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Concentrations of major and trace elements in the soils, edible parts of crops and urine of farmers in agroecological communities

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ABSTRACT. The relationship between soil and health is important for populations that depend on the local environment to meet their nutritional needs. This study aimed to evaluate the concentrations of major and trace elements in the soils, edible parts of crops and urine of farmers in agroecological communities. We collected samples of soil, edible parts of crops and urine of farmers and family members in 23 crop fields in agroecological communities from northeast Brazil. These samples were analyzed to concentrations of Al, As, Ca, Cr, Cu, Fe, Hg, Mg, Zn, and Pb in urine and discriminant analysis and principal component analysis were used to assess the data. Concentrations of potentially toxic elements in soils and crops of agroecosystems were below regulatory levels. Farmers living in agroecological communities present most of the essential and toxic elements in urine within the reference ranges. In general, results showed that urinary concentration of toxic elements among farmers and their families were below allowable limits, which may be due to the agroecological practices.

Keywords: heavy metals; agroecossystems; biomonitoring.

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Introduction

Several major (Ca, Cl, Mg, P, K, Na, S, N, C, O, and H) and trace (Co, Cr, Cu, Fe, Mn, Mo, Se, Zn, F, and I) elements are essential for human nutrition, although excessive concentrations of some of these elements may cause toxicity problems. On the other hand, elements such as As, Cd, Pb, Hg, and Al have no biological functions and are only toxic (Selinus, 2004). The concentrations of major and trace elements in crops are primarily dependent on the geological parent material of the soil, although anthropogenic sources may contribute to increases in these values.

Agroecology is the study of ecological processes that operate in agricultural production systems. Production systems based on agroecology are characterized by the use of environmentally friendly technologies that promote natural processes and biological interactions that enhance synergy so that diversified lands can support their own soil fertility, crop protection and productivity (Altieri, 2002).

The exposure routes of humans to essential and toxic elements are the ingestion of food, water and soil, inhalation and dermal absorption. The ingestion of food and soil, the latter especially by children, have been identified as the main exposure routes for essential and potentially toxic elements in humans (Khillare et al., 2012). The assessment of human exposure to chemicals can be performed by measuring the chemicals or their metabolites in human specimens, such as blood or urine. Urine has been regarded as a good biological indicator to evaluate the nutritional status due to its advantages over other biological matrices, such as its ease of sampling and effective indication of exposure to trace elements and various diseases (Horng et al., 2002; Parsons & Barbosa, 2007). Several studies have suggested that urine, as a non-invasive matrix, is preferred for the biomonitoring of potentially toxic elements (Moon et al., 1999; Barbosa, Tanus-Santos, Gerlach, & Parsons, 2005; Berglund et al., 2005). In the present work, we hypothesize that the concentration of elements in the urine of people living in communities that produce their own food are closely related not only to the food itself but also to the soils and agricultural practices.

Although agroecological practices are widely regarded to protect human health and the environment, there have been very few studies assessing the validity of this assumption. This study aimed to evaluate the urinary concentration of major and trace elements among farmers and family members in order to correlate it with the concentration of these elements in the soils and food produced by agroecological communities. Both essential (Cu, Zn, Cr, Fe, Ca, and Mg) and toxic (Pb, As, Hg, and Al) elements were assessed.

Material and methods

Study sites

We selected farmers using agroecological practices from six communities from seven municipalities of Pernambuco State, northeast Brazil (Figure 1). In each community, three or four crop fields were selected, with a total of 23 production areas with diversified agroecological approaches, namely, the use of agroforestry, organic farming and vegetable gardens.

