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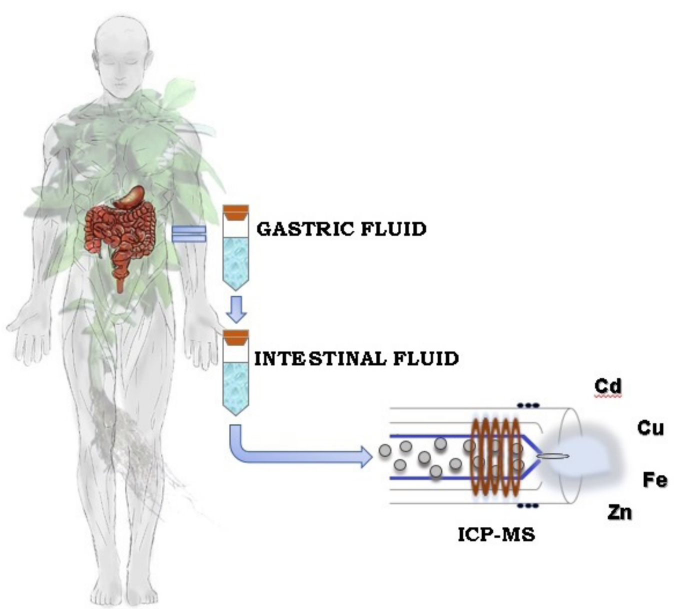
ARTICLE

In Vitro Bioaccessibility of Cd, Cu, Fe and Zn in Basil (*Ocimum basilicum* L. Grecco a palla) after Cadmium Intoxication

Sofia da S. Martins[1], **Giselle V. de Sousa**[1,2], **Vânia de Lourdes G. Teles**[1,3], **Letícia M. Costa***[1]

[1]*LEAQUAA, Universidade Federal de Minas Gerais, Instituto de Ciências Exatas, Departamento de Química, Av. Presidente Antônio Carlos, 6627, Pampulha, PO Box 702, CEP 31270-901, Belo Horizonte, MG, Brazil.*
[2]*LASP, Instituto de Ciências Biológicas, Universidade Federal de Viçosa, 35570-00, Florestal, MG, Brazil*
[3]*Centro de Tecnologia da Universidade Federal de Alagoas, Av. Lourival Melo Mota, S/N,Tabuleiro do Martins Maceió, AL, Brazil*



Cadmium is a toxic element which can be accumulated in the edible parts of plants compromising an entire food chain with serious damage to the living organisms, presenting synergistic and antagonistic effects with the elemental bioaccessibility. In this work, a simulated gastrointestinal digestion was performed to assess the *in vitro* bioaccessibility of Cd, Cu, Fe and Zn in basil samples after Cd intoxication. The hydroponic cultivation was made in a Hoagland solution at different concentrations (0, 1.5 and 3.0 µmol L^{-1}). Elemental concentration was achieved using a microwave-assisted acid digestion after the growing up of the plants in vermiculite pots by 15 days. The *in vitro* gastrointestinal procedure was applied in fresh and lyophilized leaves followed by a clean-up step in a sonoreactor cup horn using 1 mL of the extract, 100 µL of HNO_3 and 500 µL of H_2O_2 by 5 minutes. The results showed that Cd bioaccessibility was statistically different at 95% confidence level ($p < 0.05$) for the lyophilized and fresh leaves samples. The *in vitro* bioaccessibility increased with concentration in the contamination treatment. Moreover, a high positive correlation was observed between Cd-Fe and Cu-Zn, and a negative correlation between Cd-Zn and Fe-Zn in lyophilized and fresh leaves, respectively, suggesting that the absorption of essential elements was affected by Cd.

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INTRODUCTION

Cadmium is the third major contaminant in the environment, after Hg and Pb. It can be accumulated in edible parts of plants, and its bioaccumulation in the food chain is highly dangerous to all living organisms with negative long-time effects on human health.[1–3] Some foodstuffs, such as green leaves vegetables, fish and meat, may accumulate different levels of cadmium,[4] which is mainly reabsorbed in the human body by gastrointestinal, pulmonary and dermal systems.[1]

In plant tissues, several metal transporters and channels are involved into Cd reaching the root cells until its final accumulation in the edible parts, even when the element is present at low concentration.[3,5-7] Basil can uptake Cd from soil and water through their root cells, translocate and accumulate in the aerial part that contains edible parts for animals and humans.[6,8] Despite the fact that Cd is a non-essential element for plants, it can influence the uptake of nutrients.

