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Determination of As, Cd, Cu, Fe, Ni, Pb and Zn in Soybean Seeds and their Correlation with Relevant Biochemical Parameters to assess Food Quality

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Soybean (*Glycine max*) represents one of the most important crops in Uruguay, mostly processed for animal feed, while a smaller percentage is processed for human consumption. Soy foodstuffs are also a source of trace elements (TEs). Cu, Fe, Ni, Zn, As, Cd and Pb were determined in soybean seeds batches and correlations within them and with biochemical parameters (superoxide anion content, superoxide dismutase enzyme and viability of the analyzed seeds) were studied. Analytical determinations of Cu, Fe and Zn were performed by flame atomic absorption atomic spectrometry (FAAS) while As, Cd, Ni and Pb were determined by electrothermal atomic absorption spectrometry (ETAAS). For sample preparation, a microwave assisted digestion was carried out using diluted acid (3.5 mol L^{-1} HNO$_3$). The concentrations of the essential elements where in the following ranges: 12 – 24 mg kg^{-1} for Cu, 60 - 125 mg kg^{-1} for Fe, 5.4 – 17.9 mg kg^{-1} for Ni and 33 – 50 mg kg^{-1} for Zn. Cd and As content was < 0.2 and 0.3 mg kg^{-1} respectively, whereas Pb exceeded slightly the admitted limit in five samples (above 0.2 mg kg^{-1}). Positive correlations were found for Fe:Cd:Pb ($p<0.005$), Cu:Fe, As:Cd:Pb and Zn:Ni ($p<0.05$). A novel highly significant positive correlation ($p < 0.005$) between Cd content and seed quality parameters related to seed germination was found. This suggests that the concentration of Cd do not produce negative effects in the development of the seedlings, despite this Cd and Pb levels must be monitored to guarantee food safety.

Keywords: Soybean seeds, essential trace elements in food, food safety

INTRODUCTION

Some trace elements (TEs) are essential micronutrients for human beings. The requirement is no more than a few milligrams per day, but deficiencies, excesses, or imbalances in their supply from dietary sources can have an importantly deleterious influence. Some of the most relevant are copper (Cu), iron (Fe) and zinc (Zn). The essentiality of Fe and Cu resides in their capacity to participate in one-electron exchange reactions. Systemic Cu deficiency can generate anemia, ataxia, diminished growth, alterations in bone mineralization, diminished immune response and Menkes disease, among others. Moreover, it is well known that Fe deficiency can mainly produce anemia [1]. The primary influence of Zn in biological systems resides in its presence in ca. 300 enzymes. Zn has particularly relevant roles in growth, reproduction, immune and neuronal functions [2].

These elements must be incorporated through the diet but nowadays, due to frequently dietary disorders, TEs deficiencies have become a matter of concern. In the case of Nickel (Ni), it is essential for plants and bacteria [3-5] but there is no evidence of the effect of Ni deficiency in humans. On the other hand, TEs such as arsenic (As), lead (Pb), and cadmium (Cd) are potentially harmful to human and animal health [6]. Humans exposed to As may develop skin lesions, neuropathy, gastrointestinal diseases, cardiovascular diseases, cancer, and other ailments. Exposure to Pb due to contaminated food may cause changes in the neurologic system, leading to loss of neurological function. Acute Cd exposure can cause stomach irritation,

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while the long-term intake of low levels of Cd can cause kidney disease and bone fragility [7,8]. Therefore, regional regulations established maximum limits for As, Cd and Pb in several foodstuff for safety reasons. Then, it is important to monitor these TEs to ensure food safety.

Crops such as rice, wheat and soybeans are the basis of human diet in many countries and are widely used in food and feed. Soybean is one of the most important crops in terms of cultivated area in Uruguay covering more than 60% of the total agricultural area [9]. Besides, studies on the transfer of heavy metals from soil to crops have shown that soybean may accumulate more potentially toxic elements than other crops [10]. Salazar et al. evaluated the content of Cd, Pb and Zn in agricultural soils, the transfer of these elements to the plant and its relation to crop quality. They found that concentration values for Pb and Cd in both soils and soybeans, at several sites in Argentina, were above the maximum permissible levels. This information alerted about the possible presence of these elements in seeds that are imported from Argentina to Uruguay as raw material for food as well as for planting purposes [10].

