

## DIETARY INTAKE AND TROPHIC TRANSFER OF CADMIUM AND LEAD FROM THREE DAILY CONSUMPTION VEGETABLES IN QUITO, ECUADOR

Ingesta diaria y transferencia de cadmio y plomo de tres vegetales de consumo diario en Quito, Ecuador

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(Received: November 2021; accepted: October 2022)

Key words: cadmium, *Drosophila melanogaster*; lead, health risk, natural products.

### ABSTRACT

The presence of toxic metals in vegetables of daily consumption is a concern for human health. Little is known about the concentration of toxic metals in vegetables sold in markets in Quito, Ecuador, and the corresponding risk to the population. This study aimed to measure the concentration of cadmium (Cd), and lead (Pb) in tomatoes, carrots, and lettuce, to estimate the risk for human health, and to determine the trophic transfer factor using *Drosophila melanogaster* flies. Vegetable samples were obtained from four local markets in Quito to measure the concentration of Cd and Pb (expressed in mg/kg of dry weight) in the vegetables by microwave-assisted acid digestion and atomic absorption spectrophotometry. *Drosophila melanogaster* flies were fed with contaminated vegetables to determine the trophic transfer factor of metals in the trophic chain. Our results showed the highest accumulation of Pb and Cd occurred in lettuce. In all cases, the Pb and Cd concentrations exceeded the maximum levels established by the Food and Agriculture Organization. However, the daily intake of metals and the health risk index showed no risk from daily consumption. Pb's trophic transfer factor was excessively high in the tomato-fed flies, showing transference and accumulation in the flies. Cadmium concentration in tomato-fed flies was also the highest among the three vegetables. The trophic chain transfer of toxic metals from vegetables to *Drosophila* has been observed through a high accumulation of these metals in flies, and this suggests a negative impact on both human health and ecosystems.

Palabras clave: cadmio, *Drosophila melanogaster*, plomo, productos naturales, riesgo en la salud.

### RESUMEN

La presencia de metales tóxicos en las verduras de consumo diario es una preocupación para la salud humana. Poco se sabe sobre la concentración de metales tóxicos en las verduras que se venden en los mercados de Quito, Ecuador, y el correspondiente riesgo para la salud de la población. Este estudio tuvo como objetivo medir la concentración de cadmio (Cd) y plomo (Pb) en tomates, zanahorias y lechugas, para estimar el índice de riesgo para la salud humana y determinar el factor de transferencia trófica de los metales a moscas *Drosophila melanogaster*. Se obtuvieron muestras de vegetales

de cuatro mercados locales en Quito para medir la concentración de Cd y Pb (expresada en mg/kg de peso seco) en los vegetales mediante digestión ácida asistida por microondas y espectrofotometría de absorción atómica. Los adultos de *Drosophila melanogaster* fueron alimentados con los vegetales contaminados para determinar el factor de transferencia trófica de los metales. Nuestros resultados mostraron que la mayor acumulación de Pb y Cd ocurrió en la lechuga y que, en todos los casos, las concentraciones de Pb y Cd excedieron los niveles máximos establecidos por la Organización para la Agricultura y la Alimentación. Sin embargo, la ingesta diaria de metales y el índice de riesgo para la salud no mostraron riesgo por el consumo diario. El factor de transferencia trófica de Pb fue excesivamente alto en las moscas alimentadas con tomate, mostrando transferencia y acumulación en las moscas. La concentración de Cd en moscas alimentadas con tomate también fue la más alta entre las tres verduras. La transferencia de estos metales de las verduras a las moscas *Drosophila* fue observada a través de su alta acumulación, lo que sugiere un impacto negativo tanto para la salud humana como para los ecosistemas.