Aerial parts of *Ocimum basilicum* L. are employed as a vegetable source for human consumption given their nutritional value in terms of natural minerals, trace elements and several characteristics of the herb, such as antioxidant, anti-aging, anti-inflammatory, anticarcinogenic, antimicrobial, cardiovascular, among others.[9] The economic relevance and global spread of basil is due to its multiple uses in cooking of different countries, both in its natural and dehydrated form, and in folk medicine and as essential oil in the cosmetic industry.[10–12]

Only a fraction of the mineral present in the food matrix is bioaccessible and can be absorbed by the human body.[13] Bioaccessibility is defined as the soluble fraction of a mineral that is released from its matrix food into the gastrointestinal tract, and refers to the maximum bioavailability and proportion of a contaminant ingested with food that is absorbed by the intestine, entering the systemic circulation with toxic effects.[14] It is typically evaluated by a sequential analysis with simulation digestion using artificial gastric and intestinal juices.[14,15] Recently, several studies have evaluated the bioaccessibility of mineral and trace elements by simulated gastrointestinal digestion in different food matrices, such as vegetables,[16–18] rice,[19,20] fruits,[21] yerba mate tea[22] and in rice.[23] The knowledge of the in vitro bioaccessibility of metals in vegetables is relevant for nutritional information and health risk assessment. The release of minerals in the gastrointestinal digestion process depends on different factors since in the aqueous solutions minerals with chemical similarities can compete for transport proteins or other uptake mechanisms, as well as for chelating organic substances, facilitating or hindering absorption.[24]

Therefore, it is pertinent to evaluate how bioaccessibility of toxic elements interfere with the absorption of nutrients by the human body during simulation of the gastrointestinal digestion. In this sense, the aim of this study was to investigate the influence of the lyophilized and fresh leaves of basil on the in vitro bioaccessibility of Cd, Cu, Fe and Zn, and the correlations established with Cd and these nutrients.

MATERIALS AND METHODS

Sample

Basil seeds (*Ocimum basilicum* Grecco a palla), purchased at a local market of Belo Horizonte, MG, Brazil, were germinated on Germitest paper for three days. Aluminium foil sheets with holes spaced at 2 cm were adapted into 1,000 mL pots to allocate the rootlets, which were cultivated in 5% v v^{-1} Hoagland nutrient solution.[25] Each pot contained thirteen seedlings, and their solutions were supplemented with 1, 1.5 and 3.0 µmol L^{-1} of Cd (as $Cd(NO_3)_2.4H_2O$) in triplicate. A control experiment was also performed without Cd supplementation. The nutrient solution and Cd supplementation were changed every 3 days, and after 15 days of hydroponic cultivation, the plants were transferred to vermiculite pots, irrigated daily with ultrapure water and kept on this substrate for another 15 days. Then, the leaves were harvested after 30 days of the beginning of the cultivation. Fresh leaves were split in two parts: one was used immediately while the other part was separated for freeze-drying.

Chemicals, materials and instrumentation

Reagents were of analytical grade, and all the solutions were prepared using ultrapure water (resistivity > 18.2 MΩ cm) obtained by a Direct-Q system (Millipore, SAS-67120, Malssheim, France). All glassware, plastic bottles and microwave vessels were cleaned overnight with 10% v v^{-1} HNO_3 and then washed with deionized water. Nitric acid (65% m m^{-1}) purified by a DuoPUR system (Milestone, Sorisole, Italy), and hydrogen peroxide (30% m m^{-1}) (Merck, Darmstadt, Germany) were used for the microwave digestion and ultrasound extraction. Hydrochloric acid (37% m m^{-1}, (Merck, Darmstadt, Germany), pepsin, pancreatin, bile salts, and sodium bicarbonate ($NaHCO_3$) employed in the in vitro gastrointestinal digestion were provided by Sigma- Aldrich.

A Milestone Ethos 1 − Advanced Microwave Digestion System oven (Sorisole, Italy) was employed for the basil leaves acid digestion. The leaves were frozen in a 0222E24, MODULYOD-230 Thermo Electron Corporation freeze-dryer (Asheville, USA) at 600 W. A thermostatic bath Dubnoff Quimis® (model 0226M2) and shaking table (model TS-2000ª VDRL SHAKER) were used in the in vitro gastrointestinal digestion procedure (IVG). All samples of IVG were pre-treated in a cup-horn ultrasonic processor (VCX 505, Sonic& Materials INC, USA), operating at 500W and 20 kHz before introduction in the ICP-MS.

