SJSS
SPANISH JOURNAL OF SOIL SCIENCE

Assessment of arsenic toxicity in spiked soils and water solutions by the use of bioassays

Evaluación de la toxicidad del arsénico en suelos y soluciones contaminadas mediante el uso de bioensayos
Avaliação da toxicidade de arsénio em solos contaminados e em soluções aquosas com recurso a bioensaios

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Received: 13.07.2012 | **Revised:** 26.09.2012 | **Accepted:** 05.10.2012

ABSTRACT

Arsenic (As) has become a serious environmental problem due to its extensive use and its potential high toxicity. Furthermore, it tends to accumulate in soils because of its low mobility in this medium. In this study, an assessment of potential As toxicity based on bioassays with *Vibrio fischeri* and *Lactuca sativa* was made in soil and water solutions of laboratory-contaminated samples. Soils spiked with 100 ppm of arsenic registered a reduction in As solubility in the soil solution over time, while for the longest incubation periods (8 weeks), the toxicity responses in all of the samples proved negligible for these bioassays. In spiked water solutions with increasing concentrations (0, 0.1, 1, 10, 12.5, 25, 50, and 100 ppm As), significant reductions in root elongation and luminescence were found in lettuce and bacteria bioassays, respectively. The effective concentrations (EC50) of As were 1.52 ppm for *L. sativa* and 4.98 ppm for *V. fischeri*; this indicated that the *L. sativa* bioassay was more sensitive to arsenic concentrations in spiked solutions.

RESUMEN

El arsénico es un elemento que causa serios problemas medioambientales debido a su uso extensivo y a su alta toxicidad, tendiendo a acumularse en suelos debido a su baja movilidad en este medio. En este estudio realizamos la evaluación de la toxicidad potencial de arsénico a partir de bioensayos con Vibrio fischeri y Lactuca sativa en suelos y soluciones acuosas contaminados en laboratorio. Los suelos contaminados con 100 ppm de arsénico registraron una fuerte reducción de la solubilidad de este contaminante con el tiempo, obteniéndose una respuesta a la toxicidad prácticamente nula a las ocho semanas de incubación. En las soluciones acuosas contaminadas con concentraciones crecientes de arsénico (0, 0,1, 1, 10, 12,5, 25, 50 y 100 ppm As) se produjo una reducción significativa en la elongación de raíces y en la luminiscencia en los bioensayos con lechuga y bacterias, respectivamente. La concentración efectiva de arsénico que reduce un 50% la variable respuesta (EC50) fue de 1,52 ppm para el ensayo con L. sativa y 4,98 ppm para el ensayo con V. fischeri, indicando que el bioensayo con L. sativa fue más sensible a las concentraciones de arsénico en las soluciones contaminadas.

RESUMO

O Arsénio (As) constitui um grave problema ambiental devido à sua ampla utilização e elevado potencial tóxico. Para além disso, este elemento tende a acumular-se nos solos devido à sua baixa mobilidade. Neste estudo, fez-se uma avaliação do potencial de toxicidade do As em solos e soluções aquosas de amostras laboratoriais contaminadas com este elemento, recorrendo a bioensaios com Vibrio fischeri e Lactuca sativa. Os solos contaminados com 100 ppm de arsénio registaram uma redução da solubilidade do As na solução do solo ao longo do tempo, enquanto que para os períodos de incubação mais longos (8 semanas), as respostas à toxicidade em todas as amostras neste tipo de bioensaios foi insignificante. Em soluções aquosas contaminadas com concentrações crescentes de arsénio (0, 0,1, 1, 10, 12.5, 25, 50, and 100 ppm As), observaram-se reduções significativas no elongamento das raízes e na luminiscência respetivamente na alface e nos bioensaios com bactérias. A concentração efetiva de As (EC50) apresentou os valores de 1,52 ppm para a L. sativa e 4,98 ppm para a V. fischeri, o que indica que os bioensaios com L. sativa foram mais sensíveis às concentrações de As nas soluções contaminadas.

