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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 where observed when As and F concentration surpassed 0.6 mg As L^{-1} and 25 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 mg As kg^{-1} and less than 5 mg F kg^{-1}.

1. Introduction

Arsenic (As) is a normal constituent in groundwater around the world, with concentrations ranging from 0.01 mg l^{-1} to 2100 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 Taiwán (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 mg l^{-1} to 250 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^{-1}), As *L* (0.6 mg As l^{-1}), As *M* (10 mg As l^{-1}), As *H* (50 mg As l^{-1}) and As *VH* (200 mg As l^{-1}), 5 F levels F *VL* (4.5 mg F l^{-1}), F *L* (9 mg F l^{-1}), F *M* (25 mg F l^{-1}), F *H* (50 F mg l^{-1}) and F *VH* (200 F mg l^{-1}) 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^{-1} 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^{-1} of organic carbon (Walkley and Black method), 7.6 pH, 32.8 mg kg^{-1} available phosphorus (Kurtz and Bray method) and 0.38 dSm^{-1} EC_S (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_3/H_2O_2 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 (Anawar 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^{-1})/As or F concentration in roots (mg kg^{-1})

BCF = As or F concentration in roots/shoots/beans (mg kg^{-1})/As or F bioavailable concentration in soil (mg kg^{-1}).

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^{-1} 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^{-1} (Treatments F *VL*, F *L* an 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

Table 1
Water, As and F balance at the end of the experiment. Water data are in ml per pot and As and F data are in mg per pot.

Component	Treatment				
	VL	*L*	*M*	*H*	*VH*
Water[i] added	21,205	20,957	20,092	17,286	15,921
Water[i] drained	2365	2300	2272	1020	766
Water in pots[ii]	772	769	763	763	760
Water[iii] evapotranspired	18,068	17,888	17,057	15503	14,395
As added	6.36	12.57	201.10	866.30	–[vi]
As drained[iv]	0.19	0.63	19.11	103.96	–[vi]
As stored in plant[v]	0.02	0.02	0.06	0.04	–[vi]
As stored in soil	6.35	11.92	181.93	762.30	–[vi]
F added	95.42	188.61	502.29	818.82	3184.20
F drained[iv]	3.82	11.31	53.23	89.82	517.29
F stored in plant[vii]	0.03	0.05	0.08	0.15	0.17
F stored in soil	91.57	177.25	448.98	728.85	2666.74

[i] Average of As, F and As+F treatments.
[ii] Difference in water content in the pots before seeding and after harvest, plus water content in plants at time of harvest. Average of all treatments.
[iii] Estimated.
[iv] Calculated from concentration of As and F in leachates measured at the start, around middle time and the end of the experiment.
[v] Average of As treatments.
[vi] Irrigation was cut down after plants dies.
[vii] Average of F treatments.

Table 2
Available As and F concentrations in soil at harvest for each treatment after irrigation. Letters indicate differences between treatments for each element ($p<0.05$).

	Treatments					
	C	*VL*	*L*	*M*	*H*	*VH*
As ($mg\,kg^{-1}$)						
As	0.6 ± 0.16a	1.5 ± 0.18b	2.1 ± 0.20b	7.9 ± 0.90c	15.2 ± 2.20e	78.3 ± 7.20g
As+F		1.3 ± 0.20b	2.2 ± 0.30b	7.1 ± 0.80c	14.3 ± 2.50d	70.1 ± 8.10f
F ($mg\,kg^{-1}$)						
F	7.1 ± 0.81a	10.2 ± 0.31ab	11.19 ± 0.45ab	15.7 ± 2.93b	24.1 ± 3.28d	43.9 ± 6.86f
As+F		10.8 ± 0.81ab	12.8 ± 0.88ab	19.4 ± 1.47c	30.2 ± 5.04d	48.8 ± 2.58f

Table 3
Mean values for pods number and weight (g DM $plant^{-1}$) and bean number and weight (g DM $plant^{-1}$). LSD: least significant difference.