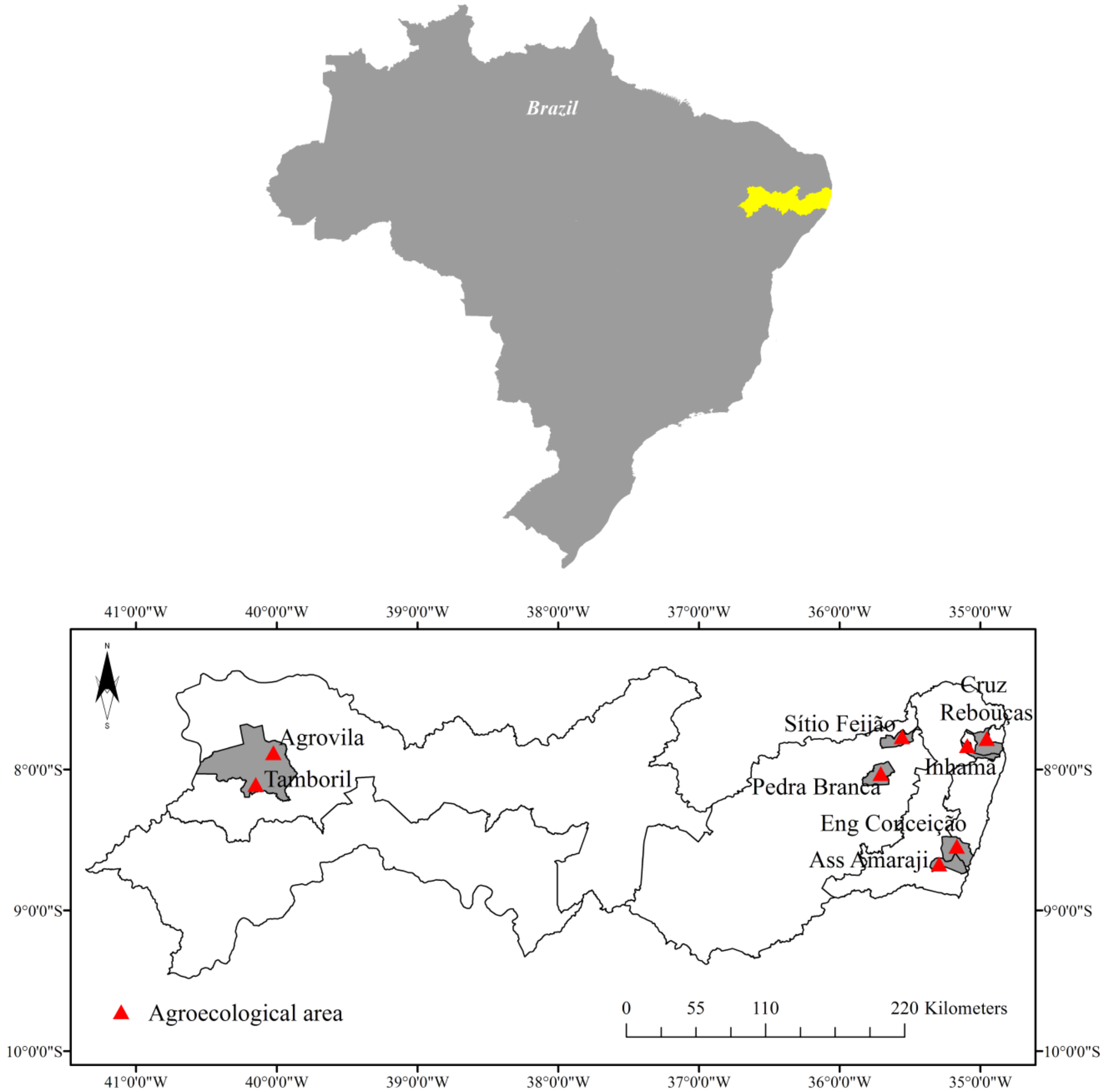

Figure 1. Sampling sites of agroecological systems in the state of Pernambuco, Brazil.

Sampling of soil, edible parts of crops and urine

Three composite soil samples (0 - 20 cm) were collected in each studied area; each composite sample consisted of fifteen randomly collected simple samples. Samples of the agricultural products produced and consumed by the farmers were also collected. Several samples of the edible parts of crops were randomly collected (Khillare, Jyethi, & Sarkar, 2012). According to the seasonal availability, we collected samples from different food groups, including the following vegetables, tubers and fruits: green onion (*Allium* *schoenoprasum*), coriander (*Coriandrum sativum*); kale (*Brassica oleracea* L.); gherkin (*Cucumis anguria* L.); bell pepper (*Capsicum annuum*); okra (*Abelmoschus esculentus* L.); sweet potato (*Ipomoea batatas*); purple yam (*Dioscorea alata* L.); avocado (*Persea americana*); acerola (*Malpighia punicifolia* L.); banana (*Musa sapientum*); cocoa (*Theobroma cacao*); caja (*Spondias mombin* L.); star fruit (*Averrhoa carambola*); red mombin fruit (*Spondias purpurea*); guava (*Psidium guajava*); soursop (*Annona muricata*); orange (*Citrus sinensis*); lemon (*Citrus limon*); passion fruit (*Passiflora edulis*); and sugar apple (*Annona squamosa*).

For the urine sampling, farmers were divided into three groups: women (⩾ 18 years old), men (⩾ 18 years old), and children and teenagers (5 - 17 years old). For the children and teenagers, sampling was carried out with the consent of a legal guardian. Urine samples were collected in 80 mL fully transparent plastic bottles (universal collector) that had been previously sterilized by ionizing radiation. People were instructed to collect samples from the first morning urine, after disregarding the first jet, according to the procedures approved by the Research Ethics Committee of the University of Pernambuco (UPE), Brazil. All tubes were identified and transported in a cooler to the laboratory, where they were immediately frozen at - 4°C in a freezer.

