Toxicity assessment using *Lactuca sativa* L. bioassay of the metal(loid)s As, Cu, Mn, Pb and Zn in soluble-in-water saturated soil extracts from an abandoned mining site

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Abstract

Purpose We used the different soluble-in-water concentrations of As, Cu, Mn, Pb and Zn from contaminated soils in an abandoned mining area (anthropogenic origin) to assess the phytotoxicity of the abandoned site using the results obtained with a *Lactuca sativa* L. bioassay.

Material and methods The study has been carried out on potentially polluted samples from the Rodalquilar mining district (southern Spain). The area was sampled according to the different metallurgical treatments for gold extraction used in each one: dynamic cyanidation and heap leaching. The saturation extracts were obtained by filtering each saturated paste with a vacuum-extraction pump, in which measurements of metal(loid) concentrations, pH and electrical conductivity were made. The variables evaluated in the bioassay, defined as toxicity indices ranging from -1 (maximum phytotoxicity) to >0 (hormesis), were seed germination (SG) and root elongation (RE) of lettuce seeds.

Results and discussion In areas with a low degree of contamination, the most sensitive toxicity index is RE, whereas in highly contaminated areas, both RE and SG are good estimators of soil toxicity. According to these results, samples from the western area showed moderate to low toxicity, which was closely related to water-soluble As concentrations. Samples from the eastern area had a high degree of toxicity in 40% of the soils.

Conclusions The comparison of the two indices (SG and RE) defined using the *L. sativa* L. bioassay indicates that, for areas with a low degree of contamination, the most sensitive toxicity index is RE, whereas in highly contaminated areas, both RE and SG are good estimators of soil phytotoxicity. Unsupervised pattern recognition methods such as HCA and PCA enabled us to conclude that the low/moderate phytotoxicity of the soils is related to the extraction process used for the recovery of gold (mainly dynamic cyanidation in tanks located in the eastern area) and to the As and Pb contents.

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1 Introduction

Metalliferous mining areas and the metallurgical operations carried out in them are considered to be one of the main

sources of metal(loid) contamination in soils (Alloway 1995). The abandonment of the mine leads to decay over time, especially in places where waste is accumulated (abandoned cavities, mineral dumps, tailing ponds, leaching piles, etc.), thereby favouring the transport of potentially pollutant particles by means of meteorological agents such as wind or rainwater. This is a significant source of contamination in soils surrounding the mines and alluvial soils downstream.

The Rodalquilar mining district (Almería, SE Spain) is an abandoned mining area in which gold and other metal (Pb–Zn–Cu–Ag–Au) bearing minerals were exploited, mainly in the form of sulphides or native elements. Rodalquilar was exploited for different ores (Pb, Zn, Cu, Mn, Au) at different times (Hernandez 2005). The main ore mined in the area was Au (from 1864 to 1990), and the method used to extract the gold from the ore changed over time. Initially, the process involved amalgamation with Hg in situ or close to the mine entrance (until 1930). This method was replaced with a process using cyanidation tanks (1930–1970, approximately), and in the last phase of mining activity, heap leaching was used (1990–1991). As a result of this activity, the site contains two large tailings, situated in the eastern area (waste from the cyanidation tanks process) and in the western part (from the heap leaching process). Arsenic is another element that is strongly associated with the primary ore mineralogy of the El Cinto deposits. Although this element is not particularly important in terms of its abundance, it appears in a wide variety of toxic mineralogical expressions in this mining district and is consequently an important element to be considered from an environmental point of view.

Soil is an essential part of the ecosystem and one of the most frequent media to receive contamination (Martín et al. 2008). When contaminants get into the soil, they can be stored, transformed or transferred to other media. Soils also have a very high response capacity in relation to other ecosystem elements, such as water, air and/or living organisms. The presence of potentially contaminant elements in soils therefore means that a thorough environmental risk assessment (ERA) must be performed.

Soil contamination studies are becoming an increasingly important part of ERA. In ERA processes, there are several predictive and descriptive phases (Rutgers et al. 2001) with special relevance for the toxicity characterization of contaminated media. In these phases, many different toxicity assays can be applied to the study of contaminated soils (Sheppard et al. 1992; Tarradellas et al. 1997; Fernández et al. 2006; Parvez et al. 2006; Boekhold 2008; Thomas et al. 2009). In addition, for the protection of ecosystems, the use of toxicity bioassays has become a key factor in supporting the regulatory framework regarding the declaration of contaminated soils (Tarazona et al. 2005).