## INTRODUCTION

Fresh fruits and vegetables are considered a major source of nutrients in the human daily diet but can also be a source of contaminants, such as toxic metals or other toxic components present in pesticides (Tricker and Preussmann 1990, D'Mello 2003, Mahmood and Malik 2014). Consumption of contaminated food has been identified as a major pathway of human exposure to contaminants, and over 90% of all existing ingestible contaminants are present in food (Fries 1995, Järup 2003, Järup and Åkesson 2009, Cherfi et al. 2014, Duan et al. 2020). Thus, the Food and Agriculture Organization (FAO) the World Health Organization (WHO) and the European Commission have established maximum levels (ML) of metals in food to control the quality of food and production processes.

Many studies have shown that prolonged intake of toxic metals may produce liver, kidney, cardiovascular, nervous, and bone disorders (WHO 1992, Järup et al. 2000, Järup 2003, Zaidi et al. 2005). Further, about 30% of human cancers have been associated with diet-related carcinogenic contaminants (Tricker and Preussmann, 1990, Sá et al. 2016, Duan et al. 2020, van Gerwen et al. 2022).

The daily intake of metal (DIM) refers to the accumulation of a contaminant in an organism resulting from its uptake from said organism's diet. Therefore, measuring an organism's DIM can calculate the health risk index (HRI) to evaluate a metal's potential toxic effect from daily consumption.

The presence of cadmium (Cd) in high levels may produce toxic effects in plants, such as a reduction of metabolic activity of plants and induce oxidative damage, chlorosis, rolling of leaves and

stunting, lipid peroxidation, alter the uptake of other minerals, and inhibits the nitrate reductase activity in shoots (Edelstein and Ben-Hur 2018, Patra et al. 2020). Therefore, because Cd can accumulate in several plant species, there is an increased risk of its consumption and subsequent transference to higher levels of the trophic chain, including humans (Cambra et al. 1999, Sharma et al. 2015, Fajana et al. 2020). In humans, Cd is considered a nephrotoxic metal (Thomas et al. 2013). Dietary Cd exposure has been associated with gastrointestinal problems, severe toxic effects on the kidney, liver, testis, ovaries, and the nervous and cardiovascular systems (Järup and Åkesson 2009), testicular and germ cell damage (Marettová et al. 2015), and prostate cancer (Julin et al. 2012). Lead (Pb) can cause hematological, neurological, and gastrointestinal effects, renal failure, physiological disorders, and carcinogenic effects according to the Agency for Toxic Substances and Disease Registry (ATSDR 2020).

Vegetables can accumulate toxic metals from the contaminated air, soil, or water (Yadav et al. 2013, Alghobar and Suresha 2017, Gupta et al. 2019, Romero-Estévez et al. 2023). Toxic metals are absorbed through the roots and transported further into other parts of the plant, such as leaves, fruits, and flowers, where they can form deposits (Singh et al. 2010, Gupta et al. 2019). Moreover, the aerial organs of plants have absorption mechanisms through foliar transfer after the deposition of atmospheric particles on the surface (Shahid et al. 2016). Thus, this exposition route can be considered an essential way of contamination, principally in areas with high levels of metals in the atmosphere. The trophic transfer factor (TTF) allows for the observation of the transference of metals in the trophic chain and

can be used to demonstrate the risk to all organisms in a contaminated ecosystem.

*Drosophila melanogaster* is an insect model that has been used traditionally in genetic and developmental biology studies; however, nowadays is also used in biomedical (Pasini et al. 2010, Martín-Bermudo et al. 2017, Wang et al. 2018) and toxicological research (Rand 2010, Vecchio et al. 2013, Ong et al. 2015, Chifiriuc et al. 2016, Affleck and Walker 2019). In diverse studies, *Drosophila* has been used, with excellent results, to analyze the effect of metals (Soares et al. 2017, Sundararajan et al. 2019, Li et al. 2020), nanoparticles (Vela et al. 2020, Sarkar et al. 2021), antioxidants (Yi et al. 2021), chemical products (Soares et al. 2017, Nesterkina et al. 2020, Senthilkumar et al. 2020), and drugs (Kruger and Denton 2020, Matinyan Gonzalez et al. 2021, Matinyan Karkhanis et al. 2021). In this study, we use *Drosophila melanogaster* features to observe the transference and accumulation of metals through oral exposure under controlled conditions.

