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Effects of high arsenic and fluoride soil concentrations on soybean plants

Efecto de altas concentraciones de arsénico y fluor en el suelo sobre plantas de soja

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Abstract. Arsenic (As) and Fluoride (F) are present in many soils, affecting crops and posing risks in the food chain. We performed pot experiments on spiked soils enriched in these elements either individually or simultaneously, over a wide range of concentrations. Soybean biomass production, grain yield, As and F accumulation and distribution within the plant, and the antioxidant response to these stresses were analyzed. Arsenic was more toxic than F. At As levels >35 mg/kg and F levels >375 mg/kg, yield loss reached 60% and 30%, respectively. At the highest dose of As plants died within 2 weeks, whereas F showed no lethality. When they were applied simultaneously, detrimental effects were more important. As and F in plants increased in all soybean organs although grains presented the lowest concentrations. Antioxidant enzymes were enhanced in plants but this increase was not high enough to cope with the oxidative damage.

Keywords: Soil contamination; Toxic elements in crops; Grain contamination; Oxidative stress; Soybean.

Resumen. Arsénico (As) y Fluor (F) están presentes en muchos suelos, afectando a los cultivos y presentando riesgos en la cadena alimenticia. Nosotros llevamos a cabo un experimento en macetas con suelo enriquecido en ambos elementos, en un amplio rango de concentraciones, tanto individual como simultáneamente. Se determinaron la producción de biomasa de soja, su rendimiento en granos, la acumulación de As y F y su distribución dentro de la planta, así como la respuesta antioxidante de las planta a ambos estreses. El As fue más tóxico que el F. Con concentraciones de As mayores a 35 mg/kg y de F mayores a 375 mg/kg, las pérdidas de rendimiento alcanzaron un 60% y 30%, respectivamente. Las plantas de soja murieron dentro de las 2 semanas frente a la dosis mayor de As, mientras que el F no mostró ser letal en ninguna concentración. Los efectos detrimentales fueron más importantes cuando As y F fueron aplicados simultáneamente. La concentración de As y F en plantas se incrementó en todos los órganos de la soja, aunque los granos presentaron la concentración más baja. La concentración de enzimas antioxidantes se incrementó en las plantas pero este incremento no fue suficiente para resistir el daño oxidativo.

Palabras clave: Contaminación de suelos; Elementos tóxicos en cultivos; Contaminación de granos; Estrés oxidativo; Soja.

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INTRODUCTION

Arsenic (As) is widely distributed in all types of rocks of the earth crust. In addition, it is a significant constituent in groundwater in many countries, in concentrations that range from 0.5 µg/L to over 120 mg/L (Smedley & Kinniburgh, 2002). As a consequence of rock weathering, water use and other anthropogenic inputs, it also occurs in soils, most often as inorganic As (V), in concentrations ranging from 0.1 to 90 mg/kg. The world average is around 5 mg/kg. However, As concentration can exceed 20 g/kg in contaminated areas (Mandal & Zuzuki, 2002; Dahal et al., 2008). Fluoride (F) is also found in rocks, groundwater and soils (Wenzel & Blum, 1992). The origin of F in soils is similar to that of As. Under uncontaminated conditions, the F concentration in soils is around 15-20 mg/kg, whereas in contaminated soils concentrations may reach up to 1500 mg/kg (Cronin et al., 2000). The use of groundwater for irrigation and/or its capillary rise could result in increased, As- and/or F-rich concentrations in soils.

Soils with high concentrations of each element may negatively affect crop production and food safety, a phenomenon that has been documented in several countries (Cronin et al., 2000; Heikens et al., 2007; Brammer & Ravenscroft, 2008; Dahal et al., 2008). Plants exposed to increased As levels suffer oxidative stress and show toxicity symptoms, such as germination inhibition, a decrease in chlorophyll and photosynthesis, reduced height, tillering or ramification, decreases in root and aerial biomass growth and yield, and even plant death (Abedin et al., 2002; Rahman et al., 2007; Pigna et al., 2008). Fluoride has been less extensively studied regarding its phytotoxicity. However, lower root growth, reduced biomass production and yield loss have been found in different crop and pasture species (Cronin et al., 2000; Loganathan et al., 2001; Jha et al., 2009). Under normal growth conditions, plants maintain an equilibrium between production and scavenging of reactive oxygen species (ROS), avoiding the damages caused by their accumulation. This equilibrium may be perturbed when plants are subjected to stresses, such as the accumulation of toxic elements. To resist these toxic oxygen intermediates, plant cells and their organelles have antioxidant defense systems. A great deal of research has established that the induction of the cellular antioxidant machinery includes enzymatic and non-enzymatic antioxidants. In plants, As and F cause considerable stress (Stoeva et al., 2005) but the biochemical responses to this stress are insufficiently studied (Hartley-Whitaker et al., 2001).