Most bioassays applied to contaminated soils are based on the evaluation of the toxic effect of the soil solution or of the soil itself on a living organism (animals, algae, plants and bacterial bioassays). Plant biomarkers in particular offer a variety of advantages, such as a large array of assessment endpoints (germination rate, biomass weight, enzyme activity, etc. (Ferrari et al. 1999)). Although some authors (Farré and Barceló 2003) have stated that tests based on the growth response of plants require a long time (4–6 days) for root-length measurements, the bioassay using Lactuca sativa L. (lettuce) studies is considered a simple, quick and sensitive way of evaluating potential environmental risk (Gopalan 1999) and can be successfully applied in soil with a high concentration of heavy metals of natural and anthropogenic origin (Inaba and Takenaka 2005; Escoto et al. 2007). The test is based on an analysis of the phytotoxic effects of contaminants in the germination phase of the seeds and in the development of the seedlings during the first 5 days of growth (120 h of exposure).

The aim of this work was to evaluate the phytotoxicity associated with soluble-in-water concentrations of As, Cu, Mn, Pb and Zn in metal-contaminated soils from an abandoned mining area by applying unsupervised pattern recognition techniques such as hierarchical agglomerative cluster analysis (HCA) and principal component analysis (PCA) to the normalized data set obtained from the L. sativa L. bioassay.

2 Materials and methods

2.1 Sampling

The area (Fig. 1) is characterized by the presence of small hills composed of different volcanic materials. The main area of mining activity was Cerro del Cinto. These hills are surrounded in the lower areas by porous sediments produced by the erosion of the adjacent reliefs. In the eastern part of this area (Rodalquilar Valley), some small rivers or streams descend through the tailing pond where the wastes produced in the process of gold extraction by dynamic cyanidation in tanks were left abandoned. These watercourses behave in a particular way due to the arid climate of the area. Normally, they have very little or no water, but occasionally, during periods of strong rains, they reach high levels, producing torrents and overflows. They carry the sediment from the tailing pond downstream across the valley. In the western area, a pile of waste (closely related to the heap leaching process used for gold extraction) still remains today near agricultural land. The often windy weather (wind erosion) and the arid climate mentioned above can carry the sediments from the pile across the basin.

A set of 19 soil samples (12 from the eastern area and seven from the west) were selected for this study (see
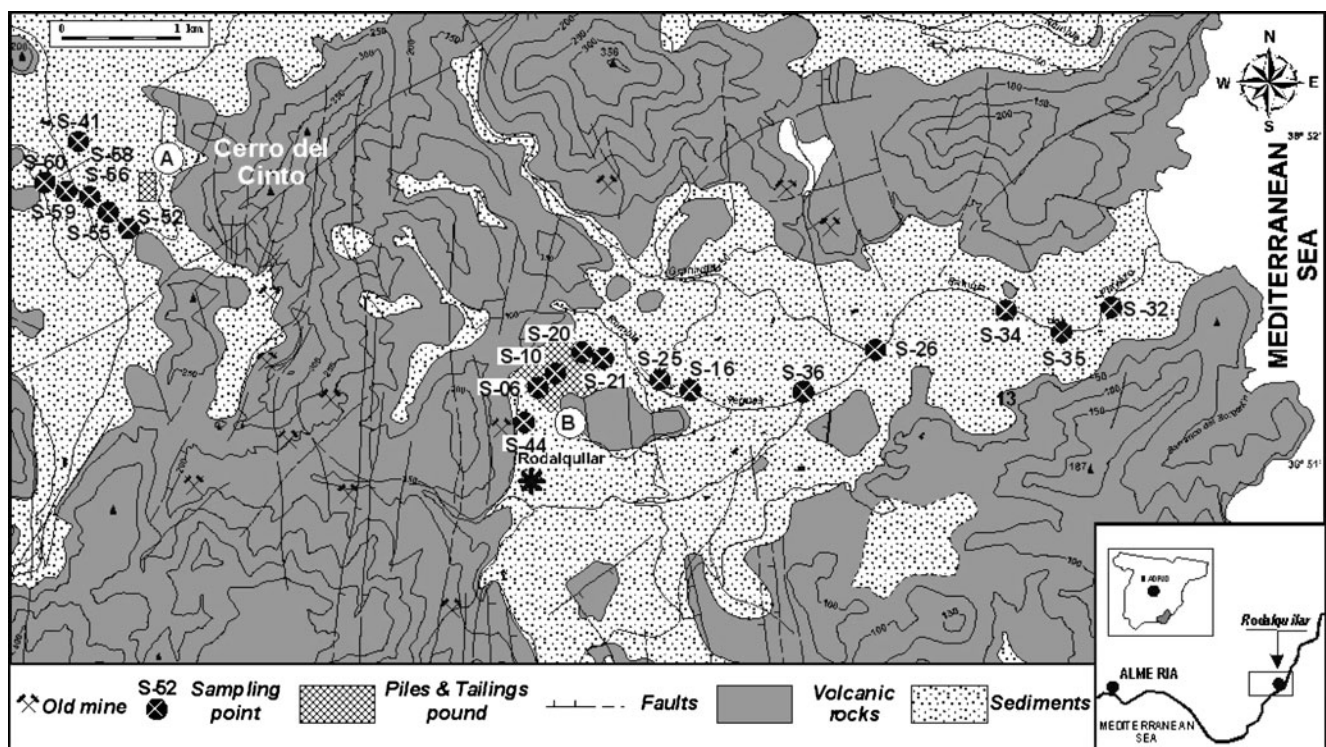
Fig. 1 Location of the study area and sampling point distribution

