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Effects of elevated CO_2 concentrations and fly ash amended soils on trace element accumulation and translocation among roots, stems and seeds of *Glycine max* (L.) Merr.

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ABSTRACT

The carbon dioxide (CO_2) levels of the global atmosphere and the emissions of heavy metals have risen in recent decades, and these increases are expected to produce an impact on crops and thereby affect yield and food safety. In this study, the effects of elevated CO_2 and fly ash amended soils on trace element accumulation and translocation in the root, stem and seed compartments in soybean [*Glycine max* (L.) Merr.] were evaluated. Soybean plants grown in fly ash (FA) amended soil (0, 1, 10, 15, and 25% FA) at two CO_2 regimes (400 and 600 ppm) in controlled environmental chambers were analyzed at the maturity stage for their trace element contents. The concentrations of Br, Co, Cu, Fe, Mn, Ni, Pb and Zn in roots, stems and seeds in soybeans were investigated and their potential risk to the health of consumers was estimated. The results showed that high levels of CO_2 and lower concentrations of FA in soils were associated with an increase in biomass. For all the elements analyzed except Pb, their accumulation in soybean plants was higher at elevated CO_2 than at ambient concentrations. In most treatments, the highest concentrations of Br, Co, Cu, Fe, Mn, and Pb were found in the roots, with a strong combined effect of elevated CO_2 and 1% of FA amended soils on Pb accumulation (above maximum permitted levels) and translocation to seeds being observed. In relation to non-carcinogenic risks, target hazard quotients (TQHs) were significant in a Chinese individual for Mn, Fe and Pb. Also, the increased health risk due to the added effects of the trace elements studied was significant for Chinese consumers. According to these results, soybean plants grown for human consumption under future conditions of elevated CO_2 and FA amended soils may represent a toxicological hazard. Therefore, more research should be carried out with respect to food consumption (plants and animals) under these conditions and their consequences for human health.

1. Introduction

In recent decades, anthropogenic activities have substantially increased the concentrations of some heavy metals and other trace elements in various ecosystems [1]. Among the various anthropogenic sources are processing and manufacturing industries, cement production, road networks, vehicle exhausts, coal and fuel combustion, waste incineration and fertilizer application to agricultural soils [2–5]. Trace elements have been the subject of numerous investigations with regard to human health and environment, mainly because they can bioaccumulate and biomagnify in the environment, thus causing toxic effects and reduction of crop yields [6,7]. The accumulation of these elements in agricultural soils is an aspect of great concern with respect to environmental and food safety. Contaminated soils have a reduced quality in terms of physical and chemical properties that determine the metal retention capacity [8].

The use of FA in agriculture has been based on its liming potential and supply of nutrients such as Ca, Na, K, Mg, B, S and Mo, which alleviate nutrient deficiency in soils thus promoting plant growth [9,10]. However, FA normally also contains high proportions of heavy metals and other trace elements [11–13]. Many studies have mentioned that low levels of FA amendment to soils may cause an increase in the growth and yield of crops; whereas high levels could cause adverse effects on crops such as corn, soybean, barley, cabbage, apple, alfalfa and sugar beet [14–16]. Thus, the addition of FA to soils in agricultural production areas may generate either positive or negative effects on crops depending on the type of FA and the amount applied to the soil [17]. Furthermore, food consumption has been

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identified as the major source of metal exposure in humans [18].

The soil–plant transfer of trace elements is a very complex process governed by several factors, both natural and anthropogenic. Various parameters control the processes of mobility and availability of elements, which in general are of a geochemical, climatic or biological origin [19]. Among these, an important factor for plant physiological processes such as stomatal opening and water availability is atmospheric CO_2 concentration, which has increased along with pollutants in the present scenario of socio-economic development since the beginning of industrialization, and is expected to rise in the future. In general, crops grown under elevated CO_2 have shown an increase in biomass, leaf photosynthetic rate and carbohydrates [20–23]. However, only a few studies have been conducted with respect to food safety for crops grown under elevated CO_2 and heavy metal enriched soils, which have been mostly approached from the viewpoint of phytoremediation [24–28]. However, to date there are no studies that combine the effect of elevated CO_2 and soil amended with fly ash on a crop of great economic importance such as soybean. Therefore, the purpose of this study was to evaluate the combined effect of elevated atmospheric CO_2 concentrations and FA amended soil on the uptake of trace elements in soybean plants and on their accumulation in different plant organs. A special emphasis was placed on the accumulation in seeds and its potential consequences for food and feed safety.

2. Materials and methods

2.1. Plant material, experimental conditions and chemical characteristics of fly ash

Sixty plants of *Glycine max* (L.) Merrill (advanced line of conventional J001730; MG 5 short; soybean breeding program INTA Marcos Juárez, Argentina), with three replicates for each of the five soil treatments, were grown from seed to maturity in environmentally controlled chambers at the University of Hohenheim, Germany (Vötsch - Bio Line, Type VB 151,415 with CO_2 and dosing adjustment device IR system 3600) under the climatic conditions of the city of Córdoba (Argentina). Three seeds of *G. max* were planted in 4-L pots on 17 February 2007. The substrate used was prepared from a standard soil LD80 (macronutrients [mg L^{-1}]: 124–185 N, 120–179 P_2O_2, 190–284 K_2O; pH: 5.5–6.1; salinity [g L^{-1}] 0.8–1.4) and sand at a 3:1 (v/v) ratio. This standard substrate (S) was gradually enriched in heavy metals through the incorporation of FA from a coal-fired power plant in the Stuttgart region provided by the EnBW (Energie Baden-Württemberg) electricity. Two CO_2 concentrations (400 ppm/ambient and 600 ppm/elevated) and five soil-treatments (fly ash FA/standard substrate S): 0, Control (0% FA/100% S); 1 (1% FA/99% S); 2 (10% FA/90% S); 3 (15% FA/85% S) and 4 (25% FA/75% S) were applied. To avoid chamber and placement effects, plants were moved from one chamber to the other on a weekly basis and the pots were randomly mixed when put into the chambers. Also the CO_2 treatments were switched weekly. All pots were watered daily with deionized water. One week after planting, each pot was thinned to a single plant. After another week, all pots were fertilized with 50 mL of Hoagland solution to assure adequate micronutrient supply. The harvest was made at the maturity (R8) stage as defined by Fehr and Caviness [29].

