

Toxicity of arsenic in relation to soil properties: implications to regulatory purposes

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Abstract

Purpose The present work evaluates the influence of different soil properties and constituents on As solubility in laboratory-contaminated soils, with the aim of assessing the toxicity of this element from the use of bioassays to evaluate the soil leachate toxicity and thereby propose soil guideline values for studies of environmental risk assessment in soil contamination.

Materials and methods Seven soils with contrasting properties were artificially contaminated in laboratory with increasing concentrations of As. Samples were incubated for 4 weeks, and afterwards, soil solution (1:1) was obtained after shaking for 24 h. The soil leachate toxicity was assessed with two commonly used bioassays (seed germination test with *Lactuca sativa* and Microtox[®] test with *Vibrio fischeri*).

Results and discussion The relationship between soluble As and soil properties indicated that iron oxides and organic matter content were the variables most closely related to the reduction of the As solubility, while pH and CaCO₃ increased As solubility in the soil solutions. Toxicity bioassays showed significant differences between soils depending on their properties, with a reduction of the toxicity in the iron-rich soil (no observed effect concentration (NOEC)=150 mg kg⁻¹) and a significant increase in the highly carbonate samples (NOEC between 15 and 25 mg kg⁻¹).

Conclusions Soil guideline values for regulatory purposes usually set a single value for large areas (regions or countries) which can produce over- or underestimation of efforts in soil remediation actions. These values should consider different

levels according to the main soil properties controlling arsenic mobility and the soil leachate toxicity.

Keywords Arsenic · EC50 · Soil guideline values · Soil properties · Toxicity

1 Introduction

Arsenic (As) is a ubiquitous element in the environment which appears in concentrations that vary widely from one area to another. Its presence is associated with both natural and anthropogenic factors, and the high toxicity often threatens human health and even global ecosystems (Nriagu et al. 2007). Arsenic has properties intermediate between metals and non-metals, although its electronegativity and ionization energy are more characteristic of non-metals. Under natural conditions, this element has a complexation chemistry rather similar to phosphorus (CCME 2001) and tends to appear in anionic forms (Moreno-Jiménez et al. 2009).

The mean value of As in the earth's crust is 1.8 mg kg⁻¹, while concentrations in natural soils are generally below 10 mg kg⁻¹ (Adriano 2001; Mench et al. 2009). In soils, As usually accumulates due to its low mobility in this medium (Mench et al. 2009; Beesley and Marmiroli 2011), and it is often found as inorganic species because organic compounds have lower solubility (Nriagu et al. 2007; Martínez-Sánchez et al. 2011). In the normal range of pH-Eh soil conditions, arsenates are more common than arsenites (Bowell 1994), the presence of these species being essential to control toxicity in soil, sediment or water (Dobran and Zagury 2006). The As concentration in soil varies widely because it depends on the initial concentration (background) in parent material, natural geochemical cycles and soil type (Díez et al. 2007); therefore, the mean values of As in natural soils, according to the literature, range between 0.1 and 80 mg kg⁻¹ worldwide

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(Alloway 1995; Adriano 2001; Bohn et al. 2001; Kabata-Pendias and Mukherjee 2007).

Arsenic can be taken up by plants together with other micronutrients, but so far no conclusive results are available concerning the possible biological role of As in living organisms, although concentrations between 1 and 1.7 mg kg⁻¹ have been reported as normal values in plants (Kabata-Pendias 2011). Mobility, bioavailability and therefore the potential toxicity or deficiency of most elements for plants and microorganisms in terrestrial system are controlled largely by soil properties (Sheppard and Evenden 1988). The main soil properties affecting As availability are as follows: Fe oxides, pH, calcium carbonate, clay content, cation-exchange capacity and organic matter content (Lock and Janssen 2001; Smolders et al. 2003, 2004; Rooney et al. 2006; Song et al. 2006).

In recent decades, new methods have been developed to determine the impact of As on human health and the risk of accumulation of As in agricultural crops (Bhattacharya et al. 2007). Biological assays for toxicity determination show direct responses of organisms experimentally exposed to potentially polluting elements, and thus are indicative of the potential impact of pollutants on living organisms (Petänen et al. 2003). In the European Union, environmental risk assessment (ERA) is conducted primarily to assess the effects on human health and on the terrestrial, aquatic and atmospheric resources (Song et al. 2006). The use of bioassays is essential for ERA and is applied to determine the potential risk of contamination to living organisms and ecosystems. In soils, these bioassays can be divided into two groups: those using the liquid phase (soil solution extracts, pore water, leachates, etc.) and those using the solid phase of the soil that serves as a substrate for living organisms (Farré and Barceló 2003; Martín et al. 2010). Lors et al. (2011) comparing bioassays in the solid phase as well as in soil extracts found a high correlation between the two phases, indicating that soil extracts usually reflect the toxicity of the solid phases. However, the correlation between the total and soluble forms of a pollutant is not always direct, and in the case of As, its solubility and toxicity are usually more tightly controlled by the soil properties than by the total amounts present in the sample (Martín et al. 2011; Martín Peinado et al. 2012). In this sense, bioassays using the liquid phase extract can more readily reflect the behaviour of mobile phases, evaluating the short-term risk of dispersion, solubilization and bioavailability of pollutants in the environment.

Laboratory studies, using artificially contaminated soil, may differ in the composition of the soil solution against in situ contaminated soil. However, laboratory experiments tend to overestimate the solubility and availability of metals in relation to field contaminated soils (Smolders et al. 2009), so that the toxicity level defined by these studies usually overrates the effects, which can raise the safety threshold for the environmental risk assessment.