In this work, in addition to determine the contents of TEs (four essentials and three potentially toxic) in soybean seeds, correlations between these contents and biochemical parameters related to oxidative stress such as superoxide dismutase activity, basal superoxide anion level and non-enzymatic activity, and seed quality (vigor and germination) were carried out [11,12]. The results are presented for the first time to asses' food safety and commercial and economic aspects of these crops.

MATERIALS AND METHODS
Reagents

Commercial standard solutions 1000 mg L^{-1} of As(V), Cd, Cu, Fe, Ni, Pb and Zn provided by Merck (Germany) were used. Calibration solutions were prepared by dilution of the stock solution of each element, using 0.1% v/v nitric acid (HNO$_3$) prepared from concentrated HNO$_3$ (67% v/v) provided by Merck (Germany).

Ultrapure water, ASTM Type I (18.2 MΩ cm resistivity) was obtained from a Millipore® (Brazil) Direct-Q 5 purifier.

All glassware remained submerged overnight in 10% v/v HNO$_3$ and after that it was rinsed exhaustively with ultrapure water before use.

Chemical matrix modifier for Cd and Pb determination were prepared from stock solutions of Pd(NO$_3$)$_2$ and Mg(NO$_3$)$_2$ provided by Merck (Germany) containing 10000 and 20000 mg L^{-1} respectively. For As determination a permanent modifier was prepared from a stock solution of Nb(NO$_3$)$_5$ 1000 mg L^{-1} provided by Sigma- Aldrich (Switzerland). All other reagents were of analytical reagent grade or better.

Reagents and solvents for the determination of biochemical parameters were commercially available research-grade chemicals and were used without further purification [13].

Analytical determinations
Trace elements

Analytical determinations of Cu, Fe and Zn were performed by flame atomic absorption atomic spectrometry (FAAS) using a spectrometer Perkin Elmer AAnalyst 200 (USA) operated at the analytical lines of Cu (324.7 nm), Fe (248.3 nm) and Zn (213.9 nm). Photron (Australia) hollow cathode lamps were used as recommended by the manufacturer. Flame composition was acetylene (2.5 L min^{-1}) and air (10.0 L min^{-1}).

As, Cd, Ni and Pb were determined by electrothermal atomic absorption spectrometry (ETAAS) using a spectrometer Thermo Scientific iCE 3500 (United Kingdom) equipped with auto-sampler module (GFS33) and employing Zeeman-based correction. A transversely heated graphite tube furnace module (GFS35Z), from Thermo Fisher Scientific, was used. Photron (Australia) hollow cathode lamps, operated at the

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193.7 nm (As), 228.8 nm (Cd), 232.0 nm (Ni) and 283.3 nm (Pb), were used. The spectrometer was controlled with the commercial software SOLAAR from Thermo Scientific (United Kingdom). Integrated peak-area was used as signal for evaluation and quantification. All the determinations were performed using pyrolytically coated graphite tubes from Thermo Scientific. Argon 99.998% provided by Linde (Uruguay) was used as the purge and protective gas. The heating programs employed for the analytical determinations are showed in Table I. These conditions were optimized and reported in a previous work [14].

Table I. ETAAS optimized temperature programs for the determination of As, Cd, Ni and Pb

Stage	Temperature (ºC)	Ramp rate (ºC s^{-1})	Hold time (s)
Drying 1	100	10[a,c,d]/5[b]	30
Drying 2[b]	140	15	20
Pyrolysis	1200[a]/350[b]/1000[c,d]	15[a]/10[b]/150[c,d]	15[a]/0[b]/20[c,d]
Atomization	2200[a]/1500[b]/2500[c] /1800[d]	0	3
Cleaning	2600	0	3

[a]As, [b]Cd, [c]Ni, [d]Pb

For Cd and Pb determinations the chemical matrix modifier used was: 10 µL of solution containing 5 µg of $Pd(NO_3)_2$ and 3 µg of $Mg(NO_3)_2$. For Cd two drying steps were required using conditions presented in Table I [15]. Sample injection volume was 20 µL for both elements. For Ni determination, no chemical modifier was required, and sample injection volume was 30 µL.

Since As determinations required a special procedure using a permanent modifier, graphite tubes were treated with niobium, according to Machado et al. by pipetting 50 µL of a 1000 mg L^{-1} $Nb(NO_3)_5$ solution and then submitting the tube to the following temperature program: [temperature/ramp time/ hold time]: drying (100 °C / 10 s / 60 s), atomization (2700 °C / 0 s / 5 s). The entire procedure was repeated six times (to obtain an amount of 300 µg of permanent modifier on the tube). Then the temperature program was as shown in Table I. The injection volume was 30 µL. In all cases argon flow rate was 0.2 L min^{-1} [14].