An inductively coupled plasma mass spectrometer 7700 (Agilent Technology, Tokyo, Japan) was used for determination of Cd, Cu, Fe and Zn. A collision cell (He gas) was employed to correct isobaric interference and the instrumental parameters were: RF power 1.55 kW, plasma gas flow 15 L min^{-1}, auxiliar gas flow 1 L min^{-1}, nebulization gas flow 1.05 L min^{-1}, He flow rate in the collision cell 1.2 L min^{-1}, Micro-Mist nebulizer and Scott type – double pass nebulization chamber. The measurements were performed in He mode at an integration time, sweeps, replicates and resolution equal to 0.3 s, 100, 3 and <1, respectively. The monitored isotopes were ^{111}Cd, ^{63}Cu, ^{56}Fe and ^{66}Zn. Reference Cd, Cu, Fe and Zn solutions were prepared daily by an adequate dilution from a 10 µg mL^{-1} multi-element standard solution (ESI, M1-ICPMSE-100, USA). The calibration curve was prepared with six levels from 0 to 100 µg L^{-1} and 0 to 75 µg L^{-1} for microwave-assisted digestion and IVG, respectively.

Sample preparation

Fresh leaves from each plant were washed and dried on paper towels. For the total sample obtained for each Cd treatment (0, 1.5 and 3.0 µmol L^{-1}), 200 mg of fresh leaves were immediately subjected to IVG and the rest was frozen and lyophilized for at least 24 h. The lyophilized samples were weighed, macerated with mortar and pestle, and stored for the IVG and microwave-assisted acid digestion procedures.

Microwave-assisted acid digestion was used to determine the total concentration of the elements. A mass of 15 mg of leaves was weighed into PTFE-vessels in triplicate for each Cd treatment (0, 1.5 and 3.0 µmol L^{-1}). Volumes of 3.5 mL of HNO_3, 3.5 mL of H_2O and 1.0 mL of H_2O_2 were added separately to each vessel, which was submitted to a heating program in the microwave oven: 10 min until reached 165 °C, 20 min at 165 °C following by 30 min ventilation. Final volume was adjusted to 10 mL and the quantification was done by ICP-MS.

Determination of Cd, Cu, Fe and Zn bioaccessibility after the in vitro gastrointestinal digestion

The IVG simulation was developed according to the procedure described by the US Pharmacopeia.[26] The simulated gastric and intestinal solutions were daily prepared. The simulated gastric fluid was prepared by solubilization of 0.2 g of NaCl and 0.32 g of pepsin in deionized water, with subsequent addition of 0.7 mL of concentrated hydrochloric acid. The volume was filled up to 100 mL and the pH adjusted to 1.2.[15,27] The intestinal fluid was prepared by diluting 0.68 g of K_2HPO_4, 1 g of pancreatin, 1.25 g of bile salts and addition of 7.7 mL of NaOH 0.2 mol L^{-1}. Final volume was completed to 100 mL and the pH adjusted to 6.8 - 7.0 using 3% m v^{-1} $NaHCO_3$.[27]

3.5 mL of gastric fluid was added in 200 or 15 mg of fresh and lyophilized leaves, respectively. The mixture was submitted to slow stirring (approximately 60 rpm) at 37 °C in a thermostatic bath for 2 hours. Then, the tubes were transferred to an ice bath for 5 minutes in order to stop the enzymatic activity.

Subsequently, for intestinal digestion, 0.4 mL of $NaHCO_3$ + 3 mL of intestinal fluid was added to the gastric digest. Again, the solution was stirred in a thermostatic bath for 2 h at 37 °C, with constant stirring. [27]

Each assay was performed in triplicate (including blanks), and the final volume was adjusted to 10 mL using ultrapure water following centrifuged for 10 minutes at 10000 rpm at 4 °C. The clean-up step at sonoreactor cup horn was performed with 1 mL digested, 100 µL HNO_3 and 500 µL H_2O_2 by 5 minutes. Final volume was filled up to 2 mL with ultrapure water and the samples were centrifuged for 5 min at 12,000 rpm to prevent clogging of the ICP-MS nebulization system.

The following formula described by Leufroy (2012)[28] was adopted to obtain the percentage (%) of bioaccessibility of the elements:

$$\%\text{Bioaccessibility} = \left(\frac{\text{Fraction of element released}}{\text{Total element concentration}}\right) \text{x } 100$$

in which the fraction of the element released in the simulated digestion is compared to the total amount of the element.

