

Metal Input in Lettuce Grown in Urban Agricultural Soils

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Abstract

Urban agriculture plays an important role in supplying produces to big cities; however, the quality of water used for irrigation can hinder this activity. Hence, the purpose of this study was to evaluate metal inputs, as well as their transfer and translocation factors, in lettuce (*Lactuca sativa* L.) crops cultivated in an urban plot. The research was conducted during the dry and rainy seasons. In the former, crops were irrigated with treated wastewater, whereas during the latter, crops were maintained just with rainwater. Composite samples for soils and plants were collected from the same plot during two crop cycles in 2013. Some edaphic variables were measured. Total metal concentration was determined, for both, soils and lettuce plants (leaves and roots). Water soluble and exchangeable soil metal fractions were also analyzed. A multivariate analysis of variance was performed to test for differences between seasons, among the variables analyzed. There were significant differences in edaphic characteristics between seasons. However, there was no difference in total metal content, except for Mn. Concentration of soluble metals was lower than exchangeable metal concentration, for both seasons. There was no correlation in total metal concentration between soils and plants. Transfer factor values were higher for Cd, Mn and Zn for the dry season, while for Cu, Fe and Pb were higher during the rainy season, as well as the translocation factors for all metals. Soil characteristics, together with transfer and translocation factors, showed temporal variations, leading to different metal concentrations in the edible lettuce tissues between the two analyzed crop cycles. The incorporation of metals is particular for each site, season and crop management type. Our results indicate that the metal concentration in lettuce tissues places no harm to human health. However, management strategies for urban agriculture must consider specific studies for each site.

Keywords

Transfer Factor, Translocation Factor, Seasonal Variation, Wastewater Irrigation

1. Introduction

In order to achieve food security, it is essential to increase food production. Urban agriculture is a resourceful practice that can help to accomplish this necessity, guaranteeing both food quality and quantity [1]. However, different activities that take place in the cities cause environmental alterations and accumulation of waste materials that contaminate soils, water and air. Pollutants may have an adverse effect on crop development, and they may also enter the food chain, via incorporation into the plant, resulting in an important route of exposure for those who consume these produces [2]. These problems have prevented health authorities to approve the consumption of crops that have grown in urban agriculture parcels [3] and those that have been irrigated with wastewater. The use of wastewater in agricultural lands has been practiced for more than 400 years in many parts of the world [4] [5] [6]. Nonetheless, its use over long periods of time can cause the accumulation of metals in soils, and their transfer to plants. [7]. The risk involved in growing crops irrigated with wastewater demand systematic evaluations to make the correct decisions and avoid negative effects on the consumers health [8] [9] [10].

Metal mobility and phytoavailability in soils are influenced by different variables like pH, redox potential, texture, quantity and quality of soil organic matter, mineral composition, temperature and water regime [11]. In general, the magnitude of the mobile metal fraction can be inferred by the characteristics of the soil. Those soils with neutral to slightly basic pH, and with high contents of clay and organic matter, are expected to have low metal mobility/availability, and low incorporation rates into plants [12] [13] [14] [15].

Several studies report the lack of significant correlations between soil characteristics, soil metal content and crop metal concentrations [16] [17] [18] [19] [20]; however, the soil labile fraction (soil solution and exchangeable fractions) correlates with metal absorption [11] [21]. Metal concentration in plants is the result not only of soil characteristics, but also the quality of water used for irrigation and the characteristics of the plants themselves. Plant properties linked to metal absorption include species, root absorption capacity, root selectivity, active and passive transfer processes, ionic interactions, rhizosphere physiology and plant-associated microorganisms [22]. Soil properties vary between seasons [23] and also among soil management treatments [24] [25]. This variability together with the plant characteristics will define the way in which metal transfers and translocation into plants will occur [26]. The complexity and the many variables involved in the metal transfer from soil to plants have to be considered for every

particular soil-plant system, in order to establish urban agricultural that is hazard free to human health [11].

According to FAO [27] just 19 percent of the urban agriculture areas (Chinampa System) in Mexico City is still in use, this represents 19,213 tons of food, from which 7453 tons correspond to lettuce cultivation, making this crop economically and nutritionally important. The use of treated wastewater for irrigation has undoubted benefits; nevertheless, its pollutants content may have serious long-term implications for food security. In this study we evaluated the variability of metal input in lettuce (*Lactuca sativa* L.) cultivated in an urban agricultural soil, irrigated with treated wastewater during the dry season, and rainwater through the rainy season. The objectives were: 1) to estimate the variability of some edaphic parameters and metal content in the soil (Cd, Cu, Ni, Fe, Mn, Pb, and Zn), in the dry and rainy seasons; 2) to correlate extractable metal concentrations (total and labile fraction) with metal contents in lettuce tissues (*Lactuca sativa* L.) and 3) to estimate transfer and translocation factors in the dry and rainy seasons. The information obtained will be used to design strategic management practices to minimize metal incorporation into crops and reduce the potential risk to human health.