In Quito city, there are few studies about contamination by toxic metals in vegetables consumed by the population (Romero-Estévez et al. 2020) and how human health could be affected. However, although the population of Quito, both locals, and foreigners, could be exposed daily to contaminants possibly coming from the consumption of natural products, there is no previous information related to the presence of contaminants and the possibility of its transfer to higher trophic levels. On the other hand, Ecuadorian soils are rich in some metals, including Cd and Pb, and this natural presence contributes to its incidence in crops (Barraza et al. 2017, Romero-Estévez et al. 2020, Romero-Estévez et al. 2023). In addition, considering that tomato, lettuce, and carrots are commonly consumed in local recipes as salads, sandwiches, juices, among others, this study aimed to determine the concentrations of Cd and Pb in three vegetables of common daily consumption in Quito, tomatoes (*Solanum* sp.), carrots (*Daucus* sp.), and lettuce (*Lactuca* sp.) to calculate the HRI and TTF values and demonstrate the transference of Cd and Pb in the trophic chain.

## MATERIALS AND METHODS

### Vegetable sampling

Fruits and vegetables grown in Ecuador's northern, central, and southern provinces are sold in wholesale markets from Quito. Four wholesale open markets from Quito, which provide the products to

smaller markets, stores, and restaurants, were selected to obtain the samples. Three of the most common vegetables in Quito city were selected to determine the Cd and Pb content. Several samples of each vegetable were purchased from different vendors in the market. Approximately 10 kg of each vegetable was obtained to form three composite samples: tomato, lettuce, and carrots. The samples were washed with high-quality reagent water (resistivity 18.2 MΩ·cm) to eliminate the impurities; then, the samples were cut into small pieces, homogenized, and freeze-dried.

### Concentrations of Cd and Pb in vegetables and flies samples

To analyze the vegetable and homogenized fly samples, the method described by Romero-Estévez et al. (2020) was validated for each matrix. Approximately 0.5 g (0.1 mg precision) of each vegetable and fly sample was weighed in teflon vials, then 5 mL of 70% nitric acid (Fisher Chemical, Certified ACS plus, CAS# 7697-37-2, Fair Lawn, NJ, USA) and 3 mL of 30% hydrogen peroxide (Fisher Chemical, Certified ACS plus, CAS# 7722-81-1) were slowly added. Acid digestion was performed using a Mars 6 microwave (CEM, Matthews, NC, USA). Sample analysis was performed in triplicate using fortifications of 1.0 mg/kg for Cd and 15.0 mg/kg for Pb, prepared from a previously calculated amount of original standards of 1000 µg/mL (Inorganic Ventures, Christiansburg, VA, USA) that were added to non-fortified samples of each matrix (vegetables and flies), to evaluate the inexistence of matrix variation considering the result obtained in the non-fortified samples as an initial value. Blanks were impossible to measure because there were no cadmium- and lead-free samples. All samples presented quantifiable values of the metals from the beginning. The standards of each calibration curve, samples, fortifications, and blanks were prepared using analytical grade reagents and high-quality reagent water. Total concentrations of Cd and Pb were measured using a flame atomic absorption spectrophotometer (AAnalyst 400, Perkin Elmer Inc., Waltham, MA, USA), the parameters are described in **table I**. The results are presented in mg/kg of dry weight.

The method's reliability and performance were evaluated through relative standard deviation (% RSD) and the recovery rates of fortifications using the criteria established by the Association of Official Analytical Chemists (AOAC) in the Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals (AOAC 2002). A precision of 8 % for repeatability and

**TABLE I.** ANALYTICAL CONDITIONS FOR THE DETERMINATION OF CADMIUM (Cd) AND LEAD (Pb).

Parameter	Cd	Pb
Wavelength (nm)	228	217
Slit (mm)	2.7/1.35	2.0/1.35
Calibration standards (mg/L)	0.01 0.05 0.1 0.3 1.0	0.3 1.0 3.0 5.0

recoveries between 75 % and 120 % for accuracy were applied. Also, a certified reference material (CRM) of tomato leaves 1573a from the National Institute of Standards and Technology was used as additional quality control.