The setting of reference levels in regulations of many countries is often controversial because a single value for large areas (regions or countries) is often set, regardless of spatial variations in soil types, and the extent of contamination or the associated environmental risk in specific areas may be misjudged (Díez et al. 2009). For example, the cleanup guidelines for arsenic in US soils for unrestricted use range from 7 to 40 mg kg⁻¹ depending on the State (Teaf et al. 2010); Dutch standards fixed the target value in 29 mg kg⁻¹ and the intervention value in 55 mg kg⁻¹ (RIVM 2000); meanwhile Soil Guideline values in UK for residential/allotments is 20 mg kg⁻¹ and for commercial/industrial use is 500 mg kg⁻¹ (DEFRA 2002). In Spain, some regions have different reference levels for different land-use scenarios (Macías and Calvo de Anta 2009). Specifically, in Andalusia (southern Spain), a preliminary approximation for establishing these levels fixed the total As thresholds for agricultural soils, natural parks and industrial areas at 50, 100 and 300 mg kg⁻¹, respectively (Aguilar et al. 1999). Anyway, most guideline values are based on natural background concentrations or in artificial standard soils, preventing about variations according soil type but lacking about additional data.

The present work evaluates the influence of different soil properties and constituents on As solubility in laboratory-contaminated soils, with the aim of assessing the toxicity of this element from the use of bioassays to evaluate the soil leachate toxicity and thereby propose soil guideline values for studies of environmental risk assessment in soil contamination.

2 Materials and methods

2.1 Soil samples

Seven soil horizons with different properties, representing most of the main soil groups in Spain, were selected (Table 1). The main parameters analysed were: pH (soil/water ratio 1:2.5), electrical conductivity (EC), texture, organic carbon (OC), water-holding capacity, cation-exchange capacity, specific surface area and calcium carbonate content (CaCO₃). The main physico-chemical properties were analysed according to the official analysis methods (MAPA 1994). Moreover, free and amorphous iron, aluminium and manganese oxides were analysed according to different procedures (Holmgren 1967; Schwertmann and Taylor 1977). All analyses were made in triplicate.

The total arsenic concentration (AsT) was determined from acid digestion in strong acids (HNO₃+HF) and water-soluble As (AsW) was determined from soil/water extracts (1:1 ratio). In all cases, As was measured by inductively coupled plasma-mass spectrometry (ICP-MS) in a spectrometer ICP-MS NEXION 300D. Instrumental drift was monitored by regularly running standard element solutions between samples. For

Table 1 Main properties of selected samples (mean, standard deviation)

Soil type IUSS (2006a, b)	Samples													
	Calcaric Cambisol (humic)		Calcaric Cambisol (humic)		Calcaric Kastanozem (antric)		Leptic Cambisol (eutric)		Leptic Regosol (eutric)		Leptic Regosol (distic)		Cutanic Luvisol (chromic)	
Soil horizon	Ah		Bw		Ck		Bw		Ah		C1		Bt	
Name of sample	H1		H2		H3		H4		H5		H6		H7	
pH (S)	7.96	0.06	8.67	0.03	8.79	0.02	6.74	0.19	7.20	0.09	5.87	0.09	7.03	0.44
EC (dS m ⁻¹)	0.08	0.04	0.07	0.01	0.07	0.01	0.04	0.01	0.09	0.01	0.05	0.01	0.05	0.01
I (mol l ⁻¹) ^a	7.3	0.6	2.0	0.1	1.7	0.1	0.9	0.1	13.0	3.1	0.5	0.1	1.6	0.3
CaCO ₃ (%)	37.11	0.44	72.39	0.86	92.32	1.80	nd	–	nd	–	nd	–	0.92	0.16
OC (%)	5.43	0.38	0.42	0.12	0.38	0.17	0.61	0.10	8.22	0.02	0.49	0.02	0.66	0.25
Clay (%)	23.61	0.90	11.79	0.44	7.70	0.58	19.05	0.38	23.79	0.12	8.31	0.12	54.76	1.13
CEC (cmol _c kg ⁻¹)	21.43	2.00	9.83	1.00	2.94	0.13	9.91	0.42	25.90	0.37	3.83	0.37	15.53	1.01
SA (m ² g ⁻¹)	0.047	0.002	0.008	0.001	0.003	0.001	0.015	0.001	0.057	0.002	0.004	0.001	0.083	0.003
As T (mg kg ⁻¹)	15.51	0.34	9.07	1.22	3.39	0.15	16.23	1.23	12.27	0.10	4.39	0.10	25.66	1.89
As W (mg kg ⁻¹)	0.021	0.001	0.007	0.001	0.010	0.001	0.077	0.015	0.014	0.001	0.010	0.001	0.010	0.001
Fe _d (g kg ⁻¹)	18.97	0.81	8.67	0.71	3.29	0.02	17.90	1.15	19.41	1.21	7.77	0.01	82.58	7.16
Mn _d (g kg ⁻¹)	0.54	0.02	0.13	0.01	0.01	0.01	0.32	0.03	0.85	0.01	0.15	0.01	0.13	0.01
Al _d (g kg ⁻¹)	2.40	0.02	1.10	0.01	0.60	0.02	1.90	0.09	1.90	0.07	0.90	0.10	5.10	0.16
Fe _o (g kg ⁻¹)	0.68	0.03	0.20	0.02	0.01	0.01	0.52	0.09	0.65	0.14	1.00	0.06	0.78	0.07
Mn _o (g kg ⁻¹)	0.06	0.01	0.02	0.01	bdl	–	0.18	0.02	0.41	0.07	0.09	0.01	0.03	0.01
Al _o (g kg ⁻¹)	1.17	0.03	0.31	0.02	0.15	0.02	0.38	0.07	0.50	0.06	0.27	0.06	0.73	0.06

nd non-detected, bdl below detection limit, EC electrical conductivity, I ionic strength, OC organic carbon, CEC cation-exchange capacity, SA specific area, AsT total arsenic concentration, AsW soluble in water arsenic concentration, Fe_d/Mn_d/Al_d free oxides, Fe_o/Mn_o/Al_o amorphous forms

