Inorganic Contaminants Evaluation in Tuberous and Leafy Vegetables Consumed in Paraíba Valley, Brazil

Rodrigo Fernando dos Santos Salazar[1], André Luís de Castro Peixoto[2], Thaís Cristina Coelho de Ornelas Salasar[3], Roberta Cattaneo Horn[4], Ritielli Berticelli[5], João Fernando Zamberlan[6], Ludmila Noskoski Salazar[7], Rafael Pivotto Bortolotto[8], Hélcio José Izário Filho[9]

[1]Department of Environmental and Sanitary Engineering, Cruz Alta University, Cruz Alta, Brazil
[2]Department of Chemical Engineering, Instituto Federal de Educação, Ciência e Tecnologia - São Paulo (IFSP), campus Capivari, Brazil
[3]Graduate Stricto Sensu Program in Comprehensive Health Care (PPGAIS), Cruz Alta University (UNICRUZ) and Unijuí University (UNIJUÍ), Cruz Alta / Unijuí, Brazil
[4]Oxidative Stress Laboratory, Cruz Alta University (UNICRUZ), Cruz Alta, Brazil
[5]Department of Environmental and Sanitary Engineering, Cruz Alta University, Cruz Alta, Brazil
[6]Graduate Stricto Sensu Program: Professional Master's in Rural Development (MPDR), Cruz Alta University (UNICRUZ), Cruz Alta, Brazil
[7]Department of Veterinary Medicine, Cruz Alta University, Cruz Alta, Brazil
[8]Graduate Stricto Sensu Program: Professional Master's in Rural Development (MPDR), Cruz Alta University (UNICRUZ), Cruz Alta, Brazil
[9]Department of Chemical Engineering (LOT), Escola de Engenharia de Lorena (EEL), São Paulo University (USP), Lorena, Brazil

Abstract— The aim of the present study is to investigate the aluminum, cadmium and lead concentrations in vegetables such as lettuce, potatoes, carrots, beets and cassava often consumed in human diet. Calcination and acid digestion methods were used to prepare the samples. The Spike test has shown that the methods were viable for almost all samples. The recovered analyte percentage ranged from 88.0% to 108.7% in the acid digestion, and from 55.1% to 109.6% in the calcination method. Comparative analyses conducted in lettuce samples have shown lead concentrations 26.4 ± 2.6 mg kg^{-1} in electrothermal atomic absorption spectrometer (ETAAS) and 33.3 ± 0.3 mg kg^{-1} in inductively coupled plasma optical emission spectrometer (ICP-OES). The herein presented results allow saying that the concentrations of the elements analyzed in each sample were above the limit set by the Brazilian Ministry of Health and within the tolerance limit set by the Codex Alimentarius.
Keywords— Al, AAS, Cd, Pb, food.

I. INTRODUCTION

Metal elements may affect the environment, as well as humans, in different ways, depending on their availability and essentiality; it happens due to their physical and chemical features. Some elements such as B, Ca, Fe, Mg, Mo, among others, are essential at concentrations between micro- and milligrams per day [1;2;3]. On the other hand, elements such as Al, Cd and Pb do not present known biological function and are well-known for their toxicity when they are inhaled or ingested in excess [4-9].

Aluminum is seen as non-essential. In addition, there is evidence that the Al^{3+} ion may be linked to health issues such as Alzheimer's disease, and that it may exacerbate intoxications in people with impaired renal function [7;10]. The Al^{3+} may be incorporated to the tissue of vegetables cultivated in soils showing high bauxite content; thus, it is a contamination liability and, consequently, a Public Health concern [7;8;9]. It is known that cadmium (Cd) mobility and toxicity mainly depend on its oxidation state. Thus, Cd may become bioavailable and harm the environment, as well as humans [3;11]. Therefore, this element has been investigated to help better understanding its toxicity mechanisms and setting threshold concentrations that cannot cause any type of harm in case of exposure [12;13]. The particulate lead, which is transported in the form of oxides, may be aspirated due to its wide distribution in the atmosphere [6;11;14]. Whereas the gastrointestinal Al absorption is

influenced by several factors, mainly age and diet [7;9]. The lead toxicity results from its capacity to interact with the essential metals of the main metabolic pathways, mainly with Zn, Ca and Fe [2;9].