### Daily intake of metal

We calculated the daily intake of metal (DIM) of Cd and Pb from tomatoes, carrots, and lettuce sold in Quito markets using the equation 1:

$$\text{DIM} = \text{C-metal} \times \text{D-food intake} / \text{B-average weight}, \quad (1)$$

where DIM is the daily intake of metals (mg/day/individual), C-metal is the toxic metal concentrations in plants (mg/kg), D-food intake is the daily intake of vegetables being consumed daily by an individual (g/day), and B-average weight is average body weight (BW, 60 kg for the adult and 35 kg for children in Ecuadorian population).

The results were compared with the tolerable daily intake for metals recommended by the FAO/WHO (Fortin 2010) and by the European Commission (SCOOP 2004). For the Hispanic population, the quantity consumed per day (g/day) for tomatoes is 59.04 for adults and 52 for children, for carrots 8.31 for adults and 2.5 for children, and for lettuce 14.5 for adults and 1 for children, based on the EPA Analysis of NHANES 2005-2010, data using <http://fcid.foodrisk.org/percentiles>. (USEPA 2018). The vegetables represent 327.3 g/day (14.6 %) of total dietary intake (FAO/WHO 2019).

### Health risk index

Health risk index (HRI) was calculated for tomato, carrot, and lettuce consumption in Quito using equation 2 (Khan et al. 2008):

$$\text{HRI} = \text{DIM} / \text{RfD}, \quad (2)$$

where DIM is the daily intake of metal and RfD (mg/kg/day) is the reference dose of the metal; an HRI value of < 1 means the exposed population is assumed to be safe. The RfD for Pb is 0.0035 and for Cd 0.001 (Ferreira-Baptista and de Miguel 2005, Sun et al. 2017, Barraza et al. 2018).

### Trophic transfer factor

*D. melanogaster* was selected for its annealed recognized use for studies of human diseases, including those related to models for testing metal metabolism and homeostasis. In addition, flies not only have a short life cycle, easy handling, and cheap maintenance, which represent many advantages in laboratory studies but also they can be raised in a large number offering a considerable number of genetic tools to evaluate metabolism function related to metal trophic transference (Ferreira-Baptista and De Miguel 2005).

To determine the transference of toxic metals through the trophic chain, we exposed *D. melanogaster* flies to a diet based only on freeze-dried vegetables.

*D. melanogaster* first instar larvae (Oregon strain) were fed with a culture medium containing freeze-dried vegetables (either tomatoes, carrots, or lettuce) during the larval stages (seven to nine days). Emerged adult flies, in fresh weight, were analyzed to measure the concentration of Cd and Pb. The methodology described by Romero-Estévez et al. (2019) was also used to also analyze the concentration of Cd and Pb in flies.

The TTF was determined to demonstrate the transference of Pb and Cd in the trophic chain from the vegetables to the flies. The TTF was calculated by equation 3 (DeForest et al. 2007):

$$\text{TTF} = \text{Metal concentration in the organism} / \text{Metal concentration in the organism's food} \quad (3)$$

## RESULTS AND DISCUSSION

The concentration of Cd and Pb was determined in tomatoes, carrots, and lettuce coming from the wholesale Quito markets. Our results showed high concentrations of Cd and Pb in all three vegetables (**Table II**). Although carrots and tomatoes presented minor concentrations of Cd and Pb, in all analyzed vegetables the metal concentrations exceeded the ML for human consumption established by the FAO/WHO (2019).