		Pods		Beans	
		Number	Weight	Number	Weight
As	C	33.2 ± 1.6	3.1 ± 0.3	53.7 ± 1.6	4.7 ± 0.3
	VL	31.0 ± 1.4	2.9 ± 0.5	55.5 ± 2.2	4.5 ± 0.4
	L	32.7 ± 1.9	3.0 ± 0.4	53.5 ± 1.9	4.6 ± 0.3
	M	14.3 ± 2.1	1.7 ± 0.5	41.8 ± 2.2	2.8 ± 0.2
	H	12.3 ± 1.6	1.5 ± 0.3	37.1 ± 2.7	1.7 ± 0.2
F	VH	Sd	Sd	Sd	Sd
	VL	31.2 ± 4.5	3.3 ± 0.4	52.5 ± 1.9	4.8 ± 0.3
	L	33.0 ± 3.2	2.9 ± 0.5	52.0 ± 2.6	4.4 ± 0.3
	M	18.3 ± 1.7	1.9 ± 0.5	51.7 ± 2.2	3.5 ± 0.3
	H	17.7 ± 1.5	1.8 ± 0.4	42.5 ± 2.1	3.4 ± 0.3
	VH	14.8 ± 0.5	1.5 ± 0.3	42.9 ± 2.3	3.2 ± 0.2
As+F	VL	32.7 ± 2.3	3.3 ± 0.5	32.6 ± 1.9	3.7 ± 0.3
	L	19.7+1.5	1.9 ± 0.4	29.7 ± 1.8	3.9 ± 0.4
	M	16.0+1.2	1.3 ± 0.3	27.3 ± 1.6	2.9 ± 0.2
	H	1.0 ± 0.1	0.9 ± 0.3	5.2 ± 0.85	1.6 ± 0.1
	VH	Sd	Sd	Sd	Sd
LSD	$p<0.05$	2.0	0.3	1.8	0.9

Sd: without data due to plant death.

the highest As concentration and the concentration order was roots > leaves > shoots > pods > beans. With few exceptions between shoots and pods, F showed the same trend (Table 4). Arsenic concentration was 10 times lower in soybean bean than in its aerial biomass and F concentration in soybean beans was more than 20 times lower than in its aerial biomass. In the present case, bean concentrations were less than 1 $mg\,As\,kg^{-1}$ and less than 5 $mg\,F\,kg^{-1}$. The TF showed differences between As and F. The As TF from roots to shoots was around 0.18–0.23 and from roots to beans from 0.10 to 0.07. The F TF tended to decrease as F concentration in the irrigation water increased: roots to shoots from 0.63 to 0.44 and roots to beans from 0.54 to 0.14. The measured BCF values tended always to decrease with increasing As in the soil: from 0.73 to 0.14 soil to shoots; 0.41–0.06 soil to beans and 4.03 to 0.84 soil to roots. Also for F this index behaved different than for As: it mostly increased as F in soil increased. The BCF was from 0.17 to 0.37 soil to shoots; around 0.12 soil to beans and from 0.27 to 0.84 soil to roots.