^a Values × 10³

calibration, two sets of standards containing the analyte of interest at five concentrations were prepared using rhodium as an internal standard. Procedural blanks for estimating the detection limits ($3 \times \sigma$, $n=6$) were <0.21 ppb for As. The analytical precision was better than ± 5 % in all cases.

2.2 Soil spiking

Soil samples were spiked in laboratory with increasing amounts of As from sodium arsenate solution (Na₂HAsO₄·7H₂O) with the following concentration range: 50–100–300–600–1,200 mg As/kg soil. These levels were chosen according to the reference values (in milligram per kilogram) proposed for Andalusia (Aguilar et al. 1999) (50=agricultural soils; 100=natural areas; 300=industrial zones, and multiplying the highest concentrations of industrial zones by 2 and 4, respectively). Furthermore, an uncontaminated sample (blank) was left, making a total of six treatments. The spiking was performed in triplicate on individual samples of 50 g of soil.

Once spiked, samples were incubated for 4 weeks at 25 ± 1 °C and 60 % humidity, with a light cycle of 10 h. The incubation period chosen allowed the stabilization of the

contaminant added, optimizing the time spent on these tests, this protocol being based on similar studies by other authors (Fendorf et al. 2004; Tang et al. 2006).

After the incubation period, a saturation extract was prepared with a soil/water ratio of 1:1. A soil solution was made after shaking for 24 h, through the extraction system of 10 cm Rhizon MOM. In the resulting extracts, the pH, electrical conductivity, and water-soluble As concentrations by ICP-MS were measured. All assays were performed within 48 h of making extracts.

2.3 Bioassays

Two toxicity bioassays were selected for this experiment:

1. Seed germination/Root elongation Toxicity Test, according to OECD (2003) and US EPA (1996) recommendations. This test assesses the phytotoxic effects on seed germination and seedling growth in the first days of growth (Torres 2003). In Petri dishes, 15 seeds of *Lactuca sativa* and 5 ml of soluble extract from As-contaminated soils were placed in an incubator at 25 ±

1 °C, and the number of seeds germinated and the length of the germinated seed roots were measured after 120 h. Two endpoints were calculated: (a) the percentage of germinated seeds (SG) in relation to the control and (b) the root elongation reduction (LsR) in relation to the control (distilled water).

2. Microtox® test (ASTM 2004) is based on the reduction of the light emitted by a nonpathogenic strain of luminescent marine bacterium *Vibrio fischeri* upon exposure to a toxic sample (Ribó and Kaiser 1987). The test was performed in a Microtox 500 analyser from Microbics Corporation, according to a modification of Microtox Basic Test for Aqueous Extracts Protocol (Martín et al. 2010; Azur Environmental 1998). The luminescence was measured before the mixture with the As-contaminated solutions (0 min). The inhibition of bioluminescence was measured at 15 min after the mixture with the different extracts coming from the As-contaminated soils. The results were expressed as the luminescence reduction in the sample in relation to the control (VfR). The values range from 0, for samples that do not exhibit toxicity, to 100 for samples with maximum toxicity.

2.4 Data analysis

After it was verified that the data distribution fit a normal distribution, significant effects were determined with an ANOVA and, for multiple comparisons, Tukey's test ($p < 0.05$) was applied. For the analysis of the influence of soil properties on As solubility and toxicity, Spearman's correlations were performed, whereas a factor analysis was made from a rotated component analysis using the Varimax with Kaiser normalization method. All these analyses were performed at a confidence level of 95 %. Statistical analyses were performed using SPSS v.15.0 (SPSS Inc., Chicago, USA).

The EC_x (effective concentration causing a $x\%$ —50 and 10 %—of reduction in the endpoints) and its 95 % confidence interval were established by the fitting to a log-logistic model (Doelman and Haanstra 1989), using the method of Marquardt (proc NLIN, SAS 9.1, SAS Institute, Cary, NC, USA). No observed effect concentration (NOEC) compared to the uncontaminated sample was calculated by analysis of variance and Tukey's test in multiple-range data from the log-logistic model (Romero-Freire 2012).

3 Results

3.1 Arsenic solubility and influence of soil properties

The water extracts taken from the contaminated samples indicated significant differences in the solubility of As between

the different treatments and soil samples, these differences being higher with the increase in the As concentration added to the soils (Fig. 1). In all treatments, the sample with the lowest As concentration in the solution was H7 (red Mediterranean soil); meanwhile, the highest solubility was found in the highly carbonate samples (H2 and H3). In the highest concentration of spiked soils (1,200 mg kg⁻¹), the As solubility also significantly differed among samples, with higher solubility in the slightly acidic and non-carbonate samples (H6 and H4) in relation to the organic horizons (H5 and H1).

The correlation analysis (Spearman) between the water-soluble As concentrations and the soil properties for each sample considered individually indicated that the soluble arsenic was significantly ($p < 0.01$) and positively related with two main variables: the total As added to the soil and the ionic strength of the soil solution, with correlation coefficients greater than 0.972 and 0.822, respectively, in all cases.

