Implications for food safety of the uptake by tomato of 25 trace-elements from a phosphogypsum amended soil from SW Spain

Santiago Enamorado [a], José M. Abril [a],*, Antonio Delgado [b], José L. Más [a], Oliva Polvillo [c], José M. Quintero [b]

[a] Departamento de Física Aplicada I, Universidad de Sevilla, EPS, c/Virgen de África n° 7, D.P. 41011, Seville, Spain
[b] Departamento de Ciencias Agroforestales, Universidad de Sevilla, ETSIA, Ctra. Utrera km. 1, D.P. 41013, Seville, Spain
[c] Agricultural Research Service of the University of Seville (SIA-CITIUS), Ctra. Utrera Km. 1, D.P. 41013, Seville, Spain

HIGHLIGHTS

- Uptake of 25 trace-elements by tomato was studied in phosphogypsum-amended soils.
- Cadmium had high transfer factors in tomato shoots (4.0) and fruits (1.5).
- These two TF values of Cd increased to 5.7 and 2.5 after PG-inputs, respectively.
- PG decreased the concentration of B, Cu, Sb, Cs, Ba, Tl and Th in fruits.
- Assessment on trace elements in PG is needed to avoid exceeding legal limits in food.

ABSTRACT

Keywords:
Phosphogypsum
Soil amendment
Heavy metals
Transfer factor
Food safety

Phosphogypsum (PG) has been usually applied as Ca-amendment to reclaim sodic soils such as those in the marshland area of Lebrija (SW Spain). This work aimed at the effects of PG amendments on the uptake of trace-elements by tomato and its implications for food safety. A completely randomized experiment was performed using a representative soil from Lebrija in a greenhouse involving six replicates and four PG treatments equivalent to 0, 20, 60, and 200 Mg ha^{-1}. Soil-to-plant transfer factors (TFs) were determined for Be, B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sb, Cs, Ba, Tl, Pb, Th and U. The highest TF in shoots was observed for Cd (4.0; 1.5 in fruits), its concentration being increased with increasing PG doses due to its content in this metal (2.1 mg Cd kg^{-1} PG). Phosphogypsum applying decreased the concentrations of Mn, Co and Cu in shoots; and of B, Cu, Sb, Cs, Ba, Tl and Th in fruits, however enhanced the accumulation of Se in fruits. Although Cd concentrations in tomato were below the maximum allowed levels in control pots (0 Mg PG ha^{-1}), PG amendments above 60 Mg ha^{-1} exceeded such limits.

1. Introduction

Phosphogypsum (PG) is a by-product of the industrial production of phosphoric acid, it being generated at a mass ratio of 5/1. Large quantities of PG are annually produced worldwide (about 170 million tons in 2006 [1]), most of them being stockpiled. For safety and economical reasons, there is a great interest in finding beneficial uses of PG, able to revalue and eliminate this waste. Use of PG in agriculture can be of particular interest because it is a Ca-source which can improve the functioning of sodic and acidic soils. Also, it can supply S and P to crops. Studies have been conducted, among others, on the effect of PG applying on the improvement of soil structure, salt balance and crop yield [2–4], control of soil erosion [5], availability of S and P [6], and N balance on reclaimed drained soils [7]. Thus, it can be supposed that agriculture could become the main worldwide sink of PG.

Depending on the origin of the phosphate rock, concentrations of some heavy metals (e.g. Cd) and natural radionuclides (e.g. ^{226}Ra) in PG can be higher than in agriculture soils by one order of magnitude [8,9]. Due to the associated potential hazards, some countries have developed regulations for the uses of PG, as the US-EPA 64 FR 5574 [10], or the Spanish Law of 19 July 2005 which allows its use as a soil amendment. The European Regulation No. 466/2001 establishes maximum levels of Cd, Pb and Hg in plant material and fruits for human consumption. Thus, some studies have been performed on the environmental impact and the radiological and alimentary safety in the agricultural use of PG [1,9,11–13]. On the other hand,

it has been found that PG reduces the leaching of As, Cd, Tl, Pb, Zn and Ni in acid soils [14,15]. Thus, this by-product is considered for soil remediation practices in metal polluted soils.

Phosphogypsum can affect plant nutrition either as a direct source of nutrients (Ca, S, P [6,16]) or varying soil geochemistry [15,17,18]. It can also enhance the concentrations in plant tissues of some hazardous elements, thus making the study of PG effects on soil-to-plant transfer of these elements a complex task of broad interest [1].

The agricultural use of PG in SW Spain is a relevant topic since the phosphoric acid industry located in Huelva generated a legacy of over 100 million tons of PG accumulated over four decades. The radionuclide and heavy metals content of this PG is well characterized [9,19]. Since the last sixties, this PG was crucial in the reclamation of the saline-sodic marshlands from SW Spain [4,20–22] where it was applied as Ca-amendment at a usual rate of 25 Mg ha^{-1} (wet weight, after being sun-dried, and with typical remaining water content of 20%). This amendment was essential to achieve a reduction of Na saturation and improvements of physical properties in soil [6,7,23]. On the other hand, there are evidences of significant enrichment of ^{226}Ra in the surface horizon of reclaimed soils plots relative to the deeper horizons and Cd in tomatoes produced in the area with higher concentrations than those found in tomatoes from other origins in Spain [1,9]. These latest are long term effects, evident after three decades of PG amendments. In farm soils under traditional practices, there may be different sources of Cd and other harmful pollutants (e.g. phosphated fertilizers, organic and inorganic soil amendments), and their cycling and interactions in agro-ecosystems are still poorly understood. Thus, it seems advisable complementing the previous works with an experimental study conducted under controlled conditions.

The aim of the present paper was to carry out such experimental study on trace-elements uptake by plant as being affected by different PG doses with a view to state its implications for food safety. Special focus was put on the soil-to-plant transfer of 25 trace-elements (Be, B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sb, Cs, Ba, Tl, Pb, Th and U) present in PG. To this end, a well characterized PG source, a representative soil from the area of Marismas of Lebrija, and a crop of great economical and social importance in SW Spain (industrial tomato) had been used. Results have been analyzed in terms of legal regulations on their potential health effects due to the consumption of tomato fruits by humans and of plant debris by livestock. Results obtained, beside their regional interest, can provide information and tools to study the potential effects of the agricultural use of PG and other hazard products on the environment and food safety.

2. Experimental

2.1. Area of study, soil and crop

The area known as Marismas de Lebrija accounts for most of 40,000 ha of originally saline-sodic soils in SW Spain. Reclamation of this area started at the end of the seventies of last century, with the installation of a tile-drain system and the application of PG amendments. After reclamation, most of the soils in the area can be classified as *Aeric Endoaquepts* [24]. More information about the area is available elsewhere [6,21,23]. The main crops are cotton, sugar beet, cereals (wheat and corn), and some vegetables. In recent years, industrial tomato is progressively replacing cotton and sugar beet in many farms. The area is also important by its extensive livestock (ovine, bovine, poultry and horse) which are fed with grass and plant debris produced locally.

Surface soil (ca. 0–30 cm) was collected in January 2005 from ten points randomly selected throughout the area (6 ha) of a commercial farm located at 37°1.2′ N, 6°7.4′ W. This farm had received during the last decades up to six PG amendments at typical doses of 20–25 Mg ha^{-1} [9]. General soil properties in the 0–30 cm horizon were characterized by Hurtado et al. [4]. Texture analysis provided 85, 372 and 543 g kg^{-1} of sand, silt and clay, respectively. Organic carbon and calcium carbonate equivalent (CCE) were 6.4 and 235 g kg^{-1}, respectively; and its cation exchange capacity (CEC) was 32 cmol$_c$ kg^{-1}. Finally, the pH determined in the 1:5 soil extract was 8.5. Soil was air-dried, homogenized and ground to pass a 2-mm screen, and 11.6 kg were added to each 15-L pot along with the perlite (0.8 kg). PG was collected from an inactive stack in Huelva (37°15.3′ N, 6°54′ W) and processed as the soil.

2.2. Experimental design and samples

Industrial tomato (*Solanum lycopersicum* L.) was grown under greenhouse conditions in pots using as substrate the soil described above, mixed with inert material (1/3, v/v ratio of expanded perlite) to ensure a viable soil aeration and hydraulic conductivity. A completely randomized experiment was performed involving six replications and four treatments: C (farm soil without any new PG addition), T1, T2 and T3 (as C, but with 1, 3 and 10 usual doses of PG, respectively). The addition of 60 g of PG to the previous substrate in each 15 L pot will represent one dose, which is the equivalent to the usual rate applied to soil in the field, 25 Mg ha^{-1}, considering that this amendment is mixed with soil to depth of 40 cm. As fertilizer, each pot received 60 g of Osmocote® supplied by Scotts España S.A. (Tarragona, Spain) with a nutrient content of 18% N, 10% P$_2$O$_5$, and 11% K$_2$O. Beside this, each plant received 1 L of a liquid foliar fertilizer Ca(NO$_3$)$_2$ during fruit development stage to avoid the nutritional disorder blossom-end-rot.

