
Arsenic biotransference to alfalfa (*Medicago sativa*)

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Abstract: The arsenic (As) concentration in the edible part of crops depends on the availability of the As present in the soil and the ability of a crop to take up As and translocate it to the target organs. The aim of this study was the determination of As concentration in soils and alfalfa (*Medicago sativa*) in field conditions and pot experiments to analyse As soil-forage transference. Results show that most of the available As is transferred.

Keywords: soil; forage; food chain contamination; arsenic.

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1 Introduction

Arsenic (As) is a toxic pollutant present in the atmosphere as well as in the aquatic and terrestrial environment. Most of the environmental problems caused by As are associated with mobilisation of As as a result of natural processes (Smedley and Kinniburgh, 2002). However, anthropogenic sources, including industrial production or use of As in smelting operations, as a wood preservative and as a feed additive for livestock, particularly poultry and swine, significantly contribute to these problems (Hingston et al., 2001; Rutherford et al., 2003; Duker et al., 2005; Li and Chen, 2005).

Arsenic contaminated soils, sediments and water supplies are the major source of food chain contamination. Arsenic can be present in terrestrial, fresh water and marine environments in various chemical forms such as arsenite –As(III)- arsenate –As(V)- methylarsonate (MA), dimethylarsinate (DMA), trimethylarsine oxide (TMAO), tetramethylarsonium cation (TETRA), arsenobetaine (AB), arsenocholine (AC) and arsenosugars (Litter et al., 2008; Bundschuh et al., 2008).

Large-scale water pollution by geogenic As in Bangladesh has recently promoted this element into an environmental pollutant of prime concern. Widespread water and crop contamination originating from the natural release of As from aquifer rocks has also been identified in different regions of the world (Smedley and Kinniburgh, 2002; Nriagu et al., 2007).

In Latin America, the most affected countries are Argentina, Chile and Mexico, however, high levels of As have been reported in Bolivia, Peru, and Ecuador and the information is scarce in other regions (Bundschuh et al., 2012).

In Argentina, the Chaco Pampean Plain covering $\sim 1 \times 10^6$ km² is one of the largest known regions with high levels of As in groundwater. High concentrations have been documented in several densely populated provinces, particularly in Córdoba and Santa Fe (Smedley and Kinniburgh, 2002; Farías et al., 2003; Pérez-Carrera and Fernández-Cirelli, 2004, 2010) where agriculture is one of the main activities.

The high-As groundwaters in the pampean plains originate from Quaternary deposits of loess (mainly silt) with intermixed rhyolitic or dacitic volcanic ash (Nicolli et al., 1989; Smedley et al., 2002). The prevalent form of As is As(V). Metal oxides in sediments (especially Fe and Mn oxides and hydroxides) are thought to be the main source of dissolved As produced by desorption at high pH. Another potential source of dissolved As is dissolution of volcanic glass.

It is well recognised that consumption of As, even at low levels, leads to carcinogenesis (Bates et al., 1992; Hopenhayn-Rich et al., 1996). An endemic disease linked to drinking water with high As levels was first described in Argentina early in the last century. This disease known as HACRE (Hidroarsenicismo Crónico Regional Endémico or Chronic Endemic Regional Hydroarsenism) has been associated with a specific form of skin cancer (Biagini et al., 1995). One of the most affected areas by HACRE is the southeast of Córdoba (Hopenhayn-Rich et al., 1996), which is also one of the most important milk production zones.

From the socioeconomic point of view, this region is one of the most important food production areas. In particular, milk production is concentrated in the provinces of Córdoba and Santa Fe, where high levels of As were reported in groundwater used for animal drinking and crop irrigation (Farías et al., 2003; Pérez-Carrera and Fernández-Cirelli, 2005, 2010; Pérez-Carrera et al., 2008).

The greatest threat to human health is from As in drinking water mostly in zones where water contains more than $10 \mu\text{g l}^{-1}$ (WHO, 2001; Huang et al., 2006). However, As intake from food may also significantly contribute to human exposures to As (Mandal and Suzuki, 2002). To this date, only few studies have examined effects of high As levels in livestock drinking water on livestock health and on As content in the milk (Pérez-Carrera and Fernández-Cirelli, 2005; Pérez-Carrera et al., 2008; Rosas et al., 1999).