For the different treatments considered individually, the solubility of As in all cases was significantly and inversely related to the free iron (Fe_a) forms, with an increasing correlation coefficient when the As concentration rose (Table 2). At lower concentrations of added As (≤ 300 mg kg⁻¹), other properties such as soil pH and CaCO₃ content significantly influenced the solubility of this element, while for the treatments with high concentrations of As (≥ 600 mg kg⁻¹), an inverse relation with OC was found.

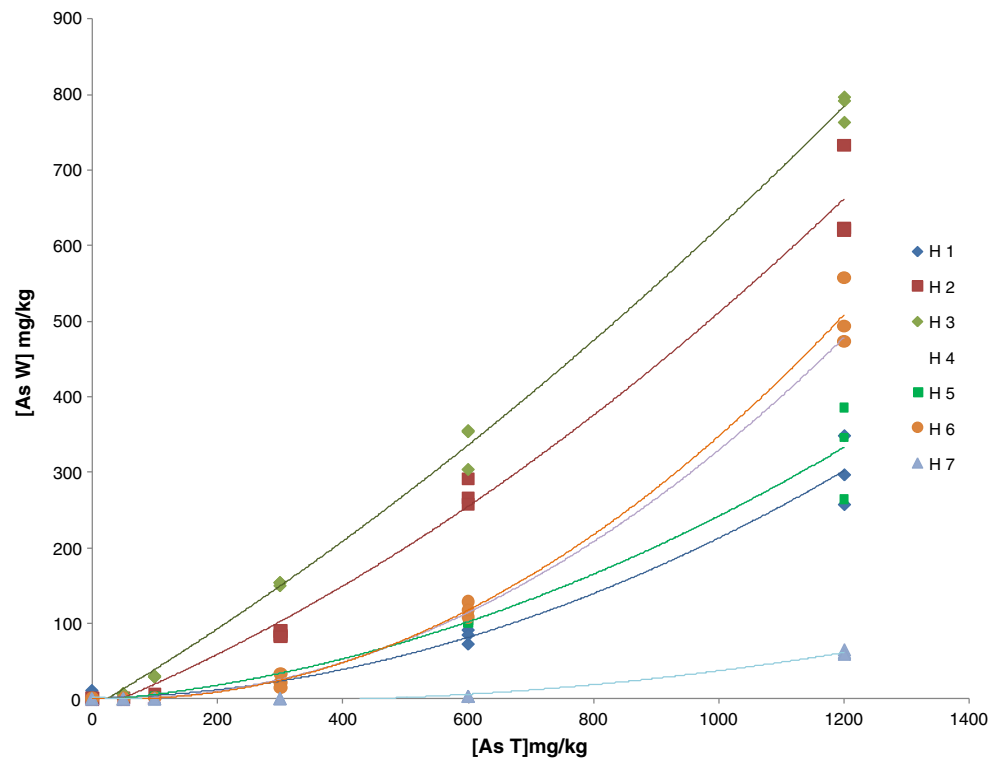
3.2 Toxicity bioassays

3.2.1 *L. sativa*

In the seed germination (SG) test, the carbonate soils (H3, H2 and H1) showed stronger inhibition of germination than the other soils, while the sample with the lowest inhibition was the red Mediterranean soil (H7). The germination was inhibited for concentrations ≥ 300 mg kg⁻¹ in sample H3, for concentrations ≥ 600 mg kg⁻¹ in samples H1 and H2, and for concentrations of $\geq 1,200$ mg kg⁻¹ in samples H4, H5 and H6 (non-carbonate soils); in sample H7, germination was not inhibited in any treatment, with germination values of 87 % at the maximum contamination level (1,200 mg kg⁻¹).

In the root elongation (RE) test, the reduction of elongation increases with the amount of As added to the samples (Fig. 2). The inhibition of elongation was stronger in carbonated soils in relation to the other samples. For the first level of contamination (50 mg kg⁻¹), the percentage of elongation in relation to the uncontaminated soils for the samples H3, H2 and H1 was 32, 76 and 88 %, respectively, whereas for the second level of contamination (100 mg kg⁻¹) it was 4, 29 and 68 %, respectively. Samples of non-carbonate soils (H4, H5 and H6) did not significantly differ, with elongation values higher than 50 % for the contamination with 100 mg kg⁻¹ and higher than

Fig. 1 Concentration of soluble arsenic (AsW) against total (AsT) for the seven samples studied in all treatments for the three replicates. Mean quadratic fits of trend lines ($R^2 > 0.980$ in all cases)



10 % for the contamination with 300 mg kg^{-1} . The soil with the lowest phytotoxicity was H7, registering root elongation values of 49 % in relation to the uncontaminated sample for the contamination level of 600 mg kg^{-1} .

3.2.2 *V. fischeri*

Reduction values at 15 min of the initial luminescence increased with the amount of As added to the soils (Fig. 3). Highly carbonate soils (samples H2 and H3) showed the greatest reduction, with luminescence values lower than 32 % in relation to the uncontaminated sample for the treatment of 100 mg kg^{-1} , followed by the organic horizons (H1 and H5) with luminescence around 60 % in relation to the uncontaminated soil, for the same contamination level. The non-carbonate soils (H4 and H6) presented a luminescence higher than 95 % for the contamination with 100 mg kg^{-1} , and around 30 % for 300 mg kg^{-1} of As added to the soil. The red Mediterranean soil (H7) again showed the least toxicity, no inhibition was detected for the contamination with 300 mg kg^{-1} , and luminescence higher than 55 % in relation to the uncontaminated soil for the treatment of 600 mg kg^{-1} of As added to the soil.

