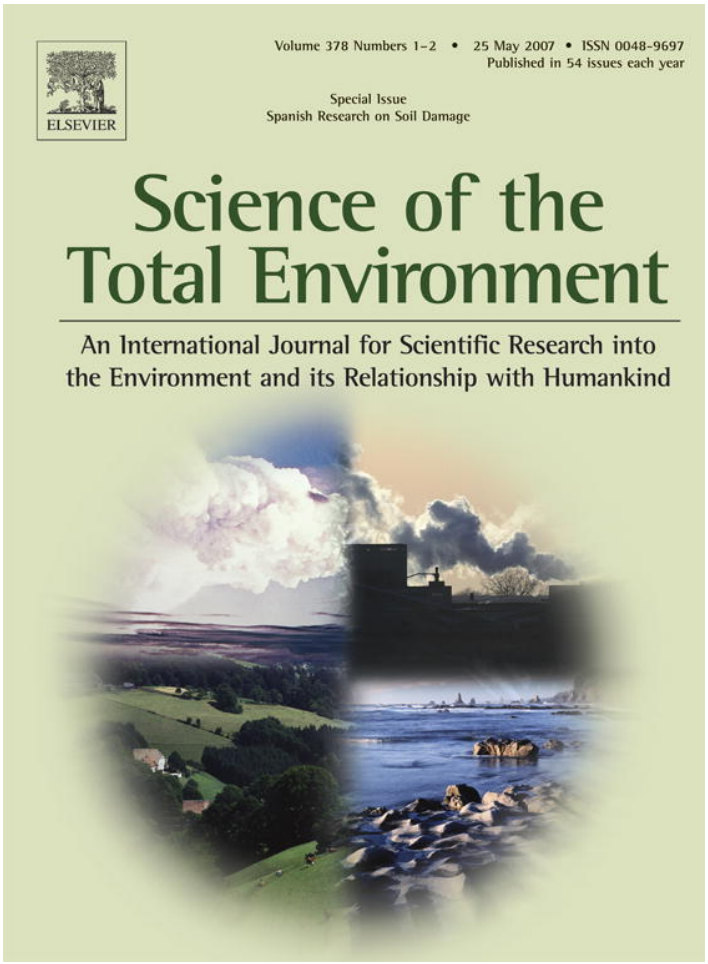

Available online at www.sciencedirect.com
ScienceDirect
Science of the Total Environment 378 (2007) 63–66
Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind
www.elsevier.com/locate/scitotenv

# Determination of phytotoxicity of soluble elements in soils, based on a bioassay with lettuce (*Lactuca sativa* L.)

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Available online 20 February 2007

**Abstract**

In this work the different concentrations of soluble elements in soils from natural (peridotitic soils) and anthropogenic (soils affected by a pyrite-mine spill) origin, are used to determine the phytotoxicity in lettuce (*Lactuca sativa* L.). The solutions are obtained from soil:water extracts (1:1), having neutral pH and high concentrations of As, Pb, Zn, Mn, Co and Ni, with values exceeding the toxic level for soil solution [Bohn HL, McNeal BL, O'Connor GA. Soil Chemistry, Wiley Interscience. Wiley & Sons, New York, 1985]. The variables evaluated are: Seed Germination (SG), Root Elongation (RE), Germination Rate (GR) and Root Necrosis (RN). The most sensitive variables in the bioassay with these solutions are GR and RN, in these cases the solution causes a reduction of 44% and 67%, respectively, in relation to control (distilled water). The test using soil–water solutions is sensitive and reproducible to determine phytotoxicity in lettuce caused by potentially pollutant elements in soils.


*Keywords:* Soil–water solution; Phytotoxicity; Soluble elements; *Lactuca sativa*

## 1. Introduction

The presence of toxic agents can be detected by the changes caused in an organism with the use of bioassays. These tests are reliable, cost effective, quick, and simple (Gustavson et al., 2000). Bioassays can also be used to measure potential environmental risks (Gopalan, 1999). The use of plants offers an advantage over other organisms because they can be more sensitive to environmental stress (Dutka, 1989), they are easy to manipulate and store, and furthermore, they offer a low-cost and good correlation in contrast with other bioassays (Fiskesjö, 1993).

Torres (2003) defined the bioassay with lettuce seeds as static and of acute toxicity, where the phytotoxic effects of a pure compound or a complex mixture in the germination of seeds and in the development of the seedlings was evaluated during the first few days of growth (120 h of exposure). Thus, the germination rate is also a variable to measure when seed viability and germination tests are conducted (Enríquez-Peña et al., 2004; Vadillo et al., 2004). The aim of the present work is to evaluate the sensitivity of lettuce in Seed Germination (SG), Root Elongation (RE), Germination Rate (GR) and Root Necrosis (RN) when lettuce is exposed to different concentrations of soluble elements in soil–water solutions, as an alternative way to determine soil toxicity.