Fig. 1), taking into account their geological context and the mining activities that were carried out in each place. The soil samples were collected at depths of between 0 and 10 cm and located in the field by GPS. In order to determine the geological background of the soils in the area, three soils were sampled every 10 to 50 cm in depth. Samples were sieved in situ to 2 mm; the gravels (>2 mm) and fine earth (<2 mm) were separated and then weighed in the laboratory, and the percentage of each fraction was calculated. All the analyses were made with the fine-earth fraction thoroughly homogenized. Samples were placed in plastic bags. The sampling process was completed with an "in situ" screening study using a portable fluorescence X-ray analyzer.

2.2 In situ estimation of metal(loid) content of soils and geochemical background

In order to estimate these analytical parameters (Table 1), we used a field portable X-ray fluorescence (PXRF) analyzer NITON XLt 792 (Niton, Billerica, USA) with a 40-kV X-ray tube with a Ag anode target excitation source and a Silicon PIN diode with a Peltier-cooled detector. Measurements were performed according to the manufacturer's instructions and the Method 6200 (US Environmental Protection Agency (US EPA) 1996). As part of the standard setup routine, the analyser was initially calibrated using the silver and tungsten shielding on the inside of the

shutter, and the source count analysis was fixed at 90 s. In all cases, the PXRF measurements were made through the bags. Prior to the field campaign, a quality control of the analyser was made in the laboratory (Martín Peinado et al. 2010).

An essential part of our study was to establish the geochemical background of the area. This was done in order to define the presence of anomalies or potentially contaminated soils in the area. In order to avoid the influence of potential contamination by surface processes (runoff water or wind erosion), the samples of the uppermost 20 cm were discarded.

2.3 Saturation extract

In all the samples, a freshly saturated paste of soil was prepared by mixing 50 g of soil sample with deionized water for 24 h. The saturation extract was obtained by filtering with a vacuum-extraction pump. In all the cases, an aliquot of this paste was used to measure the moisture percentage by heating in a laboratory oven for 24 h (Selecta, Barcelona, Spain) at $105 \pm 1°C$.

2.4 Analytical parameters of saturation extracts

– Measurements of pH. The pH values of the saturation extracts were measured potentiometrically using an XS instruments pH 510 (LabProcess, Barcelona, Spain).