3.3 Relationship between toxicity and soil properties

The soil properties with highest influence on arsenic toxicity were studied using a correlation analysis (Spearman) between the data obtained in the bioassays and the main variables analysed (Table 3). The root elongation reduction of

L. sativa in relation to the control (LsR) was significantly correlated in all treatments with AsW, and Fe_d in direct and inverse relation, respectively. Furthermore, for treatments $\leq 600 \text{ mg kg}^{-1}$, pH (pH S) and calcium carbonate content were directly and significantly related to the increase in phytotoxicity, and for treatments $\geq 600 \text{ mg kg}^{-1}$, toxicity was also significantly related to the pH (pH W) and ionic strength (I) of the soil solution.

According to luminescence reduction of *V. fischeri* in relation to the control (VfR), the variables significantly correlated to this toxicity index in all treatments were the calcium carbonate content and soil pH, in direct relation, and Fe_d in inverse relation to VfR (Table 4). Treatments $\leq 300 \text{ mg kg}^{-1}$ had higher correlation coefficients with the AsW and the free

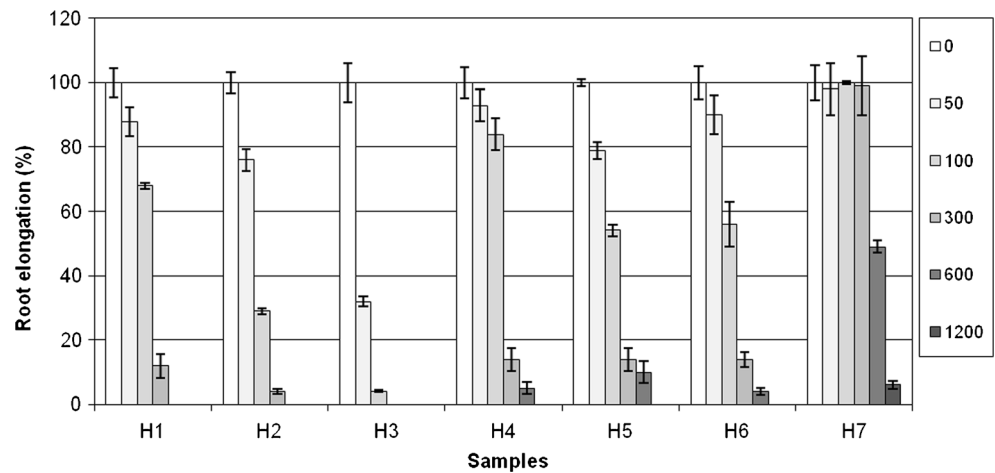
Table 2 Correlation coefficients (Spearman) between AsW and some soil properties

Treatment	OC	CaCO_3	Fe_d	pH S
50	-0.087	0.757**	-0.556**	0.703**
100	-0.206	0.826**	-0.616**	0.770**
300	-0.301	0.687**	-0.719**	0.627**
600	-0.544*	0.416	-0.866**	0.428
1,200	-0.621**	0.419	-0.910**	0.399

Treatment: 50, 100, 300, 600, 1,200 mg kg^{-1} of total As added to the soils
 OC organic carbon, CaCO_3 calcium carbonate content, Fe_d free iron oxides, pH S soil pH

* $p < 0.05$; ** $p < 0.01$

Fig. 2 The root elongation percentage in relation to the control for the different samples and different treatments of total As added (0–50–100–300–600–1,200 mg kg⁻¹). Values higher than 100 % could indicate hormesis



iron forms (Fe_d) than did treatments with higher As concentrations, with no significant relation with AsW in the treatment of 1,200 mg kg⁻¹. Furthermore, for the treatments ≥100 mg kg⁻¹, the toxicity was significantly related to the pH and ionic strength (pH W and I) of the soil solution.

The factorial analysis made with all the variables studied showed that 90.96 % of the variance was explained by a total of four components (Table 5). The responses of the toxicity bioassays (LsR and VfR) were grouped in component 2, explaining 27.16 % of the variance, with the AsT, AsW, and ionic strength (I) of the solution.

3.4 Toxicity index

3.4.1 *L. sativa*

The effective concentrations of water-soluble As (EC10 and EC50) for root elongation data between the different samples showed no significant differences. The water-soluble As concentrations that inhibited 10 % of root elongation ranged between 0.92 and 0.19 mg kg⁻¹, while the 50 % reduction of

the elongation was caused by values between 4.30 and 1.81 mg kg⁻¹. The NOEC ranged between 0.10 and 0.02 mg kg⁻¹ of soluble As, without significant differences between soil samples.

In relation to the total As (Table 6), the sample that presented the highest toxicity was the highly carbonated soil horizon (H3), wherein concentrations of 23 and 43 mg kg⁻¹ of As inhibited the root elongation by 10 and 50 %, respectively. The sample with lowest toxicity was the red Mediterranean soil (H7), with an EC10 value of close to 400 mg kg⁻¹ and EC50 of more than 620 mg kg⁻¹. The non-carbonate soil H4 also presented low toxicity, in which concentrations of 95 and 185 mg kg⁻¹ of total As in soil reduced the root elongation by 10 and 50 %, respectively. The other samples did not significantly differ, with EC10 values ranging between 30 and 51 mg kg⁻¹, and EC50 values ranging between 84 and 147 mg kg⁻¹ of total As in soil.