Plants were transferred to pots after 1–1.5 months at seedbed and allowed to produce fruits. Volumes of irrigation water during the experiment were monitored, accounting for 54.6 L for each pot. Drain water samples were collected for each irrigation episode in clean trays (previously washed with 2% HNO$_3$ analysis quality diluted in 18 MΩ deionised water). Overall, drained volumes accounted for 5% of the irrigation water.

The complete aerial parts were collected after 111 days, in June 2005, and separated into fruits and shoots (stems and leaves) parts. Aerial parts were dried at 36 °C for 48 h, and after that cut into small pieces and ground. Fruits were ground using a juice mixer, dried for 5 days as described for shoots, homogenized, and ground again. Measurements of fresh and dried weights allowed for the determination of the water content in the organic samples. At the end of the experiment, soil samples were recovered from each pot and carefully separated from roots and the inert material. After that, the soils were dried in clean steel trays and representative samples were submitted for analysis. The concentrations of 25 trace-elements, namely Be, B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sb, Cs, Ba, Tl, Pb, Th and U were determined in substrate, plant, and drainage water after the experiment. Also, these elements were determined in the PG, initial soil, perlite, fertilizers and a representative sample of irrigation water to quantify their inputs into the system.

2.3. Sample preparation and ICP-MS analysis

Irrigation and drain water samples were filtered through 0.22 μm Millipore© filters to measure analytes concentrations in the dissolved fraction. A new dilution was required in most of the samples due to the high value of the total dissolved solids (routinely higher than 0.1%). In all the cases, the sample matrix was adapted to 1% HNO$_3$.

Several digestion procedures were tested for biological matrices using certified reference material, including tomato leaves (SRM 1573a), as reported elsewhere [25]. The one selected for this work used 0.5 g of sample with 10 mL of nitric acid (69% HNO_3) of Suprapure® grade (Merck, Darmstadt, Germany). For digestions in closed-vessels, a microwave system Multiwave 3000 (Anton Paar, Graz, Austria) with rotor HF100 and software version v1.52 was used. The same digestion method was selected for the rest of solid samples (soils, PG and *Osmocote* fertilizer). It is worth noting that the selected method applied to soil samples provides a reasonable estimation of leachable/total contents of elements [26], being more representative of the bio-available fraction, and eliminating bias introduced by the variable amount of non-reactive residual material [27]. Finally, the foliar fertilizer was prepared with the adapted US-EPA 3015A method after evaporating 500 mL to 100 mL in a Teflon beaker on a hot plate at $T < 95\,°C$; 1 mL of concentrated suprapure HNO_3 was added to 9 mL of the remaining solution for digestion.

After digestion, samples were cooled down to room temperature. The aliquots were then diluted with 18 MΩ deionised water until reaching a final nitric acid concentration of ca. 1%, and filtered through 0.45 μm pore size Millipore filter.

An Inductively Coupled Plasma Mass Spectrometry system Thermo Elemental ICP-MS X7 (Thermo Fisher, Cambridge, UK) with quadrupole mass analyzer, multichannel detector (Pulse Counting and Analog Methods), auto sampler ASX-500 (CETAC, Omaha, NE, USA) and software Plasma Lab version v4.5, was used for this work. The instrument, located at the Servicio de Investigación Agraria laboratory (University of Seville, Spain), was used with a concentric Meinhard type glass nebulizer, a silica impact bead spray chamber, cooled to 3 °C by a Peltier cooler, and a standard silica torch. Standard nickel sample and skimmer cones were used. The internal standard solution was added online by a "Y" connection in the pipe where the sample is aspired by the peristaltic pump. Details on the instrumental settings, correction equations, quality controls and sample lists can be found in Enamorado et al. [25]. Methods were tested through the analysis of different certified reference materials: CRM-TMDW (water), SRM 1573a (tomato leaves) and SRM 2711 (Montana Soil).

2.4. Calculation of soil-to-plant transfer ratios

The soil-to-plant transfer factor (TF) of any given element will be operationally defined as the ratio between the element concentration in plant (shoots or fruits) and the corresponding concentration in substrate (without perlite particles) at the end of the experiment, given both concentrations on a dry weight basis. This operational definition includes the use of trace-element concentrations determined after the microwave-assisted acid digestion methods described above for sample mineralization.

2.5. Statistical analysis

Analysis of variance to see the effect of the different treatments on the concentrations of studied trace-elements in plant tissues, soil, and drainage waters was performed by using the general linear model in Statgraphics plus 5.1 software. Homogeneity of variance was assessed by means of the Cochran, Levene, and Bartlett tests; the last of which is sensitive to departures from normality. The Tukey test at 95% CL was used for comparison between means associated to four treatments (C, T_1, T_2, T_3) with six replications for shoots and tomato fruits. For soil samples we used only four replications, and three for drainage waters.

Table 1
Method Detection Limits (MDL) determined from laboratory blank solutions.

Element	m/z	MDL filtered water (µg L^{-1})	MDL organic/solids (mg kg^{-1})
Be	9	0.008	0.010
B	11	0.17	0.76
Al	27	0.16	2.9
Ti	47	0.1	2.5
V [1]	51		0.12
V	51	0.008	0.12
Cr	52	0.03	0.055
Mn	55	0.005	0.033
Fe	56	1.3	10.2
Co	59	0.002	0.012
Ni	60	0.11	0.15
Cu	63	0.01	0.33
Cu	65	0.02	0.50
Zn	66	0.06	1.4
As	75	0.01	0.020
As [2]	75		0.27
Se [3]	82		0.87
Se	82	0.1	0.88
Sr	88	0.004	0.045
Mo	95	0.006	0.26
Ag	107	0.001	0.081
Cd [4]	111	0.005	0.083
Sb	123	0.003	0.005
Cs	133	0.003	0.014
Ba	137	0.017	0.059
Tl	205	0.001	0.003
Pb [5]	208	0.005	0.024
Th	232	0.01	0.005
U	238	0.001	0.002

m/z, mass to charge ratio.
Correction equations: [1] ^{51}V: -0.352^{52}Cr-3.127^{53}Cr; [2] ^{75}As: -0.031^{82}Kr-3.1322^{77}ArCl; [3] ^{82}Se: -1.001^{83}Kr; [4] ^{111}Cd: -0.764^{106}Cd-1.073^{108}Cd; [5] ^{208}Pb: 1.00^{206}Pb + 1.00^{207}Pb

3. Results

3.1. Reagent blanks, Method Detection Limits and recovery of certified samples

Detection limits for each sample preparation method (MDL) were determined from reagent blanks by using the US-EPA 200.8 definitions (Table 1). Each reagent blank was prepared using the same amount and mixture of acids and following the same experimental procedure than the unknown samples. When reagent blanks were treated as unknown samples with subtraction of the calibration blank, only Al (2.4–5.5 µg L^{-1}), an airborne pollutant, was significantly above the instrument detection limits.

Table 2 shows the measurements of three reference certified materials (three replicates each) with the sample preparation procedures used in this work. The average recovery for filtered waters was $(99 \pm 5)\%$ (ranging from 89.7% for Ni to 113% for Fe; Fig. 1). In tomato leaves, when excluding anomalous results in Ag, Cs and Sr (non certified concentrations), the average recovery was $(92 \pm 17)\%$ (ranging from 70% for Cu to $133 \pm 20\%$ for As; Fig. 1). In soils, recoveries under 50% were obtained for Ti (30%), Ba (39%) and Sb $(47 \pm 5)\%$ (Fig. 1). Anomalous high recovery was found for Ni (180%), likely due to the isobaric interference of ^{44}Ca $+^{16}$O, which was not properly addressed through correction equations or other methods. This isobaric interference is of particular concern in Ca rich matrices such as those derived from the soil used. Then, the reported values for Ni in soils and their corresponding TF have to be considered on a semiquantitative basis. For the rest of elements, the averaged recovery was $(89 \pm 19)\%$ (ranging from 54% for Al to 116% for Pb). As expected, the acid digestion of soils with nitric acid provided a pseudo-total recovery.

Table 2
Measured concentrations in certified materials following the sample preparation procedures (filtered waters and acid digestion for solids - 0.5 g of sample with 10 mL of HNO_3).