Paraíba Valley is a São Paulo State region located on the triple border between Minas Gerais, São Paulo and Rio de Janeiro states. This region has been the target of several technical-scientific investigations focused on assessing and warning about the impact of the regional development on the quality of life of humans and on the environment [4;15;16]. Okada et al. (1997) have assessed the Cd and Pb contamination degree in milk samples from Northern Paraíba Valley. They found that 19.7% of the samples showed lead levels above the maximum limit set by the legislation in force. Soares et al. (2010) have investigated the Cd, Cr and Pb concentrations in pasteurized milk purchased in marketplaces in different Paraíba Valley cities, whereas Bayod Filho et al. (2012) have investigated the Ca, Cr, Mg and Mn concentrations in umbilical cord blood and breast milk samples. These authors have proposed analytically-validated procedures and found contamination gradient increase over the years, fact that led them to report such risk to the health departments in the affected counties.

Similarly, different studies conducted throughout the 2000s have measured the contamination impact of different metal elements on vegetables and on the agricultural soils in some Paraíba Valley cities. Salazar et al. (2006) have investigated the concentration of oligo-elements (Cu, Fe and Zn) in samples comprising vegetables consumed in Lorena County (SP). However, the way the samples were prepared did not allow validating the analytical procedures, thus compromising data reliability. Nonetheless, they could see that the investigated samples showed indications that the concentrations of some elements were above the tolerance limit set by the legislation [17]. Salazar et al. (2011) have found passive Ni contamination in soil samples and in vegetables consumed in Lorena and Taubaté counties (SP), after they properly proposed and validated simplified analytical calcination and acid digestion procedures. According to the authors, the contamination results from the inappropriate use of fertilizers presenting trace element concentration as an attempt to remediate the presence of this micronutrient [16].

In light of the foregoing, the investigation and monitoring of different elements and contaminants are demanding and increase researchers' interest in better understanding their toxicity mechanisms, as well as their interest in environmental and sanitary sensing. Therefore, the aim of the current study was to investigate the Al, Cd and Pb concentrations in vegetables often consumed in Paraíba Valley region, mainly in Taubaté and Lorena counties (SP).

II. MATERIALS AND METHODS

2.1 Sampling, reagents and solutions

The sampling consisted of lettuce (*Lactuca sativa*), beet (*Beta vulgaris*), potato (*Solanum tuberosum*), carrot (*Daucus carota*) and cassava (*Manihot utilissima*) samples purchased in street markets and supermarkets in Taubaté and Lorena counties; the tuberous samples comprised roots and skin, whereas the leafy samples comprised leaves and stems. All samples were fresh and showed no signs of putrefaction [16;18].

The solutions were prepared by employing analytical degree reagents, ultrapure water obtained from using Milli-Q (Millipore Corp, de Billerica, MA, EUA) water system at 18.2 MΩ cm resistivity, nitric and chloridric acid distilled in quartz sub-boiling (Milestone, Sorisole, Italy). To prevent contamination, the vials, glassware and polypropylene materials were washed and soaked in 10% v v^{-1} HNO$_3$ and fully washed with deionized water. All the chemical reagents were of the P.A. degree. The mineral acids used were of the Dinâmica brand. The metallic patterns used, with 1-mg mL^{-1} concentration, were of the SpecSol brand with NIST traceability, Reagent organic solvents and other chemical reagents of the Vetec brand [18].

2.2 Sample preparation

First, each vegetable sample was washed in distilled water to remove soil and other relevant debris. Subsequently, they were brushed with polypropylene bristles and washed in deionized water [16]. The lettuce shoot was carefully cut along with the stalks of each leaf, except for the main leafstalk, which was discarded along with the roots. The potato, beet, carrot and cassava root samples were grated in a polypropylene grater and placed in porcelain capsules. Then, the capsules holding the samples were taken to the oven at $120 \pm 5°C$, for 24 h, for dehydrated mass obtainment [18]. After the dehydration process was complete, the samples were placed in polypropylene beakers covered with PVC film and stored in a desiccator. Calculations were made for each dried sample analyzed in the current study in order to determine how much of these samples represented, in weight, the corresponding fresh samples (eq.1):

$$M_f = (100 \cdot M_s)/(100 - \%Moist) \qquad (1)$$

Wherein M_f is the mass of the fresh sample (g), M_s is the weighted mass of the dry sample (g) and % Moist is the mean moisture content of the sample. The samples were prepared by using adaptations of the procedures

developed by Wieteska et al. (1996) for plant sample preparation via acid digestion and calcination; then, they were subjected to further determination through ETAAS or FAAS [19].