For the NOEC, significant differences were found between the different groups of soil. Carbonate soils (H1, H2 and H3) fixed the highest value of this concentration in 25 mg kg⁻¹, while in non-carbonate soils (H4, H5 and H6) this value rose

Fig. 3 Percentage of luminescence at 15 min in relation to the control for different samples and different treatments of total As added (0–50–100–300–600–1,200 mg kg⁻¹). Values higher than 100 % could indicate hormesis

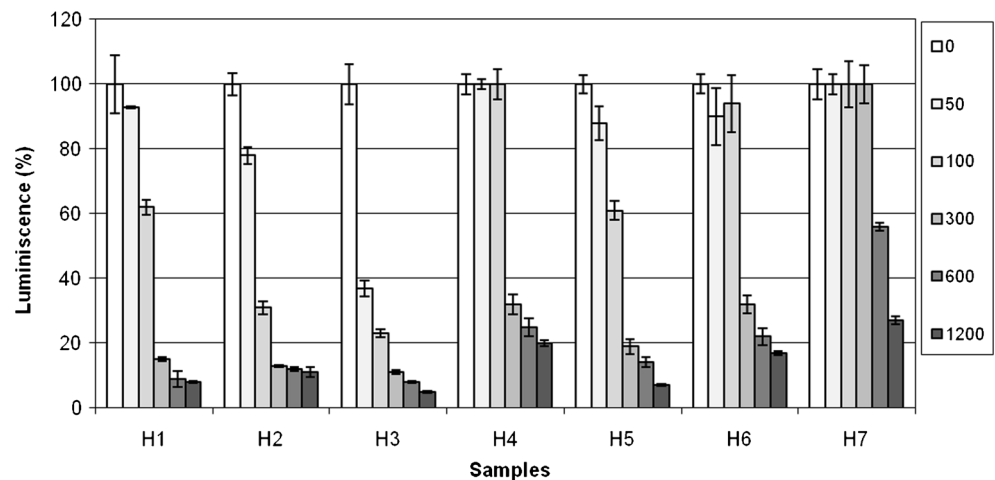


Table 3 Correlation coefficients (Spearman) between the percentage of root elongation reduction of *L. sativa* (LsR) and different variables of soils, for all treatments

	AsW	Fe _d	CaCO ₃	pH W	pH S	I
50	0.792**	-0.579**	0.641**	0.300	0.636**	0.330
100	0.941**	-0.763**	0.687**	0.233	0.601**	0.310
300	0.734**	-0.694**	0.626**	0.380	0.653**	0.257
600	0.628**	-0.596**	0.655**	0.797**	0.667**	0.449*
1,200	0.605**	-0.608**	0.214	0.509*	0.178	0.606**

Treatment: 50, 100, 300, 600, 1,200 mg kg⁻¹ of total As added to the soils
AsW soluble in water As, *Fed* free iron oxides, *CaCO₃* calcium carbonate content, *pH W* water extract pH, *pH S* soil pH, *I* ionic strength

p*<0.05; *p*<0.01

to 40 mg kg⁻¹, and for the red Mediterranean soil (H7) no phytotoxic effects were observed below a concentration of 150 mg kg⁻¹.

3.4.2 *V. fischeri*

The effective concentration EC10 of water-soluble As showed no significant differences between samples and ranged between 0.06 and 0.61 mg kg⁻¹. For the EC50, samples of carbonate soils (H1, H2 and H3) differed with respect to the other samples; in this case, the 50 % reduction in carbonate soils was caused by concentrations between 3.02 and 3.69 mg kg⁻¹, whereas for the other samples ranged between 6.78 and 15.21 mg kg⁻¹. The values of NOEC for water-soluble As were very similar for all samples with values ranging between 0.002 and 0.003 mg kg⁻¹ in all cases.

In relation to the total As (Table 7), the sample with the highest toxicity was again the highly carbonate soil (H3), wherein concentrations of 5.5 and 37.3 mg kg⁻¹ inhibited luminescence at 10 and 50 %, respectively. The sample with the lowest toxicity was also the red Mediterranean soil (H7),

Table 4 Correlation coefficients (Spearman) between the percentage reduction in luminescence of *V. fischeri* (VFR) and other soil variables, for all the treatments

	AsW	Fe _d	CaCO ₃	pH W	pH S	I
50	0.816**	-0.642**	0.644**	0.338	0.630**	0.279
100	0.936**	-0.606**	0.857**	0.465*	0.837**	0.563**
300	0.857**	-0.561**	0.863**	0.582**	0.833**	0.580**
600	0.495*	-0.447*	0.768**	0.706**	0.731**	0.712**
1,200	0.419	-0.447*	0.716**	0.586**	0.662**	0.881**

Treatment: 50, 100, 300, 600, 1,200 mg kg⁻¹ of total As added to the soils
AsW water-soluble As, *Fed* free iron oxides, *CaCO₃* calcium carbonate content, *pH W* pH of water extract, *pH S* soil pH, *I* ionic strength

p*<0.05; *p*<0.01

Table 5 Factorial analysis of rotated component matrix

	Component			
	1	2	3	4
AsT		0.953		
AsW		0.852		
pH W				0.492
pH S				0.979
I		0.689		
CaCO ₃				0.878
OC			0.977	
SA	0.881			
Clay	0.977			
Fe _d	0.969			
Al _d	0.973			
Mn _d			0.977	
CEC			0.883	
LsR		0.861		
VfR		0.847		
% ac.ex.var.	27.51	52.78	76.54	90.97

AsT total As concentration, *AsW* water-soluble As concentration, *pH W* pH of water extract, *pH S* soil pH, *I* ionic strength, *OC* organic carbon, *SA* specific area, *Fe_d/Mn_d/Al_d* free oxides, *CEC* cation-exchange capacity, *LsR* *L. sativa* reduction, *VfR* *V. fischeri* reduction, % ac.ex.var: percent of accumulated explained variance

with EC10 concentration higher than 270 mg kg⁻¹ and EC50 concentration of 765 mg kg⁻¹ of total As. The other samples did not significantly differ, with EC10 values ranging between 39 and 102 mg kg⁻¹, and EC50 values ranging between 88 and 291 mg kg⁻¹ of total arsenic in soil.

