



# Soybean as affected by high concentrations of arsenic and fluoride in irrigation water in controlled conditions



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## ABSTRACT

Arsenic (As) and Fluoride (F) are both present in many groundwater sources around the world. The use of these waters for irrigation purposes could cause problems on crop production and the food chain. The aim of this work was to investigate soybean biomass production, bean yield and As and F accumulation in the soil and the plant in controlled conditions. An experiment mimicking sprinkler irrigation with water enriched in As and F, applied individually or simultaneously was carried out. When irrigation was applied, part of the water fell inside the pot, either directly or through the leaves and increased the contents of bioavailable As and F forms in the soil. Arsenic was more toxic to soybean than F. Significant biomass and yield reductions, and As and/or F accumulation in plant tissues were observed when As and F concentration surpassed  $0.6 \text{ mg As L}^{-1}$  and  $25 \text{ mg F L}^{-1}$ . When As and F were applied simultaneously the toxic effect were additive and the detrimental effects were larger. Soybean bean yield was reduced almost 50% for As and 30% for F. Arsenic and F concentration increased in all organs but soybean beans presented lower values than concentrations hazardous to human and animal health. Bean concentrations were less than  $1 \text{ mg As kg}^{-1}$  and less than  $5 \text{ mg F kg}^{-1}$ .

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## 1. Introduction

Arsenic (As) is a normal constituent in groundwater around the world, with concentrations ranging from  $0.01 \text{ mg l}^{-1}$  to  $2100 \text{ mg l}^{-1}$  (Pais and Benton Jones, 1997). The occurrence of high-As groundwater has been documented in many countries (Smedley and Kinniburgh, 2002) including Bangladesh (Nickson et al., 1998); Vietnam (Berg et al., 2007), India (Norra et al., 2005); China and Taiwan (Wang and Huang, 1994), Chile (Cáceres et al., 1992), Mexico (Del Razo et al., 1990), United States (Welch et al., 1988), Australia (Smith et al., 2003), Germany (Heinrichs and Udluft, 1999) and Argentina (Gonzalez Uriarte et al., 2002; Smedley et al., 2005). Fluoride (F) occurrence in the earth crust is larger than As, and it is also present in groundwater around the world in concentrations varying from  $0.1 \text{ mg l}^{-1}$  to  $250 \text{ mg l}^{-1}$  (Pais and Benton Jones, 1997). Fluoride concentration is very high in groundwater located in Korea (Chae et al., 2007); China (Zhu et al., 2007); Ghana (Apambire et al., 1997); India (Yadav et al., 2012); United States (Miller et al., 1999), Mexico (Wyatt et al., 1998) and Argentina (Lavado and Reinaudi, 1983; Gonzalez Uriarte et al., 2002; Smedley et al., 2005). Very often those groundwaters are the source of water used in irrigation

systems; this is a common way both elements enter in agricultural soils, affecting negatively crop production and food safety (food chain contamination) (Senanayakea and Mukherjib, 2014). This phenomenon was documented in several countries (Cronin et al., 2000; Brammer and Ravenscroft, 2008; Dahal et al., 2008).

Plants exposed to high As concentrations show toxicity symptoms, such as germination inhibition, reduced aerial and root biomass growth and yield, and even in some cases cause death (Abedin et al., 2002; Pigna et al., 2008; Rahman et al., 2007). Fluoride has been less extensively studied regarding its phytotoxicity. However, lower root growth, reduced biomass production and yield loss have been found in different species (Cronin et al., 2000; Stevens et al., 2000).

The effect of As and/or F in irrigation water on soybean growth and yield has not been sufficiently studied, neither has the accumulation of both toxic elements in soybean plants including beans. Soybean is the fourth crop of the world, which has exceptional nutritional characteristics and ability to grow under a wide range of environmental conditions and management systems (Sadras and Calviño, 2001). Soybean is increasingly irrigated by sprinkler irrigation systems using underlying groundwater rich in both As and F, around the world and also in Argentina (Bustingorri and Lavado, 2012). The problem of As or F contaminated irrigation water on crops has been documented in the country since the 1970' decade (Reinaudi and Lavado, 1978; Troiani et al., 1987; Franco

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et al., 2012). Most studies about the effect of As and F rich irrigation water on crops, were focused mainly on the effect of As on rice cultivated using the so called flooding irrigation methods; wheat and vegetables using furrow irrigation were studied to a lesser extent, (Zhao et al., 2010). No references about sprinkler irrigation systems, using water contaminated with those elements and their toxicity in crops, were found. The aim of this work was to analyze the effect of As and F in irrigation water on soybean biomass production, bean yield and As and F accumulation and distribution within the plant. The experiment was carried out in pots mimicking sprinkler irrigation, using irrigation water enriched with As and F.

## 2. Material and methods

An 8L pots experiment was carried out following a completely randomized design with 6 replicates per treatment. For water supply, sprinkler irrigation was mimicked using artificially enriched As and F irrigation water, covering background levels and different degrees of contamination in groundwater. To contaminate the irrigation water, different concentrations of sodium arsenate and sodium fluoride were added to deionized water. Treatments included 5 As levels As VL (0.3 mg As l<sup>-1</sup>), As L (0.6 mg As l<sup>-1</sup>), As M (10 mg As l<sup>-1</sup>), As H (50 mg As l<sup>-1</sup>) and As VH (200 mg As l<sup>-1</sup>), 5 F levels F VL (4.5 mg F l<sup>-1</sup>), F L (9 mg F l<sup>-1</sup>), F M (25 mg F l<sup>-1</sup>), F H (50 mg F l<sup>-1</sup>) and F VH (200 mg F l<sup>-1</sup>) and 5 As+F levels in the water (same concentrations). A control treatment (C) was irrigated with deionized water. The Electrical Conductivity (EC) of the deionized water averaged 0.04 dS m<sup>-1</sup> and the pH 6.9 (Sparks et al., 1996). Deionized water was used in all treatments in order to standardize the chemical composition of irrigation water.