Argentina is the second largest producer of alfalfa (*Medicago sativa*), with 5.5 million cultivated hectares. In the case of milk production, alfalfa (*Medicago sativa*) is the main crop for dairy cows. Arsenic concentration in soil is the major source for the As uptake of crops (Huang et al., 2006). Much of the cultivated area is affected with high As content in soils and groundwater. Other crops have the ability to capture and accumulate As, which represents an important route of transfer into the food chain. Although As accumulation by plants has been largely studied, the relationship between As in the soil and plant uptake is still not well understood (Warren et al., 2003; Ramírez-Andreotta et al., 2013).

The As concentrations in the edible parts of crops depend on the availability of the As present in the soil and the ability of a crop to take up As and to translocate it to the target organs. The availability of soil As is determined by soil properties, notably mineral composition, organic matter content, pH, redox potential and phosphate content (Caetano and Vale, 2002; Mandal and Suzuki, 2002; Tu and Ma, 2003; Warren et al., 2003; Cao and Ma, 2004; Gulz et al., 2005). Also, the use of As compounds as herbicides or pesticides could modify the uptake of As by the agricultural products (Yongqiang and Donahoe, 2008).

The main objective of this work is to determine the As concentration in soils and alfalfa (*Medicago sativa*) in field conditions and pot experiments as well as the soil-forage transference in order to better estimate the forage contribution to the As intake by livestock.

2 Materials and methods

2.1 Sampling and samples preparation

Soil. Soil samples were obtained in dairy farms ($N=15$) located in the southeast of the Córdoba province. All soil samples were mollisols, with aptitude for agriculture activities and with similar characteristics. The samples were air dried, sieved through a 2-mm sieve mesh, and pulverised in an agate mortar. For determination of trace elements content, 0.1 g of soil were subjected to acid digestion with 10 ml of HNO_3 (c) and 10 ml of H_2SO_4 (c). After the complete oxidation of organic matter, and produced fumes of sulphur trioxide, the samples were cooled and diluted to 50 ml with deionised water previous to As determination. Extraction of As from soils (5 g) was performed with 0.05 N HCl/0.25 N H_2SO_4 (1 : 1 in vol., 20 ml) at room temperature under stirring. After filtration, samples were diluted to 50 ml with deionised water previous to As determination (available As).

Forage. In dairy farms, alfalfa (*Medicago sativa*) samples were collected and transported to the laboratory where they were carefully washed with deionised water and dried at 40°C for 24 h. The disintegration of the vegetable samples was performed by microwave digestion in closed cup (Alvarado, 1996). Arsenic content was determined in edible parts of the plants (leaves and stem).

2.2 Reagents

All reagents were of analytical grade. Working solutions were prepared by appropriate serial dilution of commercially available As stock standard solutions (Perkin Elmer, Atomic Spectroscopy Standard No. 9300281) using ultrapure water provided by a Milli-Q water purification System (Millipore, Bedford, MA, USA).

Certified reference materials for verification of the calibration procedure and validation of the analytical method were used for each type of studied matrices. In the case of soils, WQB CRM-3, and HR-1 were used (NWR) and for forage NIST-1570a, from the National Institute of Standards and Methods (NIST) was used.

2.3 Arsenic analysis

Arsenic content in soil and forage was determined by ICP-OES (Perkin Elmer, Optima 2000) following standardised methods (APHA, 1993). Determinations were performed in duplicate being the relative error <1.0% for all of them.

2.4 Cultivation of alfalfa (*Medicago sativa*)

The soils used in the experiment were collected in the study area. Soils were enriched by the addition of a solution prepared from a stock solution of As₂O₃ (Sigma Aldrich) to reach As concentrations in the soil of 25, 50 and 100 mg kg⁻¹.

The plant cultivation took place under the conditions of pot experiments in a greenhouse. Each treatment was run in triplicate. Each pot was filled with ~4600 g air-dried soil with the following characteristic:

Alfalfa (*Medicago sativa*) was grained with 25 grains per pot. Water content was adjusted daily. The water-holding capacity of the soil was never exceeded, therefore no leaching occurred.

Plants were cropped during a three month period. Samples were collected through three harvests each 35 days, at 6–10 cm from the surface according to usual handling of forage in local dairy farms.