## 2. Material and methods

Four soil samples with different element concentrations were used. Two were from soils under natural

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doi:10.1016/j.scitotenv.2007.01.007

Table 1
Main soluble-element concentrations (mg $l^{-1}$) and pH of the soil–water solutions (1:1). Mean values of three replicates (brackets: standard deviation; bold: values exceeding the toxic level according toBohn et al.,1985)

| Sample | pH | As | Pb | Zn | Mn | Co | Ni |
|---|---|---|---|---|---|---|---|
| 1V | 7.55 (0.08) | 0.035 (0.004) | **0.043** (0.005) | **0.198** (0.012) | 0.329 (0.014) | 0.003 (0.001) | 0.049 (0.007) |
| 2V | 6.88 (0.06) | **0.090** (0.007) | **0.055** (0.008) | **0.366** (0.011) | 0.781 (0.022) | **0.048** (0.005) | **0.110** (0.013) |
| 3P | 7.59 (0.09) | 0.003 (0.001) | 0.004 (0.001) | nd | 0.334 (0.011) | **0.023** (0.002) | **0.666** (0.027) |
| 4P | 7.18 (0.07) | 0.003 (0.001) | 0.003 (0.001) | nd | 0.337 (0.018) | **0.019** (0.002) | **0.387** (0.019) |

nd: not detected.

conditions, located in the Verde River Basin, Málaga (southern Spain), characterized by peridotitic material (hereafter Peridotitic Zone); and two were from soils under human influence, located in the Guadiamar River Basin, Sevilla (south-western Spain), affected by the Aznalcóllar pyrite-mine spill (hereafter Spill Zone). In all samples, a soil–water solution (1:1) was obtained by a vacuum-extraction pump after 24 h of contact. In the solutions, pH was measured potentiometrically and the soluble elements were determined by ICP-MS in a Perkin Elmer Elan 5000 spectrometer.

The methodology used in the experiment was recommended by the EPA (1996),OECD (2003) and Torres (2003). The experiment consisted in 4 samples (1V, 2V from the Spill Zone; and 3P, 4P from the Peridotitic Zone), 3 treatments per sample by diluting the initial solution (T1 = 1:1; T2 = 1:2 and T3 = 1:4), one control (distilled water) with 3 respective replications, for a total of 39 experimental units. For each unit, 25 seeds were placed in a 90 mm diameter polyethylene containers, with filter paper in the bottom as the support; afterwards, 5 ml of the different solutions were applied. The experiment was kept in a MEMERT DIN 40050-IP-20 incubator for 120 h at 24 ± 0.1 °C.

The following parameters were analyzed: (1) Seed germination (SG), corresponding to the percentage of germinated seeds after the experiment; (2) Root elongation (RE), calculated by the equation $RE=(M-C)/C$, where $M$ is the average elongation per treatment and $C$ is the average elongation of the control. Thus, when $RE=0$, there is no toxicity; if $RE<0$ the sample is toxic; and if $RE>0$ elongation is stimulated (Dutka, 1989). (3) Germination rate (GR), defined as the relationship between the number of seeds germinated and the germination time (González-Zertuche and Orozco-Segovia, 1996), and calculated by the equation $GR=\Sigma\,(n_i)/t$, where $n_i$ is the number of seeds germinated per day, and $t$ is the germination time from seeding to the germination of the last seed (GR was measured each day for three days). (4) Root necrosis (RN), defined as the percentage of the affected seeds in relation to the total; in this case, RN refers both to the root system and to the hypocotyls. In all cases, the reduction of 10% and 50% of these parameters in relation to the control were indicated.

## 3. Results and discussion

The analysis of the solutions (1:1) (Table 1), had a pH close to neutrality (6.88–7.59), exceeding the toxic levels in Pb, As, Zn, Co and Ni (Bohn et al., 1985) in most cases.

### *3.1. Seed germination (SG) and root elongation (RE) of lettuce*

The soluble elements of the samples from the Spill Zone (1V and 2V) caused no negative effects on SG, as opposed to the samples from the Peridotitic Zone (Table 2). In the latter zone, SG tended to diminish as the concentration of soluble elements in the samples increased; in this case, the toxic effect was negligible