The substrate in the pots was a mix of 30% washed sand and 70% top horizon of a sandy loam Typic Argiudoll. The particle size distribution of the substrate was 13% clay, 12% silt and 74% sand and the chemical composition of the substrate was: 12.6 g kg<sup>-1</sup> of organic carbon (Walkley and Black method), 7.6 pH, 32.8 mg kg<sup>-1</sup> available phosphorus (Kurtz and Bray method) and 0.38 dSm<sup>-1</sup> EC<sub>s</sub> (soil saturation extract) (Sparks et al., 1996). To cover the nutritional needs of plants, each pot received 2 g of triple superphosphate and 0.125 g of a mix containing all micronutrients before sowing. Every 30 days all pots had 1 g of a soluble fertilizer (25-10-10). In each pot three soybean seeds (Nidera 4613), pregerminated in dark for 48 h, were sown. Seeds were pregerminated to ensure the presence of plants in the experiment. Pots were thinned to one plant per pot after 15 days of seeding. The experiment started with the substrate in the pots wetted to field capacity and each solution was applied to each plant at a rate of 50 to 200 ml every 1–2 days. Some pots were weighted to estimate the solution addition, which varied widely according to plant growth and evolution. Drainage measurements varied from 0 to 40 ml per pot.

At pod (R3–R4) and maturity (R8) stages (70 and 130 days after sowing, respectively) plant height (main shoot only) was recorded, and vegetation samples in 3 replicates in each stage were sampled. At R8 stage roots and soil in the pots were also sampled and the numbers of pods and beans were recorded. The harvested aerial biomass was divided into leaves, shoots, pods, and beans. Roots were washed, sieved and harvested. All vegetative samples were rinsed with distilled water, dried at 60 °C for 72 h and then weighed.

Arsenic and F concentration on grinded, sieved and homogenized samples were determined in all plant material. Arsenic was extracted by HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> acid digestion and was measured by atomic adsorption (ICP-AES) (USEPA, 2006). For F content plant material was ashed at 400 °C and quantified by colorimetry (SPADNS, APHA, 1993). Soil As and F bioavailable forms were determined: As was extracted with a 1.0 M solution of sodium

acetate at pH 5 adjusted with acetic acid, filtrates were acidified and quantified by ICP-AES (Anwar et al., 2008). Fluoride was extracted with hot distilled water and determined by SPADNS (APHA, 1993). Arsenic and F in leachates were determined by the methods used for soils.

The results were evaluated using an analysis of variance (ANOVA) test. When significant differences were found, a comparison of means test (LSD) was applied. The curve fitting software, Table Curve 2D (AISN Software Inc, 2000), was used to identify the relationship between soil As and F concentration and soybean yield. To understand the behavior of different parts of soybean against toxic elements such as As and F, the translocation factor (TF), and the bioconcentration factor (BCF) (Audet and Charest, 2007) were calculated as follows:

**TF** = As or F concentration in shoots/beans (mg kg<sup>-1</sup>)/As or F concentration in roots (mg kg<sup>-1</sup>)

**BCF** = As or F concentration in roots/shoots/beans (mg kg<sup>-1</sup>)/As or F bioavailable concentration in soil (mg kg<sup>-1</sup>).

## 3. Results

When mimicked sprinkler irrigation was applied, part of the water spread over plants fell inside the pot, either directly or through the leaves. Table 1 shows the balance of water quantifying its main components. The main loss of water was evapotranspiration. Conversely, most As and F added was retained by the substrate, a fraction drained from the pots and a negligible amount was retained in plant tissues. Table 2 shows bioavailable As and F concentration in the soils at harvest. These measurements indicate accumulation of As and F in the soil, which is related to their concentration in the irrigation water. Arsenic and F showed some interactions in the soil, especially F which availability tended to increase between 10% and 20% when applied together with As (Table 2).

Up to 0.6 mg As kg<sup>-1</sup> irrigation water (Treatments As VL and As L) had no significant effect on soybean biomass production. When treatments As M and As H were applied biomass was affected and at the highest As concentration (Treatment As VH), plants survived only for 20 days and then died (Table 3). On the other hand, F had detrimental effects over soybean growth when irrigated with concentrations exceeding 25 mg kg<sup>-1</sup> (Treatments F VL, F L and F M) but even at the highest F concentration no plants died. The negative effect of As and F on soybean turn up early in crop cycle: at R3–R4 overall plant biomass in As M and As H irrigation treatments was 60% to 68% lower than control plants, respectively. Plants showed a biomass reduction of around 45% compared to equivalents F treatments (Fig. 1). Leaves were the most affected organ for As rich irrigation treatments (biomass reduction between 70% and 82%, for As M and As H treatments), whilst F irrigation treatments had a more pronounced effect over roots (ranging from 47% to 55%). Simultaneous application of As and F resulted in 15% less biomass production compared to equivalent obtained under As rich irrigation water.

Reproductive organs, biomass and number of pods and beans (Table 3) showed the same tendency as vegetative organs. Compared with VL and L treatments, pod number and weight diminished up to 45% in As M and As H treatments and diminished around 30% in F M, F H and F VH treatments ( $p < 0.005$ ). Compared also with VL and L treatments, bean number was reduced between 25% and 30% and bean yield was reduced almost 50% in As M and As H treatments and both bean number and yield were 30% in F M, F H and F VH treatments ( $p < 0.05$ ). When As and F were jointly applied in irrigation water an additional 10% of yield was lost.

Arsenic concentration in plants increased as As concentration in irrigation water (and also in soils) did. Roots showed

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