Journal of Environmental Management 218 (2018) 23–30

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

# Absorption of arsenic from soil and water by two chard (*Beta vulgaris* L.) varieties: A potential risk to human health

L.M. Yañez [a, b, *], J.A. Alfaro [a], G. Bovi Mitre [a, b]

[a] *Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, Alberdi N° 47, 4600, San Salvador de Jujuy, Argentina*
[b] *Cátedra Toxicología de los Alimentos, Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, Alberdi N° 47, 4600, San Salvador de Jujuy, Argentina*

ARTICLE INFO

*Article history:*
Received 10 February 2018
Received in revised form
8 April 2018
Accepted 10 April 2018

*Keywords:*
Arsenic
Chard
Food risk
Target hazard quotient
Jujuy
Argentina

ABSTRACT

The accumulation of arsenic (As) in vegetables poses a risk of contamination to humans via the food chain. Two chard (var. *cicla* and var. *d'ampuis*) crops were grown for 60 days in greenhouses on Aridisol soil, and irrigated with water from Pastos Chicos, Jujuy (Argentina). The soil and water used in the trial presented 49 and 1.44 mg/L As concentration levels, respectively. Total dry biomass (TDB) and total As were determined in soils, roots and leaves. The latter was quantified by atomic absorption spectrometry with hydride generation, and bioconcentration and translocation factors were determined. TDB in var. *cicla* showed statistically significant differences when the plant was cultivated in control soil and watered with the toxicant (2.04 g), as compared with the treatment without exposure (2.8 g). TDB in var. *d'ampuis* presented statistically significant differences with respect to that of the control when the plants were grown in soils with As and watered with the toxicant (3.3 g). This variety increased its biomass in the presence of As. In the two Swiss chard varieties evaluated, the largest As accumulation in root and leaves was found when they were cultivated in contaminated soil and watered with distilled water. The presence of the toxicant in the leaves exceeded the limits established by Código Alimentario Argentino, i.e. 0.30 mg/kg. Total target hazard quotient (THQ) values for As were higher than 1, suggesting that consumers would run significant risks when consuming these chard varieties. Furthermore, it was determined that the carcinogenic risk (CR) posed by this type of exposure to As exceeded the acceptable risk level of $1 \times 10^{-6}$. Based on this evidence, we may conclude that consuming chard cultivated on the evaluated site brings about considerable risks to local residents' health.



## 1. Introduction

Contamination of soil, sediments and groundwater systems with arsenic (As) is a global environmental, agricultural and public health issue due to the toxic and carcinogenic nature of this contaminant (Shakoor et al., 2015). Natural sources such as hydrothermal reservoirs and volcanic rocks, anthropogenic activities such as mining, smelting and textile manufacturing, together with the use of chemical arsenic derivatives as herbicides and fertilizers, and of As-contaminated water for irrigation, all contribute to an increasing pollution with this toxic element (Zhao et al., 2009; Niazi et al., 2016).

High As concentrations in natural waters were detected in various parts of the world. In many countries, groundwater is the main source for human consumption, as well as a vitally important resource for agricultural and livestock farming (Brammer et al., 2008). Jujuy (Argentina) is a province whose geogenic characteristics lead to high As concentrations in the water and soil of some areas in the northwest (Puna region) (Ponce et al., 2006). Quantitative water studies revealed As concentration values of 5–10.000 μg/L (Tschambler et al., 2007). Pastos Chicos, a site located at 3843 m above sea level in Susques department (23°45′58.48″ S - 66°26′13.86″ W), presents As concentrations in soil and water above the maximum allowable limits (Farías et al., 2008).

Arsenic concentration levels in crops depend on the availability of this element in the soil and the capacity of a crop to incorporate and translocate it to different organs. The solubility of As in agricultural soils can vary drastically from one place to another depending on soil conditions. Factors such as pH, redox potential, organic matter content, cation exchange capacity, water regime

* Corresponding author. Alberdi N° 47, Y4600DTA, San Salvador de Jujuy, Argentina..
*E-mail address:* lumaya12@hotmail.com (L.M. Yañez).

https://doi.org/10.1016/j.jenvman.2018.04.048
0301-4797/

(irrigation), clay content (texture), and balance and concentration of minerals, mainly iron oxides and/or calcium carbonate, may significantly interfere with As solubility in soils (Niazi et al., 2011). The As present in agricultural soils and irrigation water can reach crops (Gulz et al., 2005): this constitutes a critical point at which this toxic element is introduced into the food chain, thus causing humans and animals to become exposed to it to a certain degree (Khan et al., 2009; Rehman et al., 2016).

The contamination of vegetables, soil and water with arsenic has reached unprecedented levels over the past decade. Consequently, human exposure to arsenic has become a major health risk. Several methods have been proposed for the assessment of potential human health risks from metals and As exposure. Current non-cancer risk assessment methods are typically based on the use of the target hazard quotient (THQ), a ratio between the estimated dose of a contaminant and the reference dose below which there will not be any appreciable risk (USEPA, 2000).

Swiss chard (*Beta vulgaris* var. *cicla*) is a vegetable widely appreciated in many parts of the world for its nutritional properties, year-round availability, low cost and wide use in many traditional dishes. This member of the Quenopodiaceas family, which has green leaves and thickened stems, has a high nutritional value, with very low saturated fat and cholesterol contents and a high sodium content (Mitic et al., 2013). It makes an excellent contribution of soluble fiber, vitamins (folates, vitamin C, vitamin A, and niacin), and minerals (iodine, iron and magnesium). This vegetable also has high proportions of phytonutrients, such as carotenoids and flavonoids, which provide antioxidant protection. Chard has long been used for its beneficial effects on health, such as the stimulation of the hematopoietic and immune systems and the protection of the kidneys, liver and intestines from toxic compounds. From a pharmacological point of view, it is a crop that provides bioactive molecules with anti-diabetic, anti-inflammatory, antioxidant and anticancer activities (Ninfali and Angelino, 2013).

Agriculture is an important economic activity in Pastos Chicos, both for trade and domestic consumption. This site was chosen for the study on account of its antecedents of natural contamination with As (Farías et al., 2008), and the plant species analyzed were selected because they constitute an important part of the diet of local inhabitants. Therefore, determining As levels in chard produced in the area is of vital importance. This work aimed to evaluate total As absorption in two Swiss chard species, *Beta vulgaris* var. *cicla* and var. *ďampuis*, grown in a greenhouse with soil typical of Pastos Chicos and irrigated with local water. The ultimate goal was to evaluate the health risk associated with consuming plants from these crops.