Element	m/z	CRM-TMDW trace elements in water		SRM 1573a tomato leaves		SRM 2711 montana soil	
		Measured	Certified[#]	Measured	Certified	Measured	Certified
		$\mu g\,L^{-1}$		$mg\,kg^{-1}$			
Be	9	19.2 ± 0.2	20	0.0127 ± 0.0022			
B	11			30.2 ± 0.4			
Al	27	120.6 ± 1.3	120	567 ± 23	598 ± 12	3.53 ± 0.13[+]	6.53 ± 0.09[+]
Ti	49			24.0 ± 1.7		0.092 ± 0.006[+]	0.306 ± 0.023[+]
V	51			0.79 ± 0.04	0.835 ± 0.010	61.2 ± 0.8	81.6 ± 2.9
Cr	52	19.3 ± 0.1	20	1.65 ± 0.10	1.99 ± 0.06	35 ± 3	47[*]
Mn	55	38.5 ± 0.3	40	219 ± 6	246 ± 8	708 ± 26	638 ± 28
Fe	56	113 ± 2	100	264 ± 10	368 ± 7	2.66 ± 0.09	2.89 ± 0.06[+]
Co	59	24.6 ± 0.2	25	0.696 ± 0.019	0.57 ± 0.02	9.80 ± 0.03	10[*]
Ni	60	53.8 ± 0.6	60	1.64 ± 0.06	1.590 ± 0.07	37.1 ± 0.6	20.6 ± 1.1
Cu	65	18.5 ± 0.2	20	3.29 ± 0.14	4.70 ± 0.14	106 ± 3	114 ± 2
Zn	66			25.9 ± 0.9	30.90 ± 0.7	322 ± 7	350 ± 5
As	75	80.6 ± 0.2	80	0.149 ± 0.022	0.112 ± 0.004	59 ± 3	105 ± 8
Se	82	9.5 ± 0.2	10		0.054 ± 0.003	1.3 ± 1.2	1.52 ± 0.14
Sr	88	264 ± 2	250	76.9 ± 1.8	85[*]	260.0 ± 2.4	245.3 ± 0.7
Mo	98	96.8 ± 1.1	100	0.362 ± 0.015		0.96 ± 0.14	1.6[*]
Ag	107	2.00 ± 0.03	2.0	0.029 ± 0.018	0.017[*]	5.0 ± 0.5	4.63 ± 0.39
Cd	111	10.30 ± 0.16	10	1.47 ± 0.07	1.52 ± 0.04	46.3 ± 2.1	41.70 ± 0.25
Sb	123	10.40 ± 0.07	10		0.063 ± 0.006	9.1 ± 0.4	19.4 ± 1.8
Cs	133			0.017 ± 0.0020	0.053[*]	5.25 ± 0.06	6.1[*]
Ba	137	50.00 ± 0.04	50	55.3 ± 2.0	63[*]	285 ± 7	730 ± 40
Tl	205	10.00 ± 0.01	10	0.041 ± 0.026		2.37 ± 0.07	2.47 ± 0.15
Pb	208	36.2 ± 0.3	40	0.548 ± 0.0255		1350 ± 30	1162 ± 31
Th	232			0.095 ± 0.004	0.120[*]	14.6 ± 0.4	14[*]
U	238	10.10 ± 0.18	10	0.0293 ± 0.0021	0.035[*]	2.05 ± 0.08	2.6[*]

Measured values are reported as the mean and standard deviation (*n* = 3). Non reported values were under their respective MDL (Table 1).

[#] Certified values provided with ±0.5% uncertainties.

[*] Reference values.

[+] Values given as percentage.

3.2. Results on multi-elemental analysis

The measured element concentrations in soil, irrigation water, PG, perlite, and fertilizers used in the experiment are summarized in Table 3. Concentrations of B, Cd and U in PG and of Mo in the perlite were roughly one order of magnitude higher than those found in soil.

Table 4 shows results on water content determinations in shoots and tomato fruits. Table 5 summarizes the measured element concentrations in soil, shoots (aerial non generative part of plants) and tomato fruits (in mg kg^{-1} dry mass) at the end of the experiment, and the total amounts lixiviated through the drainage waters (μg),

for the four treatments. There was not always an increased concentration of trace-elements with increasing PG doses. In shoots and fruits, Cd was the only element which significantly increased at increased PG doses. In other cases, such as Cu, increased PG doses resulted in decreased concentrations in plant tissues (Table 5).

3.3. Soil-to-plant transfer factors

Transfer factors (TF) for shoots and tomato fruits can be evaluated, for all the elements and treatments, from raw data in Table 5. For the sake of brevity only TF for control pots are reported in Fig. 2 and Table 6. They varied up to three orders of magnitude for
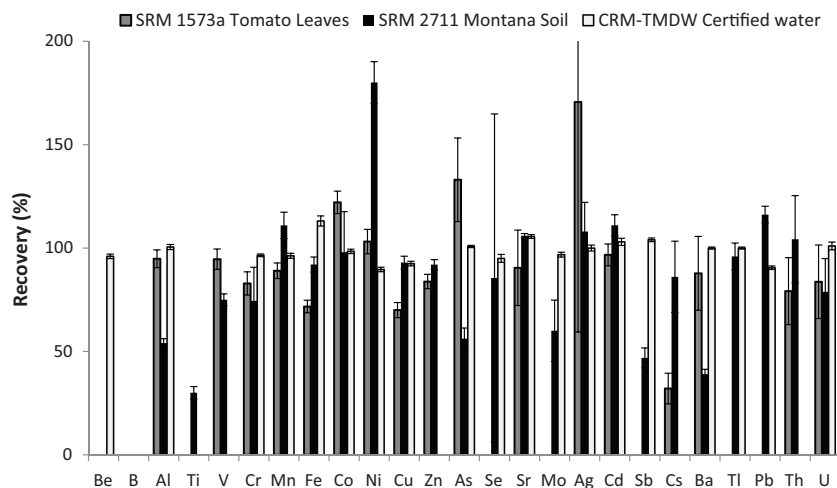

Fig. 1. Summary of recoveries for certified reference materials of different nature obtained with the sample preparation methods used in this work: tomato leaves, soil and water. Results represent the average (*n* = 3) and standard deviation.

Element	Soil[*]	Phosphogypsum	Osmocote	Inert material	Irrigation water	Ca(NO$_3$)$_2$
	mg kg^{-1}				µg L^{-1}	
Be	1.92 ± 0.04	0.42 ± 0.05	0.11 ± 0.01	N.D.	0.005 ± 0.0008	0.094 ± 0.005
B	49 ± 6	3120 ± 160	88 ± 64	N.D.	6.7 ± 0.2	78 ± 1
Al	36,100 ± 1300	3040 ± 30	350 ± 20	64 ± 1	56 ± 2	N.D.
Ti	113 ± 21	39 ± 6	6.0 ± 0.7	10.0 ± 1.3	0.018 ± 0.002	1.8 ± 0.6
V	81 ± 2	N.D.	5.4 ± 1.2	0.88 ± 0.3	0.89 ± 0.03	1.1 ± 0.1
Cr	66 ± 6	18.3 ± 0.3	8.5 ± 0.2	N.D.	1.01 ± 0.02	0.41 ± 0.02
Mn	782 ± 29	0.28 ± 0.03	290 ± 12	3.6 ± 0.4	0.31 ± 0.01	52 ± 1
Fe	32,600 ± 1100	366 ± 9	1700 ± 50	160 ± 30	35 ± 3	N.D.
Co	14.1 ± 0.2	1.47 ± 0.01	6.8 ± 0.6	0.023 ± 0.001	0.100 ± 0.004	13.2 ± 0.2
Ni	41 ± 1	12 ± 2	15.0 ± 1.4	N.D.	N.D.	112 ± 2
Cu	27 ± 1	5.2 ± 0.5	6.9 ± 0.6	0.15 ± 0.03	7.9 ± 0.5	13.0 ± 0.2
Zn	89 ± 2	11 ± 2	120 ± 8	3.0 ± 0.6	214 ± 2	N.D.
As	12.8 ± 0.6	2.6 ± 0.2	0.62 ± 0.02	0.15 ± 0.05	0.48 ± 0.03	0.25 ± 0.05
Se	0.9 ± 0.8	2.8 ± 0.7	0.8 ± 0.1	0.8 ± 0.1	0.37 ± 0.10	0.64 ± 0.06
Sr	289 ± 11	538 ± 2	520 ± 30	0.47 ± 0.12	64 ± 1	720 ± 2
Mo	0.16 ± 0.05	0.88 ± 0.15	104 ± 10	2.5 ± 0.6	0.43 ± 0.01	0.51 ± 0.05
Ag	0.16 ± 0.02	0.20 ± 0.01	0.022 ± 0.002	100 ± 8	0.0011 ± 0.0001	0.017 ± 0.005
Cd	0.19 ± 0.01	2.14 ± 0.07	0.31 ± 0.03	0.022 ± 0.002	N.D.	0.650 ± 0.005
Sb	0.027 ± 0.010	0.411 ± 0.013	0.117 ± 0.003	0.23 ± 0.01	0.193 ± 0.012	0.106 ± 0.005
Cs	6.11 ± 0.17	0.020 ± 0.006	0.16 ± 0.01	N.D.	0.0070 ± 0.0001	0.072 ± 0.005
Ba	179 ± 8	91 ± 2	27 ± 1	N.D.	13.2 ± 0.8	27 ± 1
Tl	0.38 ± 0.02	0.025 ± 0.005	0.070 ± 0.003	27 ± 1	0.0090 ± 0.0005	N.D.
Pb	36 ± 4	0.030 ± 0.002	0.42 ± 0.01	0.0037 ± 0.00	1.62 ± 0.02	2.50 ± 0.13
Th	7.86 ± 0.22	2.42 ± 0.02	2.7 ± 0.1	0.42 ± 0.01	0.003 ± 0.001	0.09 ± 0.01
U	1.72 ± 0.07	16.0 ± 0.2	3.7 ± 0.2	N.D.	0.075 ± 0.002	0.070 ± 0.002

Amounts added at each pot: 11.6 kg of soil; 0.060 kg of Osmocote; 0.060 kg for each PG dose (0, 1, 3 and 10 doses for C, T$_1$, T$_2$ and T$_3$, respectively); 0.80 kg of inert material, and 54.6 L of irrigation water. 1 L of Ca(NO$_3$)$_2$ as foliar fertilizer at pre-fruit stage.