Data treatment

The differences between bioaccessible fraction in lyophilized and fresh leaves for each Cd treatment were tested by a *t*-test ($p < 0.05$) using GraphPad Prism 5.01. The correlation plot for total concentration of elements in leaves and bioaccessibility were calculated by the software R[28] applying Pearson test with significance of regression for $p < 0.05$.

RESULTS AND DISCUSSION

The evaluated Cd concentrations (0, 1.5 and 3.0 µmol L^{-1}) were below the effective concentration that inhibits 50% of basil roots enhancement (EC_{50} = 3.6 µmol L^{-1} of Cd), as reported by Teles et al. (2022).[6] Previous studies reported bioaccumulation of Cd preferentially in the root, but the element can also be translocated until the leaves.[5,6,8]

To obtain samples with healthy aspects which would not be rejected by consumers, the samples were grown in vermiculite, an inert substrate, to complete the development cycle, after 15 days of hydroponic culture with Cd supplementation. Figure 1 (A, B and C) shows the seedlings at the end of 15 days in the hydroponic cultivation, while Figure 2 presents the samples after 30 days grown in vermiculite, respectively.

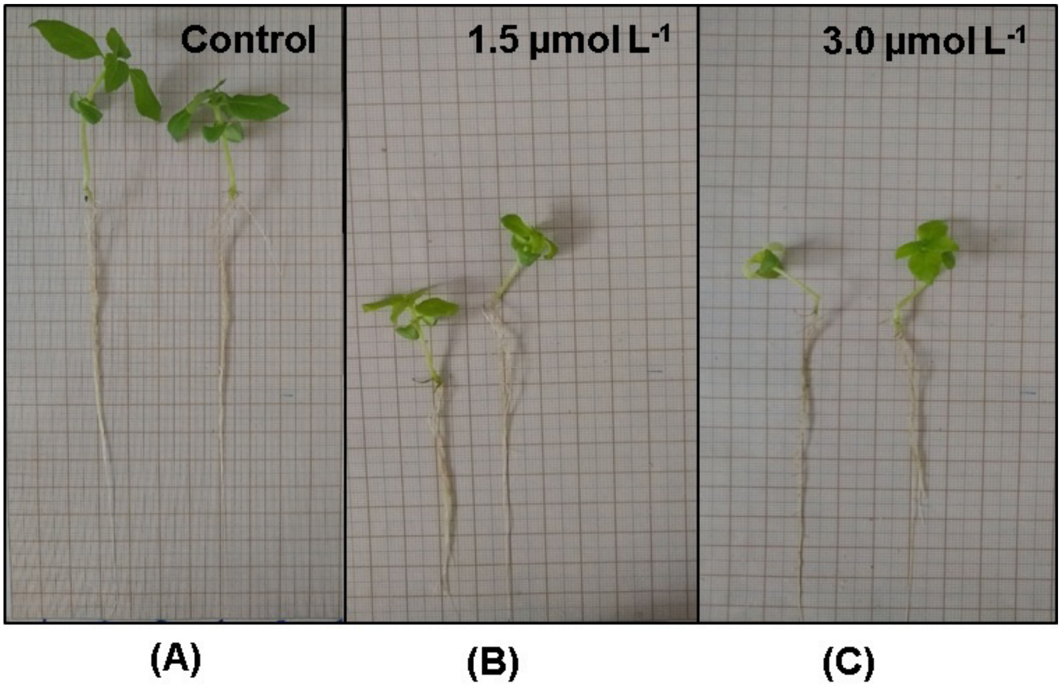

Figure 1. Basil samples after 15 days of hydroponic cultivation in Hoagland's solution and Cd supplementation at different concentrations 0 μmol L^{-1} - control (A), 1.0 μmol L^{-1} (B) and 3.0 μmol L^{-1} (C) for immediate transplanting into vermiculite substrate.

