0.17*^{Bb}	0.09^{bc}	0.27^{Aa}	0.10^{Abc}
	F2	63	0.01	0.04^{Ac}	0.04^{Ac}	0.08^{Abc}	*0.43*^{Aa}	0.09^{bc}	0.14^{Ab}	0.05^{Ac}
	F4	70	0.01	0.06^{Ab}	0.03^{Ab}	0.09^{Ab}	0.05^{Cb}	0.06^{b}	*0.40*^{Aa}	0.08^{Ab}

Concentrations exceeding the thresholds of EC Reg. 1881/06 are marked in italics; interactions of the same plant on different soils are expressed in capital letters, while interactions of different plants on the same soils are expressed in lower case.

Table 6. Estimation of the hazard quotient for adults (to 70 years).

PTEs	Hot spots	Chicory	S.E	Lettuce	S.E	B.L. Lettuce	S.E	Radish	S.E	Rocket 1st	S.E	Rocket 4th	S.E	Spinach	S.E.
As	A7	0.11	0.03	0.03	0.00	0.20	0.05	0.04	0.00	0.02	0.00	0.04	0.01	0.04	0.00
	C13	0.23	0.07	0.08	0.02	0.13	0.02	0.02	0.01	0.02	0.01	0.02	0.01	0.12	0.04
	F2	0.22	0.07	0.07	0.03	0.11	0.05	0.05	0.01	0.02	0.01	0.04	0.01	0.06	0.02
	F4	0.19	0.05	0.05	0.03	0.18	0.03	0.05	0.00	0.01	0.00	0.05	0.00	0.17	0.08
Cu	A7	0.06	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.05	0.00
	C13	0.05	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.05	0.00
	F2	0.06	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.05	0.00
	F4	0.05	0.01	0.02	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.06	0.00
Cr	A7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zn	A7	0.04	0.00	0.02	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.13	0.00
	C13	0.11	0.01	0.03	0.00	0.04	0.00	0.00	0.00	0.02	0.00	0.02	0.00	0.15	0.01
	F2	0.17	0.01	0.06	0.01	0.04	0.00	0.01	0.00	0.02	0.00	0.02	0.00	0.20	0.01
	F4	0.07	0.01	0.04	0.01	0.04	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.16	0.01
Cd	A7	0.21	0.04	0.08	0.02	0.10	0.02	0.01	0.00	0.01	0.00	0.03	0.00	0.14	0.01
	C13	0.36	0.03	0.06	0.01	0.06	0.01	0.00	0.00	0.01	0.00	0.02	0.00	0.06	0.00
	F2	0.58	0.06	0.32	0.02	0.08	0.01	0.01	0.00	0.01	0.00	0.03	0.00	0.12	0.01
	F4	*19.07*	2.24	*7.06*	0.99	*5.67*	0.46	0.14	0.00	0.28	0.01	0.83	0.15	*11.27*	0.39
Pb	A7	0.03	0.00	0.02	0.00	0.04	0.01	0.01	0.00	0.00	0.00	0.03	0.00	0.03	0.00
	C13	0.04	0.01	0.03	0.00	0.06	0.00	0.02	0.00	0.01	0.00	0.02	0.00	0.06	0.01
	F2	0.03	0.00	0.02	0.00	0.04	0.00	0.06	0.01	0.01	0.00	0.01	0.00	0.03	0.00
	F4	0.04	0.01	0.01	0.00	0.04	0.00	0.01	0.00	0.01	0.00	0.03	0.01	0.05	0.01

Values exceeding unity are marked in italics.

Table 7. Estimation of the hazard quotient for children (from 3 to 10 years).