Chemical analyses and quality control

Soil samples were dried at room temperature and subsequently ground and passed through a 2 mm mesh sieve. In order to determine the concentrations of trace and major elements, soil samples were further macerated in agate mortar and passed through a 0.3 mm mesh stainless steel sieve in order to avoid trace element contamination. The edible part of the crops was washed three times in distilled water and dried at 65°C. The material was then macerated in a mortar; leafy vegetables were milled in grinding mill.

Soil and crop samples were digested in Teflon vessels with 9 mL of HNO_3 and 3 mL of HCl in a microwave oven (USEPA, 2007). All of the extracts were transferred to 50-mL certified flasks (NBR ISO/IEC) filled with ultrapure water and filtered in a slow filter paper. High-purity acids were used in the analysis (Merck PA).

Urine samples were defrosted to room temperature and homogenized. We pipetted 2.4 mL of the sample and transferred to 15 mL sterile Falcon tube, in which we added 9.6 mL of 5% HNO_3 solution and ultrapure water for maintaining a 5-fold urine dilution (Goullé et al., 2005).

We determined the concentrations of Al, Ca, Cr, Cu, Fe, Mg, Pb, and Zn by optical emission spectrometry (ICP-OES/Optima 7000, Perkin Elmer). As and Hg were determined by an atomic absorption spectrophotometer (Aanalyst 800 Perkin Elmer) coupled with a hydride generator (FIAS 100/Flow Injection System/Perkin Elmer).

Quality control of the analysis was carried out using samples of multielement reference solutions (spikes), which were prepared from 1000 mg L^{-1} standard (TITRISOL®, Merck). In general, good recoveries of elements were obtained in the spike for almost all the elements, ranging between 87 and 106%.

Statistical analysis

Results were evaluated and discussed using univariate and multivariate statistical procedures. For the univariate procedure, we used descriptive statistics. To evaluate the relationship between the concentrations of the chemical elements in the urine and soil or urine and crops, the Spearman correlation analysis was used (non-parametric correlation test). The nonparametric statistical method was chosen after verifying that the variables, even when transformed, did not follow a normal distribution. For the multivariate procedures, discriminant analysis (DA) and principal component analysis (PCA) were used.

Results and discussion

Trace and major elements in soils and edible parts of crops

The concentration of trace and major elements in the soil varied widely (Table 1). This result is likely because the studied areas had different soil management processes and were located in different geological contexts influencing the natural content of elements in the soil. The mean concentration of elements in the soils followed the order Al > Fe > Ca > Mg > Cr > Zn > Pb > Cu > As > Hg, which reflects the abundance of elements in the Earth's crust. The potentially toxic trace elements presented concentrations within the quality reference values established for the state of Pernambuco (CPRH, 2014), except for Cu and As. However, the highest Cu and As concentrations found do not pose a risk to human health, according to the Brazilian guidelines for trace elements in soil (CONAMA, 2009).

Table 1. Mean concentrations of trace and major elements, essential and toxic, in soils of agro-ecological systems.

	Metal concentration						
	Mean	Minimum	Maximum	SD	CV	QRV	PV
	mg kg^{-1}				%	mg kg^{-1}	
Cu	8.47	1.50	50.40	9.85	116	5	60
Pb	9.38	2.21	40.34	7.95	85	13	72
Zn	11.70	2.66	41.10	8.90	76	35	300
Cr	18.51	8.19	35.72	8.77	47	35	75
Hg	0.04	0.01	0.10	0.03	62	0.1	0.5
As	0.76	0.06	2.50	0.82	107	0.6	15
	g kg^{-1}				%		
Al	19.31	9.68	39.38	9.62	50	-	-
Ca	1.12	0.31	4.10	0.88	79	-	-
Mg	0.94	0.08	2.72	0.74	79	-	-
Fe	11.77	3.21	21.44	5.11	43	-	-

SD: standard deviation; CV: coefficient of variation; QRV: Quality reference value (CPRH, 2014); PV: prevention value (CONAMA, 2009); Values not established.

The concentrations of trace and major elements in the group formed by vegetables and tubers presented the following order: Ca > Mg > Fe > Al > Zn > Cu > Cr > Pb > Hg >As (Table 2). For the fruits, the order was the same, except Al and Fe switched places.