For the NOEC, no significant differences were detected between samples. In all cases, the NOEC was between 5 and 15 mg kg⁻¹ and did not reflect the previously observed differences in solubility or toxicity of As between different samples.

Table 6 Effective concentrations (EC50 and EC10) for the *L. sativa* test of total As in soil

	Total As (mg kg ⁻¹)					
	EC50	95 % C.I.		EC10	95 % C.I.	
H1	147.24	132.65	163.43	62.15	50.69	76.22
H2	83.57	79.62	87.72	42.62	37.96	47.86
H3	43.02	37.89	48.84	23.17	15.39	34.88
H4	185.49	163.49	210.46	94.68	75.41	118.87
H5	123.83	111.73	137.24	37.92	30.19	47.62
H6	126.20	107.68	147.90	47.17	35.39	62.87
H7	621.64	571.38	676.31	394.95	293.71	531.08

95 % CI confidence interval for the mean 95 %

Table 7 Effective concentrations (EC50 and EC10) for *V. fischeri* test of total As

	Total As (mg kg ⁻¹)					
	EC50	95 % C.I.		EC10	95 % C.I.	
H1	152.65	136.86	170.27	58.21	47.72	71.02
H2	87.84	76.26	101.18	38.82	22.05	68.33
H3	37.30	30.97	44.92	5.51	3.43	8.87
H4	290.64	231.37	365.09	101.88	63.83	162.62
H5	154.87	134.73	178.01	44.38	33.35	59.06
H6	242.20	202.34	289.92	57.54	37.87	87.42
H7	767.54	701.38	839.94	332.14	279.57	394.60

95 % CI confidence interval for the mean 95 %

4 Discussion

4.1 Arsenic solubility and soil properties

Soil properties have a critical influence over the speciation, bioavailability and solubility of As (Bissen and Frimmel 2003; Juhasz et al. 2003). From the correlation analysis between the concentrations of water-soluble As and the different properties of the soils studied (Table 2), we determined that the variables having the strongest influence on the solubility of this element were the following: (a) properties related to the soil extract (pH and ionic strength of the solution) and (b) soil constituents (mainly iron oxides and organic carbon content).

Arsenic is described as one of the few metalloids that can move within the normal pH range (6.5–8.5) under oxidizing as well as reducing conditions (Dzombak and Morel 1990). In our soils, we detected an increase in As solubility with the rise in pH (also strongly related to the CaCO₃ content). The causes of this increased mobility could be associated with desorption of Fe oxides and mobilization of soil carbon under these conditions (Wang and Mulligan 2006; Klitzke and Lang 2009). Furthermore, the positive correlation between the solubility of As and the ionic strength found in our samples has been described as a key factor in increasing the mobility of heavy metals in the soil (Acosta et al. 2011), and it has been found that a rise in the ionic strength of aqueous solutions boosts the effectiveness of decontamination in soils affected by As (Deliyanni et al. 2003).

In terms of recuperation of metal(oid)-contaminated soils, one of the most widely used techniques is the increase of soil pH by liming techniques (Bolan and Duraisamy 2003). However, according to our results, when As is one of the contaminants, this liming should be done with caution since, under certain conditions, a higher pH may bring on the solubilization of certain forms of As. Our results agree with Simon et al. (2005, 2010) who studied the recovery and liming of soils after the accident at the Aznalcóllar mine (Seville,

Spain) and recommended not to raise the pH above neutrality so as not to increase As mobility.

Otherwise, the results of correlation between water-soluble As and soil constituents indicated a highly significant inverse relation with free iron oxides (Fe_d). In this respect, the Fe oxides and Fe hydroxides of soil have been widely described as the main active constituents in soil-As retention (Fitz and Wenzel 2002). Many remediation technologies have been developed using iron compounds as the most effective way to immobilize As both from soil as well as in water-contaminated areas (Simón et al. 2010; Mohan and Pittman 2007).

Also, we found a highly significant inverse relationship between soluble As and clay content and specific area, in agreement with the findings of several authors who have suggested that the presence of Fe is closely linked to the large surface areas and small particle sizes (Warren et al. 2003; Yamaguchi et al. 2011). Although, the high correlation coefficients between Fe oxides and clay content (0.938) and the specific area (0.929) may be indicative of the effects of collinearity between these variables which have to be carefully analysed to establish adsorption models.

According to Yang et al. (2012), As distribution between the soluble and solid phase is directly related to the total As, pH and OC. These results agree with those observed in this work, although the correlation with OC appears only for the highest concentrations of added As (1,200 mg kg⁻¹). This relationship between As adsorption by humic acid is well known, although the precise retention mechanisms remain controversial (Kumpiene et al. 2008), and some experiments indicate that the presence of organic matter causes the opposite effect, competing with As for adsorption sites on iron oxide surfaces (Redman et al. 2002).

4.2 As toxicity

In this work, the toxicity determined by the root elongation of *L. sativa* is considered to have a higher degree of sensitivity than does the luminescence of *V. fischeri* when studying As toxicity in soils, in agreement with other authors (Estepa 2011). Moreover, values of EC50, EC10 and NOEC both for total as well as for water-soluble As are more uniform (narrow confidence interval) and more differences between samples are detected, indicating higher reliability and sensitivity than with the *V. fischeri* bioassay. In addition, the seed germination test with *L. sativa* is the least sensitive endpoint to the As concentrations because it requires very high values to inhibit germination, and changes that occur between the different treatments are very abrupt (Bagur González et al. 2011).