Rice, followed by wheat, has been the most studied field crop regarding As toxicity. Both cereals are the main staple crops in areas contaminated with this element (Zhao et al., 2010). Neither the effect of As and/or F on soybean growth and yield nor the accumulation of both toxic elements in soybean plants including grains have been studied enough. Sheppard (1992) mentioned some old papers dealing with As effects on soybean. According to those papers, the toxic concentrations of As in soils varied from 12.5 to 84 mg/kg. Little is known about the combined effect of As and F, and it is unknown whether the relationship between them is additive, synergistic or antagonistic. Moreover, no information is available about soybean oxidative damage induced by high As and F concentrations.

Soybean has exceptional nutritional characteristics and ability to grow under a wide range of environmental conditions and management systems. This is because thus soybean is one of the main crops in the world (Eickhout et al., 2006). The expansion of the cropped area led to the introduction of soybean in marginal lands in different countries, including Argentina, which is one of the main producers and exporters of soybean in the world. Soybean was initially grown in prime soils of the humid Pampas, which have very low As contents (Lavado et al., 2004). At present, however, it is also grown in marginal lands. In these areas, there are soils rich in both As and F. The problem of cropping in these contaminated soils was locally documented many years ago (Reinaudi and Lavado, 1978; Troiani et al., 1987) and recently (Bustingorri & Lavado, 2014). The aims of this study were to analyze the effects of As and F soil concentrations on soybean biomass production, grain yield and their accumulation and distribution within the plant. Antioxidant responses on soybean plants were also studied. Experiments were carried out in spiked soil.

MATERIALS AND METHODS

One experiment using 8-L pots was carried out following a completely randomized design with six replicates per treatment. The substrate contained 70% of the top horizon of a sandy loam Typic Argiudoll with the addition of 30% of washed sand, to reduce physical problems. The final particle size distribution was 13% clay, 12% silt, and 74% sand. Following standard techniques (Sparks et al., 1996), the composition of the substrate was: 12.6 g/kg of organic carbon (Walkley and Black method), 7.6 pH, 32.8 mg/kg available P (Kurtz and Bray method) and 0.38 dS/m EC_S (soil saturation extract). Different concentrations of sodium arsenate and/or sodium fluoride were added to the soil to achieve a wide range of total As (from 5 to 500 mg/kg) and total F levels (from 50 to 1000 mg/kg). Then, each contaminated soil was subjected to wetting/drying cycles for 3 months, allowing the interaction between the soil components and the added elements, reducing the overestimation of their toxic effects. A total of 16 treatments were carried out: 5 As levels, 5 F levels and 5 As+F levels of soil contamination, and the control (*C*) treatment (soil background levels). The treatments were termed after their As or F concentrations as *VL (very low)*, *L (low)*, *M (medium)*, *H* (high) and *VH (very high)*. Both elements were applied to obtain three treatments below and three over the permitted levels of each element in Argentina (Law 24051, 1992), which followed USEPA regulations. Treatments covered from low concentrations normally found around the world (treatment VL) to concentrations exceeding the highest concentrations found in irrigated areas (Duxbury & Zavala, 2005; Dahal et al., 2008) (treatment VH). Pots were irrigated with deionized water, maintaining the soil near field capacity throughout the experiment.

Three soybean seeds (cv Nidera 4613), pregerminated in the dark for 48 h, were sown in each pot and thinned to one plant per pot 15 days later. Each pot received a complete fertilizer before sowing, and every 30 days, a soluble fertilizer (N:P:K 25-10-10). Plant height (main shoot only) was recorded at the R3-R4 and R8 stages. Aerial biomass was harvested and divided into leaves, shoots, pods, and grains. The number of pods and grains were recorded. Roots were washed, sieved and harvested. All samples were rinsed with distilled water, dried at 60 ºC for 72 hours and then weighed. Total As and F contents were determined in sieved and homogenized samples of all plant material. Arsenic was extracted by HNO_3/H_2O_2 acid digestion and measured by atomic adsorption (ICP-AES) (USEPA, 2006). Fluoride content was ashed at 400 ºC and quantified by SPADNS colorimetry (APHA, 1993).

Total As and total F were determined on soil samples at seeding. Arsenic was determined by Instrumental Neutron Activation Analysis (INAA), while F was first extracted by NaOH fusion (Sparks et al., 1996) and then determined by SPADNS colorimetry (APHA, 1993). Soil As and F bioavailable forms were determined at harvest. Arsenic was extracted using a 1.0 M solution of acetic acid and sodium acetate, and quantified by atomic absorption (AA) according to the EPA6010 method (USEPA, 2006) ICP-AES. Bioavailable F was extracted with hot distilled water and determined by SPADNS (APHA, 1993).

A parallel experiment was carried out to quantify oxidative stress following the same experimental design as the one previously described. Leaf and root samples from treatments C, M and H (for As, F and As+F) were collected 50 days after sowing, in order to measure antioxidant defenses, following standard determinations (Balestrasse et al., 2001). Lipid peroxidation was measured as the amount of thiobarbituric acid reactive substances (TBARS) determined by the thiobarbituric acid (TBA) reaction. Extracts for the determination of catalase (CAT) and peroxidase (GPOX) activities were obtained from leaves or roots with an extraction buffer containing phosphate buffer (pH 7.4), EDTA, PVP, and Triton X-100. Catalase content was measured and GPOX activity was determined in the homogenates by measuring the increase in absorption at 470 nm. Protein concentration was evaluated using bovine serum albumin as a standard.