The chemical characteristics of the FA/S mixtures (pH, concentrations of plant-available macronutrients and metals) were analyzed at the State Institute of Agricultural Chemistry (University of Hohenheim). The pH was measured using a pH meter on a 1:5 soil:0.01 M $CaCl_2$ suspension. Plant-available N, P and K were determined by calcium lactate (Cal) and Mg by $CaCl_2$. The total metal concentrations were determined by mass spectrometry with inductively coupled plasma (ICP-MS) for As, Ca, Cd and Pb, by optical emission spectroscopy with inductively coupled plasma (ICP-OES) for Cr, Cu, Fe, Mn, Na, Ni and Zn, and using cold vapor atomic absorption (CV-AAS) for Hg. As a quality control, blanks were prepared in the same way and were run after five determinations to calibrate the instrument. The coefficient of variation of replicate analysis was calculated for different determinations. Variations were found to be less than 10%.

In soybean, the biomass of roots, stem and seeds was determined at the maturity growth stage and expressed as dry weight (DW).

2.2. Elemental analysis of soybean seeds, stems and roots

The concentrations of Br, Co, Cu, Fe, Mn, Ni, Pb, and Zn were analyzed in the dry material (60 °C) of roots, stems and seeds of *G. max* in the maturity stage, which had been previously grown as described above for different soil treatments and exposed to ambient vs. elevated CO_2 levels. The plant material was ground and then reduced to ashes at 500 °C for 4 h. These ashes were digested with HCl (18%): HNO_3 (3:1), the solid residue separated by centrifugation, and the volume adjusted to 25 mL with Milli-Q water. Then, 10 ppm of a Ge solution was added as an internal standard. Aliquots of 5 μL were taken from this solution and dried on an acrylic support. Standard solutions with known concentrations of different elements and Ge as an internal standard were prepared for the calibration of the system.

The samples were measured for 200 s, using the total reflection set up mounted at the X-ray fluorescence beamline of the National Synchrotron Light Laboratory (LNLS), Campinas, Brazil. For the excitation, a polychromatic beam (approximately 0.3 mm wide and 2 mm high) was used. For the X-ray detection, a Si(Li) detector was used with an energy resolution of 165 eV at 5.9 keV.

As a quality control, blanks and samples of the standard reference material “Hay IAEA-V-10” were prepared in the same way and were run after five determinations to calibrate the instrument. The results were found to be within ±2% of the certified value. The coefficient of variation of replicate analysis was calculated for different determinations. Variations were found to be less than 10%.

2.3. Data analysis

2.3.1. Statistical analysis

Data of trace element concentrations and biomass were subject to a two-way analysis of variance (ANOVA) to examine the individual and combined effects of CO_2 treatment, and soil quality. Tukey tests were performed as post hoc on the parameters subjected to one-way ANOVA for soybean organs, CO_2 and amended soil treatments. The ANOVA assumptions were previously verified graphically (residual vs. fitted values, box plots, and steam leaf plots).

2.3.2. Translocation factor

The translocation factor (TF) was calculated by dividing the concentration of elements in the aerial part of the plant (stem or seeds) by the concentration of these elements in roots or stems using the following formula:

$$\mathrm{TF\,r/st} = \frac{\text{element concentration in stem}}{\text{element concentration in roots}}$$

$$\mathrm{TF\,st/s} = \frac{\text{element concentration in seeds}}{\text{element concentration in stem}}$$

Values higher than one suggest that the elements were easily translocated, while values below one suggest a higher accumulation in roots or stems, respectively.

Table 1
Chemical characteristics of fly ash (FA)-amended soils before and after the CO_2 exposure experiments.[a]

CO_2 exposition	FA-amended soils treatment	Parameter pH	P (mg 100 g^{-1} DW)	K (mg 100 g^{-1} DW)	Mg (mg 100 g^{-1} DW)	N (mg 100 g^{-1} DW)	Ca (mg kg^{-1} DW)	Cd (mg kg^{-1} DW)	Cr (mg kg^{-1} DW)	Cu (mg kg^{-1} DW)	Fe (mg kg^{-1} DW)	Hg (mg kg^{-1} DW)	Mn (mg kg^{-1} DW)	Na (mg kg^{-1} DW)	Ni (mg kg^{-1} DW)	Pb (mg kg^{-1} DW)	Zn (mg kg^{-1} DW)
Before	0	5.8	27	58	32	26	14500	0.05	14.8	7.84	5650	0.019	111	73	12.8	4.96	15.1
	A 0	5.8	27	50	33	30	14159	<0.05	15.05	8.47	7150	0.018	82.5	92.3	12	4.15	29.5
	A 1%	5.8	38	74	26	19	15065	<0.05	13.4	7.83	5360	0.018	77.8	103	11.2	4.4	17.7
	A 10%	6.7	125	27	46	15	17858	<0.05	18.7	10.8	7200	0.04	107	138	15.7	6.55	22.4
	A 15%	7.1	182	41	50	24	19371	0.06	20.9	12.2	1970	0.06	106	143	16.8	7.57	41.7
	A 25%	7.8	309	29	60	14	21828	0.10	25.6	15.5	9220	0.10	126	156	20.7	9.97	40.1
After	E 0	6.4	25	42	26	17	11929	<0.05	11.5	6.57	5320	<0.05	73.0	89.0	10.6	3.96	24.3
	E 1%	5.9	43	76	38	30	15280	<0.05	14.4	7.3	6030	<0.05	76.8	113	11.6	4.49	20.0
	E 10%	6.5	96	43	39	13	14407	<0.05	15.1	8.64	5900	<0.05	80.0	128	12.8	5.55	15.8
	E 15%	7.1	180	41	52	17	18954	0.07	20.9	11.3	7890	0.07	111	165	17.3	7.76	26.4
	E 25%	7.9	236	37	66	13	19345	0.08	22.3	13.9	8010	0.08	112	187	20.5	8.36	45.9
	Fly ash	12.5	925	825	7580	3.5	45100	0.3	68.6	41.8	18900	0.25	289	387	47.8	26.7	38.2

[a] 0, Control (0% FA/100% S), 1% (1% FA/99% S), 10% (10% FA/90% S), 15% (15% FA/85% S), 25% (25% FA/75% S). A (ambient CO_2 concentrations, 400 ppm), E (elevated CO_2 concentrations, 600 ppm). FA (fly ash), S (standard substrate).