2. Methods and Materials

2.1. Study Area

Xochimilco is located in a surviving lacustrine area, within the urban Mexico City (19°19' - 19°09'N; 98°58' - 99°10'W). In this area agriculture is carried out in the traditional way called Chinampa, this is an artificial soil which is constructed by using layers of different materials like: plants, lake sediments and organic fertilizers, among others. These soils, classified as terric Anthrosols, are surrounded by canals creating an entire agroecosystem [28] [29]. The traditional chinampa agriculture has been practiced in the Valley of Mexico since pre-Hispanic times [30]; however, population growth, urbanization and the consequent need for services in the megalopolis have gradually modified the environment, threatening this agricultural system and transforming the surrounding area in such a way that chinampas are now considered as urban agriculture. Moreover, the need for drinking water in Mexico City has led to its extraction from all the Xochimilco springs that once provided clean water to the canals. Since 1959, the canals have been fed with wastewater from the surrounding treatment plants and clandestine downloads of untreated sewage residual water [31], discharging pollutants into the system, including metals [32]. This practice represents a potential risk to human and animal health, mainly through the consumption of crops irrigated with this contaminated water. It has been reported that metal concentrations in soils and vegetal tissues irrigated with water from these canals are greater than the maximum limits allowed by Mexican Secretary of Environment and Natural Resources [33] [34]; nevertheless, Chinampas are considered one of the most productive systems ever created [35]. Currently, vegetables such as lettuce, radishes, beets, onions, turnips and zucchini, as

well as a great variety of flowers are produced on 200 hectares, representing 10 percent of the acreage of vegetables in Mexico City, and these are sold for consumption in several markets of the city.

The climate in the area is temperate sub-humid with rains in summer and early autumn (600 - 800 millimeters, annual total precipitation), with an average annual temperature of $17^{\circ} \pm 7^{\circ}$ Celsius (drier and warmer, 19° Celsius, in the dry season; more humid and less warm, 17° Celsius, in the rainy season) [36] [37].

2.2. Soil and Plant Sampling and Processing

To assess the effect of water irrigation quality on the metal concentration in lettuce tissues (*Lactuca sativa* L.), composite samples were collected independently, for both soil and lettuces. The sampling was carried out in two crop cycles: dry and rainy seasons (March-May and August-October 2013, respectively). Lettuce (*Lactuca sativa* L.) is grown throughout the year with four harvest dates.

The sampled area was divided into three subplots. In each subplot, ten entire lettuce plants (root and all leaves) and the soil around their roots (approx. 0 - 15 centimeters in depth) were collected to form composite samples, for both soil and plant material. The soil ($n = 9$) was sampled three times during each growing season (every 23 days), whereas lettuce plants ($n = 3$) were sampled only in the adult stage (after 70 days). Samples were collected in an "X" design to ensure representativeness through the plot.

Soil samples were stored in polyethylene bags and lettuce samples were stored in paper bags. Both types of samples were kept in coolers and transported to the laboratory for their analysis. Soil samples were air-dried and sieved through a 2 millimeters mesh, immediately upon arrival at the laboratory. Plant material was washed with tap water to remove soil particles, submerged in an acid wash (0.1 M HNO_3) for three minutes and then rinsed several times with deionized water, to guarantee the complete removal of all the soil particles on the plant surface. Plants were divided into roots and the entire above-ground portion (hereinafter termed leaves) to form a composite sample per plot, which was then dried at 30° Celsius. Plants were weighed before and after they had been dried.

Dried soil, leaves and roots were ground independently using an agatha mortar and kept in plastic bags at environmental temperature, until analyzed.

2.3. Laboratory Analysis

We used deionized water from a Milli-Q system (Barnstead E-pure D4631, Iowa, USA; 18.0 Mohm per centimeter) in all the analysis. Containers and glassware were soaked overnight in 10 percent (volume per volume) HNO_3 , then thoroughly washed and rinsed with deionized water before their use. All chemicals were analytical grade. The quality of the analytical data was monitored by using duplicates, spikes, blanks, reference materials and certified standards.

2.3.1. Edaphic Parameters

Soil pH was measured in a soil suspension (1:2.5 weight per volume, soil: water)

with an HI8314 pH meter (Hanna Instruments, Woonsocket, RI, USA) (Method NOM-021-RECNAT-2000, [38]) using J.T. Baker buffer solutions. Electric conductivity (EC) was measured in an extract obtained from a saturated soil-paste (soil: water) [39], using a conductivity meter (HI991301, Hanna Instruments, Woonsocket, RI, USA) and HI6031 calibration solution (Hanna Inst.). Texture was determined by the Bouyoucos hydrometer method [40] and bulk density by the method described by Blake and Hartge (1986) [41]. Organic carbon (Corg.) and total nitrogen (Nt) were quantified with an elemental analyzer (Perkin Elmer 2400 Series II CHNS/O); dissolved organic carbon was measured (DOC) using an Apollo 9000 Combustion TOC Analyzer (Tekmar-Dohrmann, USA). Cation exchange capacity (CEC) was determined by ammonium acetate method ($\text{CH}_3\text{COONH}_4$ 1N pH 7) (J.T. Baker); Ca and Mg concentrations were obtained by flame atomic absorption spectrophotometer (PerkinElmer Analyst 800, Perkin Elmer Instruments, Shelton, CT USA); and Na and K values by flame photometer (Jenway, PFP7, Essex, England). Soluble cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , NH_4^+) and anions (Cl^- , SO_4^{2-} , NO_3^- , and HCO_3^-) were measured in an extract from a saturated soil-paste (soil: water) using liquid chromatography (Waters, Mod. 1525, Milford, MA, USA) with an electrical conductivity detector (Waters, Mod. 432, Milford, MA, USA); for quantifying cations we used a Metrosep C6 column (250×4.0 millimeters; Metrohm, Herisau, Switzerland), as for anions we employed an IC-PaK column (4.6×75 millimeters; Waters, Milford, MA, USA) and cations with a Metrosep C6 column (250×4.0 millimeters; Metrohm). For quality control an internal reference material Xico (Geology Institute, UNAM) was used.