The results obtained were evaluated using ANOVA. When significant differences were found, means were compared using the LSD test. The curve fitting software, Table Curve 2D (AISN Software Inc., 2000), was used to identify the relationships between As and F concentrations in the soil *versus* soybean yield, and the relationships between As and F concentrations in grains and total plant biomass.

RESULTS

Total contents of As in soils ranged from 4.8 mg/kg to 289 mg/k, while total contents of F ranged from 20 to 450 mg/kg (Table 1). Bioavailable As and F are presented in Table 2. When F was applied in high doses, As availability in the soil decreased significantly (around 20%), whereas F availability was not affected by the presence of As.

Table 1. Total As and F concentration in soil at sowing time. Letters indicate differences between treatments for each element ($p<0.05$).
Tabla 1. Concentración de As y F totales en el suelo al momento de siembra. Las letras indican diferencias entre tratamientos para cada elemento ($p<0,05$).

Level	C	VL	L	M	H	VH
		------------------------------- As (mg/kg)	--------------------------------			
As	4.8 ± 1.1 a	5.1 ± 1.2 a	6.3 ± 1.4 a	35.0 ± 4.2 b	68.0 ± 5.3 c	289± 23.5 d
As+F		5.2 ± 1.1 a	6.1 ± 1.2 a	43.5 ± 2.2 b	65.5 ± 6.2 c	276 ± 16.1 d
		------------------------------ F (mg/kg)	------------------------------------			
F	20 ± 5 a	25 ± 7 ab	37 ± 10 b	200 ± 25 c	375 ± 30 d	450 ± 35 e
As+F		25 ± 5 ab	35 ± 14 b	185 ± 27 c	350 ± 25 d	435 ± 3.8 e

Note: treatments C, VL and L were below local accepted levels, while treatments M, H and VH were over them.
Nota: los tratamientos C, VL y L estuvieron por debajo de los niveles locales aceptados, mientras que los tratamientos M, H y VH estuvieron por encima de los mismos.

Table 2. Available As and F concentration in soil, at harvest. Letters indicate differences between treatments for each element ($p<0.05$).
Table 2. Concentracón de As y F disponible en el suelo al momento de la cosecha. Las letras idican diferencias entre los tratamientos para cada elemento ($p<0,05$).

Level	C	VL	L	M	H	VH
				As (mg/kg)		
As	0.6 ± 0.2 a	1.17 ± 0.3 b	2.2 ± 0.4 b	8.5 ± 0.7 c	17.2 ± 2.8 e	86.7 ± 9.4 g
As+F		1.15 ± 0.2 b	2.3 ± 0.3 b	6.8 ± 0.9 c	15.7 ± 1.5 d	62.7 ± 6.1 f
				F (mg/kg)		
F	7.1 ± 0.8 a	12.2 ± 1.3 b	13.2 ± 1.5 b	18.7 ± 2.8 bc	27.2 ± 3.6 d	57.7 ± 4.1 f
As+F		11.8 ± 1.1 b	12.8 ± 1.4 b	21.4 ± 2.7 c	33.1 ± 4.7 e	61.2 ± 3.8 f

Early seedling growth showed no visible evidences of toxicity, except for the highest As and As+F treatments (276-289 mg As/kg). The plants under these treatments presented a limited growth and died between 20 and 25 days after sowing. The height of the plants was reduced at both stages by both As and F, but when both elements were together, the reduction appeared to be more important (Fig. 1). When soybean was grown with the lower As concentrations (*VL* and *L* treatments), biomass production did not differ from that in the control ($p>0.05$). However, when As total concentration was 35 mg total As/kg and over (treatments *M* and *H*), biomass decreased by 50% (Fig. 2). Plants grown in 200 or 450 mg/kg total soil F showed a 13% or 40%, respectively, reduction for biomass compared with values in the controls. When both elements were present together in the soil, biomass was about 15% lower than that obtained with As alone ($p<0.05$). Figure 2 shows that root biomass was the most affected plant component in all treatments, followed by leaf biomass, which decreased between 30% and 60% for As treatments, and 30% and 35% for F treatments.

Pod and grain number and weight were greatly affected by *M* and *H* As, and As+F treatments. Fluoride treatments showed slightly less detrimental effects. The weight of the individual soybean grains decreased as As and F in the soil increased. Figure 3 shows the close relationship between soybean yield and As and F concentrations in the soil. The yield loss was abrupt as As concentration increased, but less pronounced as F concentration increased ($p<0.005$). The presence of both elements further reduced the yields. Arsenic concentrations were 2-3 times greater in the roots than in shoots; the concentration order was leaves>shoots>pods>grains (Table 3). Thus, As