PTEs	Hot spots	Chicory	S.E	Lettuce	S.E	B.L. Lettuce	S.E	Radish	S.E	Rocket 1st	S.E	Rocket 4th	S.E	Spinach	S.E.
As	A7	0.13	0.04	0.03	0.00	0.20	0.05	0.05	0.00	0.01	0.00	0.02	0.01	0.05	0.00
	C13	0.26	0.08	0.08	0.02	0.13	0.02	0.02	0.01	0.01	0.00	0.01	0.00	0.16	0.05
	F2	0.25	0.08	0.07	0.03	0.10	0.05	0.06	0.01	0.01	0.00	0.02	0.01	0.08	0.02
	F4	0.22	0.06	0.05	0.03	0.18	0.03	0.05	0.01	0.00	0.00	0.02	0.00	0.22	0.11
Cu	A7	0.07	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.06	0.00
	C13	0.06	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
	F2	0.07	0.00	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00
	F4	0.06	0.01	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00
Cr	A7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	C13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	F4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zn	A7	0.05	0.00	0.02	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00
	C13	0.13	0.01	0.03	0.00	0.04	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.20	0.01
	F2	0.20	0.01	0.06	0.01	0.04	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.26	0.01
	F4	0.09	0.01	0.04	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.21	0.01
Cd	A7	0.25	0.05	0.08	0.02	0.10	0.02	0.01	0.00	0.00	0.00	0.01	0.00	0.19	0.01
	C13	0.42	0.03	0.06	0.01	0.06	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.08	0.00
	F2	0.68	0.07	0.32	0.02	0.08	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.15	0.01
	F4	*22.27*	2.62	*7.00*	0.98	*5.62*	0.45	0.17	0.00	0.12	0.00	0.35	0.06	*14.99*	0.52
Pb	A7	0.03	0.00	0.01	0.00	0.04	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.04	0.00
	C13	0.05	0.01	0.03	0.00	0.05	0.00	0.03	0.00	0.00	0.00	0.01	0.00	0.08	0.01
	F2	0.04	0.00	0.02	0.00	0.04	0.00	0.08	0.01	0.00	0.00	0.01	0.00	0.05	0.01
	F4	0.05	0.01	0.01	0.00	0.04	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.07	0.01

Values exceeding unity are marked in italics.

Table 8. Correlation between PTE contents in soils and plants.

PTEs	Soil	Tot *vs* Bioav.	Chicory	Lettuce	B.L. Lettuce	Radish	Rocket 1st	Rocket 4th	Spinach
As	Tot	-	0.7371	0.6872	–0.2728	–0.4712	–0.2276	–0.2346	0.9263
	Bioav.		-	-	-	-	-	-	-
Cu	Tot	0.9143	–0.9369	–0.2967	0.5711	*–0.9819*	0.9289	0.5678	0.5750
	Bioav.		–0.7769	–0.6576	0.2563	–0.9385	0.8545	0.3809	0.3444
Cr	Tot	–0.5040	0.8385	0.0517	–0.0045	*0.9705*	*0.9911*	*0.9828*	0.0246
	Bioav.		0.0337	0.5036	0.5229	–0.6937	–0.4106	–0.3359	0.8056
Zn	Tot	0.9263	*0.9644*	0.8178	0.9726	*0.9774*	*0.9931*	*0.9993*	0.7951
	Bioav.		*0.9929*	0.9345	0.8996	*0.9819*	0.8806	0.9320	0.9145
Cd	Tot	0.9438	*0.9999*	*0.9995*	*0.9996*	*0.9991*	*0.9996*	*0.9995*	*0.9996*
	Bioav.		0.9413	0.9344	0.9449	0.9393	0.9440	0.9430	0.9445
Pb	Tot	–0.3237	0.7993	*0.9504*	0.9795	–0.0171	0.6467	–0.0944	0.8709
	Bioav.		–0.4647	–0.1180	–0.1782	–0.4351	–0.5472	0.3103	–0.4101

Values significant per P≤0.05 are reported in italics.