N.D., non detected (under MDL)

[*] Single measurement of a representative soil sample. Associated uncertainties incorporates variability in the digestion yield of certified soil samples (Table 2).

	C	T1	T2	T3
Shoots	78.3 ± 0.2	77.4 ± 0.3	77.1 ± 0.4	77.0 ± 0.2
Fruits	86.2 ± 0.2	85.8 ± 0.3	85.5 ± 0.3	85.4 ± 0.2

Mean and standard deviation ($n = 6$).

different elements, the highest values being found for Mo and Cd in both fruits and shoots (Fig. 2). For most of the studied elements present over their MDL, transfer to shoots was higher than to fruits, except for Mo. The so obtained values in our experimental setting could be partially perturbed by the foliar application. Taking into account the concentrations of tested elements in Ca(NO$_3$)$_2$ fertilizer, the used amount (Table 3), and the dry masses of shoots

and fruits (49.1 ± 0.9 and 89.5 ± 1.5 g, respectively – mean and standard deviation for control pots), the maximum contribution of the foliar fertilizer to the TF for shoots was below 1.5% for most of the studied elements. Exceptions were Co and Ni, whose TF were increased up to 12% and 4% by this means, respectively; and, to a lesser extent, B and Sr, with contributions to TFs around 3%. The foliar fertilizer was applied at the time of the appearance of the first fruits, and owing to their concentrations, the TF for Co and Ni in fruits should be also handled with care.

Transfer factors have been published for a large variety of elements, soils and crops (Table 6 shows some of them for the sake of comparison). For most of the elements, observed TFs were comparable to those reported in the bibliography, at least in order of magnitude. It is expected that TF will depend on the state and geochemistry of the element in soil, on the plant type, and on the involved physiological processes. Thus, present results have the
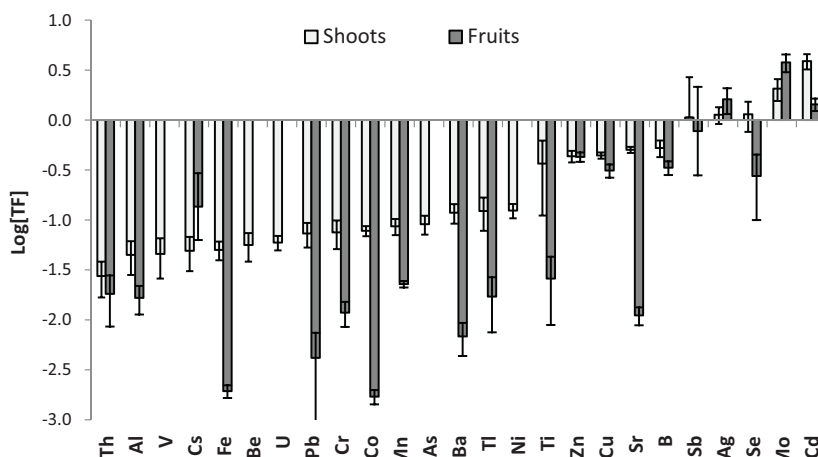

Fig. 2. Log of soil-to-plant transfer factors (as operatively defined in this work) for both shoots and fruits.

Table 5

Element concentrations in soil, shoots (aerial non generative part of plants) and tomato fruits (in mg kg^{-1} d.w.), and total amounts lixiviated through the drainage waters (µg).

		C	T1	T2	T3
Be	Soil	1.77 ± 0.11 (a)	1.6 ± 0.2 (a)	1.76 ± 0.06 (a)	1.75 ± 0.07 (a)
	Shoots	0.10 ± 0.03 (a)	0.06 ± 0.03 (a)	0.06 ± 0.02 (a)	0.060 ± 0.013 (a)
	Fruits	N.M. (a)	N.M. (a)	N.M. (a)	N.M. (a)
	Drainage Water	0.52 ± 0.05 (a)	0.20 ± 0.02 (c)	0.43 ± 0.06 (b)	0.22 ± 0.02 (a)
B	Soil	55 ± 8 (a)	54 ± 11 (a)	53 ± 2 (a)	51 ± 10 (a)
	Shoots	29 ± 4 (ab)	25 ± 4 (b)	30 ± 3 (a)	30 ± 3 (ab)
	Fruits	18.3 ± 1.1 (a)	17 ± 2 (ab)	15.8 ± 1.1 (ab)	15 ± 2 (b)
	Drainage Water	1114 ± 17 (a)	877 ± 12 (c)	1058 ± 14 (b)	567 ± 10 (d)
Al	Soil	40,000 ± 4000 (a)	39,000 ± 6000 (a)	38,900 ± 1100 (a)	38,000 ± 6000 (a)
	Shoots	1800 ± 600 (a)	1400 ± 800 (a)	1400 ± 700 (a)	1200 ± 200 (a)
	Fruits	700 ± 200 (a)	600 ± 300 (a)	450 ± 230 (a)	520 ± 110 (a)
	Drainage Water	183 ± 4 (a)	27 ± 3 (b)	190 ± 16 (a)	40.2 ± 1.2 (b)
Ti	Soil	110 ± 70 (a)	130 ± 80 (a)	113.3 ± 1.8 (a)	100 ± 80 (a)
	Shoots	42 ± 11 (a)	35 ± 13 (a)	33 ± 10 (a)	32 ± 8 (a)
	Fruits	2.9 ± 0.4 (a)	2.7 ± 0.4 (a)	2.5 ± 0.3 (a)	2.5 ± 0.4 (a)
	Drainage Water	15.1 ± 1.9 (a)	3.8 ± 0.9 (c)	10.1 ± 1.2 (b)	5.2 ± 0.9 (c)
V	Soil	69 ± 12 (a)	67 ± 16 (a)	63 ± 4 (a)	64 ± 19 (a)
	Shoots	3.2 ± 1.3 (a)	3.1 ± 1.2 (a)	3.1 ± 1.4 (a)	2.3 ± 0.8 (a)
	Fruits[a]	N.M. (a)	N.M. (a)	N.M. (a)	N.M. (a)
	Drainage Water	72.8 ± 1.5 (a)	64.8 ± 1.1 (b)	76 ± 3 (a)	45.6 ± 1.1 (c)
Cr	Soil	59 ± 4 (a)	59 ± 8 (a)	58.6 ± 1.7 (a)	58 ± 6 (a)
	Shoots	4.4 ± 1.4 (a)	3.1 ± 1.0 (a)	3.0 ± 0.6 (a)	3.2 ± 0.3 (a)
	Fruits	0.70 ± 0.19 (a)	0.52 ± 0.09 (a)	0.60 ± 0.11 (a)	0.70 ± 0.07 (a)
	Drainage Water	7.1 ± 0.4 (b)	6.1 ± 0.2 (c)	8.8 ± 0.3 (a)	4.89 ± 0.14 (d)
Mn	Soil	850 ± 30 (a)	850 ± 40 (a)	840 ± 20 (a)	834 ± 17 (a)
	Shoots	74 ± 13 (a)	51 ± 10 (b)	55 ± 9 (b)	58 ± 4 (ab)
	Fruits	19.4 ± 1.2 (a)	19 ± 2 (a)	17.5 ± 1.6 (a)	18 ± 2 (a)
	Drainage Water	12.65 ± 0.13 (b)	4.81 ± 0.11 (c)	14.5 ± 0.5 (a)	3.99 ± 0.09 (d)
Fe	Soil	32,600 ± 500 (a)	34,400 ± 1300 (a)	32,000 ± 900 (a)	32,200 ± 1100 (a)
	Shoots	1600 ± 300 (a)	1200 ± 700 (a)	1100 ± 300 (a)	1120 ± 170 (a)
	Fruits	63 ± 9 (ab)	50 ± 30 (b)	80 ± 30 (ab)	88 ± 18 (a)
	Drainage Water	780 ± 50 (a)	320 ± 30 (b)	380 ± 40 (b)	77 ± 14 (c)
Co	Soil	13.6 ± 0.3 (b)	14.4 ± 0.4 (a)	13.64 ± 0.15 (b)	13.61 ± 0.09 (b)
	Shoots	1.06 ± 0.12 (a)	0.69 ± 0.19 (b)	0.75 ± 0.12 (b)	0.79 ± 0.07 (b)
	Fruits	0.023 ± 0.004 (a)	0.025 ± 0.011 (a)	0.032 ± 0.013 (a)	0.037 ± 0.013 (a)
	Drainage Water	18.0 ± 0.7 (c)	26.6 ± 0.3 (a)	23.1 ± 0.5 (b)	9.13 ± 0.15 (d)
Ni	Soil	36.7 ± 1.2 (ab)	39.3 ± 1.7 (a)	36.7 ± 0.5 (ab)	36.0 ± 1.2 (b)
	Shoots	4.6 ± 0.7 (ab)	3.4 ± 0.6 (b)	5.3 ± 1.7 (a)	3.9 ± 0.2 (ab)
	Fruits[a]	N.M. (a)	N.M. (a)	N.M. (a)	N.M. (a)
	Drainage Water	59 ± 7 (a)	50 ± 5 (ab)	39 ± 8 (b)	22 ± 4 (c)
Cu	Soil	26.9 ± 0.5 (b)	28.8 ± 0.8 (a)	27.3 ± 1.3 (ab)	27.6 ± 0.8 (ab)
	Shoots	11.9 ± 0.8 (a)	10.4 ± 1.2 (a)	8.4 ± 1.1 (b)	6.8 ± 0.5 (b)
	Fruits	8.4 ± 1.3 (a)	9.5 ± 1.9 (a)	7.5 ± 1.3 (ab)	5.8 ± 1.2 (b)
	Drainage Water	83.3 ± 1.5 (b)	60.8 ± 0.8 (c)	102.8 ± 1.5 (a)	35.7 ± 0.5 (d)
Zn	Soil	72 ± 3 (a)	75.3 ± 1.1 (a)	75 ± 2 (a)	78 ± 10 (a)
	Shoots	32 ± 4 (a)	24 ± 3 (a)	25 ± 4 (a)	27 ± 9 (a)
	Fruits	31 ± 3 (a)	32 ± 2 (a)	28 ± 3 (a)	27 ± 5 (a)
	Drainage Water	334 ± 10 (c)	5084 ± 80 (a)	994 ± 18 (b)	379 ± 9 (c)
As	Soil	10.7 ± 0.4 (a)	11.1 ± 0.5 (a)	10.71 ± 0.18 (a)	10.8 ± 0.4 (a)
	Shoots	1.0 ± 0.2 (a)	0.8 ± 0.3 (a)	1.0 ± 0.21 (a)	0.97 ± 0.15 (a)
	Fruits[a]	N.M. (a)	N.M. (a)	N.M. (a)	N.M. (a)
	Drainage Water	26.7 ± 0.5 (b)	27.2 ± 0.9 (b)	33.3 ± 1.2 (a)	18.8 ± 0.6 (c)
Se	Soil	1.0 ± 0.2 (a)	1.1 ± 0.4 (a)	1.0 ± 0.2 (a)	1.1 ± 0.2 (a)
	Shoots	1.1 ± 0.3 (a)	0.96 ± 0.11 (a)	1.3 ± 0.2 (a)	1.3 ± 0.3 (a)
	Fruits	0.27 ± 0.16 (b)	0.32 ± 0.10 (b)	0.53 ± 0.07 (ab)	0.7 ± 0.2 (a)
	Drainage Water	30.5 ± 1.7 (c)	37.8 ± 2 (b)	49 ± 2 (a)	19.1 ± 1.4 (d)
Sr	Soil	242 ± 8 (a)	257 ± 17 (a)	246 ± 6 (a)	250 ± 10 (a)
	Shoots	266 ± 7 (b)	237 ± 7 (c)	273 ± 10 (ab)	286 ± 8 (a)
	Fruits	5.9 ± 1.1 (a)	4.9 ± 0.5 (a)	5.3 ± 0.4 (a)	5.8 ± 0.4 (a)
	Drainage Water	17,300 ± 200 (b)	16,600 ± 130 (c)	20,600 ± 200 (a)	10,130 ± 70 (d)
Mo	Soil	0.65 ± 0.11 (a)	0.71 ± 0.14 (a)	0.74 ± 0.06 (a)	0.87 ± 0.15 (a)
	Shoots	1.3 ± 0.2 (a)	1.3 ± 0.3 (a)	1.2 ± 0.2 (a)	1.14 ± 0.16 (a)
	Fruits	2.5 ± 0.3 (a)	2.5 ± 0.2 (a)	2.47 ± 0.13 (a)	2.2 ± 0.2 (a)
	Drainage Water	38.4 ± 0.8 (c)	128 ± 3 (a)	87.7 ± 0.8 (b)	19.2 ± 0.3 (d)