## 2. Materials and methods

### 2.1. Soil and water sampling

Soil samples were taken from a greenhouse belonging to Provincial Primary School N° 195, in Pastos Chicos locality (23°45′58.8″S- 66°26′14.0″W). The zig-zag sampling method was used to 20 cm depth, as established by IRAM 29481-4. The soil was stored in polyethylene bags at 4 °C until further processing for physicochemical analysis and total As determination. Soil was also collected from the same greenhouse to study As absorption in chard crops, following the procedures described later in this paper.

Water was sampled from Río Pastos Chicos (23°42′31.3″S - 66°26′42.3″ W), which runs across the site. It was kept in plastic containers, with 2 drops of concentrated $HNO_3$ per 100 ml for its preservation. The samples were taken to the laboratory and stored at 4 °C until processing for total As. Water was also collected to later irrigate the chard plants in the absorption study.

### 2.2. Physicochemical analysis of soil and irrigation water

The soil was dried at room temperature and in the shade, and then sieved with a 2 mm pore diameter mesh in order to homogenize particle size. Then pH was determined by potentiometry in distilled water (with a 1:2.5 soil/water ratio), and organic matter was determined by wet digestion using Walkley-Black method (Nelson and Sommers, 1982). The other determinations were total nitrogen according to Bremner and Mulvaney (1982), texture by the Bouyoucos hydrometer method, and phosphorus content by Bray and Kurtz's method (1945). The electrical conductivity of the saturated soil extract was measured according to Rhoades (1982), and calcium and magnesium were determined by complexometric volumetric titration with EDTA (ethylenediaminetetraacetic acid).

Water analysis consisted in the following: pH by potentiometry, electrical conductivity with a conductivity meter, calcium and magnesium by complex EDTA formation, sodium and potassium complexes by flame photometry, and carbonates, bicarbonates, chlorides, and sulfates by neutralization titration (Eaton et al., 2005).

Soil samples were processed for total As according to EPA method 3050 B (1996). Total As in soil and water was quantified using a hydride generation-atomic absorption spectrometer (HG-AAS), as described below.

### 2.3. Tests on chard exposed to arsenic

The study was carried out in a greenhouse of Facultad de Ciencias Agrarias, Universidad Nacional of Jujuy (Argentina). The chard crops were planted in soil of the type prevailing in Pastos Chicos, and irrigation water came from the river that flows across this site. As a control, a plot with soil of a similar texture to that of the contaminated substrate was planted, and distilled water was used to standardize irrigation water chemical composition (Bustingorri and Lavado, 2014).

The soil was dried at room temperature and sieved with a 2 mm pore diameter mesh to homogenize particle size. The soil was then weighed and kept in black polyethylene bags, each containing 1 kg of the substrate. The chard seeds used for the trials belonged to variety *Beta vulgaris* var. *cicla*, provided by the company Esmeral Seeds-USA, and the variety *Beta vulgaris* var. *ďampuis,* provided by Clause-France. They were washed with 3% sodium hypochlorite, triple rinsed with distilled water, and germinated in multipot trays. Once germinated, each seed was planted in a bag and kept there for 60 days with an ambient temperature, light and darkness regime, and under irrigation without leachate.

Once plants had grown, their aerial parts were cut flush with the ground and their roots were extracted for washing both with tap water and in an ultrasonic bath (model TBO24-TestLab), for 30 min. Total dry biomass (TDB) was calculated by adding the dry weight values of roots and aerial parts, which had been kept in paper bags and dried at 70 °C for 48 h.

The trials were carried out with a completely randomized design (CRD) with 15 replicates. The treatments were as follows:

1) Chard seedling in control soil, irrigated with distilled water.
2) Chard seedlings in soil with As, irrigated with water with As.
3) Chard seedlings in soil with As, watered with distilled water.
4) Chard seedling in control soil, irrigated with water with As.

Total As in root, leaves and soils was determined with a HG-AAS, as described below.

### 2.4. Translocation and bioconcentration factors

To understand the behavior of these two Swiss chard varieties, the toxic concentrations obtained were used to estimate translocation (TF) and bioconcentration (BCF) factors. Total As TF in plants was calculated as the ratio between the concentrations in the aerial part and the root. BCF was expressed as the ratio between the concentration of total As in the root and total As in the soil (Maldonado-Magaña et al., 2011).

### 2.5. Health risk assessment

In this study, the non-carcinogenic health risks associated with human consumption of chard in Pastos Chicos were assessed based on the target hazard quotient (THQ). THQ was determined by the following equation (1):

$$THQ = \frac{EFr \times EDtot \times FIR \times C}{RfDo \times BWa \times ATn} \times 10^{-3} \tag{1}$$

where THQ is the target hazard quotient; EFr is exposure frequency; EDtot is exposure duration (70 years, based on current life expectancy); FIR is food ingestion rate (g/day); C is concentration (mg/kg); RfDo is oral reference dose (mg/kg/day), which indicates the quantity of the compound per kilogram weight that a human being could ingest per day without risk; BWa is body weight (adults: 70 kg); ATn is the average time for non-carcinogens (365 days $year^{-1}$ x EDtot), assuming 70 years. The RfD value used in this study was 0.0003 mg/kg/day As, as provided by the USEPA's regional screening level (USEPA, 2015). Daily chard leaf intake by local residents (FIR) was estimated to be 200 g, with an exposure frequency (EFr) of 96 days/year (Choque, 2013).

Carcinogenic risk was estimated as the incremental probability of an individual to develop cancer over a lifetime exposure. Carcinogenic risk (CR) for As was calculated with the following equation (2):

$$CR = \frac{EFr \times EDtot \times FIR \times CSFo}{BWa \times ATn} \times 10^{-3} \tag{2}$$

CSFo is the oral carcinogenic slope factor as obtained from the Integrated Risk Information System (USEPA, 2010) database, which was 1.5 (mg/kg/day) for As. A CR lower than $10^{-6}$ is considered negligible, whereas one between $10^{-6}$ and $10^{-4}$ is generally considered acceptable, and a value above $10^{-4}$ is viewed as unacceptable (Monferran et al., 2016).

### 2.6. Total arsenic determination

Considering the matrix to digest, 0.5–1 g of dry matter was weighed and 3 ml of a suspension of the mineralizing agent, 20% w/v magnesium nitrate [$Mg(NO_3)_2$] (Anhedra-India) and 2% w/v magnesium oxide (MgO) (Anedra-Germany) were added. Then 5 ml of 50% v/v nitric acid ($HNO_3$) (Merck) was added to promote organic matter oxidation, followed by heating in a heating mantle until the reactants evaporated. Finally, the preparation was muffled at 550 °C for 24 h until white ash was formed. This was resuspended in 10% v/v hydrochloric acid (HCl) (Merck) to measure total As. The suspension was subjected to a prereduction step with a solution of potassium iodide/ascorbic acid (Merck). Arsine ($AsH_3$) was then formed by reaction with a solution of sodium borohydride ($BH_4Na$) (Merck) in an alkaline medium, together with a solution of HCl as the source of hydrogen ions.