2.3.2. Soil and Plant Metal Analysis

In all determinations we used deionized water from a Milli-Q system (Barnstead E-pure D4631, Iowa, USA; 18.0 Mohm per centimeter). Containers and glassware were soaked overnight in 10 percent (volume per volume) HNO_3 , then thoroughly washed and rinsed with deionized water before use. All chemicals were analytical grade. The quality of the analytical data was monitored using duplicates, spikes, blanks, reference materials and certified standards.

To obtain total metal values for soil and lettuce (leaves and roots), samples were ground using an agatha mortar, until they were fine enough to pass through a 400 sieve, then samples were digested using a microwave digester CEM MARS-5 (CEM corporation Matthews, NC), according to the US-EPA method 3052 (1996) [42]. To measure water-soluble and exchangeable metal fractions in soils, extracts were obtained with deionized water and 0.1 M NaNO_3 solution, respectively. Metals were measured by flame (Cu, Cr, Fe, Mn, Zn) and graphite furnace (Cd) atomic absorption spectrometer (PerkinElmer Analyst 800, Perkin Elmer Instruments, Shelton, CT USA). Metal concentrations in soils and plant extracts were calculated on dry weight basis. The standards used for metal recovery in soils were: SRM 2709 San Joaquin Soil SRM 1570a, and for vegetables, Trace Elements in Spinach Leaves (U.S. National Institute of Standards and Technolo-

gy).

2.4. Data Processing

Free ion activity was obtained from the Windermere Humic Aqueous Model (WHAM). This was used to simulate metals chemical equilibrium in soils dominated by natural organic matter [43]. Interactions between metals and organic matter are simulated as a combination of chemical and electrostatic interactions (Software WHAM 7).

Metal concentrations in soils, roots and leaves were obtained considering the dilution factor and the dry weight of the sample. In order to find the metal concentration of the whole plant (leaves and roots) we used the lettuces total biomass. Metal movement from soil to plant was estimated using two factors: a) transfer factor (soil/plant); and b) translocation factor (root/leaves).

1) Transfer factor (TrF) was calculated as (Equation (1)):

$$\text{TrF} = \text{VC}/\text{SC} \quad (1)$$

where VC is the concentration of the metal in the plant, and SC is the mean total concentration of the metal in soil [26] [44].

2) Tr Tanslocation factor (TIF) was calculated as (Equation (2)):

$$\text{TIF} = \text{ALC}/\text{RC} \quad (2)$$

where ALC is the metal concentration in the aerial part of the plants (leaves) and RC is the mean concentration in the root [45] [46].

Statistical analyses were performed with Statistica 10.0 for Windows. Prior to the analysis, data were checked for homogeneity of variance by Levene's test and for normal distribution by Kolmogorov-Smirnov test [47]. Comparisons between seasons (dry and rainy) for all soil parameters measured, for soil and plant metal concentrations and between plant organs (root and leaves) were determined by Student's t tests, U de Mann-Whitney test (T). A multivariate analysis of variance (MANOVA) was used to determine whether there were significant differences between dry and rainy seasons among the variables analyzed. Metal concentration and other edaphic variables were expressed as the mean value \pm confidence interval (95%).

3. Results and Discussion

We found, in the soils studied, high contents of Corg (9 - 13 percent) and clay (20 percent) and almost neutral pH values (6.8 - 7.7). These results suggest a low mobility and a high binding strength of metals on the soil, in addition to a low incorporation into crops [12] [13]. It can be assumed that chinampa soils possess a good buffering capacity. However, changing conditions in water temperature, quantity and quality, during the year, may induce temporary changes in some soil characteristics, increasing soil metal availability, therefore, the amount that can be absorbed by plants. Moreover, lettuce properties can also exert an influence on metal concentration in their tissues.

3.1. Edaphic Parameters

We found significant differences between seasons for all the parameters measured but Corg, Ntotal and NO₃. Values for the dry season samples were higher than those for the rainy season, with exception of CEC which presented lower values (**Table 1**).

Seasonal variation of some soil physical, chemical and biological characteristics has been previously reported [23] [25] [48] [49]. In this study the differences in soil humidity and temperature, caused by the distinctly dry and rainy seasons, generated variation in the properties and dynamics of the chinampa soils. The poor quality of the water used for irrigation, together with the elevated soil evaporation rates, during the dry months, led to the accumulation of salts in the topsoil [50] [51], as reflected in the higher values of EC, ESP, Na and pH for this season.

We found high DOC concentration values, for both dry and rainy seasons (178.7 and 128.5 milligrams per liter, respectively), these figures can be compared to those reported for forest soils (5 - 440 milligrams per liter) [52] [53]; however, data for agricultural soils lie within a range of 0 - 70 milligrams per liter, less than half the values found in the chinampa soils. The high amount of

Table 1. Soil characteristics of measured variables in the dry and the rainy season (2013) in the Xochimilco chinampa, Mexico (mean values, n = 9).