Table 5 (*Continued*)

		C	T1	T2	T3
Ag	Soil	0.122 ± 0.013 (a)	0.145 ± 0.018 (a)	0.13 ± 0.03 (a)	0.131 ± 0.008 (a)
	Shoots	0.14 ± 0.02 (b)	0.118 ± 0.015 (b)	0.14 ± 0.03 (b)	0.34 ± 0.07 (a)
	Fruits[a]	0.20 ± 0.05 (a)	0.23 ± 0.06 (a)	0.19 ± 0.04 (a)	0.20 ± 0.07 (a)
	Drainage Water	0.11 ± 0.04 (ab)	0.14 ± 0.03 (a)	0.08 ± 0.04 (bc)	0.028 ± 0.011 (c)
Cd	Soil	0.19 ± 0.02 (a)	0.21 ± 0.02 (a)	0.209 ± 0.018 (a)	0.23 ± 0.02 (a)
	Shoots	0.75 ± 0.09 (c)	0.81 ± 0.06 (c)	1.08 ± 0.18 (b)	1.32 ± 0.12 (a)
	Fruits	0.278 ± 0.019 (c)	0.35 ± 0.03 (bc)	0.44 ± 0.05 (b)	0.57 ± 0.10 (a)
	Drainage Water	8.3 ± 0.3 (c)	17.1 ± 0.3 (b)	24.4 ± 0.2 (a)	3.00 ± 0.06 (d)
Sb	Soil	0.07 ± 0.11 (a)	0.07 ± 0.07 (a)	0.04 ± 0.06 (a)	0.10 ± 0.08 (a)
	Shoots	0.075 ± 0.013 (a)	0.052 ± 0.014 (a)	0.072 ± 0.011 (a)	0.080 ± 0.011 (a)
	Fruits	0.06 ± 0.05 (a)	N.M. (b)	N.M. (b)	N.M. (b)
	Drainage Water	6.62 ± 0.07 (c)	8.74 ± 0.18 (a)	7.67 ± 0.19 (b)	3.02 ± 0.09 (d)
Cs	Soil	5.7 ± 0.6 (a)	5.9 ± 0.6 (a)	5.60 ± 0.05 (a)	5.4 ± 0.8 (a)
	Shoots	0.28 ± 0.10 (a)	0.22 ± 0.14 (a)	0.23 ± 0.12 (a)	0.19 ± 0.04 (a)
	Fruits	0.8 ± 0.7 (a)	0.002 ± 0.004 (b)	N.M. (b)	N.M. (b)
	Drainage Water	1.37 ± 0.06 (b)	0.83 ± 0.08 (c)	1.74 ± 0.18 (a)	0.56 ± 0.06 (d)
Ba	Soil	159 ± 19 (a)	162 ± 19 (a)	154 ± 10 (a)	150 ± 30 (a)
	Shoots	19 ± 4 (a)	16 ± 4 (a)	17 ± 3 (a)	16.8 ± 1.2 (a)
	Fruits	1.1 ± 0.4 (a)	1.0 ± 0.4 (ab)	0.5 ± 0.3 (bc)	0.32 ± 0.17 (c)
	Drainage Water	386 ± 6 (c)	401 ± 4 (b)	435 ± 5 (a)	217.3 ± 1.5 (d)
Tl	Soil	0.32 ± 0.04 (a)	0.33 ± 0.04 (a)	0.313 ± 0.014 (a)	0.31 ± 0.05 (a)
	Shoots	0.039 ± 0.014 (a)	0.037 ± 0.014 (a)	0.033 ± 0.009 (a)	0.028 ± 0.005 (a)
	Fruits[a]	0.005 ± 0.003 (a)	N.M. (b)	N.M. (b)	N.M. (b)
	Drainage Water	0.284 ± 0.019 (c)	1.38 ± 0.03 (a)	0.81 ± 0.03 (b)	0.138 ± 0.015 (d)
Pb	Soil	32.3 ± 0.7 (a)	30.47 ± 0.13 (b)	30.81 ± 0.10 (ab)	30.5 ± 1.2 (ab)
	Shoots	2.4 ± 0.7 (a)	1.7 ± 0.7 (a)	2.0 ± 0.7 (a)	2.3 ± 0.2 (a)
	Fruits	0.14 ± 0.11 (a)	0.14 ± 0.03 (a)	0.04 ± 0.03 (b)	0.054 ± 0.019 (ab)
	Drainage Water	20.3 ± 0.6 (c)	85.8 ± 0.8 (a)	51.7 ± 1.0 (b)	5.11 ± 0.09 (d)
Th	Soil	9.92 ± 0.19 (a)	10.1 ± 0.12 (a)	9.84 ± 0.11 (a)	9.71 ± 0.19 (a)
	Shoots	0.27 ± 0.11 (a)	0.24 ± 0.13 (a)	0.19 ± 0.11 (a)	0.17 ± 0.03 (a)
	Fruits	0.18 ± 0.09 (a)	0.024 ± 0.019 (b)	0.03 ± 0.05 (b)	N.M. (b)
	Drainage Water	3.45 ± 0.18 (c)	3.99 ± 0.07 (b)	4.68 ± 0.013 (a)	2.48 ± 0.06 (d)
U	Soil	1.63 ± 0.12 (a)	1.64 ± 0.06 (a)	1.7 ± 0.2 (a)	1.82 ± 0.11 (a)
	Shoots	0.097 ± 0.014 (a)	0.06 ± 0.02 (c)	0.064 ± 0.010 (bc)	0.089 ± 0.014 (ab)
	Fruits[a]	N.M. (a)	N.M. (a)	N.M. (a)	N.M. (a)
	Drainage Water	33.1 ± 0.5 (c)	36.4 ± 0.4 (b)	45.5 ± 0.04 (a)	28.7 ± 0.2 (d)

Concentrations correspond to mean and standard deviation of mean (n = 4 for soils, n = 6 for biological matrices and n = 3 for drain water samples).). Means followed by the same letter within a column and an element are not significantly different (Tukey's test at the 95% CL). N.M. < MDL. Treatments C, T$_1$, T$_2$ and T$_3$ as defined in the text.

value of being representative of the studied crop and soil, both of great local social and economical relevance.