Cao *et al.* (2010) (lettuce with 0.35 mg kg^{-1} f.w.) and by Guerra *et al.* (2012) (rocket salad 0.25 mg kg^{-1} f.w.) in other contaminated soils. Chromium concentrations in vegetables were significantly correlated with soil values only for radish and rocket (Table 8). HQ values were close to zero in all vegetables thanks to the very low toxicity of chromium. Indeed, HQ may be equal to 1 with unrealistic concentrations in vegetables: for children from 500 mg kg^{-1} f.w. with spinach to 12,000 mg kg^{-1} with rocket. Zn values were also very high in the soil at the site (846 mg kg^{-1}, Table 4) but the NH_4NO_3 bioavailable amounts were low and below the German DIN trigger value of 2 mg kg^{-1} (Table 5). In this case vegetables accumulated high Zn concentrations with values often higher than 10 mg kg^{-1} f.w., up to 25 mg kg^{-1} in spinach (Table 5). Similar values were also found by Ferri *et al.* (2015) (up to 18 mg kg^{-1} f.w.), thus confirming results obtained by Rocco *et al.* (2018) in other potentially contaminated sites of the same area. Zinc concentrations in plants were positively correlated with total Zn content in soils, for all vegetables, resulting not significant only for spinach (Table 8). In spite of these high concentrations, HQ values were always low, with a maximum value of 0.26 with spinach for children (Tables 6 and 7), thanks to the low toxicity of this element. Also in this case, HQ may be equal to 1 with unrealistic concentrations in vegetables: for children from 105 mg kg^{-1} f.w. with spinach to 2381 mg kg^{-1} with rocket. It must be pointed out that Zn is a beneficial microelement for human health, such that Zn deficiency is known to clinically affect several organ systems such as the epidermal, gastrointestinal, central nervous, immune, skeletal and reproductive systems (Hambidge, 2000). Therefore extensive research has been carried out to produce Zn-fortified foods by cultivating crops in Zn-rich soils (Brown *et al.*, 2010). As regards Cd, a hot spot was selected with very high concentration (13 mg kg^{-1}, Table 5), in which the bioavailable values (Table 5) were higher than the German DIN trigger value of 0.1 mg kg^{-1}. In this plot, the accumulation in vegetables was very high and exceeded the thresholds of EC Reg. 1881/06 (Root and tuber vegetables, stem vegetables = 0.1 mg kg^{-1} f.w.; leaf vegetables, fresh herbs, leafy brassica = 0.2 mg kg^{-1} f.w.), with the maximum value of 3.9 mg kg^{-1} f.w. in chicory, higher than values reported by Bandiera *et al*. (2016) (2.5 mg kg^{-1} f.w.) in other contaminated sites. Cadmium concentrations in vegetables and soil were highly correlated for all crops (Table 8). HQ values showed a potential risk for adult and children only for four crops, with values of 5.67-5.62 for baby leaf lettuce, 7.06-7.00 in lettuce; 11.27-14.99 for spinach and 19.07-22.27 for chicory, while radish and rocket salad did not represent a risk for human health, as they have a low intake rate in standard diets. Considering the high toxicity of this element, the potentially dangerous concentrations are very low (HQ=1, Table 9) and values measured in chicory, spinach, baby leaf lettuce and lettuce cultivated in this case study exceeded 19, 11, 7, and 5 times, respectively, these values for adults, and 22, 15, 7, and 5 times, respectively, for children.

Lead is ubiquitous throughout the Campania plain both due to geogenic and anthropogenic contamination (De Vivo *et al*., 2012). In this study area a selected hot spot (147 mg kg^{-1}, Table 5) showed bioavailable values below the German DIN trigger value of 0.1 mg kg^{-1} (Table 5). Nevertheless, Pb content in rocket salad and radish exceeded the thresholds of EC Reg. 1881/06 (vegetables excluding

Table 9. Potential health risk concentrations (HQ=1) mg kg^{-1} f.w.