Biochemical parameters

Biochemical parameters related to oxidative stress of the batches were performed as previously reported by our research group as follows: a) antioxidant enzymatic systems were evaluated in a buffer extract determining the superoxide dismutase (SOD) activity using the method based on the inhibitory effect of SOD over the reduction of nitrobluetetrazolium by the superoxide generated by the xanthine/xanthine oxidase system; b) basal superoxide anion level was determined by a spectrophotometric method in the same extract as in a); c) non-enzymatic antioxidant activity was determined as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of an ethanolic extract following the Brand-Williams method. A Thermo Scientific Evolution 60 spectrometer was used for spectrophotometric measures [13].

Soybean quality analysis consists mainly in three *in vitro* tests: germination, vigor and viability by tetrazolium test. Results of these tests were provided by a Uruguayan laboratory that performs tests according to the International Seed Testing Association (ISTA) rules [16]. Seeds are classified as "normal" according to ISTA rules when germination is > 80%, considering several standard parameters of growth, these seeds can be sold to farmers for planting. Vigor test is performed to detect significant differences related to physiological quality of batches of seeds, thus complementing the information of germination tests. Sometimes it happens that certain batches of seeds with high percentages of germination have different behavior when they grow in field.

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This is explained by the fact that seeds lose vigor before losing their ability to germinate. Viability tests counts percentage of viable embryos through different standard techniques [13].

Samples

Sixteen batches of soybean seeds (*Glycine max* (L.) Merrill) were obtained from a local distributor (Agropecuaria Valdense S.R.L., Colonia, Uruguay). All batches consisted of transgenic yellow soybean seeds and ranged from 0.618 to 0.864 cm of diameter. Samples were dried in an oven with forced air circulation (70 °C) and stored at 20 °C. Before sample preparation for the different assays, seeds were milled to obtain the flour.

A certified reference material (CRM) NIST-1587a of wheat flour was also analyzed, this CRM was considered adequate for trueness evaluation. This CRM did not inform Ni content and for As and Pb a concentration value is informed in the certificate but without uncertainty, thus spiked samples were also analyzed to complement trueness evaluation of the analytical methods.

Sample preparation

For trace element determinations, a microwave assisted digestion was carried out employing a microwave oven (CEM, Mars 6) provided with 12 EasyPrep Plus® vessels.

Each sample was prepared in triplicate as follows: 0.5 g of sample (flour) was accurately weighted, and 10.00 mL of 3.5 mol L^{-1} HNO$_3$ were added into each EasyPrep Plus® vessel. The program was: power 400-1800 W, 15 minutes ramp time until 200 ºC, 10 minutes hold at 200 ºC, 500 psi pressure. Reagent blanks were also run.

After digestion, TEs in samples were directly measured or when necessary a suitable dilution with ultrapure water was performed.

Assays to obtain biochemical parameters were performed with soybean seeds flour without further treatment as previously described [13].

Correlations

Associations between variables were determined via Pearson´s correlation analysis. Multiple linear correlation and linear regression analysis were carried out using MS Excel®.

RESULTS AND DISCUSION
Trace elements determinations

For all the studied analytes, the determination coefficients (R^2) for linear regression of the calibration curves were greater than 0.99 using either FAAS or ETAAS technique. Linearity (mg L^{-1}) was up to: 4.0 for Cu, 2.0 for Fe, 0.10 for Ni, 1.0 for Zn, 0.020 for As, 0.004 for Cd and 0.050 for Pb.

Detection limits were estimated for each element, according to Eurachem Guide, expressed as the element content corresponding to three times the standard deviation of a blank (3*s*; n=10) and expressed in the sample (dry basis). Quantification limits were estimated in the same way considering in this case 10 times the standard deviation (10*s*; n=10) [17].

A summary of the TEs content in each sample is presented in Table II, detection and quantification limits for each element are also shown.