DOI: 10.3232/SJSS.2012.V2.N3.05

1. Introduction

In the recent decades, heavy metal(oid)s have received considerable attention as a consequence of the increased environmental pollution from industrial, agricultural, energy, and municipal sources (Adriano 2001). Arsenic (As) has intermediate properties between metals and non-metals, although its electronegativity and ionization energy give it characteristics close to those of a non-metal, tending to form anions rather than cations (Moreno-Jiménez et al. 2009). Its toxicity and occurrence make this element one of the most serious global environmental concerns (Chowdhury et al. 2010; Smith et al. 1992). Chemically, As exists as organic and inorganic species, and it has two main oxidation states ($^{+}$III and $^{+}$V), depending on the type and amounts of sorbents, pH, redox potential (Eh), and microbial activity (Young and Mulligan 2004). Inorganic compounds are the most frequent in soil due to their water solubility, the most thermodynamically stable species within the pH range 4.0-8.0 being H_3AsO_3 of As^{III}, and $HAsO_4^{2-}$ and $H_2AsO_4^{-}$ of As^{V} (Smith et al. 1998). Toxicity of arsenic depends heavily on its chemical form (Jedynak et al. 2009), the inorganic compounds of arsenite being considered more mobile and toxic for living organisms than organic forms, and the organic As compounds the least mobile (Xu et al. 1988; Nriagu et al. 2007; Bhattacharya et al. 2007; Giacomino et al. 2010; Martínez-Sánchez et al. 2011).

KEY WORDS
Soil properties, ***Vibrio fischeri,*** ***Lactuca sativa,*** **EC50**

PALABRAS CLAVE
Propiedades del suelo, *Vibrio fischeri,* *Lactuca sativa,* EC50

PALAVRAS-CHAVE
Propriedades do solo, *Vibrio fischeri,* *Lactuca sativa,* EC50

To determine the reference levels of heavy metals, it is necessary to know their contents in soils under natural conditions (Navas and Machin 2002; Jiménez-Ballesta et al. 2010). The background concentration of As in soil is related to the lithology of the parent materials (Naidu and Bhattacharya 2006); for example, sedimentary rocks contain proportionally more arsenic (1.7 to 40 ppm) than do igneous rocks (1.5 to 3 ppm) (Bhumble and Keefer 1994). According to the degree of soil development and lithology, significant differences have been found between the background values in weakly developed soils (Entisols) over carbonate rocks (< 3.5 ppm) and more developed soils (Alfisols) over metamorphic rocks (20 to 34 ppm) (Diez et al. 2009). Also, arsenic concentrations in soils can be substantially higher than background concentrations due to natural or anthropogenic inputs (Garau et al. 2011).

Soil is a main source of trace elements for plants, both micronutrients and pollutants (Kabata-Pendias and Pendias 2001), but there is no evidence that the presence of As in this media is essential for plant growth. High As concentrations in soils can reduce crop yield, since it inhibits plant growth and under stringent conditions may be lethal to the plant (Nriagu et al. 2007). Arsenic levels above 50 ppm in soil reportedly have a negative influence on the yield or plant growth in barley, and ryegrass (Jiang and Singh 1994), tomato (Burló et al. 1999), maize, and wheat (Xiao-ke et al. 2012).

Arsenic tends to accumulate in soils due to its low mobility in this medium, although water-soluble fractions are the most ecologically relevant because they are more readily mobile and hence, bioavailable (Beesley and Marmiroli 2011; Mench et al. 2009). Kabata-Pendias and Pendias (2001) summarized the As concentrations in contaminated soils from different countries, suggesting that only a limited amount of the total As in soil is easily mobile and the greater fraction (80%) is not available for plants due to strong binding to Fe and Al mineral phases.

The mobility, bioavailability and therefore potential deficiency or toxicity of metals for plants and microorganisms is controlled largely by soil properties (Junta de Andalucía 1999). The most relevant soil properties affecting metal(oid) speciation and furthermore mobility are pH, Eh, ionic composition of soil solution, and mineral type (Bissen and Frimmel 2003). The

mobility of arsenic in soils under oxidation conditions can strongly limit its bioavailability, but under anaerobic conditions, arsenic compounds can be transformed by microbiological processes to volatile but also easily oxidize trivalent methylated forms, strongly increasing its toxicity (Kabata-Pendias and Pendias 2001). The availability and toxicity of this element in soils can also increase under acidic conditions (pH < 5) due to the rise in solubility of the iron and aluminium compounds (O´Neill 1995). On the other hand, Raven et al. (1998) indicated lower As adsorption at high pH values attributable to the more negatively charged arsenate species repulsing the negatively charged surface sites, which increases As bioaccessibility (Yang et al. 2002). Furthermore, As^{V} solubility has been reported to increase within pH ranges commonly found in soils (pH 3-8), whereas As^{III} tends to follow the opposite pattern (Beesley and Marmiroli 2011; Fitz and Wenzel 2002). Moreover, soil-particle size plays an important role in controlling the distribution and mobility of As, being clay minerals, Fe-, Al-, and Mn (hydro)oxides important sink for soluble As forms, because the surface area of the fine-grained particles is large and increase the As retention (Song et al. 2006; Nriagu et al. 2007).