Arsenic was determined with a FIAS 400 device attached to an AAnalyst 100 spectrometer (PerkinElmer brand). The technique had a 0.1 μg/L detection limit and a 0.3 μg/L quantification limit, with a linear response of up to 5 μg/L (r = 0.998). Relative error amounted to 10%, and equipment sensitivity was checked with external patterns.

### 2.7. Statistical analysis

The obtained results were submitted to an analysis of variance (ANOVA) and the means were compared with Duncan Test at a $p \leq 0.05$ significance level. All statistical analyses were conducted using Version 2008 of InfoStat program.

## 3. Results and discussion

### 3.1. Soil and water characterization

The soil presented a sandy-loam texture, with a predominant sand fraction of 73.3%, followed by 15.2% of clay, and finally 7.5% silt content. It had a moderately alkaline pH (8.26), an organic matter content of 5.12%, and a high proportion of phosphorus (72.8 mg/kg). Based on its electrical conductivity value (1.98 dS/m), the soil had the characteristics of an alkaline and slightly saline type, if Richards' criteria (1982) are considered. When Salaverry Fognoli (2014) subjected a chard crop to different salinity levels, a decrease in dry and fresh weight was recorded with 6 dS/m or 8 dS/m levels, thus showing that this type of soil was suitable for growing chard plants.

The soil in this study presented a total As concentration of 49 mg/kg, exceeding the regulatory level of 20 mg/kg of As recommended by National Law N° 24,585 for agricultural soils.

Water analysis showed a total As content of 1.44 mg/L, which was 14-fold higher than the limit allowed for irrigation water, as stipulated by National Law N° 24051, which regulates hazardous waste disposal. Similar results were reported by Buschmann et al. (2007), who determined a concentration of 1.34 mg/L of As in the Mekong River, in Cambodia. Also, Rahamn et al. (2013) analyzed groundwater samples in West Bengal, India, finding As concentration levels of 1.01 mg/L.

The water in this study had a moderately alkaline pH (8.25), which could favor As absorption, thus increasing the concentration available for crops (Prieto García et al., 2007). An electrical conductivity of 2.58 dS/m indicates very high salinity according to Richards (1954). A sodium absorption ratio (SAR) of 9.92 was found, which represents a high risk of soil salinization according to Abrol et al. (1988).

### 3.2. Growth of chard varieties when exposed to arsenic

Var. *d 'ampuis* presented statistically significant TDB differences in relation to control treatments, where plants grew in soils with As and were irrigated with water contaminated with the toxicant (3.3 g) (Fig. 1).

Total dry biomass (TDB) is a critical parameter for assessing As stress impact on plant growth (Niazi et al., 2017). The results showed that when exposed to As via irrigation and when grown in control soil, var. *cicla* chard presented statistically significant differences ($p \leq 0.05$) in TDB (2.04 g) with respect to the treatment without exposure (2.8 g) (Fig. 1). The rest of the treatments did not express significant differences in TDB. It was observed that biomass production in this variety was adequate even when exposed to the As concentrations studied.

It should be noted that when exposed to toxicant concentrations in soil and water, var. *d'ampuis* had a higher biomass production than in the control treatment. These results coincide with those of other researchers, who showed that small As amounts stimulate plant growth (Gulz et al., 2005; Spagnoletti et al., 2015). As reported

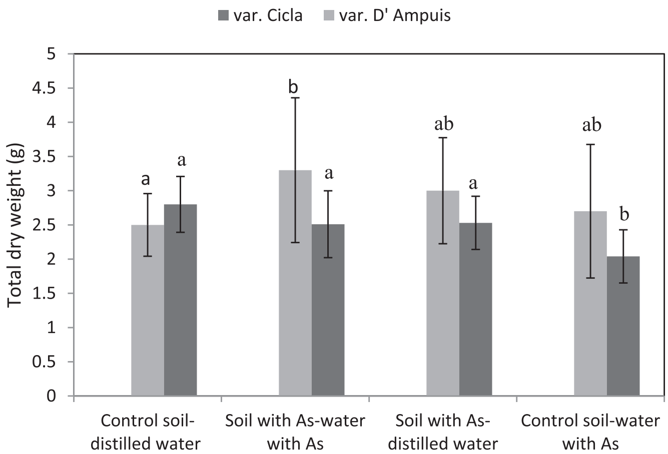


**Fig. 1.** Growth parameter in Swiss chards exposed to arsenic. Each value represents the mean of fifteen replicates ±standard deviation. The different letters within a column indicate a significant difference at $p \leq 0.05$ according to Duncan's multiple range tests.

by Hettick et al. (2016), when melon (*Cucumis melo*) was exposed to irrigation with 10 μg/L of As, it developed a greater biomass than the control. Carbonell-Barrachina et al. (1995) observed that arsenate applications at 0.2–0.8 mg/L concentrations in hydroponic culture significantly increased root and stem dry matter yield of *Spartina alterniflora* and *Spartina patens*, as compared to control plants. Several authors found that As additions stimulated growth in maize, potatoes, rye and wheat (Gulz and Gupta, 2000; Tu and Ma, 2003).

Biomass increase in var. *d 'ampuis* chard under exposure to As could be attributed to the displacement of soil phosphate ions by arsenate ions, which leads to the increase in phosphate quantities available to plants (Adriano, 2001). This availability would increase the photosynthetic rate, thus resulting in biomass gain (Gusman et al., 2013). Based on this, Pigna et al. (2009) evaluated As effects on wheat (*Triticum durum*) by subjecting the crop to irrigation with different As concentrations (0.5, 1 and 2 mg/L) and fertilizing it with phosphate. At the evaluated concentrations, these researchers found a decrease in the toxic effects on biomass by 12, 16 and 26%.

The high content of extractable phosphorus (72.8 mg/kg) in the soil under study could have had positive effects on plant growth under As stress, possibly because phosphorus favors metabolic functions. A high phosphorus concentration in plant shoots can result in downregulation in plasma As/phosphorus transporters. On the other hand, a high phosphorus content in plant cells can lead to increased competition with As (V) through different biochemical processes, where phosphorus replaces As (Niazi et al., 2017).

### 3.3. Total arsenic concentration in chard plants

Total As (TAs) concentration was determined in chard roots and leaves. The chard species evaluated presented a variable As accumulation. When var. *cicla* was cultivated in soil with As and irrigated with distilled water, the maximum As concentrations in leaves (8.21 mg/kg) and roots (257.67 mg/kg) were recorded. This treatment showed statistically significant differences with respect to the other treatments (Table 1).

When grown in soil with As and irrigated with distilled water, var. *d'ampuis* showed a behavior similar to that of var. *cicla* regarding toxicant accumulation. Arsenic quantification exhibited maximum values in leaves (18.53 mg/kg) and roots (288.08 mg/kg), with statistically significant differences from the other treatments (Table 1).