Table 2
Seed germination (SG), root elongation (RE) and root necrosis (RN) in the control and in the solutions of the samples

| | | SG (%) | | RE | | RN (%) | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD |
| Control | | 97 | 1.2 | 0 | 0 | 0 | 0 |
| 1V | 1:1 | 96 | 1.8 | −0.307 | 0.021 | nd | – |
| | 1:2 | 97 | 1.3 | −0.127 | 0.099 | nd | – |
| | 1:4 | 97 | 1.6 | −0.029 | 0.042 | nd | – |
| 2V | 1:1 | 96 | 1.5 | −0.061 | 0.042 | nd | – |
| | 1:2 | 97 | 1.3 | −0.049 | 0.027 | nd | – |
| | 1:4 | 96 | 1.4 | −0.004 | 0.006 | nd | – |
| 3P | 1:1 | 91 | 2.1 | −0.422 | 0.128 | 67 | 4.2 |
| | 1:2 | 95 | 1.9 | −0.123 | 0.071 | 49 | 2.1 |
| | 1:4 | 97 | 1.4 | −0.053 | 0.042 | 24 | 1.8 |
| 4P | 1:1 | 84 | 2.8 | −0.336 | 0.099 | 67 | 3.2 |
| | 1:2 | 93 | 2.2 | −0.250 | 0.119 | 59 | 2.0 |
| | 1:4 | 95 | 1.8 | −0.160 | 0.082 | 52 | 1.9 |
| 10% red. | | 87 | | −0.098 | | 10 | |
| 50% red. | | 48 | | −0.500 | | 50 | |

nd: not detected.
(SD = Standard deviation. 10% red.; 50% red. = reduction of 10% and 50% in relation to the control).

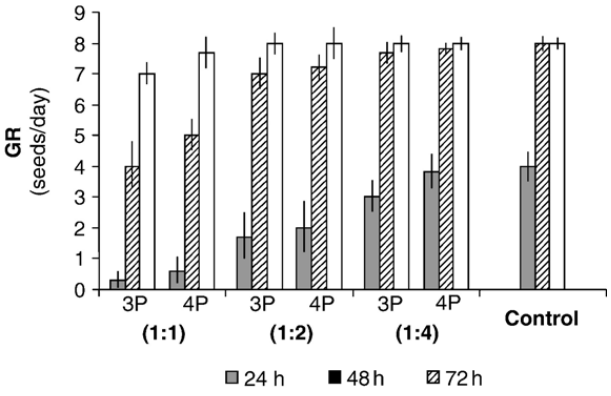


Fig. 1. Germination rate (GR) of lettuce seeds in samples of the Peridotitic Zone in relation to Control (distilled water). (Error bars representing standard deviation).

for the samples of the Spill Zone and minimum in the Peridotitic Zone (only the 1:1 solution in sample 4P caused a reduction greater than 10% in relation to control).

In all cases, RE presented negative values when lettuce seedlings were exposed to the different solutions, providing evidence of the toxic effect (Table 2). According to the values necessary for a reduction of 10% and 50% in relation to the control indicated in this table, the sample 4P presented a moderately high toxic effect in the lettuce seedlings (the values corresponding to a reduction of 10% were reached at higher dilutions than 1:4; and the reduction of 50% were close to a 1:1 solution); the samples 1V and 3P presented a moderately toxic effect (the values corresponding to a reduction of 10% were reached between the dilutions 1:2 and 1:4), while the sample 2V had a minimum phytotoxic effect (the values corresponding to a reduction of 10% were reached at concentrations higher than 1:1).

### *3.2. Germination rate (GR) and root necrosis (RN) in lettuce seedlings*

Fig. 1 shows the distribution of GR in the different treatments measured at 24, 48 and 72 h, for the samples of the Peridotitic Zone. GR clearly tended to rise with increased dilution values, and the phytotoxic effect was clearer during the first 24 h (stronger differences in relation to the control); in this way, the germination was completed for the control at 48 h, while for the samples analysed it was completed at 72 h in all dilutions, with the exception of sample 3P (1:1), which showed a higher phytotoxic effect for this parameter.

In the case of RN, samples of Peridotitic Zone presented phytotoxic effects, causing root necrosis in the lettuce seedlings. In both cases, the solution 1:1 caused the highest RN value (67%) in relation to the control, and the dilutions reduced the values, less intense in sample 4P.

## 4. Conclusions

The methodology proposed in this work is applicable in the measurement of the phytotoxicity in lettuce exposed to soluble elements coming from soil–water extracts, as an alternative way to determine soil toxicity. The method is inexpensive, quick, and reproducible.

The concentrations of soluble elements in the solutions, exerted a stronger effect on RE than on SG in lettuce, presumably due to the relatively low concentrations of the solutions used. The treatment made from a sample can have different effects on germination or root elongation in lettuce, and the definition of toxicity of the sample would correspond to the variable with the highest toxicity value registered. In this case, the most sensitive were GR and RN, causing a reduction of 44% and 67%, respectively, in relation to the control (distilled water).

The test developed enables the identification of different levels of phytotoxicity in the samples, helping to characterize areas with contaminated soils where the high concentrations of soluble elements are caused by human activity or natural conditions.

## Acknowledgement

We thank Mr. David Nesbitt for correcting the English version of the manuscript.

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