Variable	Dry season		Rainy season	
pH H ₂ O (1:2.5)	7.39*	(0.14)	6.95*	(0.06)
DOC Dissolved Corg (mg·L ⁻¹)*	178.70*	(15.16)	128.54*	(7.33)
Corg (%)	12.21	(0.72)	11.36	(0.58)
Ntotal (%)	0.90	(0.07)	0.82	(0.04)
Exchangeable cations (Cmol(+) kg ⁻¹)	75.74*	(2.29)	64.70*	(1.47)
CEC (cmol(+) kg ⁻¹)	52.04*	(1.44)	59.30*	(1.28)
ESP (%)	2.56*	(0.25)	0.89*	(0.07)
EC (dS cm ⁻¹)	1.50*	(0.15)	0.56*	(0.07)
Na ⁺ (cmol(+) kg ⁻¹)	8.90*	(0.83)	1.59*	(0.62)
NH ₄ ⁺ (cmol(+) kg ⁻¹)	1.22*	(0.14)	0.91*	(0.18)
K ⁺ (cmol(+) kg ⁻¹)	3.19*	(0.41)	1.43*	(0.34)
Ca ²⁺ (cmol(+) kg ⁻¹)	12.40*	(1.86)	4.89*	(1.01)
Mg ²⁺ (cmol(+) kg ⁻¹)	15.38*	(2.54)	4.93*	(1.25)
Cl ⁻ (cmol(+) kg ⁻¹)	6.25*	(0.90)	0.91*	(0.43)
NO ₃ ⁻ (cmol(+) kg ⁻¹)	0.22	(0.07)	0.66	(0.90)
SO ₄ ²⁻ (cmol(+) kg ⁻¹)	35.16*	(5.24)	10.70*	(2.52)
HCO ₃ ⁻ (cmol(+) kg ⁻¹)	2.05*	(0.28)	1.30*	(0.15)

Values in parenthesis: confidence interval (95%). *significant difference (p < 0.05,) between seasons. Bold: higher values.

DOC found in this research can be explained by the large volumes of compost and manure continuously applied to chinampa soils [54]. Moreover, the use of treated wastewater for irrigation during the dry season has also an important impact on these soils, by increasing DOC concentration, either by acting as a source of dissolved organic matter (DOM) or by enhancing the solubilization of soil organic matter via an increase in pH [55]. In soils studied in the Hidalgo State in Mexico, it was found that DOC was derived mainly from the wastewater used for irrigation [56]. In addition, Xiao and Zheng (2000) [57] reported that a rise in temperature results in an increase of microbial activity, boosting DOC concentrations. Irrigation with wastewater from the canals and the higher temperatures (by an average of 2° Celsius) in dry conditions, may explain the significantly higher DOC concentrations during the dry season in the present study.

Besides the influence of the seasonal variability on metal availability, crop management is also an important aspect that needs to be considered during the design of sampling strategies when soil properties are compared in time or with findings from other studies, in order to distinguish seasonal variation from long-term change.

3.2. Metals in Soil

There were no significant differences in soil total metal concentration between seasons, except for Mn, which presented higher values in the rainy season samples (Table 2). Metal concentrations in soils decreased in the following order: Fe > Mn > Zn > Cr > Cu > Pb > Cd. None of the metals measured exceeded the background levels reported in the literature or the Canadian Soil Quality Guidelines (2007) [58] (Table 2), even when the concentration of metals in the surface horizon was between 50 to 100 percent greater than in the deeper horizons of these soils (data not shown) [59]. This suggests an input to the soils, which may come from soil amendments [60], irrigation water [61] [62] and atmospheric deposition [63].

Regarding bioavailable metals, there were no significant differences between dry and rainy seasons for Cd concentrations in any of the evaluated forms; however, values for the other measured metals were significantly higher for the rainy season than those in the dry season (Table 3). In the case of Pb, the soluble fraction was not significantly different between seasons. The differences in concentration between the two seasons were, in general, greater by an order of magnitude.

Concentration of the bioavailable forms (soluble + interchangeable) in the dry season varied in the following way: Cd < Cr = Cu < Pb < Zn < Fe < Mn and in the rainy season Cd < Cr > Pb < Cu < Zn < Mn < Fe. This bioavailable fraction represents 0.1 to 10 percent of the total metal concentration in the soil (Table 3).

Contrary to what was anticipated, DOC does not determine a higher solubility of metals for the soils studied. We found no significant relationship between DOC and available metals. There are contradictory results regarding the influence

Table 2. Metal concentrations in chinampa soils in the dry and rainy seasons (mean values, n = 9).

Metal	Dry Season (mg·kg ⁻¹)		Rainy season (mg·kg ⁻¹)		Baseline soil concentration [†] (mg·kg ⁻¹)	Soil Quality Guidelines [‡] (mg·kg ⁻¹)
Cd	0.36	(0.03)	0.36	(0.02)	0.05 - 1.0	1.4
Cr	48.9	(1.6)	50.6	(0.9)	10 - 50	64
Cu	31.5	(1.2)	31.7	(1.2)	10 - 40	63
Pb	21.8	(3.4)	22.7	(3.5)	10 - 30	70
Zn	98	(13)	95	(12)	20 - 200	200
Fe	11,313	(299)	11204	(86)	1% - 5%§	-
Mn*	384	(25)	443	(25)	0.03% - 1% ^{**}	-

Values in parenthesis: confidence interval (95%); significant difference ($p < 0.05$) between seasons. Bold: higher values. [64] [†]Canadian Soil Quality Guidelines (2007) [58]. -, not reported. §Fe₂O₃, ^{**}MnO. Fe and Mn values are expressed as percentage.

Table 3. Mean concentrations of bioavailable metals in soil (soluble, interchangeable and the sum of both fractions) in the dry and rainy season, 2013, and their percentage in relation to the total metal concentration.