Considering the characteristics of the soil used in this study (with a 15.2% clay content), a pH of 8.26 and an organic matter content of 5.12% could favor As desorption from soil colloids, hence resulting in a greater amount of As bioavailable for plants (Carbonel et al., 1995).

When comparing roots and leaves in terms of As absorption, it was observed that in both varieties the root presented the highest values. This could be a signal of bioavailable As in the soil. Smith et al. (2009) reported a similar distribution of As in hydroponic chard (*Beta vulgaris* L. 'Fordhook Giant') crops, where the concentration of the toxicant in roots (207 mg/kg) was considerably higher than in aerial parts (3.13 mg/kg). In hydroponic lettuce (*Lactuca sativa* L. 'Great Lakes') crops, Smith et al. (2009) determined a distribution of As in roots (278 mg/kg) and leaves (3.18 mg/kg) similar to that found in the chard varieties evaluated in this study.

This high As accumulation in the root could be attributed to its efficient compartmentalization in vacuoles, probably associated with phytochelatins (Pickering et al., 2006). Also, Bleeker et al. (2003) reported that As (III) and As (V) induced phytochelatin production in a variety of plants. It is believed that the complexation between phytochelatins and As species would be essential for plant tolerance to inorganic As species.

The lower As concentrations recorded in roots and leaves when both chard varieties were exposed to As in water and soil, in comparison to the values obtained when chard was planted in contaminated soil and irrigated with distilled water (Table 1), could derive from an As saturation process, where plants exceed their capacity of bioaccumulation of the toxicant, thus releasing some of the As absorbed by the root to the rhizosphere, as a detoxification mechanism (Zhao et al., 2009; Gusman et al., 2013). In addition, Zhao et al. (2009) determined an active output of arsenite in several plant species grown in hydroponic culture: wheat, barley, maize, tomato, *H. lanatus* or *Arabidospsis thaliana*.

As reported by Castillo et al. (2013), As concentration in vegetables tends to increase with a higher dose of this element in irrigation water and in the soil. However, our results do not support these findings.

Under the conditions of our study, both chard varieties showed a translocation of As from the root to the leaves. In the different treatments, As concentrations in the leaves (edible part) exceeded the allowable limits established by the Código Alimentario Argentino: 0.30 mg/kg of As (CAA, Res. N° 116 and 356/2012). Thus, this represents a critical situation in which the toxicant is introduced into the food chain.

### 3.4. Total arsenic content in soils where both Swiss chard varieties were grown

TAs content in the soils after harvesting the two Swiss chard varieties is shown in Table 2. It can be observed that statistically significant differences were recorded when both chard varieties were cultivated in soils with As, as compared with the other treatments.

The increase in As concentrations in the treatments with soils contaminated with the toxicant could be due to alkaline soil conditions, which together with the sulfates and carbonates supplied by water, could produce As co-precipitation by oxyhydroxides and sulfates (García et al., 2009), or precipitations like calcium arsenate, which is less insoluble than calcium phosphate (Burriel et al., 1999).

The presence of cations in the soil and water can also alter As retention or mobilization in soils. In this sense, Smith et al. (2002) determined that the presence of $Ca^{+2}$ and $Na^{+}$ causes an increase in arsenic retention. Similarly, Stachowicz et al. (2008) described how $Ca^{+2}$ and $Mg^{+2}$ could promote phosphate and arsenate adsorption in soils. In studies conducted by Martínez Villegas et al. (2013), $Ca^{+2}$

**Table 1**
Arsenic content (mg/kg dry weight) in Swiss chard crops.

| Treatments | var. *cicla* | | var. *d' ampuis* | |
|---|---|---|---|---|
| | Root | Leaves | Root | Leaves |
| Control soil-distilled water | 8.51 ± 0.9 (a) | 0.68 ± 0.1 (a) | 16.25 ± 0.8 (a) | 1.30 ± 0.9 (a) |
| Soil with As-water with As | 194.56 ± 0.3 (b) | 4.13 ± 1.1 (b) | 160.57 ± 1.6 (b) | 5.54 ± 0.2 (a) |
| Soil with As-distilled water | 257.67 ± 35.6 (c) | 8.21 ± 1.5 (c) | 288.08 ± 54.2 (c) | 18.53 ± 3.0 (b) |
| Control soil-water with As | 11.20 ± 1.3 (a) | 1.83 ± 0.5 (a) | 36.70 ± 0.8 (a) | 2.22 ± 0.2 (a) |

Each value represents the mean of three replicates ± standard deviation. The different letters within a column indicate a significant difference at $p \leq 0.05$ according to Duncan's multiple range tests.

**Table 2**
Arsenic concentration (mg/kg) in the soils where chard crops were grown.

| Treatments | var. *cicla* | var. *d' ampuis* |
|---|---|---|
| Control soil-distilled water | 4.50 ± 0,53 (a) | 5.12 ± 0.63 (a) |
| Soil with As-water with As | 54.33 ± 1.53 (b) | 57.47 ± 3.78 (b) |
| Soil with As-distilled water | 56 ± 3.61 (b) | 50.92 ± 2.02 (b) |
| Control soil-water with As | 6.67 ± 0.45 (a) | 6.58 ± 0.95 (a) |

Each value represents the mean of three replicates ± standard deviation. The different letters within a column indicate a significant difference at $p \leq 0.05$ according to Duncan's multiple range tests.

**Table 3**
Translocation and bioconcentration factors values in chard crops.

| Treatments | Translocation factor | | Bioconcentration factor | |
|---|---|---|---|---|
| | var. *cicla* | var. *dampuis* | var. *cicla* | var. *d'ampuis* |
| Control soil-distilled water | 0.08 | 0.08 | 1.98 | 3.18 |
| Soil with As-water with As | 0.02 | 0.03 | 3.58 | 2.79 |
| Soil with As-distilled water | 0.03 | 0.06 | 4.60 | 5.66 |
| Control soil-water with As | 0.16 | 0.06 | 1.68 | 5.58 |

was found to be the main parameter of the soil that limited As dispersion in a mining zone. It is known that Ca ions can immobilize As ions when adsorbed in the soil humus, and act as adsorption sites for this toxicant.

Even though the test covered a single sowing season and lasted a limited time period (2 months), an As accumulation became evident in the treatments where the soil contained the toxicant. Irrigation with contaminated water is likely to increase As concentrations, as published by Norra et al. (2005) and Rahman and Naidu (2009). In the present experiment, the short period of time during which crops were irrigated could have affected the processes that determine As dynamics in the soil: adsorption, precipitation, complexation, etc. (Bustingorri and Lavado, 2014). During irrigation, As (III) and Fe (II) species easily oxidized to As (V) and Fe (III), respectively, and tended to coprecipitate as As-Fe oxide. Arsenic ions (V) can also adsorb onto Fe oxide particles present in agricultural soils (Neidhardt et al., 2012). In the same way as Fe, Mn can also form oxide and adsorb As (V) ions in irrigation water.