**TABLE II.** LEAD (Pb) AND CADMIUM (Cd) CONCENTRATIONS MEASURED IN VEGETABLES AND THE CORRESPONDING MAXIMUM LEVELS.

Pb Samples	Mean concentration in vegetables mg/kg (n = 3)	RSD %	Fortification recovery rates %	ML FAO/WHO mg/kg
Tomatoes	0.51	4.1	103.5	0.05
	0.65	4.7	107.6	
	0.46	3.8	96.3	
	0.52	2.9	100.7	
Carrots	2.13	1.0	105.9	0.1
	2.13	1.1	105.6	
	1.54	1.6	96.6	
	1.35	5.0	103.5	
Lettuce	5.81	1.3	96.6	0.3
	5.45	0.7	94.7	
	4.74	1.4	95.2	
	3.56	2.7	103.7	
Cd Samples	Mean concentration in vegetables mg/kg (n = 3)	RSD %	Fortification recovery rates %	ML FAO/WHO mg/kg
Tomatoes	0.65	2.4	105.3	0.05
	0.72	4.3	103.3	
	0.51	3.0	106.3	
	0.61	2.8	107.0	
Carrots	0.45	2.6	106.7	0.1
	0.49	5.1	99.3	
	0.71	4.3	104.3	
	0.74	4.4	102.3	
Lettuce	0.65	4.5	106.3	0.2
	0.72	2.4	103.0	
	1.02	4.1	105.7	
	0.99	4.8	106.7	

n: number of replicates, FAO/WHO: Food and Agriculture Organization of the United Nations/World Health Organization, ML: maximum level, RSD: relative standard deviation.

The limits of detection (LOD) were calculated by using low-level concentration standards for each metal. The obtained LOD values were 0.01 and 0.30 mg/L for Cd and Pb, respectively. The limits of quantification (LOQ) were obtained using low-level fortifications in the original non-fortified samples, and the obtained LOQ values were 0.20 and 0.50 mg/kg for Cd and Pb, respectively.

### Lead concentration in vegetables

Leafy vegetables tend to accumulate toxins and toxic metals in their foliar structures (Chiroma et al. 2007, Mahmood and Malik 2014, Zhou et al. 2016). In this study, the highest concentration of Pb (mean 4.89 mg/kg) was observed in the four samples of lettuce as compared to carrots and tomatoes (**Table II**).

In the case of tomatoes (*Solanum* genus), the accumulation of toxic metals can occur in roots but also fruits. In the analyzed samples, the highest concentration of Pb was 0.65 mg/kg, however, other studies have detected higher concentrations of Pb in tomatoes (1.968 mg/kg) (Naser et al. 2009). In carrots, the highest concentration of Pb was 2.13 mg/kg (**Table II**). Carrots are root vegetables are in direct contact with the soil, therefore, the carrots' metal concentration is associated with the soil's original composition.

The FAO determined that ML for Pb in leafy vegetables is 0.3 mg/kg, in tomatoes 0.05 mg/kg, and in carrots 0.1 mg/kg (FAO/WHO 2019). In European countries the European Commission established ML for Pb in lettuce is 0.3 mg/kg wet weight, however, concentrations between 0.004 and 0.226 mg/kg have

been detected in vegetables (SCOOP 2004, EFSA 2010). Thus, in the three vegetables in our study, the concentration of Pb exceeded the ML and is near to the concentration observed in industrialized countries (Graedel and Cao 2010, Mamtani et al. 2011, Lake et al. 2012).

All the % RSD results were acceptable (below 5.0 %) and the recoveries of the fortifications were between 94.7 % and 107.6 %. These values were in good agreement with the AOAC (2002) criteria of 75 % and 120 %. In addition, the CRM results were within the acceptance criteria in the certificate.

