

Organic vs conventional agriculture: evaluation of cadmium in two of the most consumed vegetables in Brazil

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Abstract

Cadmium (Cd) concentrations were evaluated in conventionally and organically grown foods. The samples were prepared, submitted to acid extraction and analyzed by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The mean concentration of Cd found in organic lettuce samples was 0.0811 ± 0.0367 mg kg⁻¹, while in conventional lettuce samples it was 0.1549 ± 0.0266 mg kg⁻¹. Organic carrot samples had a mean concentration of Cd of 0.1064 ± 0.0553 mg kg⁻¹, while samples of carrots cultivated by the conventional method had a mean concentration of 0.1174 ± 0.0780 mg kg⁻¹. It was observed that conventionally cultivated foods in individual evaluations presented concentrations of 1.2 to 3.1 times higher of Cd when compared to organic vegetables. The Brazilian legislation regarding the detection of Cd is established by RDC n° 42. It can be inferred that the average concentrations found in this study are within the values established by the legislation. When considering Cd exposure through vegetable consumption by evaluating the estimated daily metal intake (EDI) and the target hazard quotients (THQ), the samples did not present a potential health risk.

Keywords: vegetables; cadmium; organic; conventional; estimated daily metal intake; food safety.

Practical Application: Compare cropping systems (conventional and organic) in order to determine Cd exposure by evaluating the estimated daily metal intake (EDI) and the target risk quotient (THQ).

1 Introduction

The growth of the world population has increased anthropic activities with the purpose of providing means for survival. Industrial and agricultural areas grow in proportion to the number of inhabitants, in order to meet the needs generated. Food supply must be associated with food safety, which is a public health concern (Arisseto-Bragotto et al., 2017).

There is an increase in the consumption of vegetables that is related to the growing awareness of the nutritional value of plant foods, as they are an important source of carbohydrates, vitamins, minerals and fiber (Hadayat et al., 2018). However, studies have pointed out the risks of foods contaminated by trace-level toxic metals (Gupta et al., 2021; Kasozi, et al., 2021; Reboredo et al., 2019; Thompson & Darwish, 2019; González et al., 2019), with several studies carried out in Asia (Ahmed et al., 2019; Sawut et al., 2018; Hu et al., 2017; Hu et al., 2013), in North and South America (Araújo et al., 2019; Dala-Paula et al., 2018; Hadayat et al., 2018; Correia et al., 2018; França et al., 2017; Corguinha et al., 2015), in Africa (Edogbo et al., 2020; Hattab et al., 2019; Ametepey et al., 2018) and in Europe (Defarge et al., 2018; Hurtado-Barroso et al., 2019).

It should be noted that the food chain is an important route for human exposure to toxic metals, as these have a great capacity for bioaccumulation in plants and a long half-life of 10 to 35 years (World Health Organization, 2020; Gupta et al., 2019; Correia et al., 2018; Paltseva et al., 2018).

Human exposure to cadmium (Cd), undesirable even in trace concentrations, occurs mainly through food consumption (World Health Organization, 2020). However, there are several sources of food contamination by Cd: deposition of particulate matter with a metal associated with it from pollution caused by industrial and vehicular emissions (Gupta et al., 2019; Kibblewhite, 2018; França et al., 2017); contaminated soil (Yang et al., 2018); irrigation with contaminated water (Ahmed et al., 2019; Islam et al., 2018); use of pesticides or chemical fertilizers during cultivation (Wang et al., 2018; Reboredo et al., 2019; Parente et al., 2019), among others.

Cd is highly undesirable even in trace concentrations, as it causes adverse effects in the human body, such as disturbances in calcium metabolism, osteomalacia and osteoporosis, bone fractures, renal dysfunction, coronary heart disease and hypertension, endocrine dysfunction, cancer (lung, kidneys and prostate), mutagenicity and genotoxicity (World Health Organization, 2020; Duncan et al., 2018; El-Kady & Abdel-Wahhab, 2018; Dala-Paula et al., 2018; Barregard et al., 2016).

Due to the public's growing concern with nutritional safety and quality, the consumption of organic foods is increasing (Aitken et al., 2020). In addition to reducing pesticides, these organic foods have higher amounts of polyphenols and, in general, lower amounts of trace-level toxic metals, such as Cd (Hurtado-Barroso et al., 2019).