Comparisons between toxicity indexes and soil properties indicate that the results for inhibition in both bioassays were similar to those for water-soluble As behaviour. The soil parameters with the greatest influence were iron oxides,

calcium carbonate content, pH and ionic strength of the solution, clay content and specific area. Of these, iron oxides appear to play the most decisive role in toxicity reduction, coinciding with the extensive use of this constituent as an amendment agent in the recovery of As-contaminated soils (Hartley et al. 2004; Miretzky and Fernández 2010; Nielsen et al. 2011).

Another variable with great influence is pH of the soil solution, observing in our study that a rise in the pH in turn raises toxicity. Even though increased As availability and toxicity in soils under acidic conditions (pH <5) was generally assumed to be due to the increased solubility of iron and aluminium forms that retain this element (O'Neill 1995), As mobilization at higher pH values can also be important in certain soil types. Low As adsorption in soils with high pH values is related to a gain in more-negatively charged arsenate species, causing repulsion by the negatively charged soil-exchange sites and increasing the bioavailability of this contaminant (Yang et al. 2002). Moreover, the adsorption capacity of As by Fe oxides diminishes with rising pH, due to the decrease in positive surface charges of these oxides (Klitzke and Lang 2009), which may augment the solubility of arsenates in the normal pH range of soils (Beesley and Marmiroli 2011). In this sense, As mobilization over time after remediation actions, implying applications of amendment material, is of great concern because soil pH is one of the properties most strongly affected. In this sense, we have described remobilization of As in soils 10 years after remediation actions in the soils contaminated by the Aznalcóllar mine pollution accident (Seville, Spain) (Martín et al. 2011) indicating that the monitoring of recovered areas over the medium and long term was necessary in these cases.

4.3 Reference values

Today, for regulatory purposes, reference values for As in soils are based on the establishment of a single value for large regions (countries or communities), regardless of the variations between different soil types, without representing the potential risk of contamination (toxicity) for each scenario. In this way, the most restrictive reference values were established for agricultural soils, with large differences between countries: 10 mg kg⁻¹ in UK (Barth and L'Hermite 1987), 19 mg kg⁻¹ in Belgium (BWRHABT GG 1995) and 29 mg kg⁻¹ in Holland (RIVM 2000). Otherwise, guideline values vary across about a 1,000-fold range (0.039 to 40 mg/kg) in the USA (Teaf et al. 2010). In this study, we confirmed that the different soil types are key in controlling As mobility and thus result in different degrees of toxicity for the same total concentration of contaminant.

In relation to the concentrations of water-soluble As, the results are not conclusive because they have very wide confidence intervals, especially for lower toxicity thresholds,

Table 8 Data from the literature on reference values for total As (in milligram per kilogram) concentrations by root elongation test compared with our results

Reference	NOEC	EC10	EC50
Song et al. (2006) (<i>barley</i>)	15–125	4–207	27–458
Cao et al. (2009) (<i>lettuce and wheat</i>)	<40–200	20–156	59–426
Our study (<i>lettuce</i>)			
Carbonate soils	15–25	25–60	45–150
Non-carbonate soils	10–40	40–95	125–185
Iron-rich soil	150	400	620

causing an overlap of different values for the different samples. Considering the EC50 value as one of the most common thresholds used in ecotoxicity tests (Isnard et al 2001), in our study, for the *L. sativa* bioassay and water-soluble arsenic concentrations, we found a range of between 1.8 and 4.3 mg kg⁻¹, similar to the value 2.3 mg kg⁻¹ reported by Vaughan and Greensdale (1998) for the same type of bioassay. In the case of *V. fischeri*, our values range between 3.0 and 15.2 mg kg⁻¹, similar to those presented by Fulladosa et al. (2004) of 5.7 (at pH 8) and 20.3 mg kg⁻¹ (at pH 7) in As-contaminated solutions.

With respect to the total As concentration, the influence of soil properties and constituents lead to sharp differences between the values of the same endpoint in a given bioassay (Table 8).

The studies cited in the table indicate that the soil properties having the strongest influence in toxicity are pH and the content of sesquioxides and, as in our study, the lowest toxicity (highest values) occurs in soils with the highest iron oxide content. Soil guideline values can be defined according to the background and NOEC concentrations (Crommentuijn et al. 2000) in natural soils. In the case of arsenic, we can preliminarily define three levels: 25 mg kg⁻¹ for carbonate soils, 40 for non-carbonate soils and 150 for iron-rich soils. In any case, the variations between the different soil types are very important, so that more studies are needed to propose generic environmental quality standards defined according to the main soil properties and constituents affecting arsenic mobility and toxicity.

5 Conclusions

The solubility of arsenic in the samples studied is strongly controlled by soil properties and constituents, the iron oxides, the organic carbon content, pH and ionic strength of the soil solution being the most important variables. Also, we also found that the soil properties strongly alter the As toxicity of the soil leachate, assessed by bioassays, showing a significant reduction of the toxicity in the iron-rich soil and significant

increase in the highly carbonate samples. The broad range of responses of arsenic toxicity in relation to soil properties can produce over- or underestimation of guideline values in soil remediation actions. According to these results, soil guideline values of As should be set at different levels depending on soil properties, which could optimize the efficiency and profitability in the use of generic environmental quality standards.

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