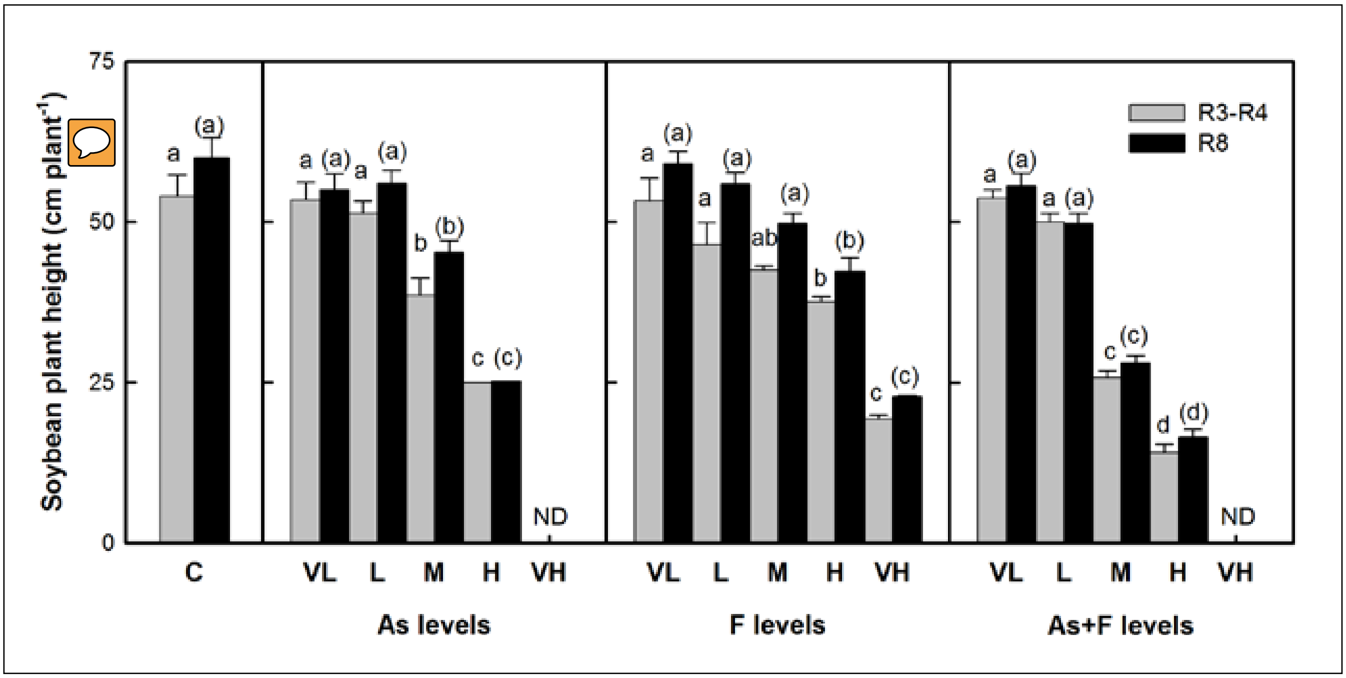

Fig. 1. Soybean plant height at R3-R4 and R8 stages as affected by As, F and As+F. Letters indicate differences between treatments for each element ($p<0.05$).
Fig. 1. Efecto de As, F y As+F en la altura de la planta de soja en los estados R3-R4 y R8. Las letras indican diferencias entre tratamientos para cada elemento ($p<0,05$).