In general, consumers believe that organic foods are safer and healthier than conventional ones (Gomiero, 2018). It is noteworthy

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that truly comparative studies between conventional and organic foods are recent (Cámara-Martos et al., 2021; Araújo et al., 2019; González et al., 2019; Gomiero, 2018; Hadayat et al., 2018; Hurtado-Barroso et al., 2019; Garcia & Teixeira, 2016; Bressy et al., 2013). Doubtful and questionable data have already been obtained in this comparison, thus, there is still controversy as to whether organic products are safer from the point of view of contamination by toxic metals at a trace level than conventionally grown products since, despite the organic label, it is very likely that environmental contamination occurs in both forms of cultivation (Siwulski et al., 2021; González et al., 2019; Gomiero, 2018; Krejčová et al., 2016).

Hattab et al. (2019), through graphite furnace atomic absorption spectrometry (GFAAS), observed that concentrations of Cd in organic lettuce showed an increase of up to 7.6 times when compared to conventionally cultivated. Unexpectedly, in organic lettuce, the value found for Cd was $0.47 \mu\text{g g}^{-1}$, higher than the maximum limits allowed in the standard guidelines Food and Agriculture Organization (2016).

Krejčová et al. (2016) evaluated essential and toxic metals in conventionally and organically grown carrot samples. In this study, it was found that there was no difference between the two forms of cultivation, and no potential damage resulting from contamination was diagnosed. Li et al. (2015) determined the concentration of Cd in several vegetables, with the highest concentrations found in lettuce leaves. Gaweda et al. (2012) evaluated trace-level toxic metals, including Cd, in carrot samples produced in conventional and organic cropping systems. The authors found concentrations of about 15-20% lower in organic cultivation compared to those produced conventionally.

Thus, establishing acceptable limits for metals in food is an important tool for ensuring the food safety of a population, regulating food production and ensuring public health (Liu et al., 2018). Several indices can be used to assess the intake and harmful effects of chronic exposure to toxic metals at the trace level, including the Provisional Tolerable Monthly Intake (PTMI), which represents the amount of the substance present in the food that can be ingested daily throughout life without adverse health effects. The PTMI for Cd is $25 \mu\text{g kg}^{-1}$ body weight/month (World Health Organization, 2021).

In this work, lettuce (*Lactuca sativa L.*) and carrot (*Daucus carota L.*) were studied, which, according to the last report of the Program for the Analysis of Pesticide Residues in Food (PARA), carried out in Brazil, presented pesticide residues not allowed for their culture, thus bringing risks to consumers (Brasil, 2020).

Given the above, the occurrence of toxic trace metals in foods, regardless of their organic or conventional origin, needs to be monitored. Therefore, the aim of this study was to compare Cd concentrations in lettuce and carrot samples grown using conventional and organic techniques, with certification seal, sold in retail markets in the North Zone of Rio de Janeiro - RJ, and assess the health risk of Cd intake through food consumption.

2 Materials and methods

2.1 Materials and reagents

All plastic material and glassware used were decontaminated for 24 hours in a 5% Extran solution (CAS No: 1310-73-2) ($v v^{-1}$)

and 48 hours in a 10% nitric acid solution ($v v^{-1}$). Subsequently, they were rinsed three times with deionized water and dried at 40°C , as described in the 3050B method (Hadayat et al., 2018; United States Environmental Protection Agency, 1996).

The reagents used in preparing the solutions and in the chemical analysis of the process are analytical grade (PA) or suprapur. The provenances of the reagents used were Extran Merck (Elmsford, NY USA), nitric acid (CAS No: 7697-37-2; Merck 65% PA) and 30% hydrogen peroxide (CAS No: 7722-84-1; Merck).

The standard solution was prepared each day of analysis by appropriate dilutions of the Multi-element standard solution (Merck) was used with appropriate dilution to prepare the calibration curve.

2.2 Collection and preparation of vegetable samples

Three samples of lettuce and carrots cultivated by conventional methods and three samples organically cultivated certified with the organic label of the Brazilian System of Organic Conformity Assessment - SisOrg (Brasil, 2014) were acquired in markets in the North Zone of the Metropolitan Region of Rio de Janeiro, Brazil, from December 2019 to March 2020, totaling 12 samples, which were analyzed in triplicate.