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Table II. Trace elements content (mg kg^{-1}) in soybean seeds

Samples	Cu	Zn	Fe	Ni	Cd	Pb	As
S01	14.9 ± 0.4	42 ± 2	99 ± 1	9.5 ± 0.5	0.027 ± 0.002	0.292 ± 0.002	0.125 ± 0.001
S02	13.9 ± 0.3	50 ± 1	105 ± 2	12.8 ± 0.4	0.015 ± 0.001	0.218 ± 0.002	0.147 ± 0.001
S03*	13.0 ± 0.2	35 ± 1	115 ± 1	9.4 ± 0.1	0.056 ± 0.001	0.539 ± 0.017	0.158 ± 0.006
S04	13.2 ± 0.8	44 ± 1	82 ± 4	14.3 ± 0.3	0.014 ± 0.001	0.165 ± 0.011	0.104 ± 0.001
S05	24 ± 3	44 ± 2	125 ± 8	13.6 ± 0.3	0.032 ± 0.001	0.416 ± 0.016	0.097 ± 0.001
S06*	12.2 ± 0.5	42 ± 3	83 ± 1	7.4 ± 0.3	0.035 ± 0.001	0.324 ± 0.009	0.164 ± 0.002
S07	14.5 ± 0.2	44 ± 2	77 ± 4	7.4 ± 0.2	0.011 ± 0.001	0.194 ± 0.003	0.108 ± 0.001
S08	15.0 ± 0.3	35 ± 1	70 ± 12	9.4 ± 0.1	0.013 ± 0.002	0.112 ± 0.008	0.151 ± 0.001
S09	12.5 ± 0.2	39 ± 1	67 ± 4	9.2 ± 0.2	0.014 ± 0.002	0.137 ± 0.010	0.060 ± 0.004
S10	14.2 ± 0.1	41 ± 2	83 ± 6	10.9 ± 0.2	0.013 ± 0.002	0.173 ± 0.011	0.144 ± 0.001
S11	14.8 ± 0.7	48 ± 3	81 ± 3	10.7 ± 0.1	0.015 ± 0.001	0.123 ± 0.009	0.092 ± 0.003
S12	14.0 ± 0.1	43 ± 1	61 ± 1	17.9 ± 0.1	0.013 ± 0.001	0.136 ± 0.004	0.111 ± 0.003
S13	17 ± 2	41 ± 1	52 ± 7	7.7 ± 0.2	0.011 ± 0.001	0.178 ± 0.009	0.106 ± 0.002
S14	14.5 ± 0.3	44 ± 2	70 ± 2	8.1 ± 0.1	<LOD	0.080 ± 0.005	0.107 ± 0.002
S15	13.2 ± 0.2	33 ± 1	60 ± 2	5.4 ± 0.3	<LOD	0.081 ± 0.001	0.092 ± 0.003
S16	12.5 ± 0.2	41 ± 1	66 ± 2	8.3 ± 0.2	<LOD	0.085 ± 0.009	0.104 ± 0.004
LOD	0.16	0.08	0.10	0.02	0.002	0.012	0.008
LOQ	0.54	0.26	0.30	0.05	0.006	0.038	0.024

Results are expressed on dry basis as mean ± standard deviation (n=3). Cu, Fe and Zn were determined by FAAS. As, Cd, Ni and Pb were determined by ETAAS. LOD: Limit of detection (3s; n=10). LOQ: Limit of quantification (10s; n=10). * Samples with germination <80%.

The maximum limits allowed by regional regulation to consider soybean seeds a safety food are 0.2 mg kg^{-1} for Cd and Pb and 0.3 mg kg^{-1} for As [18], therefore detection limits for As, Cd and Pb were adequate for monitoring food safety in soybean seeds since they are much lower than the legal limits allowed for this food.

Precision expressed as relative standard deviation (RSD) was in all cases lower than 10% (n=3 for each element in each sample replicate and n=6 for the CRM).

Trueness was evaluated by the analysis of the CRM under the same conditions and by performing a spike-recovery assay over the 16 batches of soybean seeds. Recoveries for the CRM were in the range 90-110% for Cu, Fe and Zn using FAAS and in the range 90-120% for As, Cd, Ni and Pb using ETAAS.

Results obtained using a CRM such us wheat flour, a very similar matrix, guarantee that the analytes are quantitative extracted from the matrix even using diluted acid (3.5 mol L^{-1} HNO$_3$). When the flour obtained from soybean seeds is spiked with a known amount of analyte, it can be ensured there are no losses during the analysis. Both studies guarantee the trueness of the method.

The use of diluted acid for microwave assisted digestions was reported as an efficient procedure for total digestion in several complex matrices including soybean seeds [14,19]. In this work, the use of 3.5 mol L^{-1} HNO$_3$ was successful. Clear solutions were obtained after the digestion process with good recoveries and complying with the principles of Green Chemistry.