2.2.1 Calcination method

Firstly, the calcination of dehydrated samples was carried out by weighing approximately 0.5 g of each vegetable in porcelain crucibles and, then, covering them 19. The crucibles were transferred to a muffle, which was heated at 500°C for 2 h; the heating was done at 12°C min^{-1}, with 20-minutes threshold each, at 100, 200, 300 and 400°C. The calcination residues were digested in 2.0 mL of deionized H_2O, 0.5 mL of HCl and 1.0 mL of concentrated HNO_3. The crucibles were covered with watch glass and heated on a plate, for 30 min, in sand bath, in order to assure better heating and reflux control to prevent analyte loss [16]. After the digestion and cooling were complete, the entire digestion system was properly rinsed with deionized water, the crucible solutions were quantitatively transferred to 50.0 mL flasks and supplemented with deionized H_2O.

2.2.2 Acid Digestion method

A 0.5 g of each sample was weighed in 50 mL glass beakers. Next, 5.0 mL of an acidic mixture composed of HNO_3 and $HClO_4$ (4:1 v/v) and of 2.0 mL of deionized H_2O were pipetted into each beaker. Then, each beaker was covered with watch glass and transferred to sand bath in order to allow mild digestion, as recommended when one works with this type of acid mixture. The digestion time ranged from 1 to 2 hours; the system solution was kept in continuous reflux until the digestion product became as clear as possible [19]. After the digestion and cooling processes were complete, the crucible solutions were quantitatively transferred to 50.0 mL flasks and supplemented with deionized H_2O. The Al, Cd and Pb determination values and the M_f of the vegetable samples were used to calculate the metal mass per fresh sample mass, through Equation 2:

$$m = (C/M_f) \cdot f \qquad (2)$$

Wherein the parameter "m" is the metal concentration per fresh sample mass (mg Kg^{-1} or µg Kg^{-1}); "C" is the metal concentration in the solution (mg L^{-1} or µg L^{-1}); "M$_f$" is the fresh sample mass (g); and "f" is the unit conversion factor.

Addition and analyte recovery tests were used to assess result accuracy by adding 2.0 µg L^{-1} of Cd standard, 10 µg L^{-1} of Pb standard and 30mg L^{-1} of Al standard to 0.5 g of dehydrated samples. Interlaboratory analyses of lettuce samples digested through calcination and acid digestion were referred to inductively coupled plasma optical emission spectrometry (ICP-OES) in order to give greater reliability to the spike and recovery tests performed in the current study [16;18;20;21].

2.3. Instrumentation

All atomic absorption measurements were performed in the Perkin Elmer*;* Model*:* AAnalyst 800, using a deuterium lamp (D$_2$) for background correction. The aluminum was determined through FAAS (flame atomic absorption spectrometry). The electrothermal atomic absorption spectrometry (ETAAS) system was used to determine Cd and Pb [21]. The herein used experimental conditions are described in Tables 1 and 2.

Table.1: Experimental conditions for lead determination through atomic absorption.

	Al	**Cd**	**Pb**
Wave-length (nm)	309.3	228.8	283.3
Slot opening (nm)	0.70	0.7	0.7
Lamp current (mA)	25	4	440
Sample aliquot (µL)	--	10	10
Chemical modifier aliquot (µL)	--	10	10
Determination module	FAAS	ETAAS	ETAAS
Gas flow (L min^{-1})	N$_2$O / C$_2$H$_2$ (16.0 / 7.8)	--	--
Spectroscopic buffer (%v v^{-1})	KCl (0.1)	--	--

Table.2: Graphite Furnace Programming (ETAAS) to determine Cd and Pb.

Elements	Cd				Pb			
Stages	A	B	C	D	A	B	C	D
Drying	150	1	2	25	150	1	2	25
	0	0	0		0	0	0	
Pyrolysis	800	3	2	25	900	3	1	25
	0	0	0		0	0	0	
Atomization	140	0	5	0	150	0	5	0
	0				0			
Cleaning	50	5	5	25	250	5	3	25
				0	0			0
Cooling	245	5	3	25	20	5	5	25
	0			0				0

A - Temperature (°C); **B**: Heating Ramp (s); **C**: Platform Time (s); **D**: Argon Flow (L min^{-1}).