The samples were ground and homogenized using a Mixer (Philips, Brasil) with a stainless steel blade. After that, the samples were placed in a previously decontaminated watch glass and submitted to an oven at $65^\circ\text{C} / 72 \text{ h}$ (Hadayat et al., 2018).

2.3 Sample digestion and analysis

The extraction procedure was based on the 3050B method with modifications. 0.5 g of dry sample was weighed in falcon tubes, in triplicate, using an analytical balance (Ohaus, Brazil). After adding 5 mL of 1:1 nitric acid (HNO_3), the tubes were placed in an ultra thermostatic water bath (Fanen, Brazil) at $100^\circ\text{C} / 5 \text{ h}$. After cooling to room temperature, 1 mL of hydrogen peroxide (H_2O_2) was added. After 24 hours, the samples were filtered on C41 quantitative filter paper and bulked up in 20 mL falcon tubes with deionized water. Then, the samples were centrifuged at 3,000 rpm for 10 minutes to check for the presence of supernatants. In all extraction procedures, a blank was performed.

2.4 Determination of Cd using GFAAS

After digestion of the lettuce and carrot samples, the extracts were analyzed to determine the cadmium concentration in a Graphite Furnace Atomic Absorption Spectrometer (GFAAS; AA Perkin Elmer PINAAcle 900T). The Software used to process the data was WinLab32.

In programming the equipment, the operating parameters were: wavelength (228.8 nm), slit (0.7 nm), detection level (0.002 mg L^{-1}), sensitivity (0.025 mg L^{-1}) and linear range (up to 2.0 mg L^{-1}).

The instrumental parameters used are shown in Table 1. The injection temperature was 20°C and the diluent used in the samples was 0.2% HNO_3 . The volume of modifier (palladium

Table 1. Temperature program for cadmium determination in lettuce and carrot samples.

Stage	Temperature (°C)	Time (s)		Gas flow (mL min ⁻¹) (Argon) (0-300)	Note
		Ramp	Hold		
1	110	1	30	250	
2	130	15	30	250	
3	500	10	20	250	
4	850	10	20	250	
5	1600	0	5	0	Absorbance Reading - Atomization
6	2400	1	3	250	Cleaning

nitrate and magnesium nitrate) injected was 20 µL and the sample volume injected was 10 µL.

The analytical curve (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 µg L⁻¹) was prepared on each day of analysis using Multi-element standard solution (Merck) and used for sample quantification. Linearity was obtained by linear regression using the least squares method, where R² > 0.99. The limit of detection (LOD) and the limit of quantification (LOQ) were estimated using values obtained by extrapolating three calibration curves at five concentration levels in triplicate. The values were determined according to Equations 1 and 2 (Brasil, 2003a):

$$LOD = \frac{3,3 X \sigma}{SV} \quad (1)$$

$$LOQ = \frac{10 X \sigma}{SV} \quad (2)$$

Where, σ = standard deviation of the three values of the area where each line intersects the y axis, SV = mean of the three slope values of the calibration curve (SV = slope variation).

The LOD and LOQ were 0.004 and 0.012 mg kg⁻¹ for cadmium. The limits of detection (LOD) and quantification (LOQ) of each analyte were calculated as the analyte concentration that corresponded to three and ten times, respectively, the triplicate standard deviation of the intercept of the calibration curve with the y axis, divided by the mean of the triplicates of the slope of the calibration curve.

2.5 Lettuce and carrot consumption by the studied population

In order to obtain the consumption of the studied vegetables, in the usual way over a period, the Food Frequency Questionnaire (FFQ) was used, a method commonly used to verify the association of diet and disease (Pedraza & Menezes, 2015). The FFQ allows the assessment of food consumption in the usual way over a period, being a retrospective method, which ensures that its application will not influence the results (Araujo et al., 2010).

The FFQ, created on Google Forms, a survey management application, was randomly sent to adult consumer groups and residents of the metropolitan region of Rio de Janeiro, through Whatsapp.