Table 1 Analytical parameters

Samples	Metal(loid) concentration in soil samples (over ng/g)					Metal(loid)s concentration in saturation extracts of soil samples (ng/g)					Physicochemical Parameters		
	As	Cu	Mn	Pb	Zn	As	Cu	Mn	Pb	Zn	pH	EC_a	Salinity
Eastern zone													
S-6	139,150	35,580	339,570	52,340	127,260	137.6	30.3	52.3	2.6	29.8	7.81	2.86	1,401.4
S-10	336,040	51,420	263,600	10,165	105,300	189.6	38.6	4.3	1.2	31.3	8.16	2.64	1,293.6
S-16	572,540	55,170	105,590	300,390	72,270	476.6	17.2	11.0	3.4	48.6	7.67	1.56	764.4
S-20	427,790	38,380	117,280	129,080	79,370	657.2	22.7	41.3	14.7	123.1	6.51	11	5,390
S-21	395,370	58,650	123,970	164,290	81,550	1129.3	21.0	6.1	22.6	46.8	7.04	1.5	735
S-25	599,570	66,270	191,460	341,460	94,910	416.2	21.0	53.4	3.8	67.2	7.5	2.64	1,293.6
S-26	368,650	62,840	313,700	253,180	89,130	166.7	17.9	17.8	4.3	50.9	7.37	1.3	637
S-32	353,840	91,210	426,860	411,220	185,740	554.3	113.3	44.0	3.0	34.3	8.32	4.83	2,366.7
S-34	281,400	55,060	246,800	227,140	108,290	250.2	17.1	7.8	7.4	25.3	7.6	1.35	661.5
S-35	203,440	90,870	399,080	337,050	155,670	327.0	96.1	47.1	8.9	55.5	8.17	4.83	2,366.7
S-36	512,680	72,610	385,640	569,120	134,950	630.8	48.3	22.6	142.3	199.3	7.04	1.32	646.8
S-44	66,630	33,050	486,930	97,390	105,650	31.5	47.0	14.9	1.6	16.6	7.95	2.42	1,185.8
Western Zone													
S-41	89,790	34,670	835,300	107,960	201,350	215.9	158.7	995.4	152.1	651.2	8.47	25	12,250
S-52	331,420	34,870	125,620	440,950	48,000	410.2	122.5	54.9	4.9	35.8	8.85	13.5	6,615
S-55	98,820	29,090	806,500	190,900	117,980	16.2	33.4	57.9	11.6	59.3	8.13	2.86	1,401.4
S-56	100,990	42,730	864,760	174,850	166,790	92.3	159.9	321.6	3.2	46.5	8.62	11.5	5,635
S-58	83,960	45,690	725,480	178,210	133,190	72	149.8	428.7	0.8	66.8	7.8	18	8,820
S-59	49,330	38,510	855,370	117,500	104,700	33.8	52.5	209.2	49.8	94.2	8.17	3.19	1,563.1
S-60	62,810	71,790	806,740	137,500	151,180	65	88.7	105.2	1.5	152.8	8.06	27	13,230

– Electrical conductivity (EC) of each saturation extract was measured in a Crison 522 conductimeter (Crison, Barcelona, Spain). In order to obtain the best estimate of salinity (S) from EC (Simón et al. 1994), aliquots of saturation extracts were diluted with progressively larger quantities of distilled water until the EC of the diluted extracts ranged between 0.1 and 0.3 dS m^{-1}. Then, adjusted electrical conductivities (EC_a) were estimated according to Eq. 1:

$$EC_a = (EC_d - EC_w)f \tag{1}$$

where EC_d is the conductivity of the diluted saturation extract, EC_w is the conductivity of deionized water and f is the dilution factor applied. Finally, S was calculated by multiplying the EC_a obtained by the factor 490 according to Simón et al. (1994). This expression can be used in all saturation extracts, regardless of the concentration and type of ions present. –Metal(loid) content

The concentrations of As, Cu, Mn, Pb and Zn in saturation extracts of soils were determined by inductively coupled plasma–mass spectrometry (ICP–MS). The instrument used was a Perkin-Elmer Sciex-Elan 5000 ICP–MS (Perkin-Elmer, Überlingen, Germany) equipped with quartz torch, nickel sampler and skimmer cones, a peristaltic pump (maintaining a 1 mLmin^{-1} sample uptake rate), a cross-flow-type pneumatic nebulizer and a double-pass Scott-type spray chamber. The ICP–MS operating conditions used were RF power of 1,000 W, plasma argon of 15 Lmin^{-1} and flow of nebulizer of 0.9–1 Lmin^{-1}. The data acquisition system was through peak hop transit under normal resolution. The dwell time was 50 ms, and there were three replicates.