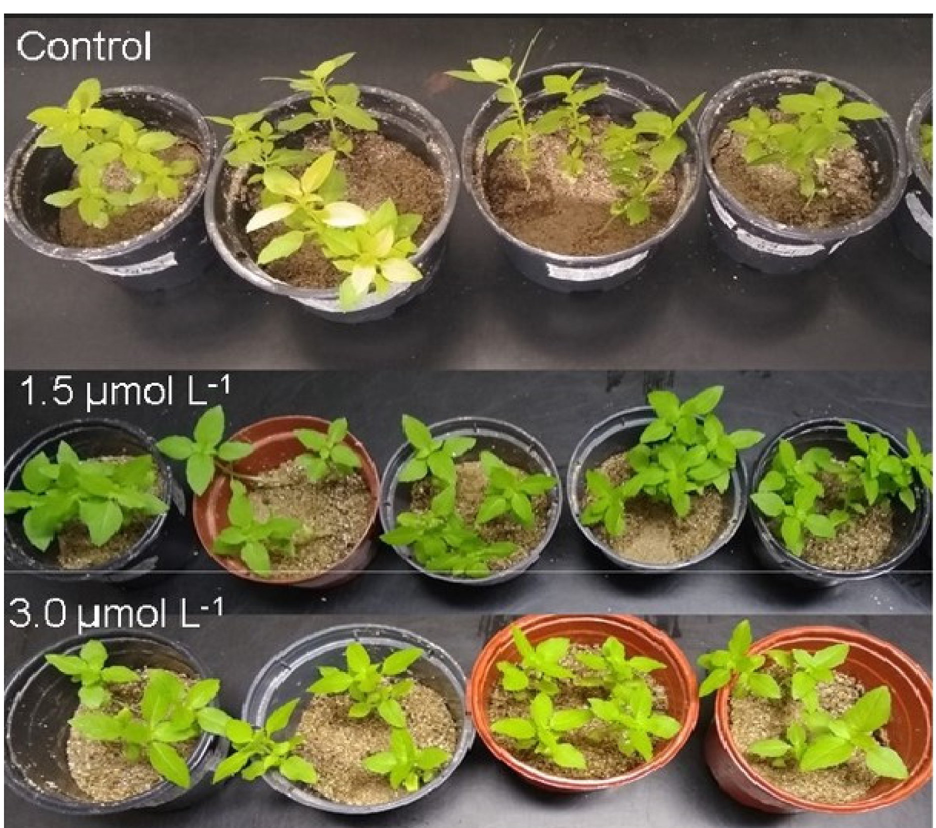

Figure 2. Basil samples after 30 days cultivation (15 days of hydroponic cultivation and 15 days in vermiculite substrate).

Some important characteristics were identified in the basil seedlings exposed to Cd (Figure 1) such as chlorosis on the leaves, stunting and reduced root length and number/size of leaves, which corroborate with the effects of Cd on plants reported in the literature.[3,10,29] These results justified the transplanting to vermiculite substrate to promote the development of the basil seedlings (Figure 2). Increasing Cd concentration compromised the accumulation of biomass in basil leaves. The biomasses in leaves cultivated with 1.0 and 3.0 µmol L^{-1} of Cd were on average 83% and 41% lower than that of control plants, respectively. Cd toxicity can be related to the stunted plant growth, negatively affecting water and nutrient uptake and translocation, photosynthesis, carbon and nitrogen assimilation, and oxidative stress.[3]

Bioaccessible fractions of minerals and their correlations

The limit of detection (LOD) and quantification (LOQ) of microwave-assisted acid digestion and IVG digestion procedures for Cd, Cu, Fe and Zn are presented in Table I. The LOD and LOQ are defined as $3.3*s/m$ and $10*s/m$, respectively, where s is the standard deviation corresponding to 10 analytical blanks and m is the slope of the calibration curve.[31] The analytical blanks of the gastrointestinal digestion have also undergone the clean-up procedure.

Table I. Limit of detection (LOD) and quantification (LOQ) of Cd, Cu, Fe and Zn in basil leaves after microwave-assisted acid digestion (MW) and gastrointestinal digestion procedures

Elements	Microwave-assisted acid digestion (MW)		Gastrointestinal digestion	
	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	LOD (µg kg^{-1})	LOQ (µg kg^{-1})
Cd	0.071	0.230	0.288	0.951
Cu	0.602	2.00	7.95	26.2
Fe	10.7	35.3	11.4	37.8
Zn	8.44	27.9	0.989	3.26

Table II displays the total concentration (µg g^{-1}) of Cd, Cu, Fe and Zn after microwave-assisted acid digestion (MW) and bioaccessible fraction. Cadmium content in basil leaves is directly proportional to the exposure concentration during cultivation. Total concentration of Cu and Zn also increased proportionally with Cd intoxication while for Fe it was observed a decreased of its concentration. According to the Brazilian legislation,[32] Cd concentrations in leafy vegetables must be lower than 0.20 mg kg^{-1}. The humidity content in the basil leaves was around 94.8 ± 0.2%. So, MW total concentration for Cd treatments of 1.5 and 3.0 µmol L^{-1} in wet weight is around 1.2 and 2.3 mg kg^{-1}, respectively with both values exceeding the legislation. However, the total amount of the elements present in food does not reflect the amount absorbed and metabolized by the human body. In fact, *in vitro* experiments may elucidate the mechanism of absorption and correlation with other essential nutrients present in the physiological concentrations that can be absorbed by the human body.