It is worth noting that due to As cumulative effect, toxicant concentrations in the soils where both chard varieties grew exceeded the regulatory levels of 20 mg/kg established by National Law N° 24585 for agricultural soils. This happened when the soils contained As and when irrigation was carried out with either contaminated or control water.

### 3.5. Translocation and bioconcentration factors of arsenic in chard varieties

There are certain factors that allow determining the capacity of plants to absorb and transfer the toxicants from the soil to their aerial parts. Arsenic translocation to the crop and through the plant is one of the key factors that cause humans to become exposed to this toxic element through the food chain (Khan et al., 2008).

Translocation factor (TF) was calculated to evaluate the ability of plants to mobilize As from root to shoot. It could be observed that, in both varieties, the control treatment showed a TF greater than treatments with soils contaminated with As. By contrast, this factor decreased in *d 'ampuis* variety when cultivated in control soil and watered with As (Table 3).

Var. *cicla* presented a 0.16 TF value when cultivated in control soil and irrigated with water with As. According to Khan et al. (2009), the toxicant may be expelled from the plant tissue when TF values approach 0.1. Similar results were obtained by García Gallegos et al. (2011) when subjecting bean and oat crops to 50, 100 and 150 mg/kg of Pb.

TF values recorded were below 1 due to a lower As accumulation in the aerial biomass, as compared to the concentrations found in the roots. According to Huang et al. (2006), the higher As concentration levels are in the soil, the lower TF becomes, since most plants avoid intoxication by expelling the toxicant.

The TF of var. *d 'ampuis* grown in control soil and irrigated with water with As was lower than the value obtained when the variety was irrigated with distilled water. By contrast, var. *cicla* showed a higher TF when watered with As.

When both Swiss chard varieties were cultivated in contaminated soil, TF was lower when they were irrigated with water containing As, as compared to what was observed under irrigation with distilled water. Similar results were published by Pineda Chacon (2016), who found that As translocation in *Cucumis melo* and *Citrullus lanatus* decreased with higher As concentration levels in irrigation water. This could be attributed to As stress, which alters cell transport systems, thus hindering As translocation (Finnegan and Chen, 2012).

The bioconcentration factor (BCF) is an important indicator of toxic transfer from soil to plants (Chang et al., 2014). The high values obtained could be attributed to the elevated As concentrations found in the roots. When chard was cultivated in soils with As and watered with distilled water, the maximum BCF values were recorded (4.60 for var. *cicla*, and 5.66 for var. *d 'ampuis*) (Table 3).

In the control treatment, var. *d'ampuis* showed a 3.18 BCF value, higher than the value obtained when the plant was both grown in soil with As and watered with As (2.79). A similar behavior was reported by Bustingorri et al. (2014), who subjected a soybean plantation to different As concentrations. These authors determined that BCF values always decreased with higher As concentration levels in soils: 0.73 to 0.14 from soil to shoots, 0.41 to 0.06 from soil to seeds, and 4.03 to 0.84 from soil to roots. Zehra et al. (2009) reported that BCF values may be higher in plants grown in uncontaminated soils than in contaminated soils, depending on the plant species, their root exudates, the physicochemical properties of the soil, and the concentration and chemical forms of the contaminant in the soil.

Kabata-Pendias and Pendias (2001) reported that root exudates (carboxylic acids, citric and malic acids) tend to acidify the medium and displace arsenate from exchange positions in the soil. In addition, plant exudates could react with the hydroxides and Fe oxides, which would alter the surfaces on which As is retained, hence resulting in its solubilization and bioavailability (Fitz and Wenzel, 2002).

According to Audet and Charest (2007), BCF values higher than 1 would be indicative of potentially hyperaccumulating species. Li et al. (2007) and Song et al. (2012) demonstrated this when they published that var. *cicla* chard presented characteristics of a hyperaccumulating crop when exposed to soil contaminated with Cd during a two-month period. Based on the results obtained in the present study and the conditions under which it was carried out, this characteristic could be attributed to both chard varieties evaluated.

### *3.6. Potential health risks associated with the consumption of local leafy vegetables*

At present, there are several methods to estimate the potential health risks of pollutants for carcinogenic and non-carcinogenic effects. Non-cancer risk assessment is typically based on the target hazard quotient (THQ) method, which is a ratio between the determined dose of a pollutant and the reference oral dose. THQ values are associated with many factors, including intake of pollutants, exposure time, body weight, and reference oral dose of the pollutant.

In the two chard species evaluated in this work, the mean THQs of As were higher than 1 in all the treatments, indicating that the consumption of this vegetable implies a potential health risk. Consumption of plants grown in As-contaminated soil and irrigated with distilled water led to THQ values significantly different from those of the other treatments (Table 4). Similar results were published by Valdez-González et al. (2014), who assessed Cd phytoavailability and human health risk of cadmium ingestion, focusing on the consumption of *B. vulgaris* L. grown in agricultural soil. The THQ for Cd was higher than 1 for well water-irrigated treatments. In India, Kumar et al. (2016) determined As contamination levels and assessed health risk posed by As and other elements present in drinking water, vegetables, and other food components in a district called the Samastipur. The THQ values were higher than 1 for As in drinking water, vegetables and rice, thus indicating a potential health risk for the local population.

The carcinogenic risk (CR) for adults ingesting As ($1.12 \times 10^{-3}$) through chard consumption was far beyond the range ($10^{-6}$–$10^{-4}$) of acceptable risk established by the US Environmental Protection Agency (USEPA, 2010). This CR value is consistent with those reported by Li et al. (2011) for Chinese food, where incremental lifetime cancer risk derived from As intake is $1.06 \times 10^{-3}$ for adults. In addition, Li et al. (2017) reported mean CR values of $1.65 \times 10^{-3}$ for adults exposed to As through consumption of vegetables.

However, it has to be taken into account that leafy vegetables contribute only a part of the total daily arsenic intake; other sources of the toxicant include drinking water, dust inhalation, and consumption of local meat, such as mutton, llama and lamb. Therefore, the potential health risks for residents are actually higher than those found in this study. Additionally, it was observed that for special populations, with a weak constitution and higher vulnerability, such as pregnant women, the potential health risks of arsenic accumulation through vegetable consumption were likely to be higher than for the average person.

These results suggest that in order to protect local residents from As toxicity, vegetable-specific and site-specific information should be offered to them, and attention should be paid to the kinds and amounts of vegetables these people usually consume.