The high concentrations of Fe, Ca, and Mg in the first group of agricultural products (Table 2) are due to the high contributions of these elements in leafy vegetables. For instance, Ali and Al-Qahtani (2012) found high Fe concentrations in leafy vegetables grown in Saudi Arabia and attributed these high concentrations to the fact that leaves absorb and accumulate more Fe than fruits. Only the maximum Cr concentration in the first group was higher than that established by the National Health Surveillance Agency of Brazil - ANVISA (1965) (0.1 mg kg^{-1}). All the other potentially toxic elements were below the maximum allowable concentrations in food established by national and international legislation.

Table 2. Mean concentrations of trace and major elements (*in natura*) in edible parts of agricultural products of agroecological systems.

	Pb	Cr	Hg	As	Cu	Zn	Al	Fe	Ca	Mg
	µg kg^{-1}				mg kg^{-1}				g kg^{-1}	
	Vegetables and tubers									
Mean	12.34	48.91	10.71	0.71	0.88	2.38	17.16	29.18	1.77	0.65
Minimum	0.00	0.00	0.00	0.00	0.20	1.65	0.78	4.79	0.09	0.14
Maximum	41.25	158.33	26.46	3.65	2.01	4.31	37.91	87.80	11.13	3.14
SD	17.98	52.57	8.33	1.39	0.56	0.88	14.35	29.28	3.80	1.01
CV	146	107	78	194	63	37	84	100	215	156
	Fruits									
Mean	17.74	8.61	7.11	0.68	0.91	2.29	4.56	3.67	0.19	0.20
Minimum	0.00	0.00	0.29	0.00	0.30	0.80	0.39	1.71	0.03	0.11
Maximum	60.00	19.51	14.59	3.42	3.80	6.69	11.89	8.71	0.41	0.52
SD	18.60	7.31	4.87	1.15	0.97	1.85	3.91	1.96	0.13	0.12
CV	105	85	69	169	107	81	86	53	66	58

SD: standard deviation; CV: coefficient of variation.

Trace and major elements in urine

The mean concentration of Cu in the urine of farmers (Table 3) was within the reference values for the populations of Brazil, France and the UK, which range from 2.2 to 18.4 µg L^{-1}, (Batista et al., 2009), 4.3 to 12.1 µg L^{-1} (Goullé et al., 2005), and 4.6 to 40.4 µg L^{-1} (White & Sabbioni, 1998), respectively. These reference values were obtained from a large number of samples distributed across several parts of the countries, where differences occur due to the environment, eating habits, and physiological parameters; therefore, reference ranges exhibit considerable variations (Batista, Rodrigues, Tormen, Curtius, & Barbosa, 2009). The mean Cu concentration in the urine of farmers demonstrated that their exposure to this element was not problematic. The mean concentrations of Cu were lower than those reported by Inoue et al. (2014) for rural residents of Hainan Island (15.1 µg L^{-1}), China.

We found that the mean Cu concentration was higher in men than in women (Table 3). On the other hand, Aguilera et al. (2008) found higher Cu concentrations in women. These gender differences in urine Cu concentrations are linked to eating habits and hormonal influences, which may affect the absorption of this element (Olsson et al., 2002; Kazi et al., 2008). Cu is an essential trace element for humans; however, it can become toxic when it accumulates in the body at high concentrations. According to Wapnir (1998), exposure to Cu is influenced by the ingestion of contaminated food and water. Cu deficiency can lead to anemia and osteoporosis in children (Kanumakala, Boneh, & Zacharin, 2002).

Table 3. Concentrations of chemical elements in urine of farmers of agroecological systems in Pernambuco, Brazil.

	Cu	Pb	Zn	Cr	As	Hg	Al	Fe	Ca	Mg
	μg L $^{-1}$								mg L $^{-1}$	
	Women (n=25)									
Mean	6.31	1.62	124.20	0.56	7.87	0.93	59.30	34.40	45.01	56.46
Minimum	ND	ND	25.00	ND	ND	0.02	ND	9.72	0.31	11.40
Maximum	17.91	5.89	440.00	2.03	13.96	3.92	180.00	84.33	256.40	188.42
SD	4.56	1.79	114.47	0.60	3.70	0.91	54.62	18.63	55.07	44.86
CV (%)	72	111	92	106	47	98	92	54	122	79
	Men (n=23)									
Mean	7.48	1.97	290.65	0.67	8.36	1.06	75.22	41.21	94.49	99.66
Minimum	ND	ND	27.50	ND	0.75	ND	ND	8.76	4.08	13.90
Maximum	18.11	3.82	1567.50	1.85	16.73	2.32	187.50	93.15	345.48	288.32
SD	4.59	1.31	317.43	0.59	3.34	0.68	51.98	22.71	88.24	60.45
CV (%)	61	66	109	88	40	65	69	55	93	61
	Children and teenagers (n=15)									
Mean	5.98	2.35	191.50	0.77	8.78	0.70	88.17	43.46	77.86	110.60
Minimum	ND	ND	27.50	ND	4.61	0.14	5.00	12.61	3.39	18.39
Maximum	11.30	3.96	505.00	1.70	15.18	1.86	195.00	90.38	231.80	201.63
SD	3.20	1.24	118.75	0.48	2.54	0.51	60.15	24.16	69.61	53.59
CV (%)	54	53	62	62	29	74	68	56	89	48

SD: standard deviation; CV: coefficient of variation; ND: not detected.