Metal	Season Soil fraction	Dry		% total concentration	Rainy		% total concentration
		(mg·kg ⁻¹)			(mg·kg ⁻¹)		
Cd	Soluble	0.003	(0.001)	0.96	0.004	(0.0004)	1.08
	Exchangeable	0.013	(0.001)	3.52	0.014	(0.001)	3.95
	Sol + Exch	0.016	(0.002)	4.48	0.018	(0.01)	5.03
Cr	Soluble	0.01*	(0.002)	0.02	0.030*	(0.007)	0.06
	Exchangeable	0.03*	(0.003)	0.05	0.04*	(0.003)	0.09
	Sol + Exch	0.04*	(0.005)	0.08	0.07*	(0.006)	0.15
Cu	Soluble	0.012*	(0.002)	0.04	0.114*	(0.007)	0.36
	Exchangeable	0.026*	(0.001)	0.08	0.211*	(0.01)	0.67
	Sol + Exch	0.038*	(0.003)	0.12	0.326*	(0.02)	1.03
Pb	Soluble	0.03	(0.004)	0.12	0.03	(0.003)	0.14
	Exchangeable	0.09*	(0.01)	0.40	0.10*	(0.01)	0.45
	Sol + Exch	0.11*	(0.01)	0.52	0.13*	(0.01)	0.58
Zn	Soluble	0.58*	(0.08)	0.59	1.28*	(0.20)	1.34
	Exchangeable	1.40*	(0.13)	1.43	2.16*	(0.27)	2.28
	Sol + Exch	1.98*	(0.19)	2.02	3.44*	(0.45)	3.63
Fe	Soluble	4.75*	(1.78)	0.04	14.26*	(1.25)	0.13
	Exchangeable	8.96*	(1.07)	0.08	37.21*	(3.79)	0.33
	Sol + Exch	13.71*	(2.81)	0.12	51.47*	(4.21)	0.46
Mn	Soluble	1.28*	(0.16)	0.33	2.17*	(0.35)	0.49
	Exchangeable	15.64*	(1.54)	4.08	43.27*	(3.11)	9.77
	Sol + Exch	16.92*	(1.62)	4.41	45.44*	(3.43)	10.26

Values in parenthesis: confidence interval (95%). *: significant differences ($p < 0.05$) between seasons. Bold: the season with the higher metal concentration.

of DOC on soil metal availability. Several authors report that DOC can favor metal solubility in soils [65] [66]. Others relate changes in metal solubility to a high percentage of DOC consisting of humic and fulvic acids, which can form complexes with metal ions and alter the solubility of both the ligand and the bound species [67]. Apparently, the slightly low pH values and the low salinity conditions (referred to as concentrations of exchangeable bases and soluble cations and anions) in our soils led to greater solubility of metals during the rainy season. The concentration of each metal (total true solution) was also higher in the rainy season ($p < 0.05$, except for cadmium) (Figure 1). The true solution contains all the free ions and simple ligand complexes.

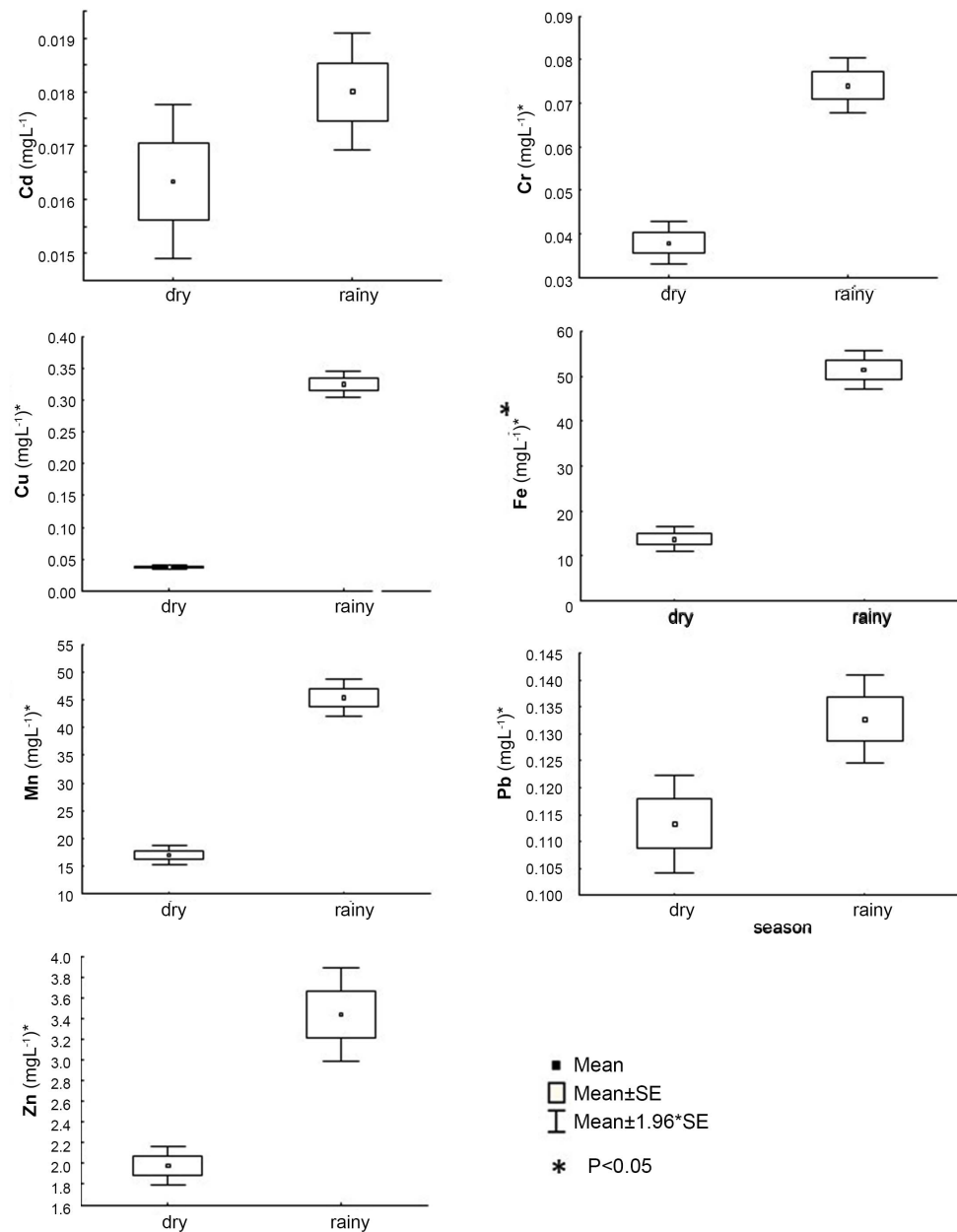


Figure 1. Concentrations by season of available metals (true solution) in the chinampa soils (n = 9).