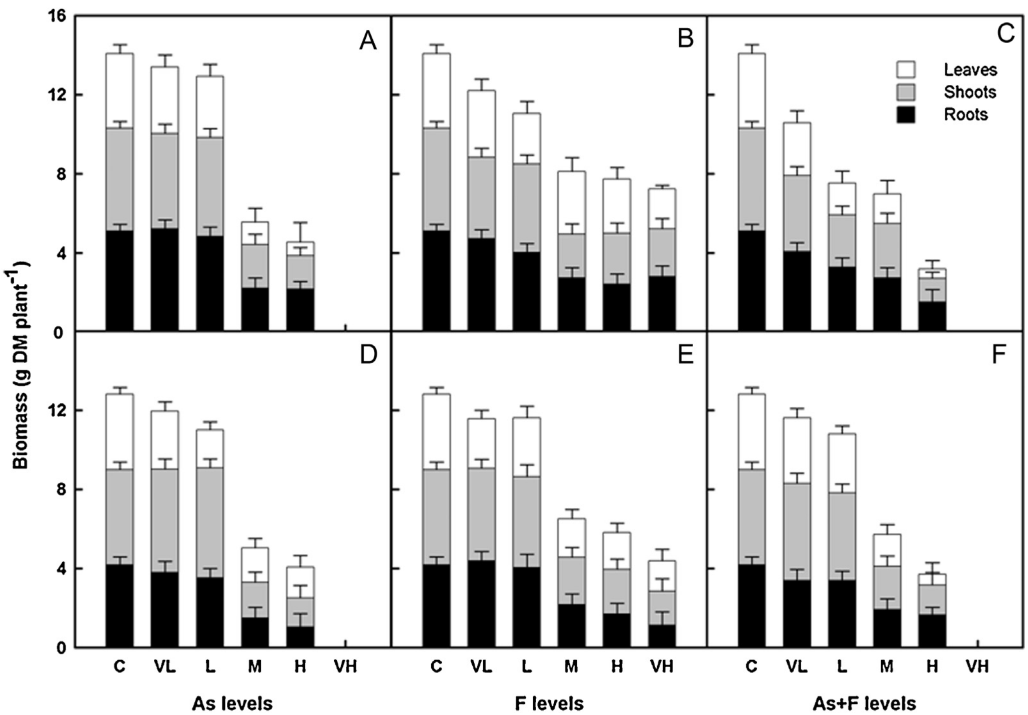

Fig. 1. Soybean roots, shoots and leaves biomass production in R3-R4 (A–C) and in R8 (D–F) under irrigation with As and /or F water. Letters indicate differences in total biomass between treatments for each element ($p < 0.05$).

Table 4
Mean values for As and F concentration in soybean plants per organ for each treatment.

		Total As concentration (mg kg^{-1})				
		Roots	Leaves	Shoots	Pods	Beans
As	C	2.42	0.75	0.44	0.36	0.25
	L	3.37	0.83	0.77	0.53	0.38
	M	7.88	4.73	1.40	0.70	0.47
	H	12.78	5.54	2.25	2.35	0.91
As+F	VH	Sd	Sd	Sd	Sd	Sd
	L	2.72	0.93	0.73	0.42	0.28
	M	7.95	3.68	0.98	0.89	0.59
	H	12.55	6.35	2.68	1.67	0.71
	VH	Sd	Sd	Sd	Sd	Sd
LSD	$p < 0.05$	0.58	0.35	0.22	0.21	0.08
		Total F concentration (mg kg^{-1})				
		Roots	Leaves	Shoots	Pods	Beans
F	C	1.9	2.35	1.205	1.36	1.03
	L	3.05	3.95	1.9	2.2666	1.067
	M	8.7	11.7	6.35	11.65	1.65
	H	22.9	18.75	14.62	14.15	2.9113
	VH	36.9	22.95	16.32	17.15	5.2
As+F	L	2.94	2.559	1.316	1.808	1.09
	M	7.995	8.2	5.989	7.314	1.1925
	H	19.1	16.5	12.18	10.51	1.75
	VH	Sd	Sd	Sd	Sd	Sd
LSD	$p < 0.05$	0.98	0.56	0.51	0.74	0.06

Sd: without data due to plant death.

4. Discussion

The application of irrigation water rich in As tends to increase its contents in soils (e.g. Norra et al., 2005) and the same with F (i.e. Troiani et al., 1987). Most results dealing with accumulation of As in soils come from Bangladesh, where irrigated vs not irrigated soils were compared (Heikens, 2006; Senanayakea and Mukherjib, 2014) and good correlations between water application and soil accumulation were mostly found. It is not easy to compare field surveys against a pot experiment because the irrigation water in the field is relatively slowly added to the soil over a period of several years. Norra et al. (2005) showed very elevated build up of As in irrigated soils but it appears that the As concentration in the pots could be higher than found in field experiments. In present case the short time the pots were subjected to irrigation surely affected the processes which determine the dynamic of As and F within the soil: adsorption, precipitation, complexation, etc. (Fitz and Wenzel, 2002; Saha and Ali, 2007). Anyway, when As concentration in irrigation water in present experiment exceeded 10 mg l^{-1} and F exceeded 9 mg l^{-1} the accumulation of both elements in available fraction decreased when compared with an hypothetical 1:1 line. The lack of proportion between As and F added and As and F measured in soil, indicate that some water percolated and As and F leached from the pot. This is shown in the As and F balance, were the drainage losses are higher as As and F concentration in irrigation water increased. The losses of both elements in the drainage water increased with time (data not shown). Soil components that adsorb significant quantities of As and F are a large range of aluminum, iron and manganese oxides; clays; carbonates and organic matter (Violante and Pigna, 2002; Wenzel and Blum, 1992).