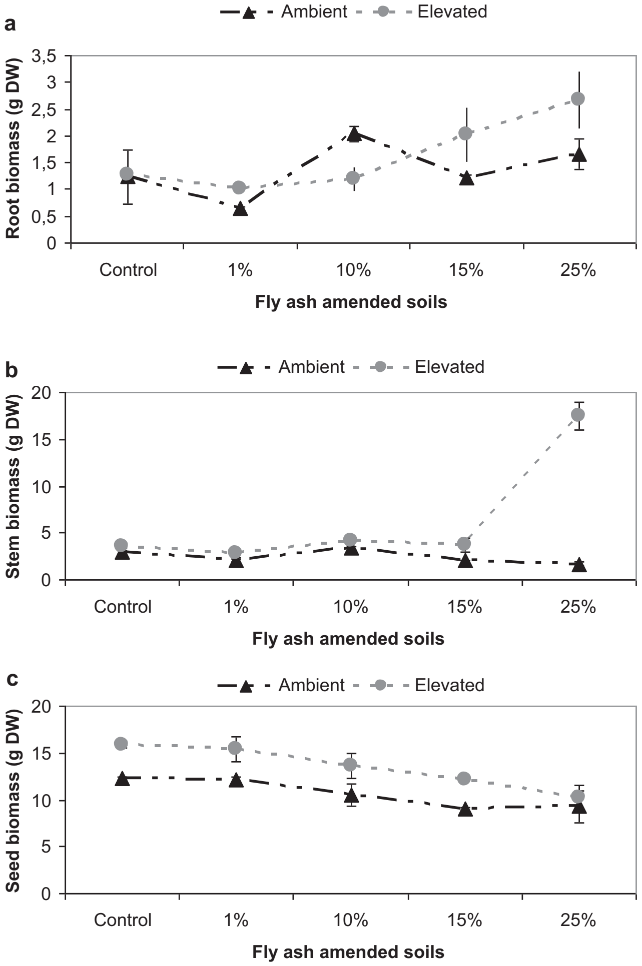

Fig. 1. Biomass of roots, stems and seeds of *Glycine max* grown under different CO_2 concentrations and at different proportions of fly ash (FA) in soils.

2.3.3. *Risk assessment*

The risk to human health resulting from consumption of soybean grown on fly ash amended soils under ambient and elevated CO_2 was calculated by employing the estimated dietary intake (EDI $\mu g\ kg^{-1}\ day^{-1}$ Bw) and target hazard quotients (THQ) described by Zheng et al. [30] and US EPA [31]. For the present study Chinese, European and Argentines inhabitants were considered as potential consumers, taking into account that Argentina exports soybean and its products mainly to China and the countries of the EU.

EDI exposure is expressed as the mass of a substance per unit body weight per unit time, averaged over a long period of time (a lifetime), and is calculated as follows:

$$\mathrm{EDI} = \frac{C \times \mathrm{Con} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{Bw} \times \mathrm{AT}}$$

where C is the median concentration of a heavy metal in soybean ($\mu g\ kg^{-1}$); Con is the ingestion rate pf soybean (g $person^{-1}\ day^{-1}$); EF is the exposure frequency (365 days $year^{-1}$); ED is the exposure duration (70 years for adults); Bw is the average body weight (65 kg for Chinese adults and 70 kg for European or Argentinean adults), and AT is the average exposure time for non-carcinogenic effects (ED × 365 days $year^{-1}$). Keinan-Boker et al. [32] report that the average daily intake of traditional soy products for a Chinese individual was 100 g $person^{-1}\ day^{-1}$, while the daily intake in Western inhabitants was less than 1 g $person^{-1}\ day^{-1}$ [32,33].

Table 2
Mean values and standard deviation (±SD) of the concentrations (in $\mu g\ g^{-1}$ DW) of Mn, Fe and Co in roots, stems and seeds of *Glycine max* at maturity grown under different CO_2 concentrations and at different proportions of fly ash (FA) in soils.