The mean concentration of Pb (Table 3) in the urine of all farmers was within the reference concentration range (< 0.03-2.96 μg L^{-1}) for the Brazilian population (Batista et al., 2009). The mean Pb concentration was lower than those found by Inoue et al. (2014) in China (4.3 μg L^{-1}) and White and Sabbioni (1998) in the United Kingdom (11.9 μg L^{-1}) and higher than those reported in other countries, such as France (0,55 μg L^{-1}) (Goullé et al., 2005), the United States (1.3 μg L^{-1}), (Komaromy-Hiller, Ash, Costa, & Howerton, 2000), and Spain (1.05 μg L^{-1}) (Castaño et al., 2012). According to Kosnett (2003), Pb contamination occurs due to the ingestion of contaminated food and beverages. According to Schifer, Bogusz, and Montano (2005), Pb concentrations in foods are higher in industrialized regions, where the metal and its compounds are widely used. Thus, it is expected that the Pb concentrations in the foods produced in agroecological communities are lower than those in the foods produced in industrialized regions, which is reflected in the farmers' health.

In a study carried out by Moreira and Neves (2008), a significant correlation between the concentrations of lead in blood and urine was observed. Thus, the authors suggested that urine can be used to replace blood in the assessment of occupational lead exposure. By applying their exposure ranges to our data, we found that only one participant among the adults had a Pb concentration within the moderate occupational exposure range (Pb concentration in the blood between 100 to 275 μg L^{-1}). Moreira and Neves (2008) failed to establish the occupational exposure range for children. Even if the adult ranges were applied to the group of children and teenagers in this work, they would all be classified as environmentally exposed (Pb concentration in the blood of < 100 μg L^{-1}).

The Pb concentrations in the urine of children and teenagers were higher than those in the urine of men and women (Table 3). This is probably due to the greater contact of children with dust and topsoil since the streets of rural communities are not paved. Several studies have shown that the ingestion of soil or inhalation of soil particles (dust) are the most significant routes of Pb exposure in children (Maisonet, Bove, & Kaye, 1997; Meyer, Heinrich, & Lippold, 1999; Paoliello et al., 2002). According to Cunha, Figueiredo, Paoliello, and De Capitani (2006), children are considered the group demonstrating the highest risk, since, compared to adults, they absorb and retain more ingested Pb. The Disease Control Center (DCC) of the United States reported that the current concerning concentration of Pb in children's blood is 100 μg L^{-1}. However, adverse effects may occur at lower concentrations.

Due to the lack of Zn reference values in urine in Brazil, the concentrations were compared with values reported in France. The mean Zn concentrations of the three groups (Table 3) were within the range established by Goullé et al. (2005) for the French population, which is 44 – 499 μg L^{-1}. The wide Zn concentration range observed in men, with a maximum concentration of 1,567.5 μg L^{-1}, was probably due to the two farmers of that group who are smokers. Schuhmacher, Domingo, and Corbella (1994) compared the urinary Zn concentrations of smokers and nonsmokers and found that smokers presented the highest values; in blood serum, in contrast, nonsmokers had higher Zn concentrations. Therefore, as confirmed by the data of our study, the use of cigarettes results in higher Zn excretion in urine. The mean Zn concentrations (Table 3) were lower than those found in the populations of the following countries: China, 470 μg L^{-1} (Inoue et al., 2014); the United States, 371.5 μg L^{-1} (Komaromy-Hiller et al., 2000); and Spain, 698.7 μg L^{-1} (Schuhmacher et al., 1994). The Zn concentrations in the group comprising children and teenagers were lower than that reported for children in Poland, which is 660 μg L^{-1} (Błażewicz, Klatka, Astel, Partyka, & Kocjan, 2013).