The experimental conditions of the inductively coupled plasma optical emission spectrometry (ICP-OES) used in the current study are shown in Table 3. The ICP-OES showed axial configuration (Vista AX, Varian, Mulgrave, Victoria, Australia); it was equipped with a charge-

coupled device (CCD) refrigerated at -35 ºC to allow measurements from 167 to 785 nm, as well as with a Cross Flow nebulizer (flow rate 1.50 mL min^{-1}) coupled with a Scott Spray Chamber (20).

Table.3: ICP-OES operating conditions (Vista AX, Varian).

Plasma Parameters	Operating conditions
Power (W)	1450
Plasma gas flow (L min^{-1})	14.50
Auxiliary gas flow (L min^{-1})	0.2
Nebulizer flow (L min^{-1})	0.54
Radio Frequency Generator (MHz)	40.0
Torch position (mm)	
Horizontal	-1.0
Vertical	2.0
Depth	1.0
Reading time (s)	25.20

Replicates	4
Wave-length,(nm)	Al(396.153), Cd(228.802), Pb(220.356)
Monitoring element	Ar (430.010)

III. RESULTS AND DISCUSSION

3.1 Validating the sample preparation procedures

The methods adopted in the current study were feasible when the recovered analyte rates ranged from 95.9% to 108.8% in the acid digestion, and from 102.7% to 122.9% in the calcination, for Al. When applying the t-test and the F-test ($p < 0.05$) it was verified there are statistically significant differences in results obtained for Al in carrot and cassava or Cd in potato and beet, Pb in beet and carrot. However, it is possible to verify that the repeatability oscillated in methodological terms and by the analyzed element. Tables 4 and 5 show the recovery results of the calcination and acid digestion procedures, respectively.

Table.4: Results of Al, Cd and Pb concentrations in the addition and recovery tests, after calcination (n = 4).

Element	Assays	Samples				
		Lettuce	*Potato*	*Beet*	*Carrot*	*Cassava*
Al	Spiked (mg kg^{-1})	45.3	30.0	54.4	30.0	38.0
	Result (mg kg^{-1})	41.3±1.4	30.8±3.9	55.3±1.7	36.9±3.9	40.6±1.0
	Recovery (%)	91.2	102.7	101.6	122.9	106.8
Cd	Spiked (µg kg^{-1})	4.6	3.0	3.3	3.0	4.6
	Result (µg kg^{-1})	4.7±0.1	3.0±0.3	3.1±0.2	2.9±0.1	3.9±0.3
	Recovery (%)	102.2	101.0	93.9	97.1	85.2
Pb	Spiked (µg kg^{-1})	28.5	10.0	14.1	10.0	13.5
	Result (µg kg^{-1})	23.9±1.0	6.3±0.4	10.1±1.2	5.5±2.5	14.8±5.9
	Recovery (%)	83.9	62.8	71.6	55.1	109.6

Table.5: Results of Al, Cd and Pb concentrations in the addition and recovery tests, after acid digestion (n=4).

Element	Assays	Samples				
		Lettuce	*Potato*	*Beet*	*Carrot*	*Cassava*
Al	Spiked (mg kg^{-1})	45.3	30.0	57.6	30.0	38.0
	Result (mg kg^{-1})	48.8 ± 0.9	32.6 ± 0.8	58.9 ± 2.0	28.8 ± 0.9	34.6 ± 1.2
	Recovery (%)	107.7	108.8	102.2	95.9	91.1
Cd	Spiked (µg kg^{-1})	4.6	2.2	3.4	2.7	4.6
	Result (µg kg^{-1})	4.4 ± 0.3	1.4 ± 0.1	2.3 ± 0.3	2.7 ± 0.3	3.5 ± 0.7
	Recovery (%)	95.5	72.3	67.6	100.9	76.1
Pb	Spiked (µg kg^{-1})	28.5	10.0	12.6	17.5	13.5
	Result (µg kg^{-1})	26.4 ± 2.6	8.8 ± 1.2	2.4 ± 0.7	16.9 ± 2.6	12.8
	Recovery (%)	92.6	88.0	108.7	96.4	94.8

According to the data in Tables 4 and 5, the two methodologies achieved high recovery in the Al analysis through FAAS, except for the Al in the carrot samples subjected to the calcination method. This result can be attributed to the KCl buffer, which was not sufficient to minimize the calcination interference and temperature.

The recovery test did not show satisfactory analytical Cd recovery (> 85%) in the digests obtained through the acid digestion applied to potato, beet and cassava samples; the recovery values were 72.3%, 67.6% and 76.1%, respectively. On the other hand, the recovery test showed satisfactory analytical results in the other digests (values above 85%) subjected to the two methodologies, fact that enables applying the acid digestion and calcination methodologies to analytically determine the Cd in the samples of interest.