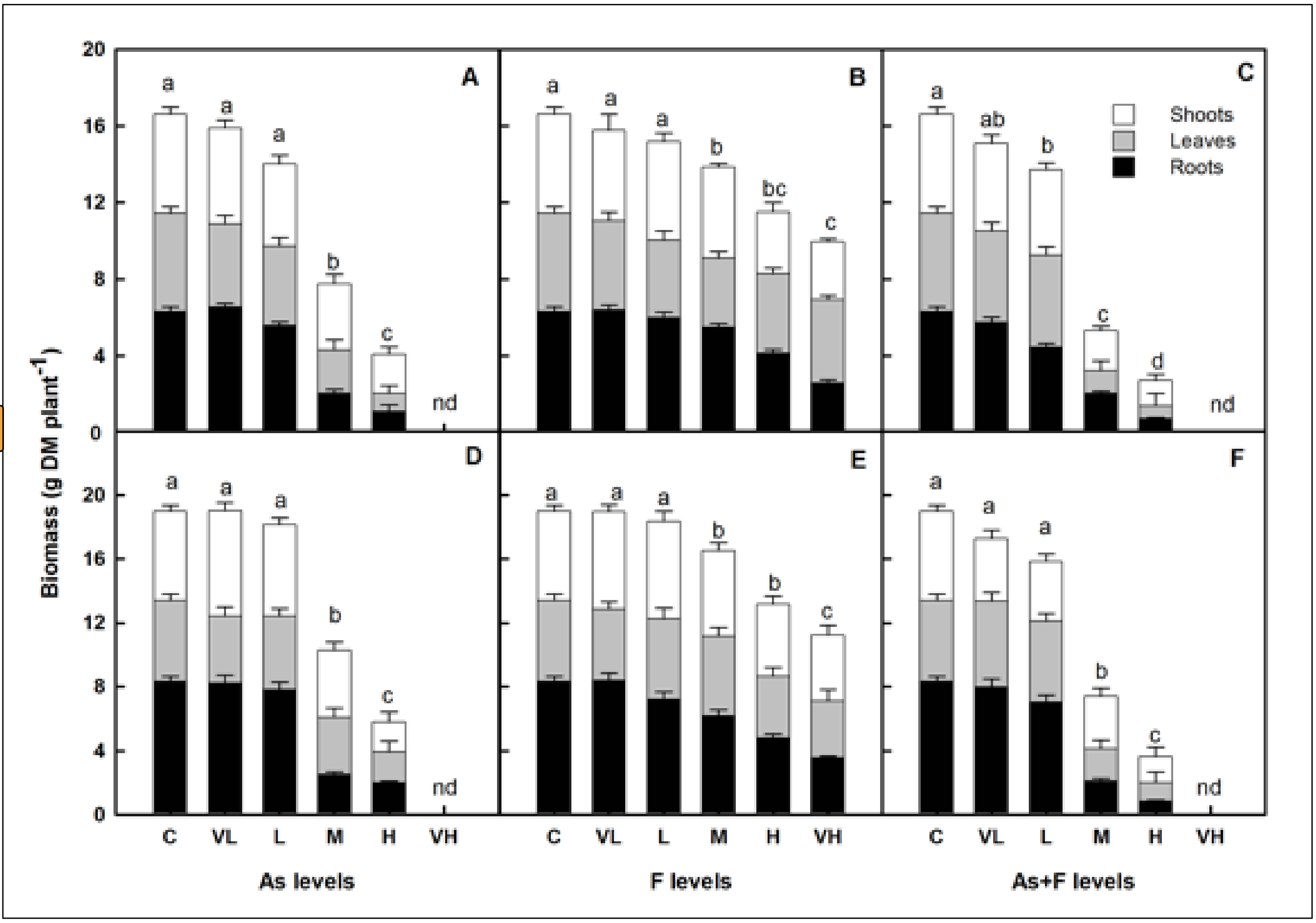

Fig. 2. Production of soybean roots, shoots and leaves in R3-R4 (A, B, C) and R8 (D, E, F) grown under different soil As and/or F concentrations. Letters indicate differences between treatments for each element ($p<0.05$).

Fig. 2. Producción de raíces de soja, tallos y hojas en los estados R3-R4 (A, B, C) y R8 (D, E, F) desarrollado bajo diferentes concentraciones de As y/o F en suelo. Las letras indican diferencias entre tratamientos para cada elemento ($p<0,05$).

Table 3. Mean and standard deviation values for As and F concentrations in soybean plant organs within each treatment.

Tabla 3. Valores medios de concentración de As y F, y desviación estándar, en los distintos órganos de soja, en cada tratamiento.

		Total As concentration (mg/kg)				
		Roots	Leaves	Shoots	Pods	Grains
	C	2.42	0.75	0.44	0.36	0.25
As	L	3.75	0.92	0.86	0.59	0.43
	M	8.95	5.33	1.61	0.79	0.53
	H	14.94	6.46	2.59	2.74	1.08
	VH	na	na	na	na	na
AS+F	L	3.08	1.05	0.83	0.48	0.32
	M	10.28	4.62	1.34	1.21	0.77
	H	15.93	7.93	3.52	2.16	0.91
	VH	na	na	na	na	na
LSD	p<0.05	0.58	0.22	0.42	0.19	0.10

		Total F concentration (mg/kg)				
		Roots	Leaves	Shoots	Pods	Grains
	C	1.9	2.35	1.32	1.36	1.03
F	L	3.35	4.34	2.09	2.49	1.17
	M	9.57	13.02	6.98	12.81	1.82
	H	25.19	21.65	16.08	15.56	3.20
	VH	40.59	28.36	17.95	18.86	5.72
AS+F	L	3.23	2.81	1.45	1.99	1.19
	M	8.79	8.87	6.59	8.05	1.31
	H	21.01	19.5	13.40	11.56	1.92
	VH	na	na	na	na	na
LSD	p<0.05	0.98	1.25	0.78	0.21	0.08