In the present study, 300 people answered the questionnaire sent electronically, where they were asked about lettuce and carrot consumption and food frequency. The semi-quantitative

FFQ initially asked “How many times have you consumed this food item in the last 3 (three) months?”, with the options ≥ 2 times/day; 1 time/day; 2-4 times/week; 1 time/week, 2-3 times/month; 1 time/month or never. Regarding the quantity, the questionnaire asked “When you consume the vegetable, how much?”. Possible answer choices were 2, 3, or 5 tbsp (tablespoons/time). According to the table for evaluating food consumption in household measures, each tablespoon represents, when used for lettuce, 8 g each, and for carrots, 12 g (Pinheiro et al., 2005).

The responses obtained were statistically analyzed (Microsoft Office - Excel version 15.0.4569/2013 integrated with Action Stat Pro 3.4.124.1308-3).

2.6 Health risk assessment of vegetable consumption

Chronic exposure to Cd from the consumption of contaminated food can be a relevant risk to human health in many regions (Åkesson et al., 2014). According to the WHO Codex Alimentarius (Food and Agriculture Organization, 2016), the daily exposure to metals can be assessed using the metal concentration values in the vegetable, the daily vegetable consumption and the average body weight of the population.

To estimate health risk from exposure to metals, several studies have used the concepts of daily dietary intake of metals (DDI) or estimated daily intake (EDI), Hazard Index (HI), Target hazard quotients (THQ), and Target Cancer Risk (TCR) (Gebeyehu & Bayissa, 2020; Gupta et al., 2019; Guo et al., 2019; Liang et al., 2019; Alam et al., 2018; Varol et al., 2017; Antoine et al., 2017).

Exposure to trace-level toxic metals through vegetable consumption can be estimated using the EDI, presented in Equation 3 (Gebeyehu & Bayissa, 2020; Guo et al., 2019; Sultana et al., 2017).

$$EDI = \frac{E_f \times E_D \times F_{IR} \times C_M \times C_F}{B_W \times T_A} \times 0.001 \quad (3)$$

Where E_f is the frequency of exposure (days in the year), E_D is the exposure time (average age of the population studied, in years), F_{IR} is the average consumption of the vegetable per day (g person⁻¹ day⁻¹), C_M is the metal concentration (mg kg⁻¹), C_F is the conversion factor from concentration to weight of fresh vegetables to dry weight (0.085), B_W is the average adult weight in the studied population and T_A is the average exposure time for non-carcinogens (365 days/year $\times E_D$). The value 0.001 represents a unit conversion factor.

The THQ assesses the non-carcinogenic risk of vegetable consumption through the EDI values ($\text{mg}\cdot\text{day}^{-1}\text{ kg}^{-1}$ of body weight) and the RfD ($\text{mg}\text{ kg}^{-1}\text{ day}^{-1}$), which is the oral reference dose. The RfD is characterized by the maximum amount accepted for consumption of metals per kg of body weight (B_w), within the safety values. According to the Integrated Risk Information System (International Toxicity Estimates for Risk Assessment, 2013), the RfD value for Cd is $0.01\text{ mg}\text{ kg}^{-1}\text{ day}^{-1}$.

In the non-carcinogenic risk assessment of vegetable consumption, a THQ result < 1 indicates that non-carcinogenic health effects are not important. However, at THQ values > 1 , there is a possibility that adverse health effects may occur in the long term (Gebeyehu & Bayissa, 2020; Gupta et al., 2019; Guo et al., 2019; Liang et al., 2019; Alam et al., 2018; Varol et al., 2017; Antoine et al., 2017).

In order to assess the potential non-carcinogenic risk through the consumption of vegetables possibly contaminated by Cd, the THQ was calculated, according to Equation 4 (Gebeyehu & Bayissa, 2020):

$$THQ = \frac{EDI}{RfD} \quad (4)$$

2.7 Statistical Analysis

To prepare the linear regression curve in the working range, with the mean, standard deviation and variance, Excel 2013 for Windows was used to evaluate the variance values. Statistical analysis was performed using Student's t test at a significance level of 5% to reveal significant differences between conventional and organic farming cultivation.