The results in the current study have also shown that both methodologies achieved high Pb recovery in the ETAAS analysis, except for potato, carrot and cassava samples subjected to calcination. These results show possible losses caused by Pb entrainment during this stage. The herein found values can be considered analytically satisfactory for all samples subjected to acid digestion pretreatment, except for the aforementioned samples and methodologies. It allows applying the acid digestion methodology to analytically determine Pb in the samples of interest.

The low lead recovery in the calcination method may be attributed to a possible Pb entrainment during the heating ramp, whereas the low cadmium recovery in the acid digestion method may due to a possible reaction of the $HClO_4$-derived chlorine, which generated volatile chlorides [14].

The interlaboratory analyses conducted in the lettuce sample showed Al concentration 19.3 ± 0.3 mg kg^{-1} when it was determined through FAAS, and 18.2 ± 0.2 mg kg^{-1} when it was determined through ICP-OES. The addition and recovery tests, as well as the interlaboratory analysis, showed similar results, fact that made it possible using the sample preparation procedures to determine Al. The interlaboratory analyses conducted in the lettuce digests showed Pb concentration 26.4 ± 0.3 µg kg^{-1} when it was determined through ETAAS, and 18.2 ± 0.2 µg kg^{-1}, when it was determined through ICP-OES. The results of the interlaboratory analysis made it possible using the acid digestion methodology and reinforced the results found in the analyte addition and recovery test used to determine Pb. With respect to Cd, it was not possible comparing the techniques, since the analyte was below their detection limit (LOD <0.001 mg L^{-1}). The fact that

Cd was not detected through ICP-OES may be explained according to the techniques. The ETAAS techniques show higher sensitivity and, consequently, better detection and quantification limits than the ICP-OES ones [8;12].

3.2 Determination of Al, Cd and Pb

Table 6 shows the Al, Cd and Pb determination values found in the vegetable samples from Lorena and Taubaté counties.

The Pb levels found in the samples from Taubaté County were higher than those found in the samples from Lorena County. After all samples were analyzed, it was possible finding alarming Pb concentrations in cassava samples from both counties, as well as in the lettuce sample from Lorena County. According to the Brazilian Ministry of Health for Al content in lettuce, potato, carrot and cassava are set up 4.1, 1.7 and 7.5 µg kg^{-1}, respectively. In relation to Cd content is recommended up to 1.7, 5.3, 4.0 and 3.7 µg kg^{-1} for lettuce, potato, carrot and cassava, respectively. Finally, for Pb content is accepted around 7.5, 0.23, 31 and 0.8 µg kg^{-1}, respectively [1].

By comparing the Al results to the tolerance limits set by the legislation, it was possible seeing that all samples showed Al levels above the allowed limits, mainly the lettuce samples, wherein the Al concentrations in Taubaté and Lorena counties were 13 and 60 times higher than the tolerance limits, respectively. It shows absorption kinetics [8;9;22] more favorable to leafy than to root vegetables. This absorption kinetics may result from air and water-related contaminations due to the geological features of the region [7;9]. Anthropogenic factors are among those mostly influencing result oscillations, since the Al concentration was higher in Taubaté County, where the industrial activity is more intense than in Lorena County. However, Lorena County is closer to the region showing the highest mining activity (bauxite extraction), fact that may have caused the lettuce samples to be exposed to Al contamination. Results shown in Table 5 allowed concluding that the Cd levels were above the tolerance limit estimated by the Brazilian Ministry of Health; however, these levels were within the tolerance limits set by the *Codex Alimentarius*. According to CODEX STAN 248 (2005), those values for Cd is above 0.2 and 0.1 mg kg^{-1} for leafy and tuberous vegetables, respectively. On the other hand, for Pb content in leafy and tuberous vegetables, the CODEX STAN 210 (2001) set up values around 0.3 and 0.1 mg kg^{-1}, respectively [23;24].

Table.6: Al $(mg\,kg^{-1})$, Cd $(\mu g\,kg^{-1})$ and Pb $(\mu g\,kg^{-1})$ determinations in vegetables consumed in Lorena and Taubaté counties (fresh mass base).