All ICP–MS standards were prepared from ICP single-element standard solutions (Merck, Darmstadt, Germany) after appropriate dilution with 10% HNO$_3$. For calibration, two sets of multi-element standards containing all the analytes of interest at five different levels of concentration were prepared using rhodium as the internal standard (based on the repeatability variation of the measurements).

2.5 *L. sativa* L. bioassay

This bioassay was applied to soil samples according to the US EPA (1996) procedure, i.e. seed germination (SG)/root

elongation (RE) acute toxicity test, OPPTS 850.4200. This test was applied because the toxicity related to the soluble-in-water fractions of contaminants reflects the behaviour of the most mobile fraction of pollutants and is strongly related to the high risk of dispersion, solubilization and bioavailability of contaminants in the environment.

The *L. sativa* L. seeds were purchased in a local nursery. For each sample, two replicates of 15 seeds were placed in 90-mm-diameter Petri dishes containing filter paper in the bottom as support. Five millilitres of saturation extract was then added, and the dishes were placed in a Memmert Din 40050-IP-20 incubator (Memmert, Bundesrespublik Deutschland, Germany) for 120 h at $24\pm0.1°C$. A blank control was prepared in the same fashion, replacing the 5 mL of saturation extract with deionized water. Finally, the number of seeds that had germinated was counted, and the length of the roots of the germinated seeds was measured.

The data were used to establish two toxicity indices:

– SG, calculated by Eq. 2: In statistical terms, this represents the normalized residual percentage of germinated seeds after the experiment.

$$SG = \frac{Germ_{sample(i)} - Germ_{control}}{Germ_{control}} \quad (2)$$

where $Germ_{sample(i)}$ is the average number of germinated seeds in saturation extract i (%), and $Germ_{control}$ is the average number of germinated seeds in the blank control (%).

– RE, calculated by Eq. 3: In statistical terms, this represents the normalized residual elongation of the root of the germinated seeds per treatment.

$$RE = \frac{Elong_{sample(i)} - Elong_{control}}{Elong_{control}} \quad (3)$$

where $Elong_{sample(i)}$ is the average length of the seed roots in the saturation extract i (cm), and $Elong_{control}$ is the length of the seed roots in the blank control (cm).

The indices are designed in such a way that their values can vary from −1 (maximum phytotoxicity) to >0. In addition, if there is a reduction of 50% in the variable studied (SG or RE) in relation to the blank control (SG_{50} o RE_{50}), this is considered as a measurement of potential "chronic toxicity". Although test results suggest that the samples are suffering "acute toxicity", the term "chronic" is used because of the long-term influence of these pollutants on the environment. This enabled us to establish the following scale: (a) 0 to −0.25 low toxicity, (b) −0.25 to −0.5 moderate, (c) −0.5 to −0.75 high and (d) −0.75 to −1 very high. RE values >0 would indicate stimulation of the growth of the seed (hormesis).

2.6 Data treatment

The statistical analysis of the data showed they were not normally distributed and were skewed (the standardized skewness and kurtosis estimated—values in brackets— varied from 0.68 [−1.26] to 2.2 [0.27] for the metal(loid) content in soils samples and from 6.51 [8.9] to 1.43 [−0.79] for the metal(loid)s in saturation extracts). Box–Cox transformation was applied in order to transform the data set into normal form (Bagur et al. 2009).

The Spearman rank-order correlations (Spearman R coefficient) were used to study the correlation structure between normalized variables (expressed as W_i) because the samples were less sensitive to outliers than the Pearson coefficients.

In this case, the statistical treatment of the data was performed using Statgraphics Plus for Windows v.5.1.

2.7 Unsupervised pattern recognition methods: HCA and PCA

HCA was made in the case of metal(loid) contents in soil samples in order to associate the grouping with the mineralogy of the studied area. The analysis was performed on the normalized data set using the Manhattan (city-block) distance to measure similarities and Ward's method as the amalgamation rules. Statgraphics Plus for Windows v 5.1 was used.

PCA was applied to the autoscaled Box–Cox transformed matrix data set from saturation extracts and the *L. sativa* L. bioassay in order to display the structure of the normalized data using information about the latent relationship within them. PCA PLS Tools from MathLab were chosen.