### Cadmium concentration in vegetables

The highest concentration of Cd (1.02 mg/kg) was detected in the lettuce samples in the current study (**Table II**). Other studies have found Cd concentrations for most leafy species ranging from 0.05 to 0.2 mg/kg, and this accumulation occurs in the leaves (Solís-Domínguez et al. 2007). Previous studies (Trebolazabala et al. 2017) have determined that tomato is a median accumulator of Cd. Cd concentrations up to 1.70 mg/kg have been detected in some *Solanum* species. In the current study, the highest concentration of Cd in the tomato samples was 0.72 mg/kg. In carrots, the highest concentration of Cd was 0.74 mg/kg, previous studies have

reported Cd concentrations from 0.02 to 0.2 mg/kg in carrots (Lee et al. 1999, Hoefkens et al. 2009, Kawada and Suzuki 2011). The ML for Cd set by the FAO/WHO (2019) for leafy vegetables is 0.2 mg/kg, 0.05 mg/kg for tomatoes, and 0.1 mg/kg for carrots.

All the % RSD results were acceptable (below 5.1 %) and the recoveries of the fortifications were between 99.3 % and 107.0 %. These values were in good agreement with the (AOAC 2002) criteria of 75 % and 120 %. In addition, the CRM results were within the acceptance criteria in the certificate.

### Dietary intake of metals and health risk index

The DIM for Pb and Cd from vegetable consumption was measured and compared with the recommended dietary intake limits (**Table III**). Also, the HRI from exposure to Pb and Cd in the diet was determined.

The calculated DIM values, in adults and children, for Pb were below the provisional tolerable weekly intake (PTWI) of 400-500 µg or 7 µg/kg of BW (FAO/WHO 2011) in the three vegetables (**Table III**). PTWI is a reference for metals with cumulative properties, while provisional tolerable monthly intake (PTMI) refers to metals that have a very long half-life in the human body.

**TABLE III.** DIETARY INTAKE OF METALS AND HEALTH RISK INTAKE FROM VEGETABLE CONSUMPTION.

Pb samples	Mean metal concentration (mg/kg)	DIM (g/day/kg)	HRI	PTWI FAO/WHO	PTMI EFSA
Tomatoes	0.53	4.43E-04 <sup>a</sup> 6.69E-04 <sup>b</sup>	1.27E-01 <sup>a</sup> 1.19E-01 <sup>b</sup>	7 µg/kg/day (SCOOP 2004)	3.6 µg/kg·day
Carrots	1.78	2.10E-04 <sup>a</sup> 0.018-04 <sup>b</sup>	5.99E-02 <sup>a</sup> 3.08E-02 <sup>b</sup>		
Lettuce	4.89	1.00E-03 <sup>a</sup> 1.19E-04 <sup>b</sup>	2.87E-01 <sup>a</sup> 3.39E-02 <sup>b</sup>		
Cd samples	Mean metal concentration (mg/kg)	DIM (g/day/kg)	HRI	Critical concentration	PTMI EFSA
Tomatoes	0.62	5.19E-04 <sup>a</sup> 7.83E-04 <sup>b</sup>	5.19E-01 <sup>a</sup> 7.83E-04 <sup>b</sup>	200 mg/kg in kidney (Solís-Domínguez et al. 2007)	25 µg/day BW
Carrots	0.59	6.95E-05 <sup>a</sup> 3.53E-05 <sup>b</sup>	6.95E-02 <sup>a</sup> 3.53E-05 <sup>b</sup>		
Lettuce	0.84	1.73E-04 <sup>a</sup> 2.04E-05 <sup>b</sup>	1.73E-01 <sup>a</sup> 2.04E-02 <sup>b</sup>	42 µg/day (SCOOP 2004)	

BW: body weight, Cd: cadmium, DIM: daily intake of metals, HRI: health risk index, Pb: lead, PTWI: provisional tolerable weekly intake, PTMI: provisional tolerable monthly intake; <sup>a</sup> adults, <sup>b</sup> children

The half-life of Pb is about one month in the blood and 20-30 years in bone (FAO/WHO 1995). The PTWI of 25 µg/kg (1500 µg Pb/week for a 60 kg person) was declared inappropriate, instead, calculating exposure limits according to the dietary habits and characteristics of the population (children, adults, women, men, smokers, etc.) was proposed (EFSA 2010). The International Agency for Research on Cancer (IARC) classified Pb compounds as probably carcinogenic to humans (Group 2) (EFSA 2010). Some estimations of exposure are shown in **table III**.