2.5 Transfer coefficient

The transfer coefficient (TC) of As from soil to plants has been defined as the ratio between the concentration of As in vegetables (mg kg⁻¹) and As concentration (total or available) in soil (mg kg⁻¹).

2.6 Statistical analysis

The average, standard deviation, minimum and maximum of As concentration in soils and forage and the ANOVA analysis were calculated using the statistical package Statistica for Windows (ver. 5.1, 1997, Statsoft, USA).

3 Results and discussion

In the studied dairy farms, the total As soil concentration in the surface layer (0–100 cm depth) showed no significant differences between the levels of As found at different depths (30, 60 and 100 cm). Values obtained ranged from 3.4 mg kg⁻¹ to 9.3 mg kg⁻¹ in spite of the similar physicochemical characteristics of the analysed soil samples. According to these results, As concentration in the studied soils was below the guidelines values considered in Argentina (20 mg kg⁻¹ for agricultural soils, Law 24051, 1993) and within the range of As concentrations in non-contaminated soils reported in various countries (Mandal and Suzuki, 2002; Smedley and Kinniburgh, 2002; Huang et al., 2006).

Arsenic concentration in alfalfa (*Medicago sativa*) leaves harvested from the same dairy farms was between 0.2 mg kg⁻¹ and 0.65 mg kg⁻¹.

There are few studies in the literature to compare these results with others previously reported. In Comarca Lagunera, Mexico, As concentration in alfalfa (*Medicago sativa*) has been reported in the range of 0.24–3.16 mg kg⁻¹ with As soil concentration between 11 mg kg⁻¹ and 30 mg kg⁻¹ (Rosas et al., 1999).

From the above results, it may be inferred that higher As soil concentrations will induce higher As leaf concentration. However, the As in soil is generally associated with clays that contain amorphous iron oxyhydroxide and aluminium and the adsorption–desorption process depends on soil physico-chemical characteristics such as redox potential, pH, soil texture, organic matter and competing ions (Aguilar et al., 2006; Marabottini et al., 2013).

Arsenic forage/soil TC values for the studied dairy farms ranged from 0.03 to 0.24, as may be expected taking into account the data dispersion observed both in soil and forage.

These results prompted us to perform pot experiments in order to be able to estimate a more accurate correlation factor that may be useful to predict As concentration in alfalfa (*Medicago sativa*) through its measure in soil.

The results of total and available As concentration in each pot are shown in Table 1. The homogeneity of the As concentration was verified by a *t*-test ($t_{12} = -0.976$, $p = 0.826$) finding no significant differences between the As concentrations (total and extractable) among the pots replicates.

Arsenic concentration in plants (roots, stems and leaves) during the first, second and third harvest for different As concentrations in soil are shown in Table 2.

It was observed that increasing the concentration of As in soil, the concentration in leaves, stems and roots also increased, demonstrating the transfer of As to the alfalfa (*Medicago sativa*).

Table 1 Arsenic concentration (mg kg^{-1}) in soil in pot experiments

Arsenic concentration	Pot	Total As	Extractable As
Blank	1	0.34	0.1
	2	1.34	0.4
	3	3.0	0.9
25 mg kg^{-1}	4	25.9	8.2
	5	24.8	7.84
	6	24.3	7.67
50 mg kg^{-1}	7	53.4	15.5
	8	49.6	14.4
	9	47.2	13.7
100 mg kg^{-1}	10	98.6	30.2
	11	104.1	31.9
	12	97.3	29.8

Table 2 Average As levels ($\mu\text{g g}^{-1}$) in leaves, stems and roots of alfalfa (*Medicago sativa*) grown in soils with 25, 50 and 100 mg As kg^{-1}

As in soil	First harvest			Second harvest			Third harvest		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
25	4.6 ± 1.0	1.8 ± 0.2	2.2 ± 0.1	2.3 ± 0.2	1.4 ± 0.2	*	1.6 ± 0.3	1.5 ± 0.3	2.9 ± 0.9
50	7.3 ± 1.6	2.8 ± 0.2	4.8 ± 2.6	4.8 ± 0.4	2.6 ± 1.0	*	3.2 ± 0.7	2.3 ± 0.1	8.3 ± 4.3
100	15.1 ± 2.5	3.7 ± 0.6	7.0 ± 0.1	8.9 ± 2.7	4.0 ± 1.2	–	–	–	–

*Root samples were not collected because there was not enough material.