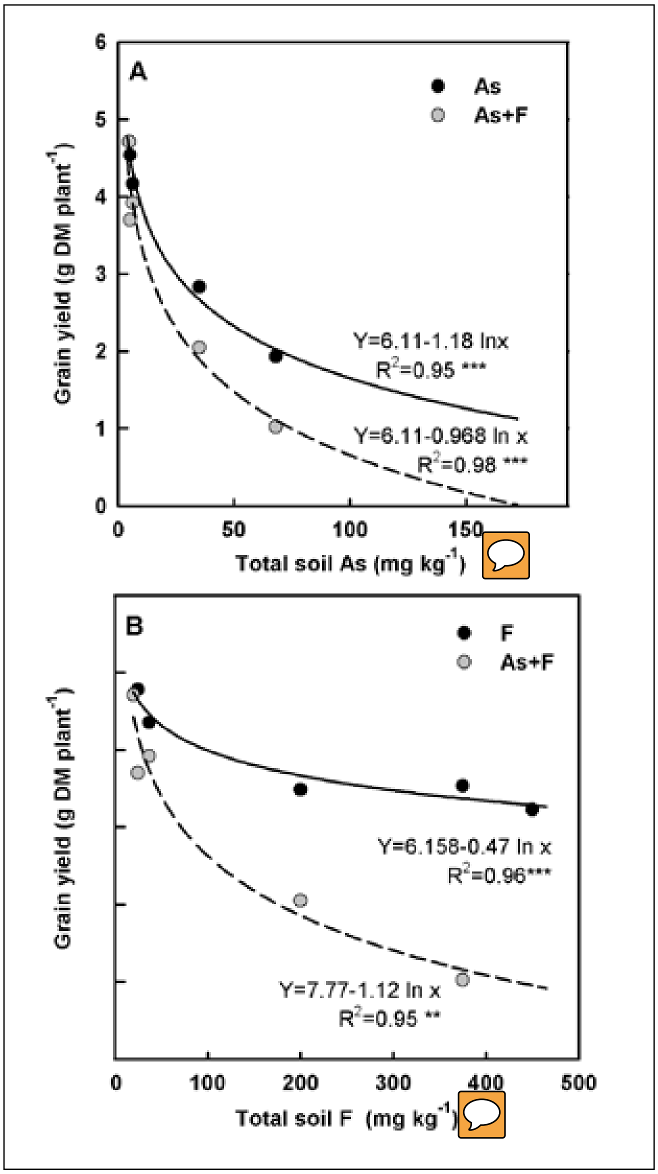

Fig. 3. Relationship between soybean grain yield and A) soil As concentration or B) soil F concentration.

Fig. 3. Relación entre rendimiento en granos de soja y A) concentración de As en el suelo o B) concentración de F en el suelo.

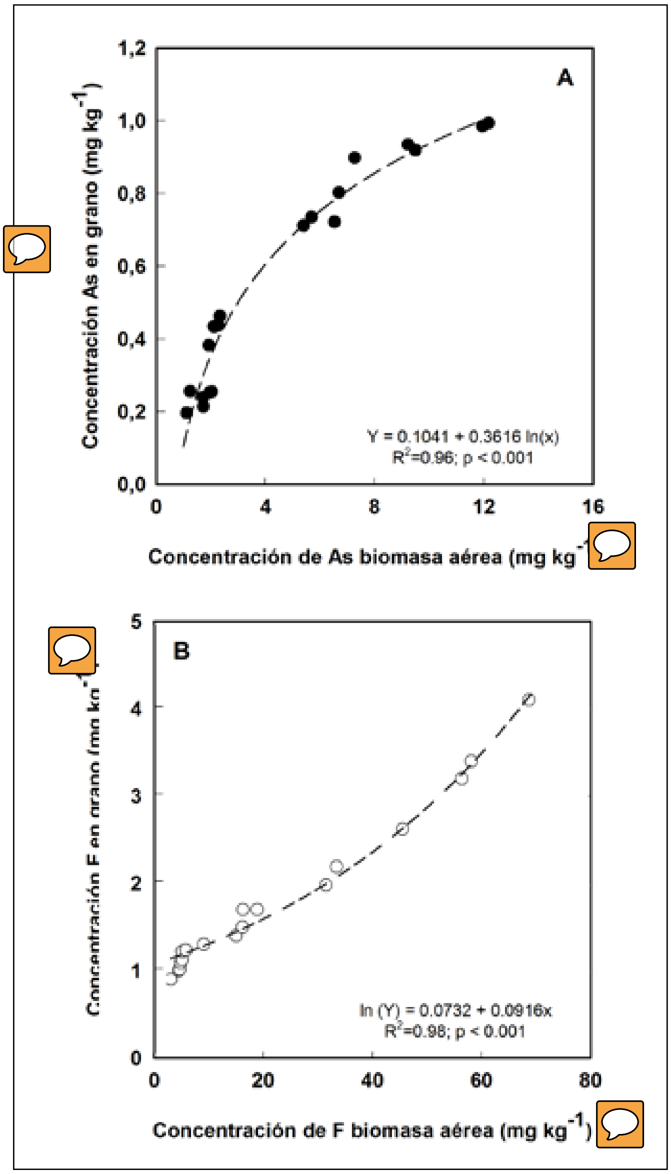

Fig. 4. Relationship between As concentration in grain vs. that in aerial biomass (A) and F concentration in grain vs. that in aerial biomass (B).

Fig. 4. Relación entre rendimiento en granos de soja vs. rendimiento en biomasa aérea (A) y concentración de F en granos vs. rendimiento en biomasa aérea (B).

grain concentrations presented values between 0.1 and 0.9 mg/kg dry matter, around 10 times lower than those in the whole plant when As concentrations were low, and 15 times lower when total plant As concentrations were high (Fig. 4A). As a result, partitioning of As to grains was not proportional. The F concentration in roots doubled that found in the aerial biomass. Fluoride presented the following concentration order: leaves>pods>shoots>grains. Fluoride concentrations were from 13 to 20 times lower in grains than in the whole plant biomass (Fig. 4B). The relation between grain and total plant F concentrations remained steady even when F values were high.