Element	Treatment	CO_2	Mean ± SD				
			Root	Stem	Seed	TF r/st	TF st/s
Mn	0	A	58.96 ± 1.30 b B	49.78 ± 0.49 c B	82.60 ± 0.54 a A	0.84	1.66
		E	118.09 ± 0.01 a A	63.65 ± 0.51 b A	57.73 ± 0.17 c B	0.54	0.90
	1	A	68.94 ± 0.26 a B	65.37 ± 0.50 b A	63.88 ± 0.21 c B	0.95	0.98
		E	108.48 ± 0.31 a A	55.07 ± 0.74 c B	89.98 ± 0.16 b A	0.50	1.63
	2	A	41.34 ± 0.31 c B	44.71 ± 0.07 b B	72.03 ± 0.06 a B	1.08	1.61
		E	113.00 ± 0.74 a A	53.89 ± 0.36 c A	101.48 ± 0.65 b A	0.47	1.88
	3	A	49.12 ± 0.52 b B	28.82 ± 0.27 c B	66.21 ± 0.48 a	0.59	2.29
		E	90.41 ± 0.50 a A	54.35 ± 0.28 c A	65.04 ± 0.75 b	0.60	1.20
	4	A	73.06 ± 0.66 a B	48.00 ± 0.01 c B	62.96 ± 0.09 b B	0.65	1.31
		E	96.96 ± 0.88 a A	68.46 ± 0.44 c A	91.46 ± 0.02 b A	0.70	1.33
Fe	0	A	2816.29 ± 54.12 a A	255.88 ± 0.87 b A	281.59 ± 0.19 b A	0.09	1.10
		E	701.60 ± 1.66 a B	242.95 ± 0.28 b B	245.24 ± 0.87 b B	0.35	1.00
	1	A	1162.84 ± 0.68 a A	278.71 ± 0.32 b B	239.24 ± 0.36 c B	0.40	0.85
		E	686.76 ± 0.99 a B	490.94 ± 0.81 c A	523.26 ± 0.38 b A	0.71	1.06
	2	A	859.45 ± 0.01 a B	258.93 ± 0.32 c A	345.64 ± 0.34 b B	0.30	1.33
		E	1861.34 ± 1.72 a A	176.02 ± 0.03 c B	515.11 ± 0.17 b A	0.09	2.93
	3	A	997.48 ± 2.68 a A	632.69 ± 0.75 c A	401.05 ± 16.26 b	0.63	0.63
		E	720.92 ± 0.76 a B	398.95 ± 0.84 b B	318.83 ± 4.61 c	0.55	0.80
	4	A	1593.27 ± 11.58 a B	297.92 ± 0.70 c A	476.68 ± 0.75 b	0.19	1.60
		E	6463.65 ± 2.98 b A	190.98 ± 0.33 c B	476.18 ± 0.57 a	0.03	2.49
Co	0	A	2.10 ± 0.04 a B	0.45 ± 0.01 c A	0.54 ± 0.01 b A	0.21	1.20
		E	3.26 ± 0.02 a A	0.33 ± 0.001 c B	0.51 ± 0.01 b B	0.10	1.54
	1	A	1.47 ± 0.001 a A	0.55 ± 0.001 b A	0.34 ± 0.001 c B	0.37	0.62
		E	1.07 ± 0.02 a B	0.40 ± 0.001 c B	1.03 ± 0.001 b A	0.37	2.57
	2	A	1.32 ± 0.001 a B	0.46 ± 0.01 b A	0.24 ± 0.01 c B	0.34	0.52
		E	1.85 ± 0.001 a A	0.35 ± 0.001 c B	0.50 ± 0.01 b A	0.19	1.43
	3	A	1.03 ± 0.01 a B	0.60 ± 0.01 b A	0.25 ± 0.01 c B	0.58	0.42
		E	1.34 ± 0.03 a A	0.70 ± 0.01 b B	0.48 ± 0.01 c A	0.52	0.68
	4	A	1.52 ± 0.01 a B	0.39 ± 0.01 b	0.41 ± 0.001 b A	0.26	1.05
		E	1.91 ± 0.001 a A	0.38 ± 0.01 b	0.36 ± 0.001 b B	0.20	0.95

Abbreviations: Soil treatments: 0 (0% FA), 1 (1% FA), 2 (10% FA), 3 (15% FA), 4 (25% FA); CO_2 treatments: A = Ambient (400 ppm CO_2); E = Elevated (600 ppm CO_2); TF r/st, translocation factor from root to stem; TF st/s, translocation factor from stem to seed. Significance of treatment effects: different lowercase letters within one line indicate significantly different element concentrations among soybean organs at $p < 0.05$. Different capital letters within one column indicate significant CO_2 effects at $p < 0.05$.

THQ gives the potential non-cancer risk for individual heavy metal and can be calculated as follows:

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{RfD}}$$

where RfD is the reference oral dose and represents an estimation of the daily exposure to which the human population is likely to be subjected to without any appreciable risk of deleterious effects during a lifetime. The RfD values used were 140, 700, 0.3, 20, 40 and 300 $\mu g\ kg^{-1}\ day^{-1}$ for Mn, Fe, Co, Ni, Cu and Zn, respectively [34]. However, as the US EPA has not yet established RfD values for Pb or Br, the ones used in this paper were 4 $\mu g\ kg^{-1}\ day^{-1}$ [35] and 120 $\mu g\ kg^{-1}\ day^{-1}$ [36], respectively.

In order to assess the overall potential for non-carcinogenic effects for more than one heavy metal, a hazard index (HI) has been calculated based on the Guidelines for Health Risk Assessment of Chemical Mixtures of EPA [31] as follows:

$$\mathrm{HI} = \Sigma\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{RfD}_1} + \frac{\mathrm{EDI}}{\mathrm{RfD}_2} + \ldots + \frac{\mathrm{EDI}}{\mathrm{RfD}_i}$$

When either TQH or HI exceeds unity, high risk of non-carcinogenic effects is implied.

3. Results

3.1. Analysis of FA-amended soils and biomass in soybean

Concentrations of macronutrients, metals and pH values in control soils and FA-amended soils before and after CO_2 exposure, respectively, are depicted in Table 1. The FA-amended soils showed that a greater proportion of FA increased the alkalinity, phosphorus and metal contents for both levels of CO_2. The available K content presented the highest values at the lowest proportion of FA-amended soil, regardless of the CO_2 treatment. Likewise, the available N content was higher in the control soil at ambient CO_2 and for 1% FA-amended soil at elevated CO_2.

The root biomass did not show a clear response pattern in ambient CO_2, and no significant differences were observed at elevated CO_2 or for the comparison between the CO_2 concentrations (Fig. 1a).

The highest values for stem biomass were observed in 0% and 10% FA treatments at ambient CO_2, while the maximum value was found at elevated CO_2 in the 0% FA treatment. The comparison between CO_2 concentrations indicated that the highest values occurred at elevated CO_2 for 10% and 25% FA-amended soil treatments (Fig. 1b).

Regarding seed biomass at both CO_2 conditions, the greatest values corresponded to the 0%, 1% and 10% FA-amended soil treatments. The comparison between CO_2 levels for seed biomass indicated that the highest values were in the 0% and 15% treatments of amended soils at elevated CO_2 (Fig. 1c).

3.2. Element concentrations in roots, stems and seeds

Tables 2–4 show the concentrations of Br, Co, Cu, Fe, Mn, Ni, Pb, and Zn in the organs of the soybean plants at the maturity stage after growth under different treatments (soil and CO_2) and also the calculated translocation factors.