	Lorena			Taubaté		
	Al	Cd	Pb	Al	Cd	Pb
Lettuce	252.6±5.7	9.9±0.9	63.9±9.0	52.4±11.4	4.1±0.5	161.3±15.8
Potato	7.7±3.4	5.8±3.5	25.93±5.11	45.6±8.9	3.9±0.8	54.4 ±8.1
Beet	26.4±3.5	15.2±1.4	31.4±1.6	59.3±12.0	8.0±2.1	63.0±1.2
Carrot	70.0±3.8	9.0±1.9	97.8±9.8	92.8±9.3	13.3±0.4	33.1±8.3
Cassava	28.9±1.3	30.4±4.3	233.6±48.1	186.9±18.6	17.8±1.9	349.6±26.2

This scenario and the concern about controlling the contamination and setting safe exposure levels can be seen in other countries, as shown in Table 7.

Table.7: Mean daily Cadmium intake and absorption by Americans [25].

Exposure Sources	Exposure	Intake	Absorption (%)	Absorption (µg / day)
Air (environment)	0.03 µg/m³	0.6	25	0.15
Cigarette (pack)	3.0 µg/pack	3.0	25	0.75
Food	--	30.0	5	1.50
Water	1.3 µg/L	2.6	5	0.13

The contamination level in the samples resulted from features found in the soil, water and in the region where the vegetables were collected. Accordingly, it was possible seeing that, in addition to the herein investigated elements, other elements may have entered the food chain and contaminated the milk consumed in the region, as Okada et al. (1997) and Soares et al. (2010) have observed for Cd and Pb. In parallel, Bayod Filho et al. (2012) and Izário Filho et al. (2014) have found that both the elements analyzed in the current study and other inorganic contaminants and nutrients can be already found in the bloodstream of unborn children and pregnant women, as well as in breast milk samples; it shows a contamination gradient that starts in the moment the fetus is formed and goes on throughout pregnancy due to the intake of doubtfully-safe food [5;25]. However, it is necessary conducting an in-depth study about the Al, Cd and Pb interaction with other elements in the human body in order to assess the deleterious effects the population circumscribed in these counties are subject to over time, as well as to help better understanding the biosorption of the herein studied and other elements by the human body [2;6;9;11;12;13;14].

The Environmental Sanitation Technology Company (Cetesb) has implemented an environmental program in the region, in 1993, in order to help monitoring lead and cadmium concentrations in sediment, water and grass samples, as a way to minimize the impact caused by anthropogenic activities on the quality of life of its inhabitants [4;15].

IV. CONCLUSION

All vegetable samples investigated in the present study showed Al, Cd and Pb levels above those allowed by the legislation. The most worrisome results refer to the Cd levels in the lettuce and cassava plants from Lorena County, whose contents were more than five and eight times higher than those allowed by the legislation, respectively. All samples showed Pb concentrations higher than those specified by the Ministry of Health. However, the Cd and Pb levels were within the tolerable daily intake limit set by the *Codex Alimentarius*. Yet, these results may work as an indication of environmental contamination in these counties, since the Al levels in the vegetables were above the values allowed by the Brazilian Ministry of Health and by international bodies. The values found in the current study represent the total concentration of the herein investigated elements. Chemical speciation studies should be conducted in order to assess the impact caused by the daily intake of these elements, as well as to determine their bioactivity and interaction mechanisms in the human body.

REFERENCES

[1] Santos, E. E., Lauria, D. C., & Da Silveira, C. P. (2004). Assessment of daily intake of trace elements due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city. Science of the total environment, (ISSN: 0048-9697), 327(1-3), 69-79. https://doi.org/10.1016/j.scitotenv.2004.01.016

[2] Llorent-Martínez, E. J., Ortega-Barrales, P., Fernández-de Córdova, M. L., Domínguez-Vidal, A. R. M. A., & Ruiz-Medina, A. (2011). Investigation by ICP-MS of trace element levels in vegetable edible oils produced in Spain. Food Chemistry, (ISSN: 0308-8146), 127(3), 1257-1262. https://doi.org/10.1016/j.foodchem.2011.01.064

[3] Abbasi, A. M., Iqbal, J., Khan, M. A., & Shah, M. H. (2013). Health risk assessment and multivariate apportionment of trace metals in wild leafy vegetables from Lesser Himalayas, Pakistan. Ecotoxicology and Environmental Safety, (ISSN: 0147-6513), 92, 237-244. https://doi.org/10.1016/j.ecoenv.2013.02.011