4. Discussion

4.1. TF and changes in element inventories in control pots

Differences found in TF between elements of the same group, such as Zn and Cd, Fe and Ni or Sr and Ba can be explained by their geochemistry, particularly by their activity in the soil solution, and by the efficiency of uptake mechanisms by plants. One of the most extreme cases is Fe, an abundant element in soil, which however is present at very low concentrations in soil solution (usually lower than 6×10^{-19} mg L^{-1} at the pH of the studied soil [31]) due to the low solubility of minerals containing it in basic soils. This low solubility restricts its uptake by plants. Depending on the mobilization strategies of plants roots, such as acidification, or the exudation of organic compounds with complexing capacity, which can be element-specific, the TF of metals could vary obviously. On the contrary, elements with increased solubility at basic soil pH, such as Mo [32], show high TF in the studied soil. The high TF observed for Cd (4.0 and 1.5 for shoots and fruits, respectively) could be related to the high Ca saturation of the soil because the latter cation can compete in the exchange complex with Cd, enhancing its release to the soil solution and thus plant uptake. This explains the increased

TF values for Cd to 5.7 (shoots) and 2.5 (fruits) with PG-inputs in treatment T3.

The largest differences in TF for the same element between fruits and shoots (Fig. 2) can be explained by its mobility in phloem. Since fruits have a lower transpiration rate than leaves (included in shoots), the TF must be obviously lower in fruits than in shoots for non-mobile elements in phloem such as Fe [33]. On the other hand, the uptake of Zn is mediated by the *nicotianamine* amino-acid [34], particularly active in tomato plants [35], promoting the phloem transport and thus the accumulation of Zn in fruit [36,37]. This explains the similar TF for shoots and fruits observed for this element (Fig. 2). A similar mechanism has been reported for Cu [38] which also shows similar TF for both plants tissues.

After crop, concentrations of Mn, Mo and Th in soil significantly (at 95% CL) increased with T3 by 9%, 300% and 25%, respectively; meanwhile the concentrations of Ni, Zn, As and Ag decreased by 10%, 19%, 24% and 16%, respectively. The foliar fertilizer did not contribute to elements inventories in substrate, but the *Osmocote* represents an obvious source of Mo (Table 3), and the added amount can explain the increase of its inventory by a factor four. Changes in the concentration of studied elements after crop can be hardly explained by a mass balance. The amounts introduced with irrigation water were negligible when compared with its inventory present in soil for all analytes, the highest inputs being those of Sb, Mo and Zn (3%, 1.2% and 1.0% of their inventories, respectively). Overall, losses through drainage were much lower than 1% of the

Table 6
Transfer factors found in the experiment for control pots versus some bibliographic data: IAEA-TECDOC-1616 [28], PNNL-13,421 [29] and ORNL-5786 [30].

	Experiment results		IAEA-TECDOC-1616[a]		PNNL-13,421		ORNL-5786	
	Vegetal tissue	Fruit	Leafy vegetables	Non-leafy vegetables (fruits, berries, ...)	Leafy vegetables	Fruit	Leafy vegetables	Fruits, seeds, tubers, etc...
As	0.005	N.M.	–	–	0.04	0.006	0.04	0.006
Th	0.009	0.018	0.0018	0.0008	0.0018	0.00025	0.00085	0.000085
Fe	0.039	0.0033	0.0010	0.0010	0.05	0.05	0.004	0.001
Al	0.045	0.017	–	–	–	–	0.004	0.00065
V	0.045	N.M.	–	–	–	–	0.0055	0.003
Cs	0.049	0.14	0.06	0.021	0.46	0.22	0.08	0.03
Be	0.056	N.M.			0.01	0.0015	0.01	0.0015
U	0.06	N.M.	0.02	0.015	0.0083	0.004	0.0085	0.004
Pb	0.073	0.042	0.08	0.015	0.01	0.01	0.045	0.009
Co	0.078	0.0017	0.17	0.14	0.23	0.007	0.02	0.007
Cr	0.08	0.014	0.0010	0.0010	0.0075	0.0045	0.0075	0.0045
Mn	0.086	0.023	0.41	0.3	0.7	0.05	0.25	0.05
Ba	0.118	0.007	0.005	0.005	0.15	0.015	0.15	0.015
Tl	0.12	N.M.	–	–	0.004	0.0004	0.004	0.0004
Ni	0.35	N.M.	0.17[b]	–	0.28	0.06	0.06	0.06
Ti	0.37	0.13	–	–	–	–	0.0055	0.003
Sb	0.4	0.72	0.00009	0.00013	0.00013	0.00008	0.2	0.03
Cu	0.43	0.32	–	–	0.4	0.25	0.4	0.25
Zn	0.43	0.43	2.4	0.4	1.3	0.9	1.5	0.9
B	0.52	0.33	–	–	–	–	4.0	2.0
Se	1.09	0.28	–	–	0.15	0.05	0.025	0.025
Sr	1.1	0.024	0.76	0.36	3.0	0.2	2.5	0.25
Ag	1.13	1.66	0.00018	0.0006	0.00027	0.0008	0.4	0.1
Mo	2.2	4.5	0.5	–	0.8	0.05	0.25	0.06
Cd	3.8	1.5	0.27	–	0.55	0.12	0.55	0.15

[a] Mean geometry (if exist disponibility).
[b] Grass.
N.M. results under MDL. – Non reported.

final inventory, the highest losses being found for Sb, Sr, Mo and Cd, with 0.8%, 0.6%, 0.5% and 0.4%, respectively. Similarly, extractions by the aerial part of the tomato plants were below 1% of the final inventory in the substrate for all the elements, except for Mo, Cd, Ag and Sb with extractions of 3.8%, 2.8%, 1.7% and 1.1%, respectively. The contribution of plant roots (not measured) and variations in the digestion yield with time remain as possible explanations for these observed changes in the concentrations of studied elements in soil.

4.2. Phosphogypsum effects on analyte concentrations in the soil–plant–water system

The added masses of PG in treatments T_1, T_2 and T_3 represented, respectively, 0.5%, 1.6% and 5.2% of the total mass of the growing substrate. Thus, only the analytes whose concentrations in PG were one order of magnitude higher than those in the soil (B, Cd and U) could appreciably change their effective concentration in the substrate. However, non-significant differences were found with our experimental settings (Table 5, at 95% CL). PG can alter the geochemistry of some elements in the soil, modifying the performance of the microwave-assisted acid digestions. This can explain the differences observed among treatments for the concentrations of Co, Ni, Cu and Pb in soil (Table 5), which were always lower than 9%.

The concentrations in shoots of 9 of the 25 studied analytes, namely B, Mn, Co, Ni, Cu, Sr, Ag, Cd and U were significantly affected by PG. Relevant amounts of Cd were supplied with PG, this being the main reason for the observed increase in Cd concentrations in shoots (Fig. 3). On the contrary, PG inhibited the uptake of Mn, Co and Cu, wherein Cu concentration in the shoots monotonically decreases with the increase of PG doses (Fig. 3). This often has been described as a result of antagonism with Ca [39,40], and some studies have suggested the potential use of PG in contaminated soils to reduce the uptake of some heavy metals by plants [41–44]. The

lower PG dose (T_1) resulted in decrease of U concentration in shoots, likely as a result of two opposite trends: the inhibition of the uptake by Ca-antagonism, and its increased availability due to its supply with PG.

Most of the elements usually show low mobility through phloem, which is the main passage of nutrients from roots to fruits; this explains their overall lower accumulation in fruits in comparison to shoots. Be, V, As and U were under their respective MDL in fruits (Table 1). The highest PG doses (T_3) decreased the concentrations of B, Cu, Sb, Cs, Ba, Tl and Th when compared with control. It is worth noting that inhibition of Sb, Cs, Tl, Th and Ba uptake cannot be statistically solved for shoots with the present experimental settings. Decreased concentrations of Mn and Co at increasing PG doses were found in shoots, but not in fruits. The Ca-antagonism can be argued as the main reason for such observed decreased concentration of metals in plant tissues. Iron concentration in fruits tended to increase with increasing PG doses, perhaps due to the supply done with the amendment; on the contrary, Pb concentration tended to decrease with increased PG doses (Table 5), as the likely result of the precipitation of Pb phosphates promoted by P supply with PG amendment [45,46].