In the case of Cd, the DIM values (in adults and children) found in the current study (**Table III**) were also below the PTWI of 1 µg/kg of BW/day (420 µg Cd/week for a 60 kg person) and the PTMI of 25 µg/kg of BW because Cd can have a long half-life in the human kidney (FAO/WHO 2011). In previous studies, a concentration of 200 mg/kg in kidney tissue has been considered a critical level for humans, however, kidney damage at 2-3 µg Cd/g of creatinine have been observed in Europeans (Järup et al. 2000, SCOOP 2004). The HRI calculated from Cd consumption was < 1, therefore, there is no risk associated with the daily consumption of tomatoes, carrots, and lettuce.

#### Trophic transfer factor of lead and cadmium

Transference of Pb and Cd from the vegetables to *D. melanogaster* flies was observed through the TTF (**Table IV**). The high TTF values for the three vegetables show that metals present in plants are transferred to insects and potentially to many kinds of organisms in the next level of the trophic chain.

Other studies have shown evidence of metal transference through the trophic chain from plants to insects. For example, metal transference has been observed in bees after 10 days of exposure, the concentration of Pb in bees was 0.3 mg/kg (Gauthier et al. 2016).

**TABLE IV.** TROPIC TRANSFER FACTOR (TTF) FOR LEAD (Pb) AND CADMIUM (Cd).

Metal	Vegetables mg/kg	Flies mg/kg	TTF vegetables to flies
Pb			
Tomatoes	0.53	10.57	20.07
Carrots	1.78	6.65	3.38
Lettuce	4.89	3.03	0.60
Cd			
Tomatoes	0.62	2.23	2.46
Carrots	0.59	0.77	1.44
Lettuce	0.84	0.79	0.86

Besides, chironomids exposed to Pb (3.5-505.5 mg/kg) for 16 days presented metal accumulation beyond 500 mg/kg in tissues. Additionally, there is evidence of the presence of Cd in bee products (honey, propolis, and beeswax) associated with bees collecting pollen from plants in contaminated areas (Bogdanov 2006, Silici et al. 2016). Previous studies have also shown that tomatoes had a higher translocation of Pb in the trophic chain (soil-plant-herbivorous insect-predator insect) with the *Cryptolaemus montrouzieri* beetle (Zhang et al. 2017). In the current study, the TTF value for the tomato-to-*D. melanogaster* was the highest of the three (20.07), which is consistent with the aforementioned observation of Pb transference in *C. montrouzieri*. These results suggest that the characteristics of each plant influence metal uptake capacity, transport from roots to other parts of the plant, and accumulation of toxic metals (Nabulo et al. 2012, Yang et al. 2016). Additionally, the larvae are in contact with the culture medium, therefore the permanent exposure to metals in the medium could increase the transference and accumulation of toxic metals. Although *Drosophila* sp. has proven to be an excellent model for observing trophic transfer of Cd and Pb by oral intake (Jiang et al. 2021), new local studies are needed in order to observe the effect of heavy metals on the gene expression.

The risk of heavy metals entering a food chain depends on their mobility and availability in the soil, pH, organic matter content, etc. (Gall et al. 2015, Romero-Estévez et al. 2023). Once ingested by plants, these contaminants can disrupt critical physiological processes and result in toxicity. Continuing to the next level of the food chain, invertebrates (as *D. melanogaster*) may involuntarily ingest metals contained in soils and plants (Gall et al. 2015). An excess of metals ingested can have a variety of impacts on invertebrate fitness, including decreased immune response, prolonged the mating latency in *D. melanogaster* females, also reducing the number of eggs laid (Xiaoyu et al. 2019) and genotoxic effects (Mutwakil et al. 1997, Doğanlar et al. 2014).