The As concentration in stem and leaves decreased along the experiment, while in roots, As concentration increased. The accumulation of As in the roots was higher because they were in direct contact with the soil throughout the experiment.

Arsenic concentration variability in leaves, stems and roots in alfalfa (*Medicago sativa*) through the harvests in pots with As soil concentration of 25 mg kg^{-1} showed significant differences in As concentration in leaves and roots through the different harvests ($F_{(2,24)} = 3.213$, $p = 0.04$).

As can be seen, As concentration in leaves was higher in the first and second harvest, while in the third harvest, As concentration was higher in root.

Arsenic concentration in leaves, stems and roots in alfalfa (*Medicago sativa*) through the harvests in pots with As soil concentration of 50 mg kg^{-1} is shown in Table 2.

The results of the ANOVA in pots with 50 mg kg^{-1} of As in soil showed significant differences between As concentration in alfalfa leaves, at different harvest time ($F_{(2,4)} = 6.68$, $p = 0.042$). However, in the case of the stem and root, the differences were not significant ($F_{(2,21)} = 2.4148$, $p = 0.113$). The largest differences in the As concentration were observed in leaves and roots.

Arsenic concentration in leaves, stems and roots of alfalfa (*Medicago sativa*) through the harvests in pots with As soil concentration of 100 mg As kg^{-1} showed significant differences between As concentration in the leaves ($F_{(2,28)} = 4.247$, $p = 0.024$).

It should be noted that in pots with As concentration in soil of 100 mg kg^{-1} most alfalfa (*Medicago sativa*) plants died after the first harvest and therefore data are missing. Signs of phytotoxicity were observed during the plant growth, including wilting and yellowing corresponding to those reported for As toxicity in plants (Adriano, 2001; Fitz and Wenzel, 2002).

Arsenic bioaccumulation in crops, in concentrations dangerous for human and animal consumption, is not common because the phytotoxic effects limit the transference (Smith et al., 1998). However, As contamination in soils can damage crops by altering their growth and yields.

In general, total As content in soil does not accurately reflect phytotoxicity. Correlation is better between plant growth and bioavailable As than between plant growth and total As (Adriano, 2001).

Table 3 presents the TC calculated considering As average concentration in stems and leaves in the first harvest and the total and available As concentration in soil.

Table 3 Arsenic transfer coefficient to alfalfa (*Medicago sativa*) considering total and available As soil concentration

Pot	TC (total As)	TC (available As)
Blank	0.11	0.37
25 mg kg^{-1}	0.34	1.09
50 mg kg^{-1}	0.30	1.03
100 mg kg^{-1}	0.26	0.84

If we consider that the TC value obtained in pot experiments for the blank considering total As concentration is more representative than the values obtained in the experiments where soils were artificially enriched with As, it may be observed that it is approximately an average of the values obtained for field measurements (0.03–0.24).

It is important to note that in artificially contaminated soils, both the TC values calculated considering the total As concentration or the extractable As concentration are similar regardless of the As content in the soil. This will allow us to estimate the value of forage As from soil As content. This estimation can be performed only when the TC has been determined in similar soils because As availability depends on soil characteristics. Differences with the blank may be attributed to the fact that spiked samples have a higher content of available As. On the other hand, the obtained results suggest that almost all available As is transferred to the plant up to a concentration in which it is toxic for the vegetable.

4 Conclusions

Arsenic soil content in the studied dairy farms showed As concentrations below the safety levels considered in Argentina for agricultural soils. Higher As concentrations in alfalfa (*Medicago sativa*) were from samples where As soil concentration was also higher, suggesting As transference to crop. From the pot experiments performed, it may be assumed that most available As in soil is transferred to the vegetable up to a concentration in which toxicity signs are observed.

A good correlation has been found between As in alfalfa (*Medicago sativa*) and As content in soil, suggesting that the latter may be a good indicator to estimate As content in forage. Therefore, the transference factor becomes a useful tool to predict the more appropriate places to cultivate alfalfa (*Medicago sativa*).

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