3.3. Metals in Plants

The metal contents in plants differed significantly between the two seasons. The total concentration in the entire plant (leaves and roots) was higher in the dry season for Cd, Mn and Zn; conversely, during the rainy season Cu, Fe and Pb concentrations were higher than in the dry season. Chromium showed no difference between seasons (Table 4).

Concentrations of Cr, Cu, Fe and Pb, for the leaves alone, were higher in the rainy season, whereas Cd content was higher in the dry season. Contents of Cd, Fe, Mn and Pb in lettuce roots were higher in the dry season, but those of Cr, Cu and Zn did not differ between the two seasons (Table 4 and Figure 2).

Table 4. Metal concentration in lettuce (*Lactuca sativa* L.) in the dry and rainy seasons of 2013.

Metal	Plant tissue	Metal (mg·kg ⁻¹)				Significant difference in metal content between leaf and root.	
		Dry season		Rainy season		Dry season	Rainy season
		mg kg ⁻¹	(95% CI)	mg kg ⁻¹	(95% CI)		
Cd	Leaf	0.17*	(0.01)	0.06*	(0.00)		
	Root	0.31*	(0.04)	0.09*	(0.01)	p < 0.05	p < 0.05
	Leaf & Root	0.19*	(0.01)	0.06*	(0.00)		
Cr	Leaf	0.07*	(0.02)	0.17*	(0.06)		
	Root	0.41	(0.15)	0.21	(0.15)	p < 0.05	-
	Leaf & Root	0.11	(0.04)	0.18	(0.07)		
Cu	Leaf	8.52*	(0.72)	12.55*	(0.29)		
	Root	13.49	(1.29)	13.41	(1.48)	p < 0.05	-
	Leaf & Root	9.17*	(0.71)	12.66*	(0.07)		
Fe	Leaf	139.11*	(10.34)	466.1*	(30.7)		
	Root	659.43*	(63.47)	447.9*	(3.9)	p < 0.05	-
	Leaf & Root	207.26*	(15.45)	463.7*	(27.1)		
Mn	Leaf	18.73	(1.26)	17.96	(1.10)		
	Root	33.93*	(1.71)	22.59*	(2.15)	p < 0.05	p < 0.05
	Leaf & Root	20.72*	(0.90)	18.57*	(0.71)		
Pb	Leaf	0.14*	(0.02)	0.59*	(0.04)		
	Root	1.20*	(0.08)	0.53*	(0.16)	p < 0.05	-
	Leaf & Root	0.28*	(0.02)	0.58*	(0.06)		
Zn	Leaf	67.61	(3.65)	37.72	(17.68)		
	Root	90.58	(13.72)	69.43	(32.88)	p < 0.05	-
	Leaf & Root	70.62*	(4.91)	41.90*	(17.25)		

In parenthesis, confidence interval (95%); *: significant difference (p < 0.05) between seasons; Bold: the season with the higher metal concentration; -: p > 0.05. Leaves and Roots concentrations were estimated by considering total biomass (leaves and roots) and the mean concentrations of metals obtained from each organ.