[4] Soares, V. A., Kus, M. M., Peixoto, A. L. C., Carrocci, J. S., Salazar, R. F., & Izário Filho, H. J. (2010). Determination of nutritional and toxic elements in pasteurized bovine milk from Vale do Paraiba region (Brazil). Food Control, (ISSN: 0956-7135), 21(1), 45-49. https://doi.org/10.1016/j.foodcont.2009.03.010

[5] Filho, L. F. B., Salazar, R. F., Alcântara, M. A., Nascimento, L. F., Filho, H. J. I., & Silva, M. B. (2012). Methodological Validation for the Determination of Ca, Cr, Mg and Mn in Umbilical Cord and Maternal Blood (City of Vale do Paraíba, São Paulo, Brazil). International Journal of Food Science and Nutrition Engineering, (ISSN: 2166-5168 (P) | 2166-5192 (O)), 2(4), 44-53. https://doi.org/10.5923/j.food.20120204.01

[6] Škrbić, B., Živančev, J., & Mrmoš, N. (2013). Concentrations of arsenic, cadmium and lead in selected foodstuffs from Serbian market basket: Estimated intake by the population from the Serbia. Food and chemical toxicology, (ISSN: 0278-6915), 58, 440-448. https://doi.org/10.1016/j.fct.2013.05.026

[7] Barnard, N. D., Bush, A. I., Ceccarelli, A., Cooper, J., de Jager, C. A., Erickson, K. I., ... & Morris, M. C. (2014). Dietary and lifestyle guidelines for the prevention of Alzheimer's disease. Neurobiology of aging, (ISSN: 0197-4580), 35, S74-S78. https://doi.org/10.1016/j.neurobiolaging.2014.03.033

[8] Sato, K., Suzuki, I., Kubota, H., Furusho, N., Inoue, T., Yasukouchi, Y., & Akiyama, H. (2014). Estimation of daily aluminum intake in Japan based on food consumption inspection results: impact of food additives. Food science & nutrition, (ISSN: 2048-7177), 2(4), 389-397. https://doi.org/10.1002/fsn3.114

[9] Stahl, T., Falk, S., Rohrbeck, A., Georgii, S., Herzog, C., Wiegand, A., ... & Brunn, H. (2017). Migration of aluminum from food contact materials to food—a

health risk for consumers? Part I of III: exposure to aluminum, release of aluminum, tolerable weekly intake (TWI), toxicological effects of aluminum, study design, and methods. Environmental Sciences Europe, (ISSN: 2190-4715), 29(1), 19. https://doi.org/10.1186/s12302-017-0116-y

[10] Djouina, M., Esquerre, N., Desreumaux, P., Vignal, C., & Body-Malapel, M. (2016). Toxicological consequences of experimental exposure to aluminum in human intestinal epithelial cells. Food and Chemical Toxicology, (ISSN: 0278-6915), 91, 108-116. https://doi.org/10.1016/j.fct.2016.03.008

[11] Khan, M. U., Malik, R. N., & Muhammad, S. (2013). Human health risk from heavy metal via food crops consumption with wastewater irrigation practices in Pakistan. Chemosphere, (ISSN: 0045-6535), 93(10), 2230-2238. https://doi.org/10.1016/j.chemosphere.2013.07.067

[12] Zhu, F., Fan, W., Wang, X., Qu, L., & Yao, S. (2011). Health risk assessment of eight heavy metals in nine varieties of edible vegetable oils consumed in China. Food and Chemical Toxicology, (ISSN: 0278-6915), 49(12), 3081-3085. https://doi.org/10.1016/j.fct.2011.09.019

[13] Huang, Z., Pan, X. D., Wu, P. G., Han, J. L., & Chen, Q. (2014). Heavy metals in vegetables and the health risk to population in Zhejiang, China. Food Control, (ISSN: 0956-7135), 36(1), 248-252. https://doi.org/10.1016/j.foodcont.2013.08.036

[14] Chen, Y., Wu, P., Shao, Y., & Ying, Y. (2014). Health risk assessment of heavy metals in vegetables grown around battery production area. Scientia agricola, (ISSN: 0103-9016 (P) | 1678-992X (O)), 71(2), 126-132. http://dx.doi.org/10.1590/S0103-90162014000200006

[15] Okada, I. A., Sakuma, A. M., Maio, F. D., Dovidauskas, S., & Zenebon, O. (1997). Evaluation of lead and cadmium levels in milk due to environmental contamination in the Paraiba Valley region of Southeastern Brazil. Revista de saude Pública, (ISSN: 0034-8910(O) | 1518-8787(P)), 31(2), 140-143. http://dx.doi.org/10.1590/S0034-89101997000200006