There are many areas of research that are being actively pursued to address the As contamination problem and the relationship with plant-soil interactions. These include new methods of screening As in the field, determining the epidemiology of As in humans, and identifying the risk of As uptake in agriculture (Bhattacharya et al. 2007). In environmental-risk assessment (ERA) the use of toxicity bioassays is essential to determine the potential risk of pollution for individual living organisms and the ecosystem as a whole. Most bioassays are based on the evaluation of the toxic effect of the solution extracted from the solid phase or by the solid phase itself over a living organism (Martín et al. 2010; Farré and Barceló 2003).

The aim of this work is to evaluate the toxicity of arsenic at different concentrations in artificially contaminated solutions using toxicity bioassays of two organisms, the bacteria *Vibrio fischeri* and seeds of *Lactuca sativa* (lettuce) as bioindicators. The potential contamination was also studied in four artificial contaminated soils with arsenic, with different ranges of physico-chemical properties, to evaluate the interaction between arsenic and soil properties, and to assess the potential toxicity of arsenic in these soils.

2. Material and Methods

Four soils were selected (**Table 1**) in the Rio Verde basin (Málaga, S Spain). In all cases, soils were low developed (genetic horizon sequence Ah-C), and have a forestry use dominated by xerophytic shrub replacing the Mediterranean forest.

Table 1. Selected soils

Sample	UTM coordinates		Soil type
	X	Y	(WRB 2007)
R1	0326133	4052376	Haplic Regosol (eutric)
M2	0325676	4045037	Haplic Regosol (eutric)
M3	0325462	4044144	Haplic Regosol (calcaric)
M4	0326380	4049951	Haplic Regosol (calcaric)

To evaluate the arsenic toxicity in soils, two bioassays were used with two different organisms: *Lactuca sativa* (lettuce), and *Vibrio fischeri* (bacterium), representative of two major groups of soil organisms (primary producers, and microbes). Assays were performed using: a) Spiked water solutions, contaminated by adding a soluble arsenic salt ($Na_2HAsO_4.7H_2O$) with increasing toxicity levels of: 0, 0.1, 1, 10, 12.5, 25, 50, and 100 ppm ; b) Spiked soils, contaminated in laboratory with 100 mg As kg^{-1} soil with the same arsenic salt. Arsenate form was selected due it is the most common specie in soils (Kabata Pendias and Pendias 2001). In the spiked soils, the assays were performed with the water-soluble forms after incubation of 1, 4, and 8 weeks. The water-soluble forms were obtained from soil-water extracts at a ratio of 1:5 (Sposito et al. 1982). In all cases, the assays were performed in triplicate.

In soil samples, the main properties and constituents were analysed according to the standard methods (MAPA 1994), and the trace-element concentrations were determined, after acid digestion, by ICP-MS in a PerkinElmer's NexION® 300 instrument. In each solution, both from the spiked soils as well as from the spiked water solutions, pH, and electrical conductivity (EC) were measured potentiometrically, and As was analysed by ICP-MS.

The seed germination/root elongation toxicity test was conducted according to OECD (2003) and U.S. EPA (1996) recommendations. This test assesses the phytotoxic effects on seed germination and seedling growth in the first days of growth (Torres 2003). Petri dishes containing 15 seeds of *Lactuca sativa* and 5 ml of soluble extract of As-contaminated solutions were placed in an incubator at 25 ±1 °C for 120 h. Finally, the number of seeds germinated was counted, and the lengths of the roots of the germinated seeds were measured. Two endpoints were measured: a) the percentage of germinated seeds (SG) in the sample in relation to the control (distilled water); and b) the average length of the seed roots (RE) in the sample in relation to the control (distilled water).