As described in shoots, Cd concentrations in tomato fruits increased with increasing PG doses due to its associated inputs (Fig. 3). A similar increase was observed for Se (Fig. 3) which was not significantly supplied by PG amendments. It has been previously reported that P and S (both supplied by PG) can compete for sorption sites with Se in soil [47] and also enhance the transport of Se within the plant system [48,49].

Results of analyte flowing through drainage waters cannot be considered representative of the actual field conditions. Nevertheless, they provide a good basis for a qualitative gradation of the potential risk of loss through leaching of different elements. The highest leachability under our experimental conditions was found for Sb, Sr, Mo and Cd, with cumulative losses through drainage
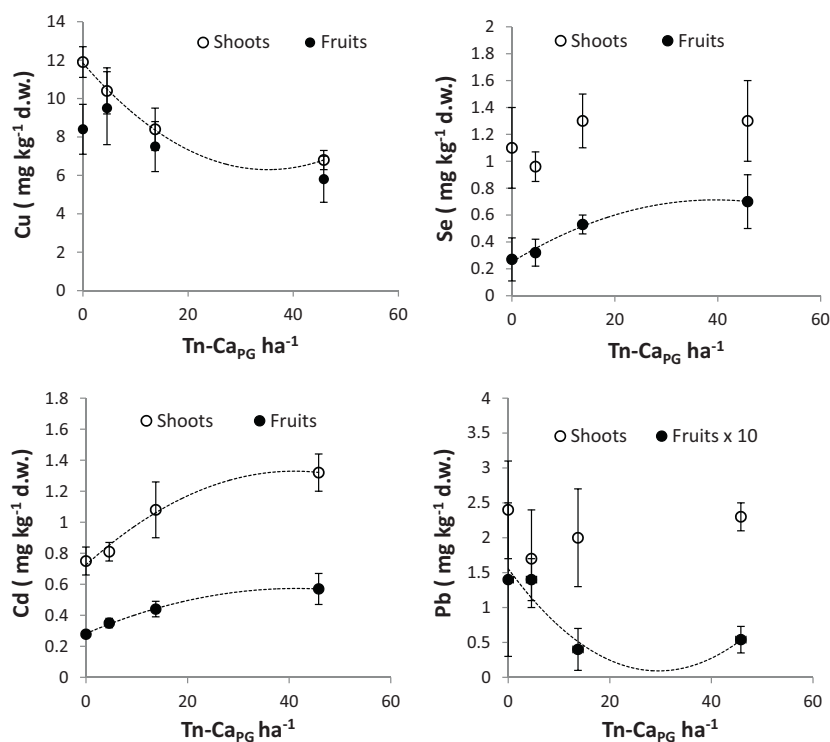
Fig. 3. Behavior of concentrations found in fruits and shoots as a function of PG dose (Tn-Ca$_{PG}$ ha^{-1}, tons of Ca supplied by PG per ha) for selected elements. Dotted lines have been added to guide the eye.

equivalent to 0.8%, 0.6%, 0.5% and 0.4% of their total inventories in substrate, respectively. Overall, the highest PG rate (T3) reduced the cumulative drained volumes by a 40% with respect to the control pots, and thus decreased the associated losses through drainage for most of the elements. This effect can be attributable to an improvement in soil microstructure due to the displacement of Na by Ca in the soil exchange complex.

4.3. Food safety

The European regulation 2005/87/CE on undesirable substances in animal feed fixed maximum allowed levels of 30 mg kg^{-1} of Pb in green fodder and 1 mg kg^{-1} of Cd in feed materials of vegetable origin, with a moisture content of 12%. The measured concentrations of Pb in tomato shoots were one order of magnitude below this limit, and values in fruits were even lower. Thus, present data on Pb levels and the potential PG effects on them do not represent any concern. Nevertheless, present levels of Cd in tomato shoots cultivated in these soils (control pots) are below, but close to the maximum allowed concentration, and thus it can be supposed that the application of few additional PG amendments would overcome such a limit.

The European regulation 1881/2006 sets maximum levels for certain contaminants in foodstuffs, being those for Pb and Cd in vegetables and fruits of 100 and 50 μg kg^{-1}, respectively (wet weight basis). Taking into account the water contents given in Table 4, the levels of Pb were not of concern. Present levels of Cd in tomato fruits cultivated in these soils (control pots) were 38.4 ± 2.7 μg kg^{-1} wet weight. Thus, as stated above for animal feed, additional PG amendments would overcome such limit for human consumption, as revealed by results with T2 and T3 treatments, with Cd concentrations in fruits of 64 ± 7 and 83 ± 15 μg kg^{-1} wet weight, respectively.

5. Conclusions

The highest soil-to-plant transfer factors (TFs) in tomato were found for Cd, Mo and Ag. Extractions by the aerial part of the tomato plant were over 1% of the final inventory in substrate only for Mo, Cd, Ag and Sb, with values of 3.8%, 2.8%, 1.7% and 1.1%, respectively. The highest leachabilities under our experimental conditions were found for Sb, Sr, Mo and Cd, being always lower than 1% of their total inventories in substrate.

Relevant amounts of Cd were supplied with PG (2.1 mg Cd kg^{-1} PG), which explains the observed increase in Cd concentrations in shoots and fruits. The highest PG doses inhibited the accumulation of Mn, Co and Cu in shoots; and of B, Cu, Sb, Cs, Ba, Tl and Th in fruits. The PG effects on Pb concentrations in shoots and fruits do not represent any concern in terms of European regulations for animal feed and human foodstuffs. Nevertheless, present levels of Cd in tomato (shoots and fruits) cultivated in these soils (control pots) were below, but close to the maximum allowed concentrations, and accumulated PG amendments greater than 16 g PG kg^{-1} soil (equivalent to three amendments with 20 Mg ha^{-1} in the field) would exceed such a limit. The assessment on trace-element content of PG, which may vary depending on the source, is needed to avoid exceeding legal limits of Cd or other pollutants in food when this by-product is used as soil amendment in agriculture.

References

[1] S.M. Enamorado Báez, J.M. Abril, J.L. Mas, R. Perianez, O.P. Polo, A. Delgado, J.M. Quintero, Transfer of Cd, Pb, Ra and U from phosphogypsum amended soils to tomato plants, Water Air Soil Poll. 203 (2009) 65–77.

[2] D.A. Mays, J.J. Mortvedt, Crop response to soil applications of phosphogypsum, J. Environ. Qual. 15 (1986) 78–81.

[3] G.L. Mullins, C.C. Mitchell, Use of phosphogypsum to increase yield and quality of annual forages. Publ. No 01-048-084, Florida Inst. of phosphate Re, Bartow, 1990.

[4] M.D. Hurtado, S.M. Enamorado, L. Andreu, A. Delgado, J.M. Abril, Drain flow and related salt losses as affected by phosphogypsum amendment in reclaimed marsh soils from SW Spain, Geoderma 161 (2011) 43–49.

[5] X.C. Zhang, W.P. Miller, M.A. Nearing, L.D. Norton, Effects of surface treatment on surface sealing, runoff, and interril erosion, Trans. ASAE 41 (1998) 989–994.

[6] A. Delgado, M.D. Hurtado, L. Andreu, Phosphorus loss in tile drains from a reclaimed marsh soil amended with manure and phosphogypsum, Nutr. Cycl. Agroecosyst. 74 (2006) 191–202.

[7] M.D. Hurtado, L. Andreu, J.M. Abril, A. Delgado, Nitrate loss from a tile-drained reclaimed marsh soil from SW Spain amended with different products, Nutr. Cycl. Agroecosyst. 91 (2011) 255–267.

[8] J.P. Bolivar, R. García-Tenorio, M. García-León, On the fractionation of natural radioactivity in the production of phosphoric acid by the wet acid method, J. Radional. Nucl. Chem. Lett. 214 (1996) 77–78.

[9] J.M. Abril, R. Garcia-Tenorio, S.M. Enamorado, M.D. Hurtado, L. Andreu, A. Delgado, The cumulative effect of three decades of phosphogypsum amendments in reclaimed marsh soils from SW Spain: 226Ra, 238U and Cd contents and tomato fruit, Sci. Total Environ. 403 (2008) 80–88.

[10] USEPA 64 FR 5574, National emission standard for hazardous air pollutants: national emission standards for radon emission from phosphogypsum stacks, Federal Register 64 (1999).

[11] P.M. Rutherford, M.J. Dudas, R.A. Samek, Environmental impacts of phosphogypsum, Sci. Total Environ. 149 (1994) 1–38.

[12] I.S. Alcordo, J.E. Rechcigl, R.C. Littell, A.K. Alva, C.E. Roessler, Agro-environmental impact of high rates of phosphogypsum applied to bahiagrass pasture on a Florida Spodisol soil, in: S. Brown, J.S. Scott Angle, L. Jacobs (Eds.), Beneficial Co-utilization of Agricultural, Municipal and Industrial By-products, Kluwer Academic Publ., Dordrecht, the Netherlands, 1998, pp. 423–430.