The mean concentration of Cr (Table 3) in this study differed from the reference values of the United States and the United Kingdom. The mean Cr concentrations in the urine of women (0.56 μg L^{-1}) was within the range established by Paschal et al. (1998) for the United States, ranging from < 0.1 to 0.7 μg L^{-1}. In men, the mean Cr concentration (0.67 μg L^{-1}) was close to the upper limit of the reference range. The group of children and teenagers showed a mean Cr concentration (0.77 μg L^{-1}) slightly above the upper reference limit. When compared with the reference values for the UK (0.04 - 0.96 μg L^{-1}) (White & Sabbioni, 1998), all groups showed mean Cr concentrations within the range.

The mean Al concentration in urine (Table 3) was above the reference concentrations established for the Brazilian population, which are between 0.22 and 17.5 μg L^{-1} (Batista et al., 2009). The mean concentration was also higher than the reference values for France, which are between 0.16 and 11.2 μg L^{-1} (Goullé et al., 2005). When compared with the reference values of the United Kingdom, which are between 1.2 and 168 μg L^{-1} (White & Sabbioni, 1998), the mean Al concentrations for all three groups were within the range. Al is toxic to the human body. The World Health Organization (WHO, 1996) reports that Al ingestion is increased when acidic foods are cooked with utensils made of Al.

The concentrations of As in the urine of farmers (Table 3) were within the reference range for the population of the United Kingdom (White & Sabbioni, 1998) and France (Goullé et al., 2005) (0.4 and 48.2 μg L^{-1} and 2.3 and 161 μg L^{-1}, respectively). The values found in this study were higher than those found by Aguilera et al. (2008) (2.11 μg L^{-1}) in Spain and lower than those reported by Komaromy-Hiller et al. (2000) (25.1 μg L^{-1}) for the United States, Calderón et al. (2001) (40.28 μg L^{-1}) for Mexico, and Nordberg et al. (2005) (56.23 μg L^{-1}) for China. Children and teenagers showed a mean As concentration higher than that of adults (Table 4). According to Zhang, Deng, Lu, and Richardson (2002), studies have shown that the expression of genes coding the methyltransferases involved in DNA methylation decreases significantly as humans age. Other authors have speculated that methylation increases over human growth and that the exposure to factors that may inhibit the methylation, such as cigarettes, alcoholic beverages, and environmental pollutants, increases with the age (Hsueh et al., 2003; Lindberg et al., 2008). The methylation of inorganic As in the human body reduces the affinity of the compound to the tissue, and urine is the main form of As elimination (Vahter, 2002).

The mean Hg concentration in the urine of farmers (Table 3) was within the reference range for the United Kingdom (White & Sabbioni, 1998), France (Goullé et al., 2005), and the Czech Republic (Batáriová et al., 2006), which established intervals of < 0.5 - 10.0 μg L^{-1}; 0.14 - 2.21 μg L^{-1}, and 0.55 - 3.45 μg L^{-1}, respectively. These mean concentrations of Hg were lower than those found by Castaño et al. (2012), with a mean concentration of 1.19 μg L^{-1}, and Komaromy-Hiller et al. (2000), with a mean concentration of 1.4 μg L^{-1}. The maximum acceptable concentration of Hg in the urine of adults in the state of New York is 20 μg L^{-1}. In contrast with the pattern for As, the mean values of Hg were lower in children and teenagers than in adults. This was also observed by Gil et al. (2006), who reported higher Hg concentrations in the urine of adults than in the urine of children.

The mean concentration of Fe (Table 3) was below the value reported by Cui et al. (2005), who compared the Fe concentration in the urine of a group of people from an uncontaminated area (310 μg L^{-1}) with those in the urine of two groups exposed to contaminated areas (190 and 180 μg L^{-1}). It is also below the mean Fe concentration (72.3 μg L^{-1}) found in a study carried out in Poland (Długaszek, Kaszczuk, & Mularczyk-Oliwa, 2011). Długaszek et al. (2011) also found higher iron concentrations in men than in women. This gender difference is because women are more susceptible to iron deficiency caused by menstrual blood flow, especially those of childbearing age (Tefferi, 2003). According to the WHO (2001), Fe deficiency anemia is the most prominent nutritional disease worldwide, affecting 20-30% of the world population; women are the highest risk group (Coad & Conlon, 2011).

Ca is an essential element and one of the most abundant nutrients in the human body (Azin, Raie, & Mahmoudi, 1998). The calcium concentrations in the urine of framers were 45.01, 94.49, and 77.86 mg L^{-1} for women, men and children and teenagers, respectively (Table 3). These concentrations were lower than those found in Nanning, China (Cui et al. 2005), and Warsaw, Poland (Długaszek et al., 2011). For men and children, we found higher concentrations of Ca than those reported in Hyderabad City, Pakistan (Afridi et al., 2008). The Ca concentrations presented high variability, with a coefficient of variation of 122% for women. The concentration of Ca in urine is related to many factors, such as daily food consumption, eating habits, malnutrition, absorption and elimination processes, renal diseases, thyroid and parathyroid gland functions, bone metabolism, stress, excessive aldosterone, smoking, alcohol and medications that interfere with the absorption of this element and promote an increase in its excretion (Siener & Hesse, 2002; Długaszek et al., 2011).