The Microtox test (ASTM 2004) was based on the reduction of the light emitted by a non-pathogenic strain of luminescent marine bacterium *Vibrio fischeri* upon exposure to a toxic sample (Ribo 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 (AZUR Environmental 1998; Martín et al. 2010). 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 As solutions. The percentage of luminescence reduction in the samples (Red 15) was calculated by comparing the value at 0 with that at 15 min. Control sample (distilled water) was measured under the same conditions as the polluted samples. Reduction of luminescence above the control indicates toxicity, while reduction of luminescence below the control indicates stimulation of bacterial activity (hormesis).

Statistical analyses were performed by using SPSS v.15.0. Significant effects were determined by ANOVA (Duncan test; $p < 0.05$). Bartlett and Shapiro-Wilk tests were applied to check homoscedasticity and normality, respectively. The EC50 (effective concentration causing 50% reduction in the endpoints) and its 95% confidence interval were determined by the fitting to a log-logistic model (Doelman and Haanstra 1989).

3. Results and Discussion

3.1. Spiked soils

Some of the most important soil properties (**Table 2**) were in the following ranges: pH (6.4 to 8.6), OM (0.4 to 6.0%), calcium carbonate (0 to 37%), clay (13.1 to 36.6%). The concentration in trace elements were also analysed (**Table 3**), and the range for the different elements were within the normal range for the soils in the area (Escoto et al. 2007). These soils were artificially contaminated in laboratory by adding 100 ppm As, and the soil-water extracts were taken after 1, 4, and 8 weeks, respectively.

Table 2. Main soil properties of the samples

Soil	pH	EC dS m^{-1}	OM %	$CaCO_3$ %	Clay %	Al_o %	Al_d %	Fe_o %	Fe_d %
R1	7.08	0.05	3.76	nd	36.59	0.73	1.02	0.76	3.51
M2	6.41	0.03	3.15	nd	13.10	0.35	0.68	0.39	2.68
M3	8.57	0.13	0.43	5.10	26.29	0.42	0.97	0.54	3.12
M4	7.89	0.09	6.02	37.29	24.84	0.65	0.91	0.70	3.04

(EC: electrical conductivity; OM: organic matter; Al_o/Fe_o: amorphous forms; Al_d/Fe_d: free oxides; nd: not detected).

Table 3. Total trace-element concentration of the samples

Soil	As	Mn	Ni	Cr	Zn	Pb	Co	Cu
				ppm				
R1	42.9	347.4	13.0	34.0	53.7	37.3	7.0	18.1
M2	50.7	121.1	28.6	77.4	49.3	22.1	5.6	16.1
M3	18.2	546.0	140.2	176.0	94.9	30.9	21.6	23.6
M4	8.9	601.5	355.3	267.1	105.7	39.5	37.7	21.9

The soluble As concentration of the artificially contaminated soils showed significant differences according to the soil type and in the course of incubation periods (**Figure 1**). Soil with lower concentrations of soluble As was R1, while M3 showed the highest values, indicating that the solubility of arsenic in the studied soils was not related either to the pH or to the $CaCO_3$ content. In all cases, the concentration of soluble As significantly diminished over time. After the addition of 100 ppm of As to the soils, the soluble concentrations ranged between 0.03 and 0.003 ppm in one week of the incubation period; these values of soluble As reduced significantly (between 22 to 74%) after eight weeks of incubation.

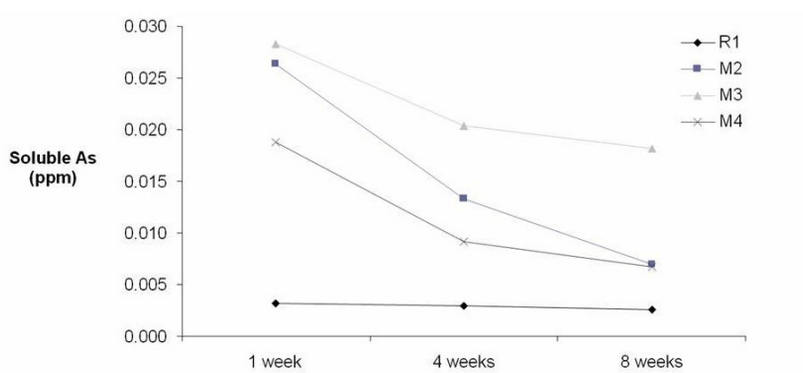

Figure 1. Soluble As concentrations in the different soils in the three incubation periods.