In this study, the IVG digestion was performed according to Bertin et al. (2016)[15] and Nascimento (2011)[27] with the addition of the clean-up step by sonication of the extracts in the cup horn reactor in order to prevent clogging of the ICP-MS nebulization system. The results showed that the Cd bioaccessible fraction differed statistically ($p < 0.05$) in lyophilized and fresh leaves and as well as contamination level (1.5 µmol L^{-1} and 3.0 µmol L^{-1}). The bioaccessible fraction in lyophilized leaves is higher than in fresh ones and directly proportional to the Cd concentration.

The bioaccessibility of Cd in samples was approximately 51% and 45% in the contaminated leaves at 1.5 µmol L^{-1} and 3.0 µmol L^{-1}, respectively (Table II). No significant difference (*t* test: $t_{experimental} < t_{critical}$, $p < 0.05$) was found in the bioaccessible Cd concentration for the gastrointestinal extracts when comparing lyophilized and fresh samples with the increase of Cd concentration. This result showed that the treatment with 3 μmol L^{-1} of Cd was not statistically different from those obtained with 1.5 μmol L^{-1}, even increasing twice the concentration level.

The Cd daily intake for lyophilized and fresh leaves at 1.5 μmol L^{-1} was calculate as 0.2 and 0.095 μg kg^{-1}, respectively, using the bioaccessible fraction related to the body weight of an adult (60 kg) that regularly consumes 1 g of basil leaves (aromatic herb added to food), as described by Schmite et al. (2019)[22]. Similarly, for lyophilized and fresh leaves at 3.0 μmol L^{-1} of Cd were 0.35 and 0.2 μg kg^{-1}, respectively. Considering that the acceptable monthly dose of Cd is 25 μg kg^{-1} (60 kg body weight),[22] the values of consumption per adult were 0.145 and 0.07 μg kg^{-1} and 0.262 and 0.142 μg kg^{-1} for lyophilized and fresh leaves at 1.5 and 3.0 μmol L^{-1}, respectively. These values indicated that basil leaves obtained under this condition are a food-safe product, representing only 0.29−0.60 and 0.57–1.05% for leaves contaminated at 1.5 and 3.0 μmol L^{-1}, respectively. These results were similar to those obtained by Schmite et al.[22]

Several factors can increase Cd uptake through the human gastrointestinal, such as low intakes of vitamin D, Ca and trace elements as Cu and Zn.[1] Several trace elements, such as Fe, Zn and Cu are components of enzymes, and should be supplied to the human body, preferably from the diet.[13] Our findings showed that bioaccessible fractions of elements also depend on how the samples are consumed (lyophilized or fresh leaves) as they differ statistically at 95% confidence (*t*-test, $t_{experimental} < t_{crit}$), except for Cu in the 1.5 μmol L^{-1} contaminated leaf and Fe in all samples ($p < 0.05$, Table II). The total Cu bioaccessible fraction concentration ranged from 7.4 to 16 μg g^{-1} and 12 to 39 to lyophilized and fresh leaves, respectively. The Recommended Daily Allowance (RDA) as published by U.S. Food and Drugs Administration of Cu is 0.9 mg day^{-1}.[33] Total Cu concentration ranged from 0.013 to 0.049 mg g^{-1}, showing that 1 g of basil presented 1.4 to 5.1% of RDA considering the daily intake of an adult (60 Kg). The bioaccessibility was greater in the fresh leaves when compared to the lyophilized. The total Fe bioaccessible fraction concentration ranged from 237 to 240 μg $g^{-1}$and from 262 to 313 μg g^{-1} for the lyophilized and fresh leaves, respectively. The RDA to Fe is 18 mg day^{-1} and the total concentration ranged from 0.405 to 0.759 mg g^{-1}, showing that 1 g of basil presented 2.2 to 4.2% of RDA. The Fe bioaccessible was greater in the fresh leaves (daily intake for 60 Kg adult).

For Zn, the bioaccessible fraction concentration varied from 70 to 158 μg g^{-1} and 37 to 53 μg g^{-1} for the lyophilized and fresh leaves, respectively. The Zn bioaccessibility in the lyophilized leaves was higher than that obtained for fresh leaves (*t* test $p < 0.05$) at all levels of Cd contamination. The RDA of Zn is 11 mg day^{-1} and the total concentration ranged from 0.074 to 0.234 mg g^{-1}, showing that 1 g of basil presented 0.67 to 2.13% of RDA. On the other hand, the bioaccessible fraction was smaller to fresh leaves when compared to the lyophilized (daily intake for 60 kg adult) (Table II).