The mean concentrations of Mg in the urine of farmers (Table 3) were higher for the groups of men and children and teenagers than the concentrations found by Afridi et al. (2008) and Długaszek et al. (2011). Urinary excretion is the main form of eliminating absorbed Mg, with 1/3 of the Mg ingested daily excreted in the urine (Alpers, Clouse, & Stenson, 1988). Considering a Mg elimination rate of 90 mg day^{-1} per the ingestion of 270 mg day^{-1} and a mean production of 1 L day^{-1} urine, women were the most affected group in our study. A total of 80% of the women had Mg concentrations in their urine that were lower than the appropriate level, followed by men (52%) and children and teenagers (33%). Mg deficiency can be classified into primary and secondary forms, in which the insufficient consumption of foods rich in Mg, the excessive consumption of sugar and fat, protein-energy malnutrition, and Mg-deficient parenteral nutrition contribute to the primary deficiency. Secondary deficiency is affected by alcoholism, decreased Mg absorption, diarrhea, laxative abuse, malabsorption syndrome, vomiting, increased renal excretion, tubular disease, glomerulonephritis, metabolic and endocrine disorders, medications, pregnancy, and physical and mental stress (Barbagallo, Belvedere, & Dominguez, 2009).

Discriminant analysis

To verify the differences in the concentrations of trace and major elements in the urine of different groups (men, women and children), discriminant analysis (DA) was applied. For this analysis, in addition to the concentrations of the elements in the urine, we added information about the body mass and height (to calculate the body mass index - BMI), age, and smoking status of each farmer. The three groups showed different behaviors (Figure 2), with an accuracy greater than 80% (Table 4).

Based on DA, we carried out principal component analysis (PCA) by groups of individuals (Figure 3). Data were standardized, and eigenvalues superior to unity were selected. Additional variables were added to the PCA, namely, BMI, age and smoking habit.

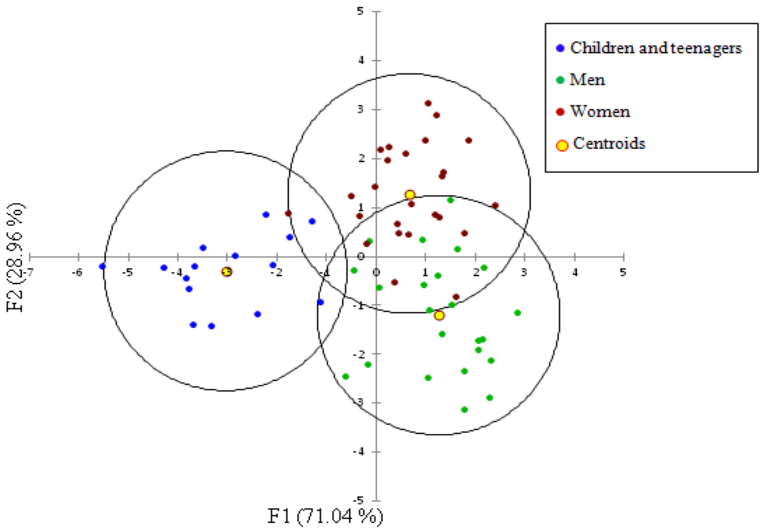

Figure 2. Distribution of groups of farmers by discriminant analysis.

Table 4. Confusion matrix between children, men and women.

Group	Children	Men	Women	Total	Correct (%)
Children	14	0	1	15	93.33
Men	0	19	4	23	82.61
Women	1	2	22	25	88.00
Total	15	21	27	63	87.30

Based on the PCA for the group of men (Figure 3a and b), it was possible to reduce the variables to three components, which together explained 57.56% of the total variation in the data. The overlap of Zn and the complementary variable smoking (Figure 3b) in men confirmed the link between higher urinary Zn excretion and the smoking habit. The readily observed overlap between Ca and Mg (Figure 3a) was due to the proximity of the mean concentrations of the elements in the urine of men, with values of 94.49 and 99.66 mg L^{-1}, respectively (Table 3).

For women, the PCA revealed little influence of complementary variables (BMI, age, and smoking habit) on the concentrations of trace and major elements in urine (Figure 3c and d). The three components together explained 65.52% of the total variation in the data.