Studies using laboratory-contaminated soils should be carefully interpreted because the soil-solution composition may have little relationship to that of field-contaminated soils. A positive viewpoint is that laboratory spiked soils generally overestimate metal availability in the field (Smolders et al. 2009), so that the toxicity levels defined by these studies increase the safety threshold for the environmental-risk assessment.

The lettuce-germination test using the soil solutions showed no differences according to the incubation period. Seed germination (SG) was higher than 95% in all cases, with no significant differences with respect to the control. This indicates that the seed-germination index is not useful when the As concentration in the solution falls below 0.03 ppm. Root elongation (RE) differed very slightly between samples and the three incubation periods (**Table 4**). In no case, there were significant differences in relation to the control. Despite the different As concentration of the solutions, the reduction in the RE in relation to the control was negligible, with values below 10% for all samples and in all incubation periods. According to the As concentration of the solutions and the data obtained in the lettuce bioassays, these results agree with the values reported by Bohn et al. (1985), who gave the value of 0.04 ppm as the toxic level for As in the soil solution.

Table 4. Root elongation (cm) of lettuce seeds in the three incubation periods

	1Week		4 Weeks		8 Weeks	
	RE	sd	RE	sd	RE	sd
R1	7.22	0.51	6.86	0.60	7.55	0.72
M2	7.01	0.56	7.03	0.70	7.40	0.89
M3	7.31	0.49	7.09	0.34	7.72	0.86
M4	6.73	0.73	6.48	0.55	7.66	0.91
Control	7.24	0.49	7.02	0.61	7.63	0.86
p value	0.33		0.46		0.24	

(sd: standard deviation; *p* value in the Tukey test).

The Microtox test showed that, in most cases, the reduction in the luminescence was not statistically significant in relation to the control (**Table 5**). There were no differences according to the soil type at 1 and 4 weeks of incubation period. In the last incubation period (8 weeks), sample M4 showed significant differences in relation to the control and to the other samples, and the lowest value in the reduction of the luminescence indicated that the soil solution of this sample stimulated bacterial activity (hormesis).

Table 6 presents the trace-element concentrations, pH, electrical conductivity (EC) of the soil solutions, and the percentage of reduction of *Vibrio fischeri* (VfR) and *Lactuca sativa* (LsR) bioassays in relation to the control. There were strong differences in the VfR and LsR for the same sample in all incubation periods. The percentage of reduction was moderate to low, with maximum reduction of 30% for M2 sample at 4 weeks of incubation and positive reduction values, indicating hormesis. At 8 weeks of incubation, the VfR and LsR values indicate negligible contamination in all studied samples.

Table 5. Percentage of reduction in luminescence at 15 min (Red 15) of *Vibrio fischeri* in the three incubation periods

	1 Week		4 Weeks		8 Weeks	
	Red 15	sd	Red 15	sd	Red 15	sd
R1	69.26	5.80	58.43	6.71	58.13a	1.56
M2	65.56	1.82	68.33	11.76	65.04a	1.34
M3	59.62	2.59	64.89	11.44	59.24a	2.60
M4	57.71	2.43	53.80	7.41	42.15b	1.55
Control	58.64	7.14	54.56	6.59	57.94a	7.14
p value	0.52		0.44		0.01	

(sd: standard deviation; *p* value in the Tukey test).

Table 6. pH, electrical conductivity (EC; dS m^{-1}), soluble trace-element concentrations (ppm) of soil solutions and % reduction in *Vibrio fischeri* (VfR) and *Lactuca sativa* (LsR) bioassays in relation to the control

1 Week	pH	EC	As	Mn	Ni	Cr	Zn	Pb	Co	Cu	VfR	LsR
R1	7.71	0.17	0.003	0.002	0.033	0.003	0.005	bdl	bdl	0.007	-26	-5
M2	6.99	0.11	0.026	0.006	0.002	0.001	0.033	0.003	bdl	0.006	-17	-8
M3	8.39	0.21	0.028	0.002	0.001	0.001	0.008	bdl	bdl	0.005	-2	-4
M4	7.89	0.27	0.019	0.003	0.010	0.001	0.071	bdl	0.001	0.005	+2	-12