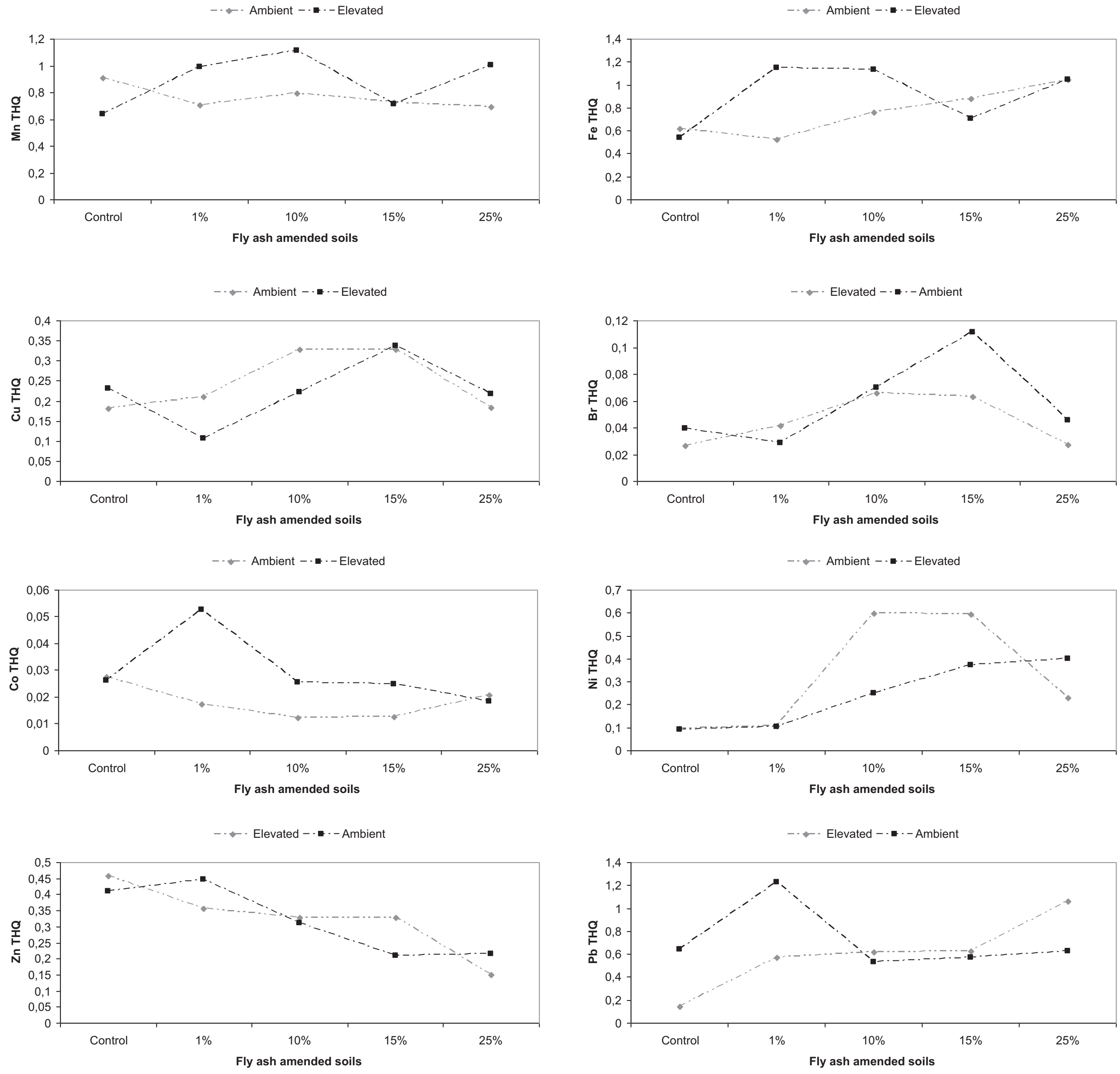

Fig. 2. Target hazard quotients (THQ) of selected elements through soybean consumption for Chinese inhabitants.

A two-way ANOVA showed significant effects of all the individual factors and their interactions (data not shown). Results of the comparison performed among amended soil treatments are not shown in Tables 2–4 because they did not provide a clear response pattern.

Significant differences were observed for most of the trace elements among the root, stem and seed compartments for the different concentrations of fly ash amended soils and CO_2. In most cases, the comparison between CO_2 treatments revealed higher concentrations of Br, Co, Cu, Fe, Mn, Ni, and Zn at elevated CO_2 than at ambient levels, while the Pb concentration was higher at ambient CO_2.

In most treatments, the highest concentrations of Br, Co, Cu, Fe, Mn, and Pb were found in the roots. Regarding Ni, a stronger accumulation in roots was observed for the control and 1% fly ash amended soils. In seeds, the highest Ni concentration corresponded mostly to 10%, 15% and 25% FA amended soils. With respect to Pb, it is interesting to note that the Pb levels in seeds were higher at ambient CO_2 for treatments with moderate or high fly ash concentrations in soil (10%, 15% and 25%) whereas at elevated CO_2, the highest Pb value in seeds was observed at low FA concentrations in soil (1%).

Regarding the Zn concentrations, differential accumulation according to CO_2 treatment showed no clear pattern of response.

3.3. Translocation factor

TF r/st values higher than one were observed for Ni in the amended soil treatments with no clear pattern of response in relation to CO_2 treatment, indicating that the addition of fly ash to soils substantially affected the translocation of Ni from roots to shoots. Moreover, TF st/s values were unusually high for Ni, Zn, and Cu when the proportion of FA in soil was 10% or 15% (Table 3).

Table 3
Mean values and standard deviation (±SD) of the concentrations (in $\mu g\ g^{-1}$ DW) of Ni, Cu and Zn in roots, stems and seeds of *Glycine max* at maturity grown under different CO_2 concentrations and at different proportions of fly ash (FA) in soils.