PTEs	Chicory	Salad*	Adult (to 70 years) Radishes	Rocket	Spinach
As	0.13	0.19	0.58	1.01	0.14
Cu	16.69	24.82	77.87	135.29	18.54
Cr	625.71	930.81	2920.00	5073.36	695.24
Zn	125.14	186.16	584.00	1014.67	139.05
Cd	0.21	0.31	0.97	1.69	0.23
Pb	1.46	2.17	6.81	11.84	1.62
EPTs	**Chicory**	**Salad***	**Children (3-10 years) Radishes**	**Rocket**	**Spinach**
As	0.11	0.19	0.49	2.38	0.10
Cu	14.28	25.03	65.07	317.44	13.93
Cr	535.59	938.57	2440.29	11903.83	522.55
Zn	107.12	187.71	488.06	2380.77	104.51
Cd	0.18	0.31	0.81	3.97	0.17
Pb	1.25	2.19	5.69	27.78	1.22

*Lettuce and baby leaf lettuce.

Table 10. Hazard Index value for adults (A; to 70 years) and children (C; from 3 to 10 years).

Hot spots	Chicory		Lettuce		B.L. Lettuce		Radishes		Rocket 1st		Rocket 4th		Spinach	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C
A7	0.46	0.53	0.16	0.16	0.39	0.39	0.07	0.09	0.04	0.02	0.11	0.05	0.39	0.52
C13	0.79	0.92	0.22	0.21	0.31	0.31	0.05	0.06	0.06	0.03	0.09	0.04	0.44	0.59
F2	*1.06*	*1.24*	0.49	0.49	0.28	0.28	0.13	0.16	0.06	0.02	0.11	0.05	0.45	0.61
F4	*19.42*	*22.69*	*7.19*	*7.13*	*5.95*	*5.90*	0.21	0.25	0.31	0.13	0.93	0.40	*11.71*	*15.57*

Values exceeding hazard thresholds are marked in italics.

leafy brassica = 0.10 mg kg^{-1} f.w.; leafy brassica, excluding fresh herbs = 0.30 mg kg^{-1} f.w.) not only in the hot spot but also in the other plots with lower soil Pb concentrations. Indeed, only for lettuce and baby leaf lettuce was there a significant correlation between concentrations in vegetables and soils (Table 8). However, HQ values were low for all crops, with maximum values of 0.063 in radish for adults and 0.078 in spinach for children (Tables 6 and 7), well below unity. Pb concentrations in vegetables representing a potential risk for human health (*e.g.* 2.15 mg kg^{-1} in lettuce) were considered attainable in contaminated agricultural areas (Kabata-Pendias, 2011), but the values measured in this study area were much lower, thus excluding any risk for consumer health and confirming the findings of Agrelli *et al.* (2017) in a broad-based survey made on vegetables produced in the same area.

A key aspect was the cumulative effect of single non-carcinogenic effects of PTEs: even though an element does not give an HQ>1 it could contribute to an overall potential disease if added to the effect of other PTEs.

As shown in Table 10, many vegetables grown in the high Cd plot (F4) showed an HI>1, but also chicory grown on F2 showed an overall HI>1. In both cases the highest contribution to the overall hazard index was due to Cd followed by As. In all other cases, the cumulative risk was well below unity.

Conclusions

Environmental characterization of an agricultural area affected by illegal disposal of tannery sludge highlighted very high concentrations of Cr and Zn in the soil. However, both our analysis of the direct risk for workers and people who could frequent this site and of the indirect risk for consumers of vegetables cultivated in this soil excluded any risk for these two elements thanks to their low to moderate toxicity.

Instead, our analysis made by using highly precautionary parameters showed that the potential risks for consumers were due to Cd, even in plots in which its soil total content was low. The legal methodology for carrying out environmental characterization, based on a few samples per hectare and the comparison between the total content of PTEs in soils and the screening values reported in specific tables, does not allow the suitability of a potentially contaminated site to be evaluated for agricultural purposes. This estimation could be improved by comparing the readily bioavailable PTE contents with trigger values reported in German guidelines, as proposed by Rocco *et al.* (2018), and then by biological assays with specific metal-accumulating crops with which a realistic risk assessment of food chain contamination can be achieved, even in soils showing low total contents of PTEs.

In conclusion, the proposed methodology aims to fill a gap in the Italian environmental legislation that fails to give due consideration to the agricultural use of soils and the consequent risk for consumers due to contaminant intake from food crops.

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