**Table 4**
Estimated target hazard quotients (THQ) in the chard crops.

| Treatments | var. *cicla* | var. *d'ampuis* |
|---|---|---|
| Control soil-distilled water | 1.69 ± 0.18 (c) | 3.26 ± 2.34 (b) |
| Soil with As-water with As | 11.17 ± 4.27(b) | 13.88 ± 0.43 (b) |
| Soil with As-distilled water | 18.90 ± 6.15 (a) | 46.40 ± 7.35 (a) |
| Control soil-water with As | 4.57 ± 1.18 (bc) | 5.56 ± 0.57 (b) |

Each value represents the mean of three replicates ± standard deviation. The different letters within a column indicate a significant difference at $p \le 0.05$ according to Duncan's multiple range tests.

## 4. Conclusions

The results of this research provide new information on water and soil contamination and the potential risk of As introduction into the food chain when certain vegetables grown in the Andean zone of the Province of Jujuy (Argentina) are consumed by local residents.

The two varieties of chard evaluated in this work showed effective As mobilization when this toxicant was present both in the soil and in irrigation water. This As was absorbed and concentrated in roots and leaves. The greatest As transfer was observed in crops growing in contaminated soils and receiving irrigation with distilled water.

The roots of the two evaluated varieties were the organs that showed the highest As accumulation in all exposure treatments. Average As concentration levels in the leaves exceeded the maximum allowable limit established by the Código Alimentario Argentino in all treatments. In fact, they exceeded by 2–62 this limit, which should be taken as a warning of the health risks consumers of these chard varieties are running. The bioaccumulation factor was high enough to characterize both chard varieties as "hyperaccumulative vegetables".

It was also found that chard planting would reduce As content in soils towards the end of harvest if the initial concentration in the soil did not exceed 10 mg/kg of soil. However, this preliminary result should be confirmed with new trials designed for this specific purpose. In the health risk assessment of the population of Pastos Chicos and on the basis of the THQ value, it was confirmed that human health would be affected if inhabitants ate food containing the As concentrations recorded in this study. Indeed, the Carcinogenicity Index obtained in this work exceeded the maximum value recommended by International Organizations (WHO, 2004; USEPA, 2010).

It will be necessary to do further research in order to determine which chemical As species is present in the two chard varieties and to assess its bioavailability. The findings presented in this work are unprecedented in the Andean region in Jujuy, and point to the need to promote research both on the presence of As in the environment and on its impact on human health.

## Acknowledgements

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and the PIO Project CONICET-UNJU Nº 14020140100136CO. We would also like to thank Dr. María Julia Amoroso for helping us write this paper.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2018.04.048.

## References


Abrol, I.P., Yadav, J.S.P., Massoud, F.I., 1988. Salt Affected Soils and Their Management. United Nations, Rome. FAO Soils Bulletin 39.

Adriano, D.C., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals. Springer-Verlag, New York.

Audet, P., Charest, C., 2007. Heavy metal phytoremediation from a meta-analytical perspective. Environ. Pollut. 147, 231–237.

Bleeker, P.M., Schat, H., Vooijs, R., Verkeij, J.A.C., Ernst, W.H.O., 2003. Mechanisms of arsenate tolerance in *Cytisus striatus*. New Phytol. 157, 33–38.

Brammer, H., Kassam, A., Meharg, A., Ravenscroft, P., Keith, R., 2008. Arsenic Pollution. A Global Problem. The Royal Geographical Society (With IBG).

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Sci. 59, 39–46.

Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen total. In: Page, A.L. (Ed.), Methods of Soil Analysis. Part 2, second ed. AmericanSociety of Agronomy (ASA), Madison, Wisconsin USA.

Burriel, F., Lucena, F., Arribas, S., Hernández Mendez, J., 1999. In: Thompson (Ed.), Química Analítica Cualitativa. Paraninfo, (Madrid).

Buschmann, J., Berg, M., Stengel, C., Sampson, M.L., 2007. Arsenic and manganese contamination of drinking water resources in Cambodia: coincidence of risk areas with low relief topography. Environ. Sci. Technol. 41, 2146–2152.

Bustingorri, C., Lavado, R.S., 2014. Soybean as affected by high concentrations of arsenic and fluoride in irrigation water in controlled conditions. Agr. Water Manag 144, 134–139.

Carbonel, Barranchina, A.A., Burló Carbonel, F.M., Mataix Beneyto, J.J., 1995. Arsénico en el sistema suelo-planta. Significado Ambiental. Editorial Espagrafic. Universidad de Alicante, España, p. 139.

Castillo, N.S., Franco, M.L., González, M.J., Santillán, J.M., Vázquez, M., Botto, I.L., 2013. Effects of irrigation with rich-arsenic water on an arugula *(Eruca sativa L.)* crop. Ediciones Digitales del SeDiCi. Universidad Nacional de La Plata. Augm Domus 5, 29–41. Available on. http://revistas.unlp.edu.ar/index.php/domus/issue/current/showToc.

Chang, C.Y., Yu, H.Y., Chen, J.J., Li, F.B., Zhang, H.H., Liu, C.P., 2014. Accumulation of heavy metals in leaf vegetables from agricultural soils and associated potential health risks in the Pearl River Delta. South China. Environ. Monit. Assess. 186, 1547–1560.

Choque, D., 2013. Determinación de arsénico total e inorgánico en alimentos de una localidad de la Puna jujeña-Argentina (Tesis de grado). Facultad de Ciencias Agrarias, Universidad Nacional de Jujuy, Argentina.

Código Alimentario Argentino (CAA), 2012. Productos alimenticios. Reglamento técnico Mercosur sobre límites máximos de contaminantes inorgánicos en alimentos. Capítulo III. Available on. http://www.anmat.gov.ar/alimentos/codigoa/Capitulo_III.pdf.

Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., 2005. Standard Methods for the Examination of Water and Wastewater, twenty-first ed. APHA, Washington DC.

EPA, 1996. Method 3050 B: Acid Digestion of Sediments, Sludges and Soils. Revision 2. Washington, DC.

Farías, S.S., Escalante, J., Servant, R.E., Bianco de Salas, G., Bovi Mitre, M.G., Ávila Carreras, M.E., Ponce, R.I., 2008. Survey of arsenic in drinking water and assessment of the intake of arsenic from water in Argentine Puna. In: Bundschuh, J., Birkle, P., Matschullat, J., Armienta, M., Mukherjee, A., Bhattacharya, P. (Eds.), Natural Arsenic in Groundwaters of Latin America. CRC Press, London, pp. 397–407.

Finnegan, P.M., Chen, W., 2012. Arsenic toxicity: the effects on plant metabolism. Front. Physiol. 3–182.

Fitz, W.J., Wenzel, W.W., 2002. Arsenic transformations in the soil-rhizophere-plant system: fundamentals and potential application to phytoremediation. J. Biotechnol. 99, 259–278.