Age and BMI influenced the Ca and Mg in the urine of children and teenagers (Figure 3e). This association is desirable, since children and teenagers require a greater amount of energy than adults, with an appropriate vitamin and mineral supply (Greer & Krebs, 2006), due to the growth phase. The three PCA components explained 66.73% of the total variation in the data. For the first component (Figure 3f), there was greater expressiveness for the potentially toxic trace elements Cu and Pb. This result corroborates the increased exposure of children to these elements, possibly because their high contact with dust and surface soil makes them more vulnerable to contamination (Sapcanin, Cakal, Pehlic, & Jancan, 2016).

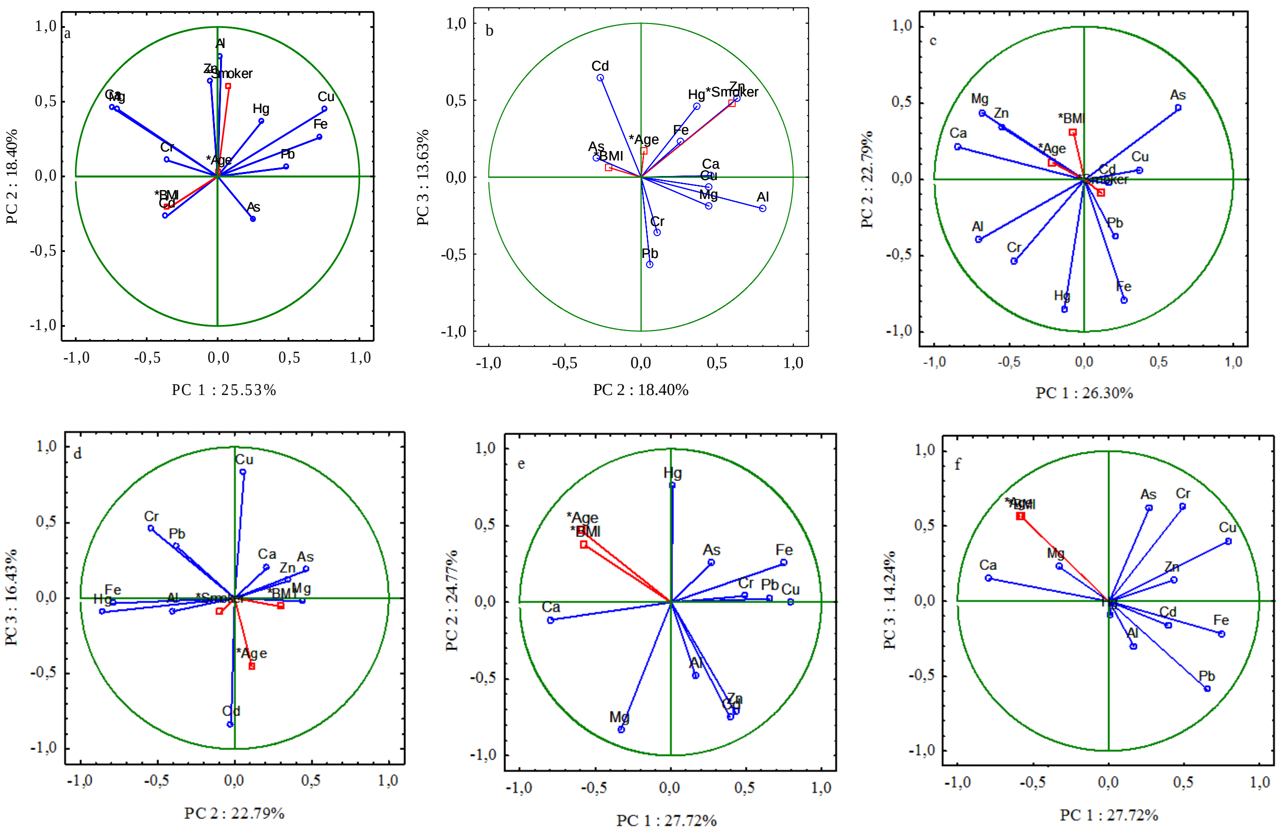

Figure 3. Concentrations of chemical elements in urine of farmers of agroecological systems and their relations with body mass index, age and smoking habits. a and b (men), c and d (women), e and f (children and teenagers).

Conclusion

The concentrations of essential and toxic elements in the urine of farmers living in agroecological communities were within the reference ranges of Brazil and other countries. Children and teenagers were more susceptible than adults to the influence of elements in soil due to their higher direct contact with the soil. In general, the results showed that the urinary concentrations of toxic elements among farmers and their families were below the allowable limits, which may be due to the agroecological practices in the studied communities. PCA analysis demonstrated an association between the smoking habit and the Zn concentration in urine.

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