[13] R. El-Mrabet, J.M. Abril, R. Periáñez, G. Manjón, R. García-Tenorio, A. Delgado, L. Andreu, Phosphogypsum amendment effect on radionuclide content in drainage water and marsh soils from Southwestern Spain, J. Environ. Qual. 32 (2003) 1262–1268.

[14] J. Aguilar-Carrillo, L. Barrios, F. Garrido, M.T. García-González, Effects of industrial by-product amendments on As, Cd and Tl retention/release in an element-spiked acidic soil, Appl. Geochem. 22 (2007) 1515–1529.

[15] M.P. Rodríguez-Jordá, F. Garrido, M.T. García-González, Potential use of gypsum and lime rich industrial by-products for induced reduction of Pb, Zn and Ni leachability in an acid soil, J. Hazard. Mater. 175 (2010) 762–769.

[16] R.P. Soratto, C.A.C. Crusciol, Dolomite and phosphogypsum surface application effects on annual crops nutrition and yield, Agron. J. 100 (2008) 261–270.

[17] I. Mariscal-Sancho, R. Espejo, F. Peregrina, Potentially toxic effects of phosphogypsum on palexerults in Western Spain, Soil Sci. Soc. Am. J. 73 (2009) 146–153.

[18] Z. Shah, I. Haq, A. Rehman, A. Khan, M. Afzal, Soil amendments and seed priming influence nutrients uptake, soil properties, yield and yield components of wheat (Triticum aestivum L.) in alkali soils, Soil Sci. Plant. Nutr. (2013), http://dx.doi.org/10.1080/00380768.2012.762634.

[19] J.M. Abril, R. Garcia-Tenorio, G. Manjon, Extensive radioactive characterization of a phosphogypsum stack in SW Spain: 226Ra, 238U, 210Po concentrations and 222Rn exhalation rate, J. Hazard. Mater. 164 (2008) 790–797.

[20] L. Andreu, F. Moreno, N.J. Jarvis, G. Vachaud, Application of the model MACRO to water movement and salt leaching in drained and irrigated marsh soils, Marismas, Spain, Agric. Water Manage. 25 (1994) 71–88.

[21] F. Moreno, F. Cabrera, L. Andreu, R. Vaz, J. Martín-Aranda, G. Vachaud, Water movement and salt leaching in drained sand irrigated marsh soils of southwest Spain, Agric. Water Manage. 27 (1995) 25–44.

[22] L. Andreu, R. Vaz, F. Moreno, F. Cabrera, Evolución de la salinidad de las Marismas de Lebrija, in: IV Simposio sobre el agua en Andalucía, Vol. III, Instituto Tecnológico Geominero de España, Madrid, Spain, 1996, pp. 33–42 (in Spanish).

[23] R. Domínguez, M.C. Del Campillo, F. Peña, A. Delgado, Effect of soil properties and reclamation practices on phosphorus dynamics in reclaimed calcareous marsh soils from the Guadalquivir Valley, SW, Spain, Arid Land Res. Man. 15 (2001) 203–221.

[24] Soil Survey Staff, Keys to soil taxonomy, 11th edn, USDA-Natural Resources Conservation Service, Washington, DC, 2010.

[25] S.M. Enamorado-Báez, J.M. Abril, J.M. Gómez-Guzmán, Determination of 25 Trace Element Concentrations in Biological Reference Materials by ICP-MS following Different Microwave-Assisted Acid Digestion Methods Based on Scaling Masses of Digested Samples, ISRN Analytical Chemistry 2013 (2013) 1–14, Article ID 851713.

[26] D.D. Link, P.J. Walter, H.M. Kingston, Development and validation of the new IPA microwave assisted beach method 3051A, Environ. Sci. Technol. 32 (1998) 3628–3632.

[27] Y. Gelinas, R.M. Barnes, D. Florian, J.P. Schmit, Acid leaching of metals from environmental particles: expressing results as a concentration within the leachable fraction, Environ. Sci. Technol. 32 (1998) 3622–3627.

[28] IAEA TECDOC 1616, Quantification of radionuclide transfer in terrestrial and freshwater environments for radiological assessment. Chemistry Unit, Agency's Laboratories, Seibersdorf. International Atomic Energy Agency, Vienna, 2009.

[29] PNNL-13421, Pacific Northwest National Laboratory. L.H. Staven, K. Rhoads, B.A. Napier, D.A. Strenge, Compendium of Transfer Factors for Agricultural and Animal Products, 2003.

[30] ORNL-5786. C.F. Baes, R.D. Sharp, a.L. Sjoreen, R.W. Shor, A review and analysis of parameters for assessing transport of environmentally released radionuclides through agriculture. Oack Ridge National Laboratory, Oack Ridge, Tennessee 37831, 1984.

[31] W.L. Lindsay, Inorganic equilibria affecting micronutrient in soils, in: J.J. Mortvedt, F.R. Cox, L.M. Shuman, R.M. Welch (Eds.), Micronutrients in Agriculture, Soil Science Society of America, Madison, WI, 1991, pp. 90–112.

[32] H. Marschner, Mineral Nutrition of Higher Plants, Academic Press, 1995.

[33] A. De Santiago, A. Delgado, Predicting iron chlorosis of Lupinus albus L. in calcareous Spanish soils from various iron extracts, Soil Sci. Soc. Am. J. 70 (2006) 1945–1950.

[34] M. Takahashi, Y. Terada, I. Nakai, H. Nakanishi, E. Yoshimura, S. Mori, N.K. Nishizawa, Role of nicotianamine in the intracellular delivery of metals and plant reproductive development, Plant Cell. 15 (2003) 1263–1280.

[35] A. Pich, G. Scholz, Translocation of copper and other micronutrients in tomato plants (Lycopersicon esculentum Mill.): nicotianamine-stimulated copper transport in the xylem, J. Exp. Bot. 47 (1996) 41–47.

[36] V. Page, U. Feller, Selective transport of zinc, manganese, nickel, cobalt and cadmium in the root system and transfer to the leaves in young wheat plants, Ann. Bot. -London 9 (2005) 425–434.

[37] O. Riesen, U. Feller, Redistribution of nickel, cobalt, manganese, zinc and cadmium via the phloem in young and in maturing wheat, J. Plant Nutr. 28 (2005) 421–430.

[38] U.W. Stephan, G. Scholz, Nicotianamine-mediator of transport of iron and heavy-metals in the phloem, Physiol. Plantarum 88 (1993) 522–529.

[39] G. Ouzounidou, M. Moustakas, L. Symeonidis, S. Karataglis, Response of wheat seedlings to Ni stress: effects of supplemental calcium, Arch. Environ. Con. Tox. 50 (2006) 346–352.

[40] M. Li, L.J. Zhang, L. Tao, W. Li, Ecophysiological responses of Jussiaea rapens to cadmium exposure, Aquat. Bot. 88 (2008) 347–352.

[41] C.G. Campbell, F. Garrido, V. Illera, M.T. García-González, Transport of Cd, Cu and Pb in an acid soil amended with phosphogypsum, sugar foam and phosphoric rock, Appl. Geochem. 21 (2006) 1030–1043.

[42] R. Pérez-López, J. Castillo, M. Sarmiento, J.M. Nieto, Assessment of phosphogypsum impact on the salt-marshes of the Tinto river (SW Spain): role of natural attenuation processes, Mar. Poll. Bull. 62 (2011) 2787–2796.

[43] T.K. Udeigwe, P.N. Eze, J.M. Teboh, M.H. Stietiya, Application, chemistry, and environmental implications of contaminant-immobilization amendments on agricultural soil and water quality, Environ. Int. 37 (2011) 258–267.

[44] R. González-Núñez, M.D. Alba, M.M. Orta, M. Vidal, A. Rigol, Remediation of metal-contaminated soils with the addition of materials–Part I: characterization and viability studies for the selection of non-hazardous waste materials and silicates, Chemosphere 85 (2011) 1511–1517.

[45] Q.Y. Ma, T.J. Logan, S.J. Traina, Lead immobilization from aqueous solutions and contaminated soils using phosphate rocks, Environ. Sci. Technol. 29 (1995) 1118–1126.

[46] S. Pinho, B. Ladeiro, Phytotoxicity by lead as heavy metal focus on oxidative stress, J. Botany (2012), Article ID 369572, 10 pp.

[47] Z. He, X. Yang, Z. Zhu, Q. Zhang, W. Xia, J. Tan, Effect of phosphate on the sorption, desorption and plant-availability of selenium in soil, Fert. Res. 39 (1994) 189–197.

[48] N. Gad, H. Kandil, Influence of cobalt on phosphorus uptake, growth yield of tomato, Agric. Biol. J. N. America 1 (2010) 1069–1075.

[49] N. Gad, N.M.K. Hassan, Influence of cobalt and phosphorus on growth, yield quantity and quality of sweet potato (Ipomoea batatas L.), J. Appl. Sci. Res. 7 (2011) 1501–1506.