[16] Salazar, R. F. D. S., Alcântara, M. A. K. D., & Izário Filho, H. J. (2011). Evaluation of sample preparation methods and optimization of nickel determination in vegetable tissues. Revista brasileira de Ciência do Solo, (ISSN: 1806-9657), 35(1), 241-248. http://dx.doi.org/10.1590/S0100-06832011000100022

[17] Salazar, R. F. S., Afonso, S. S., dos Santos, D. G., Oishi, S. S. & Izário Filho, H. J. (2006). Determinação de oligoelementos (Cu, Fe, Zn) e

contaminantes inorgânicos (Al, Cd, Ni, Pb) em hortaliças consumidas na cidade de Lorena. *Revista Univap*, *13*(24), 34-37. https://www.researchgate.net/publication/235663925 _Determinacao_de_oligoelementos_Cu_Fe_Zn_e_co ntaminantes_inorganicos_Al_Cd_Ni_Pb_em_hortalic as_consumidas_na_cidade_de_Lorena

[18] Menegaes, J. F., Backes, F. A. A. L., Bellé, R. A., Swarowsky, A., & dos Santos Salazar, R. F. (2017). Evaluation of potential phytoremediation of chrysanthemum in soil with excess copper. Ornamental Horticulture, (ISSN: 2447-536X), 23(1), 63-71. https://doi.org/10.14295/oh.v23i1.915

[19] Wieteska, E., Zióek, A., & Drzewińska, A. (1996). Extraction as a method for preparation of vegetable samples for the determination of trace metals by atomic absorption spectrometry. Analytica Chimica Acta, (ISSN: 0003-2670(P) | 1873-4324(O)), 330(2-3), 251-257. https://doi.org/10.1016/0003-2670(96)00187-0

[20] Mukaeda, L. E., Taguchi, S. P., Robin, A., Izario, H. J., Salazar, F. S., & dos Santos, C. (2012). Degradation of Y2O3-stabilized ZrO2 ceramics in artificial saliva: ICP analysis of dissolved Y3+ and Zr4+ ions. In Materials Science Forum, (ISSN: 0255-5476(P) | 1662-9752(O)), 727, 1136-1141). Trans Tech Publications. https://doi.org/10.4028/www.scientific.net/MSF.727-728.1136

[21] Peixoto, A. L. D. C., Salazar, R. F. D. S., Barboza, J. C. D. S., & Izário Filho, H. J. (2018). Characterization of controlled landfill leachate from the city of Guaratinguetá-SP, Brazil. Revista Ambiente & Água, (ISSN: 1980-993X), 13(2), Epub Apr 05. http://dx.doi.org/10.4136/ambi-agua.2136

[22] AI-Ashmawy, M. A. (2011). Prevalence and public health significance of aluminum residues in milk and some dairy products. Journal of food science, (ISSN: 1750-3841), 76(3), T73-T76. https://doi.org/10.1111/j.1750-3841.2011.02064.x

[23] Alimentarius, C. (2001). Codex maximum levels for Cadmium in Cereals, Pulses and Legumes. Joint FAO/WHO Standards, CAC/GL, p. 39-2001. http://www.fao.org/tempref/codex/Meetings/CCFAC/ ccfac33/fa01_28e.pdf

[24] Alimentarius, C. (2001) Maximum Level for Lead - Codex Stan 210 – 2001*, Rev. 1-2003*. Joint FAO/WHO Standards, CAC/GL. http://www.wdfxw.net/goDownFiles.aspx?key=3339 2885

[25] Kim, K., Melough, M., Vance, T., Noh, H., Koo, S., & Chun, O. (2019). Dietary Cadmium Intake and Sources in the US. Nutrients, (ISSN: 2072-6643), 11(1), 2. https://doi.org/10.3390/nu11010002

[26] Izário Filho, H. J., Baccan, N., Kus, M. M., Soares, V. A., Salazar, R. F., Alcântara, M. A., & Nascimento, L. F. (2014). Determination of essential and toxic elements in maternal milk collected in Taubaté, São Paulo State, Brazil. Journal of Food, Agriculture & Environment, (ISSN: 1459-0255(P) | 1459-0263(O)), 12(1), 88-92. https://doi.org/10.1234/4.2014.4136