In South America, several studies show the presence of cadmium, lead and other heavy metals in aquatic insects and marine invertebrates, whose environment present high levels of contamination by heavy metals (Lango-Reynoso et al. 2010, Valdés et al. 2014, Barriga-Sánchez and Aranda Pariasca 2018). In the case of vertebrates, small mammals primarily gain metals through ingestion, inhalation, grooming, skin absorption, or placental transfer during pregnancy (Gall et al. 2015). The extent of metal transfer in mammals depends on the degree of exposure, mammal species, diet, season, sex, age, and the metal being

considered (Gall et al. 2015). High levels of heavy metals were observed in milk from cows fed alfalfa grown on contaminated soil (Castro-González et al. 2018). Studies report higher levels of metal accumulation in carnivorous or omnivorous mammals than in exclusively herbivorous ones (Sánchez-Chardi et al. 2007, Vermeulen et al. 2009, Gall et al. 2015). These studies show the accumulation of heavy metals in soil and plants, and their transfer through the food chain in both invertebrates and vertebrates, suggesting that humans are exposed to the same risk.

In industrialized countries, the presence of high concentrations of metals in plants cultivated in areas associated with industrial activities has been demonstrated (Gan et al. 2017, Li et al. 2019, Zhou et al. 2019). Some studies have been conducted in Ecuador concerning the concentration of toxic metals in areas associated with petroleum activities (Barraza et al. 2018) and in economically important fruits like cacao (Chavez et al. 2015, 2016, Barraza et al. 2017, Argüello et al. 2019) and banana (Romero-Estévez et al. 2019). Our study thus provides important information as a punctual first approach to the health of adults and children in the Ecuadorian population related to the foods they consume regularly. It is of concern that children are exposed to heavy metals through food. Several studies reveal that small doses of metals can be toxic, causing damage to children's brain development (Bocquet et al. 2021) and increasing the carcinogenic risk (Alves et al. 2019, Wang et al. 2019). Our investigation also shows the importance of researching this field in developing countries, like Ecuador, where there is a lack of information on the risk of human exposure to severe contamination produced by mining, oil extraction, industrial pollution, and excessive use of pesticides or chemicals fertilizers in agriculture.

The aforementioned industrial and extractive activities produce accumulations of metals in soil, water, and air. Metal transference moves from an abiotic medium to plants and humans through the trophic chain. In the current study, we observed that metals accumulated in the plants could be transferred to the *D. melanogaster* flies through the culture medium. In the same way, metals could be transferred from food to humans or other organisms in the next level of the trophic chain.

## CONCLUSIONS

Although dietary intake of metals could vary substantially according to each person's habits (smokers,

non-smokers) and dietary characteristics (vegetarians and, vegans, etc.), food is the most important source of metal intake for humans (Järup 2003). Therefore, consumption of contaminated food represents a potential risk for related diseases. The current study's results show the potential risk for the Quito population due to the consumption of contaminated vegetables and the potential risk for all living organisms in a contaminated ecosystem.

A high concentration of Cd and Pb was detected in carrots, lettuce, and tomatoes consumed by the Quito population. High TTF of Cd and Pb from vegetables to flies evidence that transference of toxic metals occurs after oral exposure, therefore organisms in the upper levels of the trophic chain, including humans, could be adversely affected by consumption of vegetables contaminated due toxic metals.

The assessment of the real values of consumption of metals and index of health risks is influenced by the potential content of different heavy metals in crops, the differences between the diet associated with local customs, and the multiple heavy metals exposure paths, among other factors as the wide diversity of vegetables available in Ecuador. Thus, this study is a first approach for future research related to the calculation of health risk indices and trophic transfer with the aim of establishing public policies that ensure health protection of consumers.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the Pontificia Universidad Católica del Ecuador (PUCE) and the Centro de Estudios Aplicados en Química (CESAQ-PUCE) for all the support during this research.

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