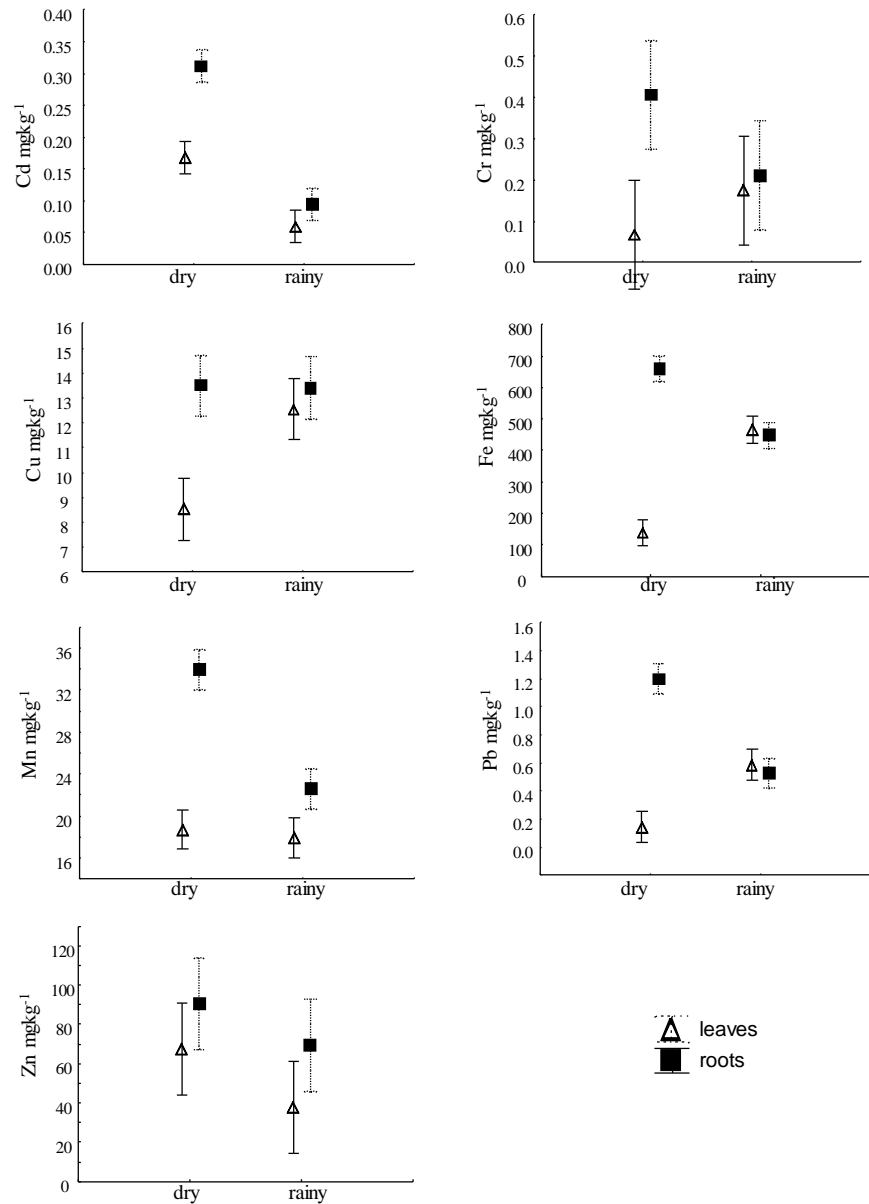


Figure 2. Metal concentrations in roots and leaves of lettuce (*Lactuca sativa* L.) in two seasons (Vertical bars: 0.95 confidence intervals).

There was no correlation between the total metal concentration in the soil and that of plants (leaves and roots), neither was between the labile metal fraction in the soil and the total concentration in plants. Similar results were reported by other authors [26] [33] [68] and these may be the effect of ion competition, which in turn affects the uptake rate of free ions [16] [69].

3.4. Soil-Plant Relationships: Transfer and Translocation Factors

In the context of food security, it is necessary to estimate the quantity of metals entering crops, particularly if an edible part is involved. It is also important to study the variables that enhance the flow of metals into the plant and their

transfer to different organs; however, this is not an easy task because of the large number of factors involved. To evaluate the metal uptake from soils to lettuce and its translocation from roots to leaves (the edible part), we calculated the transfer factor (TrF, **Figure 3**) and translocation factor (TIF, **Figure 4**), for each metal.

Metal TrF in the dry season, behaved as follows: Zn > Cd > Cu > Mn = Fe = Pb = Cr, with the order of magnitude being almost the same for each metal. In contrast, in the rainy season, the TrF for Cu exceeded that for Cd. During this

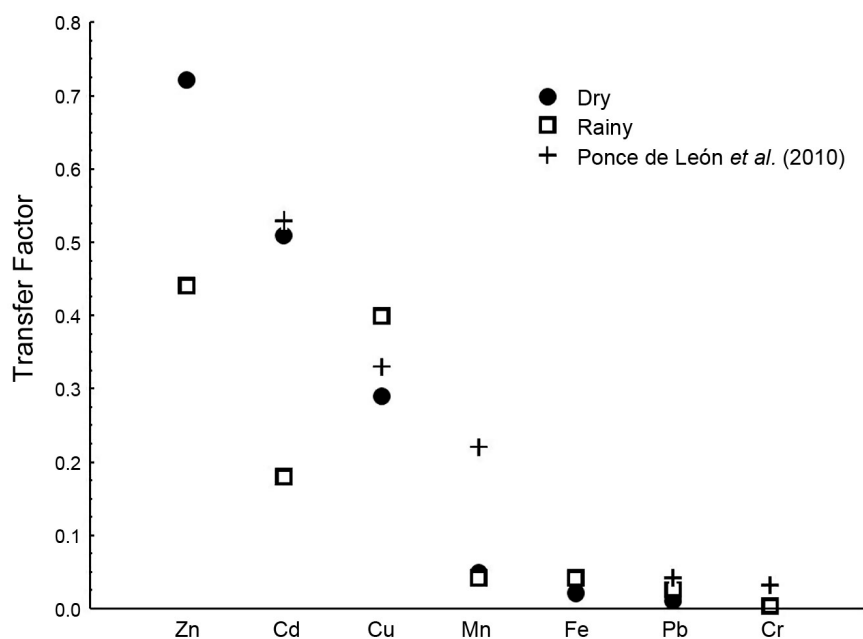


Figure 3. Transfer factors (dry weight) in lettuce in the dry and rainy seasons (2013) and those measured by Ponce de León *et al.* (2010) [33] in the same study area.

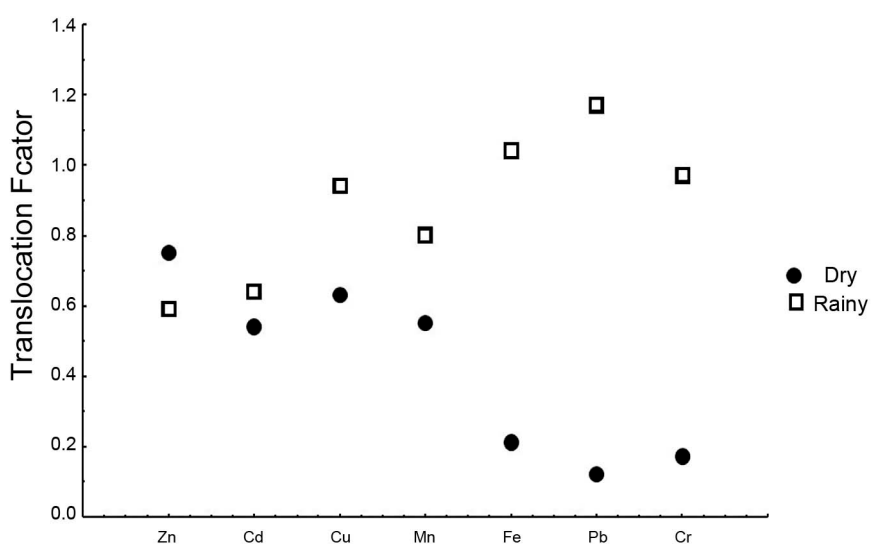


Figure 4. Metal translocation factor from roots to leaves in lettuce (*Lactuca sativa* L.) in the dry and rainy seasons.