3 Results and discussion

3.1 Cd contents in organic and conventional vegetables

According to Brazilian legislation (Brasil, 2013), leafy vegetables such as lettuce and tubers such as carrots should contain Cd concentrations lower than $0.20\text{ mg}\text{ kg}^{-1}$ and $0.10\text{ mg}\text{ kg}^{-1}$, respectively. Table 2 shows the mean concentrations of Cd, amplitude and standard deviation in $\text{mg}\text{ kg}^{-1}$ found in the studied vegetables.

When comparing the mean concentrations obtained with the acceptable limits by RDC n° 42 (Brasil, 2013), both the organic

Table 2. Mean concentration of Cd, amplitude and standard deviation in $\text{mg}\text{ kg}^{-1}$ in samples of organic and conventional vegetables.

	Conventional vegetables		
	Mean ($\text{mg}\text{ kg}^{-1}$)	Standard deviation ($\text{mg}\text{ kg}^{-1}$)	Amplitude ($\text{mg}\text{ kg}^{-1}$)
Lettuce	0.1549	0.0266	0.0707 - 0.2542
Carrot	0.1174	0.0780	< LQ - 0.3135
	Organic vegetables		
	Mean ($\text{mg}\text{ kg}^{-1}$)	Standard deviation ($\text{mg}\text{ kg}^{-1}$)	Amplitude ($\text{mg}\text{ kg}^{-1}$)
Lettuce	0.0811	0.0367	0.0208 - 0.1576
Carrot	0.1064	0.0553	0.0296 - 0.2236

lettuce samples and the conventional lettuce samples presented values below the established limits. However, two samples of conventional lettuce had concentrations of 1.2 ($0.2378\text{ mg}\text{ kg}^{-1}$) and 1.3 ($0.2542\text{ mg}\text{ kg}^{-1}$) times higher than the established limit. This fact may be associated with some increase in lettuce exposure to possible sources of Cd.

Hadayat et al. (2018), evaluated concentrations of several metals, including Cd, in a total of 120 samples of potato, lettuce, tomato, carrot and onion, conventionally and organically cultivated in California, USA. The mean concentrations of Cd found were $9.17\text{ }\mu\text{g}\text{ kg}^{-1}$ and $15.3\text{ }\mu\text{g}\text{ kg}^{-1}$ in organic and conventional foods, respectively. However, all values were below the concentrations allowed by Food and Agriculture Organization (2016). This represents a 1.7 times greater contamination by Cd in conventional foods when compared to organic ones, which corroborates the results of the present study.

In this study, the concentrations of Cd in carrots cultivated conventionally showed a concentration of Cd approximately 10% higher when compared to the concentrations of samples of organic carrots. Comparing the mean concentrations obtained with the acceptable limits by RDC n° 42 (Brasil, 2013), both the organic carrot samples and the conventional carrot samples showed values below the established limits. However, observing the individual samples, three samples of conventionally cultivated carrots presented concentrations from 1.4 to 3.1 times higher (0.1428 , 0.1730 and $0.3135\text{ mg}\text{ kg}^{-1}$) than the established limit.

The difference in Cd concentration found between carrot cultures agrees with the study carried out by Gawęda et al. (2012), who, when evaluating Cd concentrations between organically and conventionally produced carrots, found that organic carrots contained up to 28% less Cd than conventionally grown carrots.

In the present study, it was observed that lettuce, a leafy vegetable, had a higher concentration of Cd than carrots, which were tubercles. In the study by Douay et al. (2013), lettuce showed a greater tendency to accumulate Cd (8.6 times), when compared to potatoes, for example, which is also a tuber.

Hu et al. (2017) and Sultana et al. (2017) concluded that leafy vegetables accumulate higher concentrations of metals, probably due to the high rate of transpiration performed by the plant, in order to maintain the growth and moisture content of these plants, thus offering a greater risk to health when compared to tubers and fruits.

3.2 Quantitative result of lettuce and carrot consumption

In total, 231 individuals answered all FFQ questions. Based on the answers, it was possible to calculate the average consumption of those vegetables from that respondent population and the amount consumed. When asked about the amount of lettuce and carrot consumption, most of the respondent population (37% and 60%, respectively) consume 16 g of lettuce at a time and 24 g of carrots at a time.