Evidences of oxidative stress were found for the selected treatments (Fig. 5). When compared with control plants, higher levels of TBARS were found in As treatments in leaves, which were less notorious in roots. The As *M* and *H* treatments showed the largest increase in TBARS (50%) in leaves. Fluoride-treated plants presented less notorious changes in leaves (less than 40%) and almost no TBARS increase in roots. The activities of the antioxidant enzymes GPOX and CAT were also enhanced by As and F, especially in leaves. GPOX activity in As *M* and *H* treatments showed a 3-fold increase respect

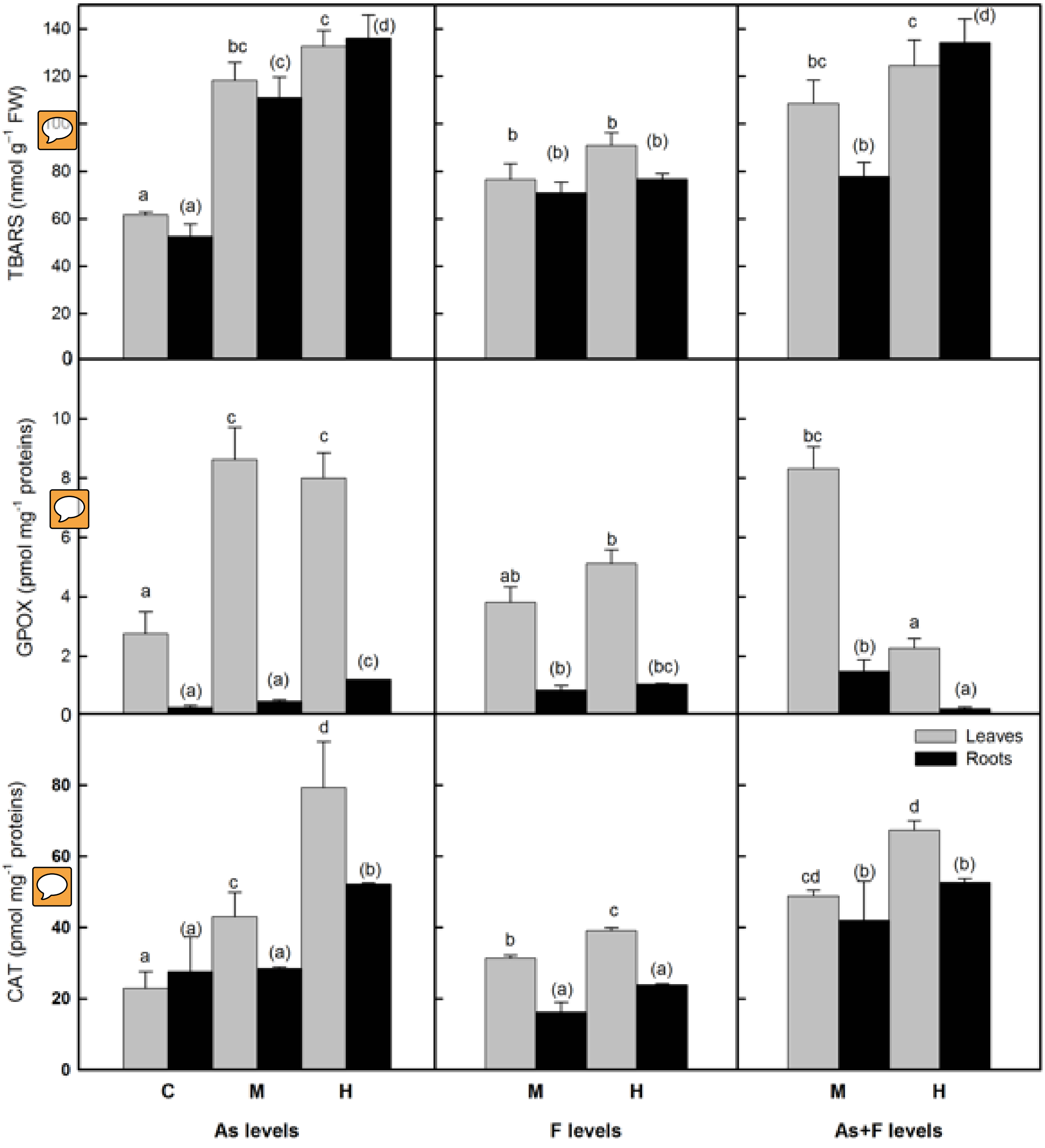

Fig. 5. Mean and standard deviation values of lipid peroxidation (TBARS); GPOX and CAT activities on soybean leaves and roots for As and/or F treatments. Letters indicate differences between treatments for roots and leaves individually ($p<0.05$).