Element	Treatment	CO_2	Mean ± SD			TF r/st	TF st/s
			Root	Stem	Seed		
Ni	0	A	5.94 ± 0.04 a	1.45 ± 0.01 b A	1.23 ± 0.001 c A	0.24	0.85
		E	3.67 ± 0.08 a	0.99 ± 0.001 b B	1.19 ± 0.01 b B	0.27	1.20
	1	A	2.43 ± 0.05 a B	1.23 ± 0.01 c B	1.41 ± 0.01 b A	0.50	1.15
		E	2.69 ± 0.01 a A	1.76 ± 0.03 b A	1.31 ± 0.01 c B	0.65	0.74
	2	A	4.61 ± 0.07 b A	0.89 ± 0.01 c B	7.74 ± 0.001 a A	0.19	8.70
		E	1.76 ± 0.01 b B	1.04 ± 0.01 c A	3.26 ± 0.001 a B	0.59	3.13
	3	A	0.88 ± 0.01 b B	0.88 ± 0.01 b B	7.72 ± 0.01 a A	1.00	8.77
		E	2.90 ± 0.11 b A	3.13 ± 0.01 b A	4.83 ± 0.06 a B	1.07	1.54
	4	A	1.44 ± 0.001 b B	3.08 ± 0.07 a B	2.98 ± 0.01 a	2.14	0.97
		E	2.83 ± 0.02 A	4.67 ± 0.03 A	5.18 ± 2.23	1.65	1.10
Cu	0	A	6.14 ± 0.03 b A	8.39 ± 0.08 a A	4.71 ± 0.01 c B	1.37	0.56
		E	3.27 ± 0.01 c B	4.20 ± 0.001 b B	6.02 ± 0.01 a A	1.28	1.43
	1	A	5.10 ± 0.001 c B	7.07 ± 0.01 a A	5.48 ± 0.01 b A	1.39	0.77
		E	10.65 ± 0.03 a A	3.86 ± 0.001 b B	2.76 ± 0.001 c B	0.36	0.71
	2	A	10.19 ± 0.09 a B	4.37 ± 0.01 c A	8.49 ± 0.02 b A	0.43	1.94
		E	18.25 ± 0.01 a A	4.21 ± 0.02 c B	5.73 ± 0.001 b B	0.23	1.36
	3	A	12.30 ± 0.03 a B	4.05 ± 0.001 c B	8.49 ± 0.01 b B	0.33	2.09
		E	14.88 ± 0.04 a A	5.86 ± 0.001 c A	8.73 ± 0.03 b A	0.39	1.49
	4	A	5.21 ± 0.03 B	5.04 ± 0.02 B	4.73 ± 0.001 B	0.96	0.88
		E	11.84 ± 0.97 a A	6.21 ± 0.01 b A	5.64 ± 0.03 b A	0.52	0.91
Zn	0	A	63.44 ± 0.16 b B	27.43 ± 0.01 c B	88.97 ± 0.60 a A	0.43	3.24
		E	88.95 ± 0.41 a A	42.17 ± 1.15 c A	79.70 ± 0.22 b B	0.47	1.88
	1	A	108.13 ± 0.95 a A	39.48 ± 0.09 c A	69.20 ± 0.30 b B	0.36	1.75
		E	78.00 ± 0.74 b B	25.00 ± 0.22 c B	86.99 ± 0.65 a A	0.32	3.50
	2	A	47.32 ± 0.33 b B	14.04 ± 0.17 c B	64.03 ± 0.73 a A	0.30	4.56
		E	125.96 ± 0.80 a A	32.03 ± 0.02 c A	60.35 ± 0.03 b B	0.25	1.88
	3	A	36.07 ± 0.74 b B	12.89 ± 0.13 c B	63.79 ± 0.28 a A	0.36	4.95
		E	120.73 ± 0.81 a A	18.07 ± 0.06 c A	40.54 ± 0.69 b B	0.15	2.24
	4	A	42.15 ± 0.83 a A	24.25 ± 0.07 c B	29.15 ± 1.03 b B	0.57	1.20
		E	35.66 ± 0.69 b B	25.00 ± 0.03 c A	41.91 ± 0.94 a A	0.70	1.68

Abbreviations: Soil treatments: 0 (0% FA), 1 (1% FA), 2 (10% FA), 3 (15% FA), 4 (25% FA); CO_2 treatments: A = Ambient (400 ppm CO_2); E = Elevated (600 ppm CO_2); TF r/st, translocation factor from root to stem; TF st/s, translocation factor from stem to seed. Significance of treatment effects: different lowercase letters within one line indicate significantly different element concentrations among soybean organs at $p < 0.05$. Different capital letters within one column indicate significant CO_2 effects at $p < 0.05$.

The TF st/s values for Pb were higher than one in control samples and at the lowest concentration of FA in soil (1%) (Table 4), with the TF st/s values for Fe and Co higher than one in the 0%, 1%, 10% and 25% FA amended soil treatments, without a clear trend apparent with respect to CO_2 levels (Table 2).

Regarding Mn, Zn and Br, there was an efficient translocation from stem to seeds, independent of the levels of atmospheric CO_2 (TF st/s > 1) (Tables 2 and 4). Finally, the TF r/st for Cu was higher than one only in the control treatments and at the lowest FA amended soil treatment (1% FA) (Table 3).

Table 4
Mean values and standard deviation (±SD) of the concentrations (in $\mu g\ g^{-1}$ DW) of Br and Pb in roots, stems and seeds of *Glycine max* at maturity grown under different CO_2 concentrations and at different proportions of fly ash (FA) in soils.