season, leaf & root concentrations were higher for Cu, Fe and Pb, and the TrF indicates that Cu is absorbed to a greater extent with the rain regime; however, in the dry season the concentrations were higher for Cd, Zn and Mn, with the highest TrF for the first two (**Table 4, Figure 3**). It is known that the uptake rate generally increases with increasing concentration of free ions in pore water [16]. Our results show that this behavior is consistent for Cu, but not for Cd and Zn, which exhibit higher TrFs in the dry season.

The TrFs found are comparable to those measured by Ponce de León *et al.* (2010) [33] in the same studied area (samples collected in December and February). However, Uwah *et al.* (2011) [70] report higher TrF values for Cd (2.23 and 2.75), Pb (1.05, 1.64), and Fe (0.71, 0.79) and similar amounts for Cu (0.44, 0.95) and Zn (0.55, 0.47). Nevertheless, the agricultural region where the study was performed, although also irrigated with sewage water, is more arid (higher temperatures and less rainfall) than our study site and has sandy soils with low organic matter content.

Translocation is an important process in calculating trace metal concentrations in edible plant organs and it is element specific [71]. TIF values for all the metals measured, but Zn, were higher in the rainy season than in the dry season. Zn, Cd, Cu and Mn had similar TIF values. However, the differences in TIFs found for Fe, Pb and Cr were much greater in samples from the rainy season than those from the dry season. Although the TrF values for these three elements were very small and almost identical for both seasons, these are not reflected in the TIFs, which are completely different between seasons. There were higher concentrations of metals in the lettuce edible part in samples from the rainy season than those from the dry season, especially for Fe, Pb and Cr. The relative magnitudes of the TIFs differed between the two seasons: in the dry season, $Zn > Cu > Mn > Cd > Fe > Cr > Pb$; in the rainy season, $Pb > Fe > Cr > Cu > Mn > Cd > Zn$.

Plants suffer from osmotic stress during the dry season as a result of the high salt concentration in the wastewater used for irrigation, causing a decrease in soil osmotic potential and reducing the plant ability to absorb water [72]. In order to preserve water, the plant keeps its stomata closed, maintaining transpiration rates at their lowest and preventing water and nutrient movement into and within the plant. These processes can explain the low translocation factor for Fe, Pb and Cr during the dry season and are reflected in the lettuce biomass (wet weight).

Lettuces harvested during the dry season weighed 40 percent less (wet weight) and were smaller than lettuces gathered in the rainy season, the latter incorporated more water and metals in their tissues; however, dry biomass was the same in both seasons. Although TIF was higher in the rainy season, only Pb and Fe values were slightly higher than 1, so lettuce can be considered a metal accumulator plant in the rainy season for Pb and Fe [73]. Nonetheless, in the dry season the plant limits metal translocation and maintains most of them in the roots,

acting as an excluder and keeping low levels in the aerial tissues.

TIF values are not constant for a given element and plant species. As mentioned before, metal speciation and uptake depend on a variety of factors: the plant genotype, the availability of the metal, the water content of the soil, and the temperature and humidity of the environment [74]; these may explain the differences in metal concentration values found between the dry and the rainy seasons.

Although it is important to know the physical and chemical characteristics of the pollutants, as well as the effects of changing environmental conditions on metal speciation and its accumulation in soils over time, this information is insufficient to know the likely uptake of the metal and the factors that promote its translocation into the plant [16]. The processes that regulate metal translocation are correlated with water transport and transpiration rates [75] [76]; therefore, in assessing the relationship between transpiration and metal solubility and movement, it is important to consider the species present at the time of absorption or translocation. In a study focused on Cd, Akhter and Macfie (2012) [76] reported that an increment in transpiration rates sometimes caused an increase in the accumulation of Cd in plants, including lettuce; however, the proportion of total metal transported to the leaves varied according to the plant species; this suggested that factors controlling the specific internal distribution of Cd compounds are more important than transpiration in the translocation of Cd to epigeous organs.

These differences can be attributed to the variation of plant responses to metals in soils [76], and they reinforce the argument that the uptake and accumulation of metals in the various organs of a plant depend on the correlation of many factors, such as plant species, developmental stage, edaphic characteristics, and soil organic matter [71] [77] [78] [79].

4. Conclusions

This study shows that lettuces grown in the chinampa soils of Xochimilco, Mexico City, accumulate metals but their uptake and transport differ between the two crop cycles analyzed, probably because of the difference in the quality of the water used for irrigation (wastewater and rainwater). More important, the metal concentration present in the plant tissues does not represent a threat to human health. During the dry season, the transfer of metals from the soil to the plant was higher than in the rainy season, but the root-to-leaf translocation was lower than in the rainy season.

To assess the local effects of metals in urban agriculture, it is necessary to perform site-specific studies. Moreover, it is important to gather information on the differences among the four lettuce harvests produced annually in these chinampas. It is critical to consider metal variability and availability in order to design sampling strategies which allow us to estimate long-term changes or to compare results among different studies.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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