3.3 Estimated Daily Intake and Target hazard quotients

Both EDI and THQ were used in our study, following the models used by Gebeyehu & Bayissa (2020). FFQ data indicated

Table 3. Parameters and variables used in the calculation of EDI and THQ and comparison of EDI found for consumption of conventional and organic lettuce and carrots, with the MTDI for Cd.

Parameter	Reference	Vegetable		EDI (mg day ⁻¹ kg ⁻¹ bw)			
		Lettuce	Carrot	Lettuce		Carrot	
E _f (days)	-	120	120	Conv	Org	Conv	Org
E _D (years)	Instituto Brasileiro de Geografia e Estatística (2019a)	76.3	76.3	4.8 x 10 ⁻⁶	2.5 x 10 ⁻⁶	5.4 x 10 ⁻⁶	4.9 x 10 ⁻⁶
F _{IR} (g day ⁻¹)	QFA	24	36	THQ			
C _M (mg kg ⁻¹ conventional vegetable dry weight)	This study	0.1549	0.1174	Lettuce		Carrot	
C _M (mg kg ⁻¹ organic vegetable dry weight)	This study	0.0811	0.1064	Conv	Org	Conv	Org
C _F	Gebeyehu & Bayissa (2020)	0.085	0.085	4.8 x 10 ⁻⁴	2.5 x 10 ⁻⁴	5.4 x 10 ⁻⁴	4.9 x 10 ⁻⁴
B _w (kg)	Instituto Brasileiro de Geografia e Estatística (2019b)	65.9	65.9				
TA (days)	-	9156	9156				
RfD (mg kg ⁻¹ day ⁻¹)	-	0.01	0.01				

Conv: conventional. Org: organic

that, on average, respondents consume lettuce and carrots 10 times a month. Considering the annual consumption, it can be said that, on average, respondents consume lettuce and carrots 120 times a year. Therefore, for the E_f parameter, the value of 120 was used.

Table 3 presents the values of each variable of the equations, as well as the results obtained for EDI and THQ. The EDI values found in this study were estimated based on the average of Cd concentrations in lettuce and carrot samples grown in conventional and organic (C_M) form. The C_F or concentration conversion factor for fresh vegetable weight to dry weight, was 0.085 (Gebeyehu & Bayissa, 2020).

EDI values are below the tolerable daily intake (MTDI) for the metal, which according to the literature is between 0.02 to 0.07 mg day⁻¹ (Shaheen et al., 2016; Basha et al., 2014; Zheng et al., 2007).

When comparing the EDI values found in conventional and organic vegetables, it can be seen that the EDI from the consumption of conventional lettuce is almost twice as high compared to the consumption of organic lettuce.

In view of these results, it can be inferred that contamination by Cd in the studied vegetables presents a non-carcinogenic risk in the long term. However, it is noteworthy that the THQ for lettuce is twice as high in conventional cultivation compared to organic. It is important to point out that even with THQs below the acceptable standard, the cumulative effect of consumption can result in adverse effects on consumer health (Gebeyehu & Bayissa, 2020).

4 Conclusion

The present study was carried out to assess the concentration of Cd in commonly consumed vegetables. In general, the concentrations of Cd in the studied vegetables were below the limit allowed by Brazilian legislation and Food and Agriculture Organization (2016). In the present study, it was found that the conventionally cultivated leafy vegetable bioaccumulates a

more expressive concentration of cadmium, despite the average concentration being lower than that established by Brazilian legislation.

THQ values were less than 1, which suggests an acceptable level of risk where non-carcinogenic health effects are not important.

It is noteworthy that the present study evaluated a metal, Cd, through the consumption of only two vegetables, lettuce and carrot, which may underestimate the risks of consuming food contaminated by metals, since the population may be exposed through from several other sources at the same time, as discussed in the present study, thus covering a potential health risk for the exposed population. It should also be taken into account that chemical contaminants can act synergistically, which would increase health risks.

Based on this study, further evaluation is recommended to study the concentrations of other toxic metals at the trace level, in order to establish and adopt measures to reduce their concentrations in vegetables and, ultimately, prevent avoidable health problems.

Thus, given the above, it is expected that agricultural policies pay more attention to organic, agroecological and low-input agriculture, and for this, it is necessary to invest in research and innovation, since, in general, it can be said that organic agriculture can provide important benefits for human health and the environment.

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