Element	Treatment	CO_2	Mean ± SD			TF r/st	TF st/s
			Root	Stem	Seed		
Br	0	A	6.79 ± 0.01 a A	6.31 ± 0.01 b A	2.06 ± 0.07 c B	0.92	0.33
		E	3.30 ± 0.01 b B	5.13 ± 0.001 a B	3.09 ± 0.09 c A	1.55	0.60
	1	A	5.71 ± 0.01 a A	4.98 ± 0.001 b B	3.20 ± 0.02 c	0.87	0.64
		E	5.48 ± 0.01 a B	5.40 ± 0.01 b A	2.20 ± 0.36 c	0.98	0.41
	2	A	5.98 ± 0.01 a B	4.04 ± 0.04 c B	5.13 ± 0.04 b B	0.67	1.27
		E	8.05 ± 0.07 a A	4.73 ± 0.01 c A	5.46 ± 0.001 b A	0.59	1.15
	3	A	6.95 ± 0.01 a B	4.91 ± 0.001 b B	4.93 ± 0.08 b B	0.70	1.00
		E	12.27 ± 0.41 a A	7.07 ± 0.01 c A	8.67 ± 0.01 b A	0.58	1.23
	4	A	3.56 ± 0.01 b B	8.22 ± 0.01 a A	2.12 ± 0.01 c B	2.30	0.26
		E	9.75 ± 0.32 a A	5.03 ± 0.13 b B	3.57 ± 0.05 c A	0.51	0.71
Pb	0	A	4.30 ± 0.01 a B	1.59 ± 0.01 b A	0.37 ± 0.01 c B	0.37	0.23
		E	4.58 ± 0.001 a A	0.97 ± 0.001 c B	1.67 ± 0.01 b A	0.21	1.72
	1	A	3.66 ± 0.01 a A	2.55 ± 0.001 b A	1.48 ± 0.01 c B	0.70	0.58
		E	1.62 ± 0.01 b B	1.46 ± 0.01 c B	3.20 ± 0.001 a A	0.90	2.19
	2	A	23.07 ± 0.02 a A	1.91 ± 0.01 b B	1.61 ± 0.001 c A	0.08	0.84
		E	7.12 ± 0.01 a B	2.58 ± 0.01 b A	1.37 ± 0.02 c B	0.36	0.53
	3	A	22.99 ± 0.01 a A	1.89 ± 0.001 b B	1.62 ± 0.01 c A	0.08	0.86
		E	9.55 ± 0.01 a B	2.21 ± 0.01 b A	1.49 ± 0.001 c B	0.23	0.67
	4	A	18.69 ± 0.01 a A	1.67 ± 0.001 c B	1.65 ± 0.06 b A	0.09	0.98
		E	9.36 ± 0.01 a B	2.06 ± 0.01 b A	1.63 ± 0.03 c B	0.22	0.79

Abbreviations: Soil treatments: 0 (0% FA), 1 (1% FA), 2 (10% FA), 3 (15% FA), 4 (25% FA); CO_2 treatments: A = Ambient (400 ppm CO_2); E = Elevated (600 ppm CO_2); TF r/st, translocation factor from root to stem; TF st/s, translocation factor from stem to seed. Significance of treatment effects: different lowercase letters within one line indicate significantly different element concentrations among soybean organs at $p < 0.05$. Different capital letters within one column indicate significant CO_2 effects at $p < 0.05$.

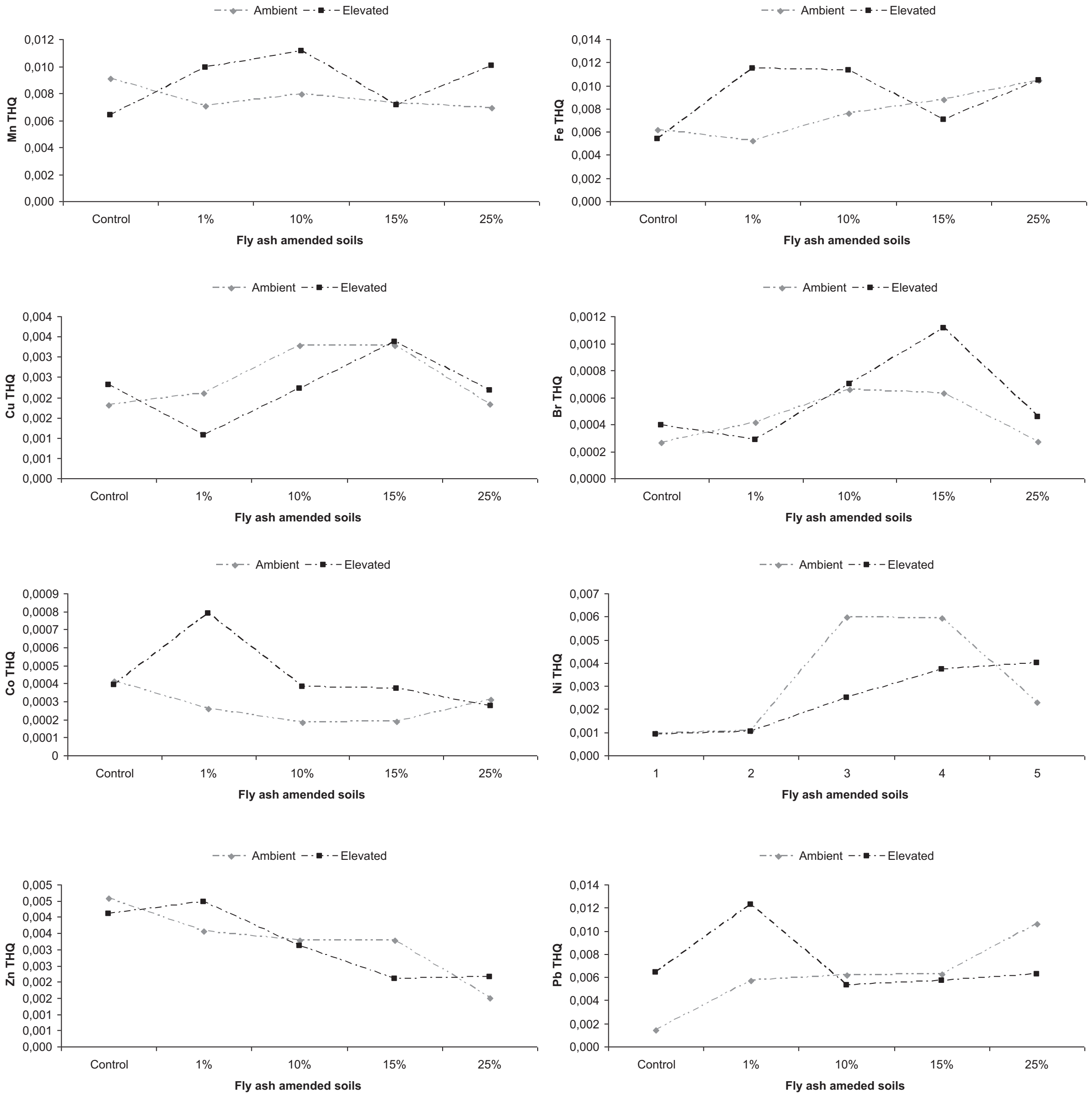

Fig. 3. Target hazard quotients (THQ) of selected elements through soybean consumption for European or Argentines inhabitants.