The essential TEs content in soybean seeds obtained are in accordance with those reported by several authors in the literature [11,19-21]. The concentration of the essential elements in the analyzed samples

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where in the range: (12.2 – 24.0) mg kg^{-1} for Cu, (60 - 125) mg kg^{-1} Fe, (5.4 – 17.9) mg kg^{-1} for Ni and (33 - 50) mg kg^{-1} for Zn. All ranges are in good agreement with values reported for Argentinian and Brazilian crops.

Other authors have found statistically significant differences in the metallic content between transgenic and non-transgenic soybean seeds, being concentrations of Cu and Fe higher in transgenic seeds by 40 and 20% respectively [22-23]. Soybean seeds composition is dependent on numerous factors, including soil characteristics and water source composition. Once these factors are controlled during the growth, it can be expected that differences in concentrations should be related only to genetic modification. In this case, all samples were from transgenic origin, so it was not possible to perform such a comparison.

Regarding non-essential elements, all samples comply with the regional regulation established for Cd and As (<0.2 and 0.3 mg kg^{-1} respectively). Therefore, soybean seeds analyzed can be considered as safe for human consumption. However, five samples showed levels of Pb that exceeded the maximum limit admitted of 0.2 mg kg^{-1}. These are only few batches to take conclusions about food safety, but they provide evidence that there must be rigorous controls of toxic elements in food. Coincidentally with the fact that Pb is toxic to living organisms, two of the samples with high levels of Pb presented poor germination (<80%), particularly sample S03 whose germination was <10%. Germination data of these same batches was previously reported by our research group [13].

Correlations within trace elements contents

The set of data presented in Table II was used to perform the correlations within TEs, where particularly novel information was presented regarding potentially toxic elements such as As, Cd and Pb. Plants have developed mechanisms to prevent their own toxicity by regulating the transportation of the toxic elements with chelation or sequestration. Different strategies are performed to deal with high concentration levels of TEs in the environment [24-25]. The uptake and efflux of metals ions at cellular level must be strictly coordinated with the requirements of the whole plant to maintain homeostasis [26].

Table III shows Pearson´s correlation coefficients between TEs content. Significantly high positive correlations were found for Fe:Cd:Pb (p<0.005), while for Cu:Fe, As:Cd, As:Pb and Zn:Ni the correlations were also positive but slightly lower (p<0.05).

Table III. Pearson´s correlation coefficients between TEs content in soybean seeds samples

	Zn	Fe	Ni	Cd	Pb	As
Cu	0.2415	**0.4328***	0.2609	0.1331	0.3238	-0.1680
Zn		0.2582	**0.4419***	-0.1752	-0.0537	-0.0487
Fe			0.2762	**0.7356****	**0.8164****	0.3685
Ni				0.0592	0.1064	-0.0033
Cd					**0.7543****	**0.4960***
Pb						**0.4544***

* p<0.05 and **p<0.005

It seems that there are important agonist interactions between potentially toxic elements, as they are positively related. In the case of Cd and Pb, it could be explained by the fact they are assimilated from the soil by the same family of ATPases, divalent cation transporter class enzymes [26].

According to Cd and Fe interactions, many studies on plants showed that Cd may displace Fe from EDTA complexing agents, leading to diminished Cd bioavailability and increasing the resistance of the plant. The inhibition of the uptake of essential elements may contribute to Cd toxicity. But also, the Cd efflux as a resistance strategy could lead to the efflux of other metal ions as well. Recent studies found no inhibition of

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Fe uptake by Cd toxicity, in fact, in this work both elements resulted to be positively related, probably due to the presence of specific transporters for each one. Several authors reported metal efflux proteins P-type ATPases for Cd and metal uptake proteins Yellow Stripe1-Like (YSL) for Fe [26-27].

Correlations between trace elements contents and biochemical parameters

Biochemical parameters, previously reported by the authors [13], are shown in Table IV. Pearson´s correlation coefficients between TEs and biochemical parameters in soybean seeds samples are presented in Table V. Regarding TEs content and biochemical parameters a moderate negative correlation between Cu and superoxide anion was found. This is in accordance with the fact that Cu integrates numerous enzymatic detoxifying systems responsible for controlling the oxidative stress.