Previous studies showed that Cd interferes with the uptake and translocation of different nutrients and positive correlations between Cd-Fe and Cd-Cu and weak negative correlations between Cd-Zn were observed to basil leaves.[6] Then, Pearson correlation (Figures 3 A and B) of the bioaccessibility in lyophilized and fresh leaves was used to find out which elements positively correlated with one another (blue) and those elements that showed negative correlations with each other (red).

Table II. Total concentration (μg g^{-1}) of Cd, Cu, Fe and Zn after microwave acid digestion (MW) and bioaccessibility (%) in the lyophilized and fresh leaves after *in vitro* gastrointestinal digestion (n = 3) ± sd

Cd treatment (μmol L^{-1})	Elements	MW total concentration (μg g^{-1})	Lyophilized leaves		Fresh leaves		Daily intake* μg kg^{-1} (body weight)	
			Bioaccessible fraction (μg g^{-1})	Bioaccessibility (%)	Bioaccessible fraction (μg g^{-1})	Bioaccessibility (%)	Lyophilized leaves	Fresh leaves
0	Cd	<LOQ	<LOQ	-	-	-	-	-
	Cu	12.6 ± 1.0	7.38 ± 0.46	58.6 ± 5.9	11.8 ± 2.3	93.6 ± 19.7	0.123	0.197
	Fe	759 ± 46	250 ± 7 [a]	32.9 ± 2.2	262 ± 10 [a]	34.5 ± 2.5	4.17	4.37
	Zn	74.3 ± 5.3	70.3 ± 2.3	94.9 ± 7.5	36.6 ± 16.0	49.5 ± 2.1	1.17	0.610
1.5	Cd	23.3 ± 1.2	11.9 ± 0.7	51.3 ± 4.0	5.7 ± 0.6	24.7 ± 2.9	0.198	0.095
	Cu	20.4 ± 2.3	15.6 ± 1.8 [b]	76.4 ± 12.2	20.2 ± 4.8 [b]	99.2 ± 26.3	0.260	0.337
	Fe	428 ± 27	240 ± 11 [c]	56.0 ± 4.4	276 ± 69 [c]	64.5 ± 16.6	4.00	4.60
	Zn	123 ± 16	98.3 ± 12.6	79.7 ± 14.6	42.5 ± 0.46	34.4 ± 4.5	1.64	0.708
3.0	Cd	46.3 ± 1.9	21.0 ± 0.9	45.4 ± 2.7	11.4 ± 1.2	24.6 ± 2.7	0.350	0.190
	Cu	49.0 ± 5.3	13.9 ± 1.0	28.4 ± 3.7	38.7 ± 8.4	78.9 ± 19	0.232	0.645
	Fe	405 ± 27	237 ± 29 [d]	58.4 ± 8.1	313 ± 64 [d]	77.2 ± 17	3.95	5.22
	Zn	234 ± 16	158 ± 38	67.7 ± 16.7	53.2 ± 5.0	14.6 ± 2.2	2.63	0.89

*Based on the bioaccessible fraction of element to calculate the intake dose by an adult of 60 kg body weight who regularly consumes 1g of basil.
Equal letters to bioaccessible fraction indicate no significant difference by *t*-test ($p < 0.05$).