3.4. *Risk assessment*

The results of the THQ for each element corresponding to an individual Chinese adult and an individual European or Argentinean adult are shown in Figs. 2 and 3, respectively. The THQ values were less than one in the European or Argentine individuals, while for a Chinese individual the THQ values were also smaller than one except for Mn (elevated CO_2 – 10% and 25% FA treatments), Fe (ambient CO_2 – 25% FA and elevated CO_2 – 1%, 10% and 25% treatments) and Pb (elevated CO_2 – 1% FA treatment). Finally, the HI calculations for aggregate non-cancer risks through soybean consumption showed values greater than one in all treatments, for Chinese consumers with the following descending order: elevated CO_2 – 1% FA > elevated CO_2 – 10% FA > ambient CO_2 – 15% FA > ambient CO_2 – 10% FA > elevated CO_2 – 15% FA > ambient CO_2 – 25% FA > elevated CO_2 – 0% FA > ambient CO_2 – 1% FA > ambient CO_2 – 0% FA.

4. Discussion

4.1. *Effects of CO_2 and FA-amended soils on macronutrients, pH, elements and biomass in soybean*

As in other studies, the incorporation of FA in soils was related to an increase in alkalinity and element content in soils [17,36], probably due to FA characteristics such as the high amount of hydroxide and carbonate salts, which give FA its alkaline nature [37]. However, in the present study, the effect of the CO_2 concentration did not influence these parameters.

It should be noted that the permitted limits for metal concentrations in the soil were not exceeded [1,38,39], with the higher availability of the macronutrients K and N at the lowest concentration of FA possibly being a result of increased salinity due to the incorporation of fly ash [40].

Regarding the stem and seed biomass, these parameters were enhanced at elevated CO_2 and at low concentrations of FA amended soils, consistent with other studies which indicate that high levels of CO_2 are associated with an increase in biomass [20,41]. This results mainly from stimulation caused by photosynthesis, bearing in mind that the photosynthetic rate in *C3* plants is not saturated at current concentrations of CO_2 [42]. In addition, Singh et al. [43] reported that the application of low concentrations of FA to agricultural soils provides good conditions for plant growth.

4.2. Element concentrations in roots, stem and seeds

Taking into account that *G. max* seeds are consumed or processed, our interest was now focused on analyzing the concentrations of trace elements in seeds for the different treatments and on the translocation rates of those elements considered toxic to human health.

In general, trace element concentrations in the root, stem and seed compartments for the control samples under both CO_2 treatments showed values for this crop in agreement with the results of other authors [38,44]. In most cases, the elements analyzed revealed higher concentrations at elevated CO_2. Li et al. [28] and Wu et al. [27] also reported an increase in the accumulation of Cu and Cd in different rice varieties and Cs enrichment in Sorghum species grown under elevated CO_2 respectively. Moreover, the high element content found in the roots of the present work could have been due to complexation of metals, with the sulfhydryl groups having less translocation to the upper parts of the plant. This aspect is known to vary from one metal to another [44–47].

Except for Pb, the concentrations of elements measured in this study did not exceed the critical range in plants [48]. The high values found for Pb probably indicated a synergistic effect between elevated CO_2 and low levels of fly ash in soils, with the concentration in seeds exceeding the maximum limit for Pb established in food ($0.2\,\mu g\,g^{-1}$ DW) according to the EU directive relating to maximum levels for certain contaminants in foodstuffs [49]. In a previous study on the transfer of heavy metals into soybean and rice grown in soils with different levels of contamination, De Souza Silva et al. [50] reported that while the transfer of Pb from roots to the aerial part of the plant was low (retention in roots), it was still sufficient for this element to accumulate in *G. max* seeds at levels above those permitted. In addition, Lavado [51] showed that *G. max* may accumulate more potentially toxic elements than other crops. There are numerous studies showing the food chain to be the main source of Pb exposure in humans and mammals [52], and it is well known that lead can cause damage to the cardiovascular and central nervous systems due to its toxicity [53].

4.3. Translocation factor and risk assessment

The addition of FA to soil promoted the translocation of elements, mainly from roots to stems. However, differences in CO_2 concentration did not produce any clear variation in translocation, except for Pb, which could have resulted from a synergistic effect between elevated CO_2 and the lowest concentration of FA in soil, due to the better availability of this type of metal in control soils or at lower FA proportions in soils when the soil pH is more acidic. In addition, there might be less competition with other elements at low concentrations of FA amended soils.

The potential non-cancer risks from individual elements (THQ), in particular with respect to Pb due to its high toxicity, means that the daily intake of these metals through the consumption of soybean may have caused adverse effects on potential Chinese consumers. On the other hand, the evaluation of the potential carcinogenic effect posed by more than one element (HI) showed that while most of the individual elements did not exceed unity for THQ. The HI value suggests that soybean consumption is more likely to produce adverse effects in a Chinese individual than in an Argentine or European.

5. Conclusions

The highest Br, Co, Cu, Fe, Mn, and Pb accumulation was found in the roots. This may be related to the element retention processes of detoxification and sequestration. For Ni, the accumulation and translocation to seeds were directly associated with moderate or high levels of fly ash amended soils. Our results show that in soybeans grown under possible future conditions of elevated CO_2 (600 ppm) and 1% of fly ash amended soils, the toxic concentrations of Pb in seeds might represent a toxicological hazard for human consumption. In addition, the aggregate risk of the elements analyzed in this study for a Chinese consumer indicates that critical attention should be paid to the potential health effects due to the consumption of soybeans in conditions of elevated CO_2 and heavy metal pollution. Considering the bioaccumulation and magnification of toxic heavy metals such as Pb, the potential risk of consuming meat from cattle whose main sources of food are products derived from soybeans must be taken into account for future research, even though no potential risks resulting from the direct consumption of soy products for European or Argentine individuals were found in this study.

The results of this first study on the combined effects of elevated CO_2 and trace element polluted soils on the chemical composition of *G. max* seeds should provide a guide for further studies about yield and toxicological risk in soybean crops grown under future environmental conditions.

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