4 Weeks	pH	EC	As	Mn	Ni	Cr	Zn	Pb	Co	Cu	VfR	LsR
R1	7.48	0.14	0.003	0.037	0.140	0.045	0.026	0.001	0.004	0.006	-9	-10
M2	6.48	0.10	0.013	0.006	0.002	0.002	0.018	0.002	bdl	0.003	-30	-8
M3	8.67	0.22	0.020	bdl	0.001	0.001	0.004	bdl	bdl	0.004	-23	-7
M4	7.74	0.23	0.009	0.002	0.007	0.001	0.031	bdl	bdl	0.005	+15	-15

8 Weeks	pH	EC	As	Mn	Ni	Cr	Zn	Pb	Co	Cu	VfR	LsR
R1	7.31	0.09	0.003	0.020	0.089	0.026	0.013	bdl	0.003	0.006	+1	-1
M2	7.03	0.09	0.007	0.004	0.001	0.001	0.011	0.001	bdl	0.003	-15	-3
M3	7.49	0.21	0.018	bdl	bdl	0.001	0.006	bdl	bdl	0.002	-1	+1
M4	7.62	0.23	0.007	0.002	0.005	bdl	0.026	bdl	0.001	0.005	+40	0

(bdl: below detection limits).

The correlation analysis (Spearman) with the variables in **Table 6** showed no significant relationships between the percentage of reduction and the trace-element concentrations. Low correlation was found between the two bioassays, indicating that the use of these tests in moderate to low contaminated samples can be carefully addressed because of the different relative sensitivities of each test organism (Domene et al. 2008; González et al. 2011). In any case, in our selected soils spiked with 100 ppm of As strongly reduced the solubility of this element, lowering in most cases the toxicity below the detection levels of the bioassays used in this study.

3.2. Spiked water solutions

The lettuce-germination test using the spiked solutions showed significant differences with regard to the As concentration. The seed germination (SG) was higher than 95% in all cases (even at highest concentrations of 50 and 100 ppm), with no significant differences according to the control. This indicates that the seed-germination index was not a good endpoint for the As concentration range used in this study. The root elongation showed a significant reduction with the increase of As concentration in the solutions (**Figure 2**).

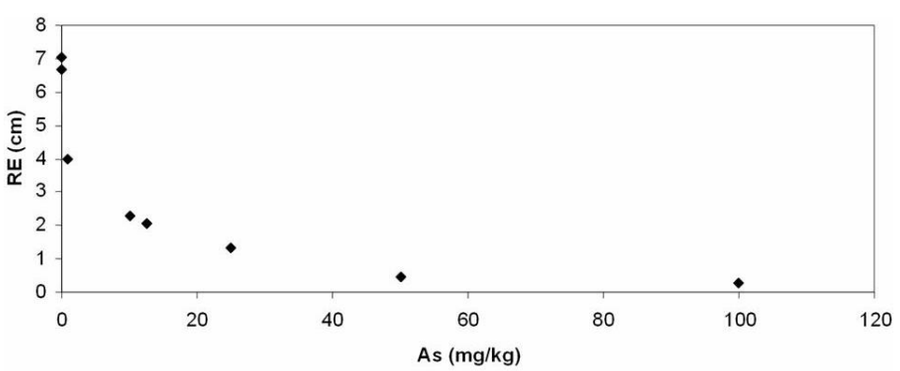

Figure 2. Root elongation (RE) in relation to the As concentration of the spiked solutions.

The percentage of reduction in the root elongation in relation to the control (distilled water) showed a significant decrease of 12% for the 0.1 ppm As concentration, and was up to 48% for the solution spiked with 1 ppm of As; the maximum reduction of 96% was reached by the solution spiked with 100 ppm of As. The Tukey test showed no statistically significant differences between the concentrations of 10 and 12.5 ppm As, and for the spiked samples above 50 ppm As. Among all the other As concentrations, the differences in root elongation were statistically significant ($p < 0.001$).

The Microtox test showed a significant reduction of luminescence with the increase of As concentration in the solutions (**Figure 3**). Values close to 60% of reduction were recorded for the solution spiked with 10 ppm As, and a slight (4%) but not significant reduction in relation to the control was measured for the 1-ppm concentration. The stimulating effect (hormesis) of the luminescence was detected for the 0.1 ppm As solution.