García, I., Diez, M., Martín, F., Simón, M., Dorronsoro, C., 2009. Mobility of arsenic and heavy metals in a sandy-loam textured and carbonated soil. Pedosphere 19, 166–175.

García-Gallegos, E., Hernández-Acosta, E., García-Nieto, E., Acevedo-Sandoval, O.A., 2011. Contenido y traslocación de plomo en avena (*Avena sativa*, L.) y haba (*Vicia faba*, L.) de un suelo contaminado. Revista Chapingo. Serie Cienc. For. Ambient 17, 19–29.

Gulz, P., Gupta, S.K., 2000. Arsenic uptake by crops. Agrarforschung 7, 3603–3665.

Gulz, P.A., Gupta, S.K., Schulin, R., 2005. Arsenic accumulation of common plants from contaminated soils. Plant Soil 272, 337–347.

Gusman, G.S., Olivera, J.A., Farnese, F.S., Cambria, J., 2013. Arsenate and arsenite: the toxic effect on photosynthesis and growth of lettuce plants. Act. Physiol. Plant 35, 1201–1209.

Hettick, B.E., Cañas-Carrell, J.E., Martin, K., French, A.D., Klein, D.M., 2016. Arsenic uptake by muskmelon (*Cucumis melo*) plants from contaminated water. Bull. Environ. Contam. Toxicol. 97, 395–400.

Huang, R.Q., Gao, S.F., Wang, W.L., Staunton, S., Wang, G., 2006. Soil arsenic availability and the transfer of soil arsenic to crops in suburban areas in Fujian Province, southeast China. Sci. Total Environ. 368, 531–541.

IRAM (Instituto Argentino de Normalización y Certificación) 29481–29484. Directivas para muestreo de sitios naturales, poco alterados y cultivados. Primera edición. Argentina.

Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants, third ed. CRC Press, Florida, USA, p. 413.

Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z., Zhu, Y.G., 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environ. Pollut. 152, 686–692.

Khan, S., Farooq, R., Shahbaz, S., Khan, A.M., Sadique, M., 2009. Health risk assessment of heavy metals for population via consumption of vegetables. World app. Sci. J. 6, 1602–1606.

Kumar, M., Rahman, M.M., Ramanathan, A.L., Naidu, R., 2016. Arsenic and other elements in drinking water and dietary components from the middle Gangetic plain of Bihar, India: health risk index. Sci. Total Environ. 539, 125–134.

Li, Y.S., Sun, L.N., Sun, T.H., Wang, H., 2007. Cadmium hyperaccumulator *Beta vulgaris* var. *cicla* L. and its accumulating characteristics. J. Agro. Environ. Sci. 26, 1386–1389.

Li, G., Sun, G.X., Williams, P.N., Nunes, L., Zhu, Y.G., 2011. Inorganic arsenic in Chinese food and its cancer risk. Environ. Int. 37, 1219–1225.

Li, L., Hang, Z., Yang, W.T., Gu, J.F., Liao, B.H., 2017. Arsenic in vegetables poses a health risk in the vicinity of a mining area in the southern Hunan. Hum. Ecol. Risk Assess. 23, 1315–1329.

Maldonado-Magaña, A., Favela-Torrez, E., Rivera-Cabrera, F., Volke-Sepulveda, T.L., 2011. Lead bioaccumulation in *Acacia famesiana* and its effect on lipid peroxidation and glutathione production. Plant Soil 339, 377–389.

Martínez-Villegas, N., Briones-Gallardo, R., Ramos-Leal, J.A., Avalos-Borja, M., Castañón-Sandoval, A.D., Razo-Flores, E., Mario Villalobos, M., 2013. Arsenic mobility controlled by solid calcium arsenates: a case study in Mexico showcasing a potentially widespread environmental problem. Environ. Pollut. 176, 114–122.

Mitic, V., Jovanovic, V.S., Dimitrijevic, M., Cvetkovic, J., Stojanovic, G., 2013. Effect of food preparation technique on antioxidant activity and plant pigment content in some vegetable species. J. Food Nutr. Res. 1, 121–127.

Monferran, M.V., Garnero, P.L., Wunderlin, D.A., de los Angeles Bistoni, M., 2016. Potential human health risks from metals and as via *Odontesthes bonariensis* consumption and ecological risk assessments in a eutrophic lake. Ecotox. Environ. Safe 129, 302–310.

National law (Nº 24051), 1993. Residuos peligrosos. Available on: http://servicios.infoleg.gob.ar/infolegInternet/anexos/10000-14999/12830/texact.htm. (Accessed 18 June 2017).

Nacional law (Nº 24.585), 1995. Protección Ambiental para la actividad minera del código de minería. Available on: https://www.entrerios.gov.ar/ambiente/userfiles/files/archivos/Normativas/Nacionales/Ley%2024585_Act_Min_EIA.pdf. (Accessed 20 June 2017).

Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon and organic matter. In: Page, A.L. (Ed.), Methods of Soil Analysis. Part 2 (2nd), vol. 9. American Society of Agronomy, Madison, WI, pp. 539–579.

Neidhardt, H., Norra, S., Tang, X., Guo, H., Stüben, D., 2012. Impact of irrigation with high arsenic burdened groundwater on the soil–plant system: results from a case study in the Inner Mongolia, China. Environ. Pollut. 163, 8–13.

Niazi, N.K., Singh, B., Shah, P., 2011. Arsenic speciation and phytoavailability in contaminated soils using a sequential extraction procedure and XANES spectroscopy. Environ. Sci. Technol. 45, 7135–7142.

Niazi, N.K., Bashir, S., Bibi, I., Murtaza, B., Shahid, M., Javed, M.T., Shakoor, M.B., Saqib, Z.A., Nawaz, M.F., Aslam, Z., 2016. Phytoremediation of Arsenic-contaminated Soils Using Arsenic Hyperaccumulating Ferns. Phytoremediation. Springer, pp. 521–545.

Niazi, N.K., Bibi, I., Fatimah, A., Shahid, M., Javed, M.T., Wang, H., Ok, Y.S., Bashir, S., Murtaza, B., Saqib, Z.A., Shakoor, M.B., 2017. Phosphate-assisted phytoremediation of arsenic by *Brassica napus* and *Brassica juncea*: morphological and physiological response. Int. J. Phytoremediat 19, 670–678.

Ninfali, P., Angelino, D., 2013. Nutritional and functional potential of *Beta vulgaris cicla* and *rubra*. Fitoterapia 89, 188–199.

Norra, S., Berner, Z.A., Agarwala, P., Wagner, F., Chandrasekharam, D., Stüben, D., 2005. Impact of irrigation with as rich groundwater on soil and crops: a geochemical case study in West Bengal Delta Plain, India. Appl. Geochem 20, 1890–1906.