Table IV. Biochemical parameters

Samples	Superoxide* (Absorbance$_{560}$)	SOD activity * (U mg^{-1} protein)	DPPH assay* (mg antioxidant *per* g dry seed)	Germination (%)	Vigor (%)	Viability (%)
S01	0.011	49.7	4.6	93	64	99
S02	0.020	30.7	5.9	80	50	88
S03	0.018	20.3	5.1	20	33	96
S04	0.011	47.1	5.4	86	31	89
S05	0.010	44.5	7.0	80	44	89
S06	0.028	36.4	5.1	70	60	98
S07	0.012	80.4	6.5	97	74	99
S08	0.020	25.4	5.2	70	45	80
S09	0.024	67.7	5.5	80	53	98
S10	0.012	39.2	6.4	87	59	93
S11	0.011	48.0	5.0	97	60	98
S12	0.018	33.5	5.8	87	44	99
S13	0.017	57.3	5.9	75	54	88
S14	0.010	35.2	6.0	74	34	94
S15	0.018	33.5	5.8	87	44	99
S16	0.017	34.5	5.4	71	36	90

*Results expressed as mean value (n=3) as previously reported by Cardoso *et al.* [13]. *In vitro* tests: germination, vigor and viability were performed using the tetrazolium test according to ISTA rules [16].

Table V. Pearson´s correlation coefficients between TEs and biochemical parameters in soybean seeds samples

Parameter	Cu	Zn	Fe	Ni	Cd	Pb	As
Superoxide	**-0.4508***	-0.3532	-0.1666	-0.3310	0.2061	0.0825	0.2779
SOD activity	0.1140	0.2501	-0.2750	-0.0305	-0.3220	-0.1995	**-0.5981***
DPPH assay	**0.5593**	0.2130	0.0385	0.2684	-0.2595	-0.0355	-0.2260
Germination	0.1470	0.4607	-0.2944	0.0935	**0.7549***	-0.5849	-0.4684
Vigor	0.0177	0.2633	-0.1281	-0.036	**0.6162***	-0.0515	0.0141
Viability	-0.3209	-0.0207	-0.0155	-0.2652	**0.6109***	0.1177	-0.2514

* p<0.05, **p<0.025, ***p<0.01

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The highly significant positive correlation ($p < 0.01$) between Cd content and quality parameter germination (vigor and viability to a lesser extent) would indicate that Cd content does not produce negative effects in the development of the seeds. This is in accordance with findings reported by some authors, who suggest that a slightly increased level of oxidative stress stimulates germination [28,29]. This theory is also supported by Bailly et al. who describes the need of a period of oxidative stress for germination process [30]. Small amounts of Cd could lead to this oxidative stress and stimulate germination. It is interesting to highlight that the positive correlation between Cd and the germination parameters is significantly higher than for Cu, being Cu an essential TE. On the other hand, theories about the pattern of Zn and Cd uptake, reinforces the hypothesis that plants are adapting to Cd, in certain amount, this element do not cause harm to the seed.

In the case of Pb, no significant correlations were observed with any of the studied biochemical parameters. The Pb uptake by plants might be due to unknown mechanisms. However, evidence has proved that Pb taken by the plant and translocated to the upper parts is under the form of Pb-chelate complexes like EDTA-Pb and HEDTA-Pb. Once the complex is inside the plant, it stays intact to relieve potential toxic effects and allow the plant to continue growing [31]. An interesting observation was the negative correlation between Pb and germination showing a toxic effect.

No significant correlations were observed for Ni or Zn with the studied biochemical parameters. On the other hand, there is a significant negative correlation ($p < 0.01$) between As content and SOD activity. This fact could indicate a negative effect of As over a protective mechanism against free radicals, interfering with the scavenging capacity of the SOD. It has been demonstrated that high-affinity as well as constitutive low-affinity uptake systems for As are present in plants. As(V) competes with phosphate for its uptake, and after it, reduction of intracellular As(V) to As(III) takes place by an As reductase and then it is detoxified through complex formation with thiol-rich peptides [32].

CONCLUSIONS

For the first time, correlations within TEs content in soybean seeds and with biochemical parameters related to oxidative stress and seed quality was performed. These results allowed us to increase knowledge about TEs effect in biological systems in soybean seeds.

Particularly, a significantly positive correlation for Cd and parameters like vigor and germination of seeds was found suggesting that small amounts of Cd can promote seed growth. Some of the analyzed batches had poor germination in field; these batches showed higher levels of Pb, but Pearson´s correlations did not show a negative effect on germination or vigor for the rest of the batches. Besides, high positive correlations for Fe:Cd:Pb ($p<0.005$) were found, which can be explained by natural physiological mechanisms of the plant.

Moreover, we would like to highlight from our study that Cd and Pb do not seem to have a negative influence on the proper growth of soybean seeds, but their levels should be controlled since food safety must be guarantee to consumers and the nutritional value can be altered.

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