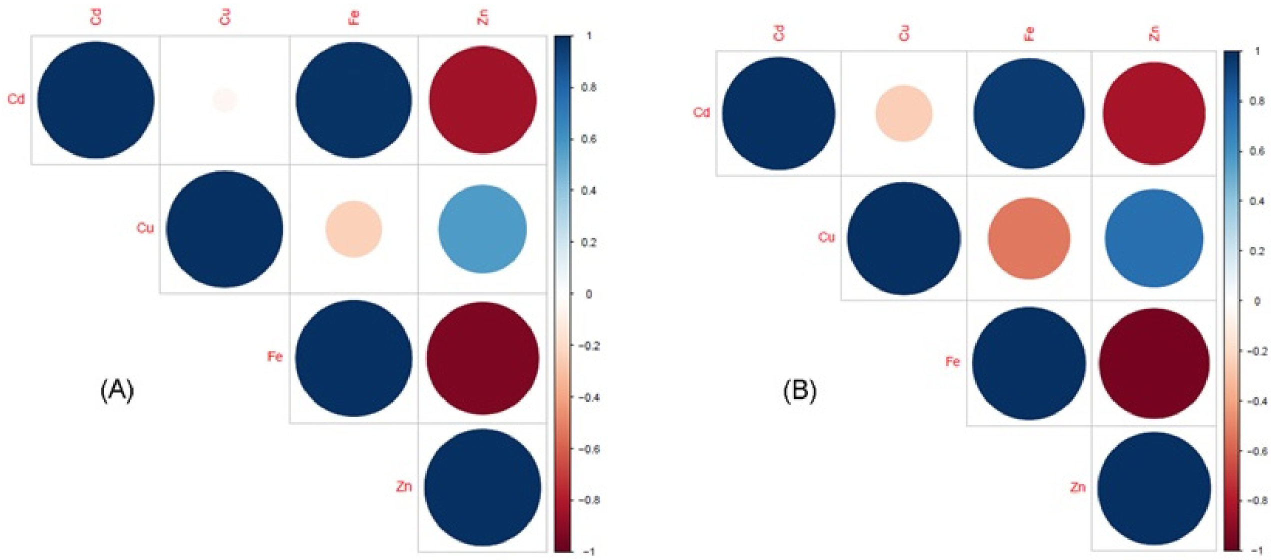

Figure 3. Correlogram matrix for bioaccessibility in basil samples, which grew at different Cd concentrations of 0, 1.5 and 3.0 μmol L^{-1} (A) lyophilized leaves (B) fresh leaves. Pearson's correlation analysis with the significance levels of $p < 0.05$.

In general, the correlograms presented similar correlations of both samples (Figure 3 A and B), demonstrating that the form of consumption did not affect bioaccessibility of elements, except for Cd-Cu ($r > -0.04$, Pearson test) in lyophilized leaves. However, a positive correlation between Cd-Fe and Cu-Zn and negative correlation between Cd-Zn and Fe-Zn were observed in lyophilized and fresh leaves, showing the strongest correlation attested by Pearson's (r > than 0.75). Indeed, *in vitro* bioaccessibility is an estimation of the *in vivo* bioaccessibility and may not reflect the fraction that is available for gastrointestinal metal absorption, once *in vivo* involves a more complex metabolism of the elements in gastrointestinal digestion. According to Sun et al. (2019),[20] *in vivo* bioavailability tests are likely more physiologically relevant and, when possible, should be employed in risk assessments of human exposure to Cd *via* rice consumption.

Our findings showed that Cd in fresh leaves negatively affects the absorption of Cu, but the opposite happens with Fe, resulting probably in a negative correlation between Cu-Fe (Figure 3B). In human health, Cu is recognized as an essential trace element with function of cofactor of many redox enzymes and immune functions as well as involved in the Fe metabolism.[34,35]

Iron is a versatile, essential element in plant metabolism and human health with function related to the synthesis of haemoglobin and myoglobin, and plays a critical role in many metabolic processes such as oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transportation.[34,35] People with low iron supplies present important metabolic parameter for cadmium uptake.[1] Our findings showed synergic effects of bioaccessibility of Cd and Fe.

Competition studies suggested that several other potentially toxic metals may share the iron intestinal absorption pathway, for example, Zn,[36] which may be related to the negative correlation between bioaccessibility of Fe-Zn in both samples of this study. In contrast to the synergistic effect of bioaccessibility of Cd with Fe, there is also an antagonistic effect between Cd-Zn. Concerning Zn, it is assumed that their molecular homology and divalent cation could be a reason for a compensatory higher Cd resorption.[1] After iron, zinc is the second most abundant metal ion in organisms[18] and it is an essential key nutrient for several biochemical activities, such as in human health. Zinc is essential for maintaining the structure and activity of many enzymes, besides playing a key role in the synthesis of nucleic acids and proteins.[34]

The results indicated that the presence of Cd causes synergic interferences with Fe and antagonic with Zn for both leaves, but antagonic with Cu to fresh leaves. In addition to the toxicity of Cd to human health, this toxic metal also influences the absorption of essential elements to the human body.

CONCLUSION

Cadmium contamination in basil samples provided a new insight for obtaining correlations of *in vitro* bioaccessibility with the essential elements Cu, Fe and Zn as a consequence of Cd uptake, absorption and translocation. In addition, it could be observed that Cd bioaccessible fraction in lyophilized leaves is greater than in fresh leaves and directly proportional to its concentration. It was also demonstrated that Cd *in vitro* bioaccessibility did not range with the increasing of its concentration in the intoxication treatments.

Conflicts of interest

The authors declare that they have no conflict of interest.

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