Pickering, I.J., Gumaelius, L., Harris, H.H., Prince, R.C., Hirsch, G., Banks, J.A., Salt, D.E., George, G.N., 2006. Localizing the biochemical transformations of arsenate in hyperaccumulating fern. Environm. Sci. Technol. 40, 5010–5014.

Pigna, M., Cozzolino, V., Violante, A., Meharg, A.A., 2009. Influence of phosphate on the arsenic uptake by wheat (*Triticum durum* L.) irrigated with arsenic solutions at three different concentrations. Water Air Soil Poll. 197, 371–380.

Pineda Chacón, G., 2016. Transferencia de arsénico en cultivares de la zona agrícola centro-sur del estado de Chihuahua. Tesis para obtener el título de Doctor en Ciencia y Tecnología Ambiental. Available on: http://cimav.repositorioinstitucional.mx/jspui/handle/1004/81.

Ponce, R., Farías, S., Bovi Mitre, G., Vélez, D., Montoro, R., 2006. Determinación de arsénico total e inorgánico en carne y vísceras de camélidos (*Lamma glama*) autóctonos de la provincia de Jujuy, Argentina. Rev. Fac. Agron. UBA (Buenos Aires, Argent. 26, 105–109.

Prieto García, F., Callejas Hernández, J., Román Gutiérrez, A., Prieto Méndez, J., Gordillo Martínez, A., Méndez Marzo, M., 2007. Acumulación de arsénico en cultivos de Haba (*Vicia faba*). Agron. Costarric. 31, 101–109.

Rahman, F., Naidu, R., 2009. The influence of arsenic speciation (As III & as V) and concentration on the growth, uptake and translocation of arsenic in vegetable crops (silver beet and amaranth): greenhouse study. Environ. Geochem. Hlth 31, 115–124.

Rahaman, S., Sinha, A.C., Pati, R., Mukhopadhyay, D., 2013. Arsenic contamination: a potential hazard to the affected areas of West Bengal, India. Environ. Geochem. Health 35, 119–132.

Rehman, Z.U., Khan, S., Qin, K., Brusseau, M.L., Shah, M.T., Din, I., 2016. Quantification of inorganic arsenic exposure and cancer risk via consumption of vegetables in southern selected districts of Pakistan. Sci. Total Environ. 550, 321–329.
Richards, L.A., 1954. Diagnosis and Improvement of Saline and Alkaline Soils. United States Salinity Laboratory Staff. Agricultural Handbook No 60. U. S. Dept. of Agriculture, Washington, D.C, p. 160.
Richards, L.A., 1982. Diagnóstico y rehabilitación de los suelos salinos y sódicos, p. 172. Limusa (ed.), México.
Rhoades, J.D., 1982. Soluble salts. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Soil Science of America, Madison, WI, pp. 167–178.
Salaverry Fognoli, M.A., 2014. Efecto de veinte niveles de salinidad del agua de riego en los indicadores agronómicos del cultivo de acelga en la cosecha (Tesis de grado). Universidad Nacional Agraria la Molina, Lima-Perú. Available on: http://repositorio.lamolina.edu.pe/bitstream/handle/UNALM/1882/F06.S343-T.pdf?sequence=1.
Shakoor, M.B., Niazi, N.K., Bibi, I., Rahman, M.M., Naidu, R., Dong, Z., Shahid, M., Arshad, M., 2015. Unraveling health risk and speciation of arsenic from groundwater in rural areas of Punjab. Pak. Int. J. En. Res. Pub. He 12, 12371–12390.
Smith, E., Naidu, R., Alston, A.M., 2002. Chemistry of inorganic arsenic in soils. J. Environ. Qual. 31, 557–563.
Smith, E., Juhasz, A.L., Weber, J., 2009. Arsenic uptake and speciation in vegetables grown under greenhouse conditions. Environ. Geochem. Hlth 31, 125–132.
Song, X., Hu, X., Ji, P., Li, Y., Chi, G., Song, Y., 2012. Phytoremediation of cadmium-contaminated farmland soil by the hyperaccumulator *Beta vulgaris* L. var. *cicla*. B. Environ. Contam. Tox. 88, 623–626.
Spagnoletti, F., Tobar, N., Chiocchio, V., Lavado, R.S., 2015. Mycorrhizal inoculation and high arsenic concentrations in the soil increase the survival of soybean plants subjected to strong water stress. Commun. Soil Sci. Plant Anal. 46, 2837–2846.
Stachowicz, M., Hiemstra, T., Van Riemsdijk, W.H., 2008. Multi-competitive interaction of as (III) and as (V) oxyanions with $Ca^{2+,}$ $Mg^{2+}$, $PO_3^{-}$ $^4$, and $CO_2^{-}$ $^3$ ions on goethite. j. Colloid Interf. Sci. 320, 400–414.
Tschambler, J., Cabrera, R., Bovi-Mitre, G., 2007. Georreferenciamiento del contenido de arsénico en aguas de la Provincia de Jujuy-Argentina. XV Congreso Argentino de Toxicología, Neuquén, Libro de resúmenes, p. 48.
Tu, S., Ma, L.Q., 2003. Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth on the arsenic hyperaccumulator *Pteris vittata* L. under hydroponic conditions. Environ. Exp. Bot. 50, 243–251.
USEPA (US Environmental Protection Agency), 2000. Risk Based Concentration Table (Philadelphia, PA; Washington, DC).
USEPA (US Environmental Protection Agency), 2010. Risk-based Concentration Table. Available on: http://www.epa.gov/reg3hwmd/risk/human/index.htm.
USEPA (US Environmental Protection Agency), 2015. Human health risk assessment. Regional Screening Level (RSL) Summary Table. Available on: http://www.epa.gov/reg3hwmd/risk/human/rbconcentration_table/Generic_Tables/docs/master_sl_table_run_JAN2015.pdf. .
Valdez-González, J.C., López-Chuken, U.J., Guzmán-Mar, J.L., Flores-Banda, F., Hernández-Ramírez, A., Hinojosa-Reyes, L., 2014. Saline irrigation and Zn amendment effect on Cd phytoavailability to Swiss chard (*Beta vulgaris* L.) grown on a long-term amended agricultural soil: a human risk assessment. Environ. Sci. Pollut. R. 21, 5909–5916.
WHO, 2004. The Global Burden of Disease Report. World Health Organization, Geneva, Switzerland. Available on: http://www.who.int/topics/global_burden_of_disease/en/.
Zehra, S.S., Arshad, M., Mahmood, T., Waheed, A., 2009. Assessment of heavy metal accumulation and their translocation in plant species. Afr. J. Biotechnol. 8, 2802–2810.
Zhao, F.J., Ma, J.F., Meharg, A.A., McGrath, S.P., 2009. Arsenic uptake and metabolism in plants. New Phytol. 181, 777–794.