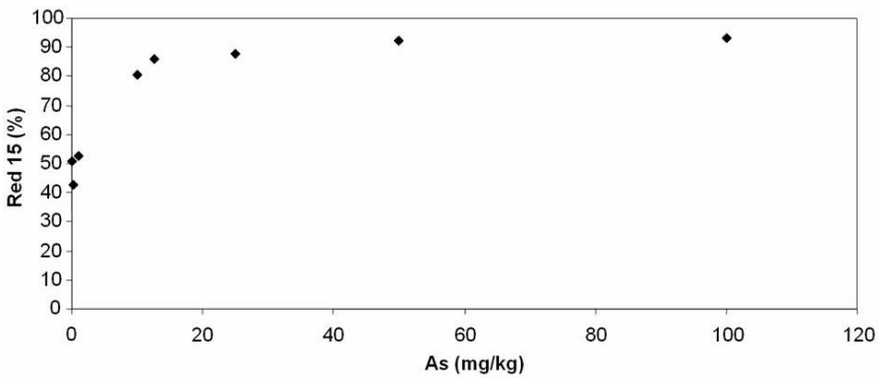

Figure 3. Percentage in reduction of luminescence (Red 15) in Microtox test in relation to the As concentration of the spiked solutions.

The reduction in luminescence presented statistically significant differences in relation to the control (**Table 7**). The Tukey test showed no significant statistical differences above concentrations of 10 ppm of As, whereas for the concentration of 0.1 ppm a significant stimulation of the luminescence was detected, with lower reduction (positive value) in relation to the control.

Table 7. Percentage of *Vibrio fischeri* luminescence reduction in relation to the control (VfR) at 15 min

As (ppm)	0	0.1	1	10	12.5	25	50	100
VfR	0 *a*	+16 *b*	-4 *a*	-59 *c*	-69 *c*	-73 *c*	-82 *c*	-83 *c*

(letters in italics indicate significant differences according the Tukey test, $p < 0.05$).

The use of the EC50 is a commonly used endpoint for the comparative assessment of the toxicity results. The determination of the EC50 threshold constitutes one of the most commonly used values for ecotoxicity tests (Isnard et al. 2001). The EC50 values in spiked water solutions (**Table 8**) were obtained from dose-response curves by a log-logistic model (Doelman and Haanstra 1989). The EC50 value of As for the *Lactuca sativa* and *Vibrio fischeri* bioassays was 1.52 ppm and 4.98 ppm, respectively. These values are slightly more toxic in the case of *Lactuca sativa* compared with the EC50 value of 2.3 ppm reported by Vaughan and Greensdale (1998) in spiked water solutions. In the case of *Vibrio fischeri*, the EC50 values are within the range of the threshold (5.7 ppm) reported by Fulladosa et al. (2004) for solutions with pH 8. In all cases, including this study, these reference values refer to a specific arsenate form, although the change to arsenite forms and the influence of the pH of the solutions should be carefully studied to gain comparative results.

Table 8. EC50 values in the contaminated solutions. CI: Confidence interval at 95%

	EC50 (ppm)	
	Value	95% CI
Lactuca sativa	1.52	1.27 – 1.83
Vibrio fischeri	4.98	3.67- 6.76

4. Conclusions

Soils spiked with 100 ppm of arsenic strongly reduced the solubility of this element, in most cases lowering the toxicity to negligible levels for the *Lactuca sativa* and *Vibrio fischeri* bioassays. Otherwise, arsenic solubility in laboratory-spiked soils changed over time, reducing its mobility by between 22 to 74% after eight weeks of incubation. These results are therefore only valid for the soils studied and in relation to their soil properties; they should be carefully interpreted when applied to field-contaminated soils in other areas.

The toxicity assessment in water solutions indicated that *Lactuca sativa* was more sensitive to As concentrations than *Vibrio fischeri*. The EC50 values of As in spiked water solutions for the *Lactuca sativa* and *Vibrio fischeri* bioassays were 1.52 ppm and 4.98 ppm, respectively.

Further studies need to be carried out with different forms of arsenic to determine the influence of the pH of the solutions and its content in amorphous components. In addition, organic matter in the soil should be addressed to gain comparative results for other soil types.

5. Acknowledgements

We express our gratitude to the Spain´s Ministry of Science and Innovation for supporting this study (Project CGL 2010-19902). Furthermore, we would like to thank David Nesbitt for correcting the English version of the manuscript.

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