The soybean biomass and yield significant decreasing by effects of As concentrations in the irrigation water, is in agreement with previous results found in rice (Panaullah et al., 2009) and in wheat (Pigna et al., 2008). Root followed by leaves biomass were the most affected organs in soybean plant, similarly that was found in other plant species (Abedin et al., 2002). Some authors found that waters with a concentration of As in the order of 0.2 mg l^{-1} caused adverse effects on rice plants and also found that As concentration in water higher than 1 mg As l^{-1} cause negative effects on bean yield. In our experiment the effects of As were shown when water exceed 0.6 mg l^{-1}. A detrimental effect of F on soybean biomass and yield was found at high concentrations (25 mg F l^{-1} and higher) in present study, being roots and shoots the more affected organs. Severe phytotoxic effects were found in rice, including a 60% total biomass reduction when F concentration in irrigation water reached 30 mg F l^{-1} (Mackowiak et al., 2003). It must be taken into account, however, that concentrations higher than 5 mg l^{-1} of F in irrigation water are not likely to be found in most agricultural conditions around the world (Smedley and Kinniburgh, 2002).

The effect of water rich in As or F applied via sprinkler irrigation is not well understood. Conversely, it has been long known that the use of Cl rich (salty) water on sprinkler irrigation poses the potential problem of salt absorption by leaves, heightening toxicity effects when compared to those found using technologies applying water over the soil surface (Bustingorri and Lavado, 2012). However, present results are not neatly different than those found with other species in field using flood irrigation or pot experiments applying both elements via roots. Arsenic and F concentration in soybean plant increased as their concentration increased in irrigation water (Table 4) as Williams and other authors found in rice (cited by Heikens, 2006). In our case a very good correlation between As in irrigation water and As in beans was found (r^2: 0.965). Similarly, a good correlation was found between rice grain and straw (Farid et al., 2005). Several authors found that As concentration in soils has little influence over rice when irrigated with As rich water (Miah et al., 2005). However, we do not discard that our results could be altered by the build up of As and F in the soil. Then, it is difficult to discriminate clearly in present experiment the influence of the irrigation system used and the soil impact on the effect of As and/or F on soybean. There was a high correlation between As in soil and As in soybean beans (r^2: 0.943). Similarly, there were also very good correlations between F content in irrigation water and F in soybean bean (r^2: 0.954), and F in soil and F in soybean beans (r^2: 0.991).

In the present case, the TF assesses the ability of plants to transfer As and F from roots to shoots and beans. TF values were less than 1 in agreement with their lesser accumulation in aerial biomass and the concentration mainly in roots. Arsenic TF was insensitive to changes in external concentration although soil concentration increased as As in irrigation water increased. Fluoride showed an apparently higher mobility in soybean and an increasing proportion of F accumulation in roots as F in water and soil increased. The bioconcentration factor (BCF), the ratio of metal concentrations in plant tissue to those in soil, is used to compare the effectiveness of the plant in concentrating elements from soil into its biomass (Audet and Charest, 2007). This index applied to present results was always lower than 1, indicating not significant As/F bioconcentration. BCF decreased with increasing As in soil, reflecting effects of As toxicity. Conversely, the BCF for F shows accumulation of F in plant tissues associated to lower damage in the plants.

The content of As in soybean beans follows the general pattern found in several crops: the edible parts are generally low as compared to those in roots and shoots. The range of As concentrations in soybean is in the same range as that found in high-As irrigation field watered rice in Bangladesh (Jahiruddin et al., 2011; Hossain et al., 2009). According to Rahman et al. plants seldom accumulate As at concentrations hazardous to human and animal health because phytotoxicity usually occurs before such concentrations are reached. In agreement, As in soybean beans subjected to the *H* treatment reached the limit of As acceptance in food (Zhao et al., 2010) but plants died at the highest As treatment (*VH*) and no beans were harvested. In the case of F, plants did not show toxicity symptoms and there is no certain threshold limit in beans that may result hazardous to human health.

5. Conclusions

The application of water by mimicking sprinkler irrigation meant the As and F built up in the soil. A pot experiment mimicking sprinkler irrigation presents limitations when compared to actual farm level sprinkler irrigation, particularly in terms of absolute results. However, in present experiments, soybean generally behaved like other crops studied under field conditions. Irrigation water with low As and F concentrations showed low concentration of both elements in plants and no detrimental effect on soybean production. Significant biomass and yield reductions, and As and/or F accumulation in plant tissues where observed when As and F concentration surpassed 0.6 mg As l^{-1} and 25 mg F l^{-1}. Arsenic resulted much more phytotoxic than F. The toxic effect of both elements was additive. Concentrations of As and F were lower than those hazardous to human and animal health.

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