Fig. 5. Valores medios y desviación estándar de actividades de peroxidación lipídica (TBARS); GPOX y CAT en hojas y raíces de soja, frente a los tratamientos As y/o F. Las letras indican diferencias entre tratamientos para cada elemento ($p<0,05$).

to controls. However, when As and F were applied together in treatment *H*, GPOX production levels were lower than those in the controls. Although GPOX in plants exposed to high F levels showed lower values than those in plants exposed to As treatments, GPOX production was doubled in treatment *VH*. Catalase activities in leaf and root production highly increased in the As *H* treatment (2.5 fold respect to control plants). In the F treatments, CAT levels increased by 30% in leaves, but no differences were found in roots.

DISCUSSION

In the present study, bioavailable fractions represented in average 30 and 23% of total As and F soil contents, respectively. Smith et al. (2008) found that bioavailable (acetate-extracted) As ranged between 8% and 26% of As total soil content in their soils, whereas Jha et al. 2009 found that soluble F was around 5% of total F added, although these authors used a weaker extraction solution. The bioavailable fraction of both elements in the present experiment could be considered somewhat high. This is in agreement with the fact that the bioavailable fraction is usually larger in spiked soils than in natural ones (Juhasz et al., 2008).

Similar to that described for rice, wheat and other crops (Abedin et al., 2002; Pigna et al., 2008; Panaullah et al., 2009), roots were the most affected organs, followed by leaves, in soybean plants. Contrasting with results obtained in rice plants (Heikens et al., 2007), soybean height was severely affected. In addition, similar to that described for rice and barley (Heikens et al., 2007), grain yield was the most sensitive parameter in soybean plants. The fact that while the addition of F decreased the bioavailability of soil As, the effect of both elements on soybean plant was additive, which was a contradictory result.

Fluoride toxicity in crops has not been thoroughly studied. Bar-Yosef and Rosenberg (1988) stated that the main differences in phytotoxic levels are due to the plant species. Few specific results have been mentioned for oat, rice, onion and pastures (Cronin et al., 2000; Loganathan et al., 2001; Jha et al., 2009). With logical differences due to the experimental conditions and species, results from other experiments agree with our present results. High concentrations of F had a detrimental effect on soybean biomass and yield, although these values (200 mg/kg of F in the soil) can be found only in highly contaminated areas (Cronin et al., 2000; Loganathan et al., 2001), and not in agricultural soils.

Arsenic and F concentrations in soybean plants were related to As and F concentrations in the soil. Similarly, Farid et al. (2005) found a highly significant correlation between As in soils and As in straw and grains of rice. The phytotoxic threshold limit (LC_{50}) for As or F is defined as the mean concentration in shoot beyond which biomass yield decreases by 50% (Jha et al., 2009). The LC_{50} in the soybean shoot was 2.59 mg As/kg and 17.95 mg F/kg. Soybean could be more sensitive than onion, which LC_{50} for bioavailable F forms was 55 mg/kg (Jha et al., 2009). Arsenic concentration in grains ranges in the same levels as in high-As irrigated rice in Bangladesh (Hossain et al., 2009). In general, the contents of As in the edible parts of most plants are generally low as compared to those in roots and shoots (Rahman et al., 2008). Also, it is hypothesized that plants seldom accumulate As at concentrations hazardous to human and animal health because phytotoxicity usually occurs before such concentrations are reached (Rahman et al., 2008). This would the case of As in this study: Arsenic in grains in the H treatment reached the limit of As in food (Duxbury & Zavala, 2005; Zhao et al., 2010). At the highest As treatment (*VH*) plants died before grains appeared. In the case of F, it is difficult to speculate whether its concentrations may result hazardous to humans because plants neither showed toxicity symptoms nor died. Also, no clear threshold limits of F in plants have been reported above which the ingestion may be detrimental to human health (Jha et al., 2009).

Cytoplasmic arsenate interferes with metabolic processes involving phosphate, giving it the potential to be toxic to plants, but it is probably reduced in the cytoplasm to arsenite (Meharg & Hartley-Whitaker, 2002). Arsenite reacts with sulfhydryl groups (–SH) of enzymes and tissue proteins, inhibiting cellular function and causing death (Ullrich-Eberius et al., 1989). TBARS formation in plants may be used as an indicator of free radical formation in the tissues. Different stresses have been associated with the production of toxic oxygen species like H_2O_2. Both As and F caused oxidative stress in the leaves, but As was more deleterious than F. This response was even more pronounced when plants were treated with both elements. Antioxidant enzymes like CAT and GPOX catalyze the rupture of H_2O_2. GPOX and CAT activities in the leaves were enhanced by these treatments, but this increase was not high enough to cope with the oxidative damage. It is interesting to note that F alone did not cause the formation of TBARS formation in the roots or changes in the antioxidant enzymes studied. Moreover, our results indicate that As behavior in roots was not affected by the presence of F.

CONCLUSIONS

When As and F concentrations surpassed 35 mg total As/kg or 375 mg F/kg, significant decreases in biomass and yield of soybean, and As and/or F accumulation, were observed. Arsenic resulted much more phytotoxic than F but together, they showed an additive effect on plant response. The present results agree with the idea that plants do not accumulate As in grains at concentrations hazardous to human and animal health because at the highest As level, plants died before harvesting. The antioxidant response could be roughly characterized for both elements, and thus the stress response in roots does not seem to fit the plant response. This issue needs deeper research.

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