3 Results and discussion

3.1 General properties of soils

The soils in the area were characterized by a low degree of development and were classified (FAO 2006) as haplic Fluvisols (in the watercourse) or haplic Regosols (outside the river bed). The main properties of the soils were a sandy-loam texture (<15% clay), low organic carbon content (<2%), no calcium carbonate content, low cation exchange capacity (<12 cmolkg^{-1}) and pH close to 7.5.

3.2 pH, electrical conductivity and salinity

As can be seen in Table 1, the saturation extracts from soil samples located in the eastern area show neutral or slightly alkaline pH values, ranging from 6.51 to 8.32. Those from the western area have moderately alkaline pH values ranging from 7.80 to 8.85.

Taking into account the relationship between EC and soil salinity classes (SSDS 1993), all the samples associated with the eastern area are either not saline or only very slightly saline. Most of the samples in the western area are moderately saline. If we observe the EC_a values, we can see that the soil salinity classes change in both areas. This may be due to dilution which diminishes the intensity of the interactions that take place in the saturation extract (ionic-pairs formation, common-ion effect, etc.), so according to Simón et al. (1994) this parameter could be considered a better estimator of the true salinity of the samples.

The salinity of the soil samples in the western area is higher than in the eastern area. This increase could be related to the differences in the extraction procedure because in the western area, the heap leaching process increases the solubility of the elements due to the formation of soluble salts after the oxidation of sulphide to sulphates. In addition, the increase in the salinity and pH of these samples could also be related to the use of fertilizers by farmers working the land nearby.

3.3 Geochemical background

There are many statistical methods to determine the background levels of soils. The most commonly used methods involve the 95 percentile (Kabata-Pendias and Pendias 2001), the median and the absolute deviation of the data with respect to the median (Tukey 1977) and the mean and the standard deviation of the data set (Hawkes and Webb 1962). In our study area, the background concentration determined by PXRF and the use of these statistical approaches gave the following levels for the main elements: Pb (23.6–26.2 mg kg^{-1}), As (21.5–23.3 mg kg^{-1}), Zn (43.3–54.9 mg kg^{-1}) and Cu (42.2–44.2 mg kg^{-1}). The comparison of these values determined by PXRF with the values described as the baseline in the soils of the province of Almería (Sierra et al. 2007), to which the study area belongs, indicated that the concentrations measured by this method are within the normal range for the region.

3.4 Metal(loid) concentrations in soils

The concentrations of As, Cu, Mn, Pb and Zn (expressed as a mean of three replicates) found in the soil samples are summarized in Table 1. The results revealed high concentrations of As, Cu, Pb and Zn in the samples. Figure 2 shows the dendrogram resulting from CA (Manhattan distance and Ward's agglomerative method) obtained by grouping the variables from the Box–Cox normalized data set. As can be seen, the variables are grouped in two clusters: cluster I in which the group of variables are related to the primary mineralogy of the area (e.g. As, Cu and Pb) and cluster II which can be attributed to elements with other
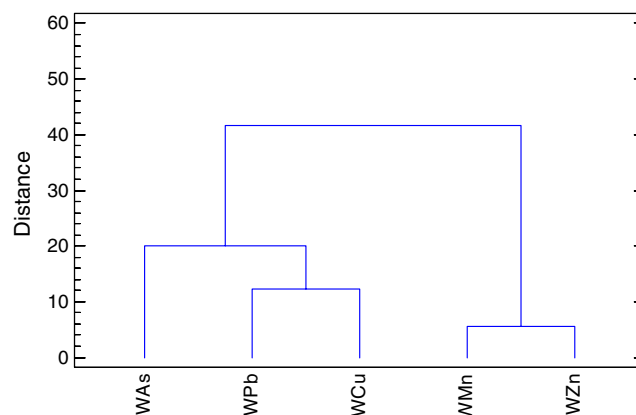

Fig. 2 Dendogram of clustering of analysed metal(loid) concentrations in soils from PXRF using Ward's method and city-block distance method

origins, i.e. the gold-extraction process, the metals present in host rocks, etc.

3.5 Metal(loid) concentrations in saturation extracts

The concentrations of As, Cu, Mn, Pb and Zn (expressed as mean values of three replicates) found in the saturation extracts exceed in most cases the reference value for water in soils (NMHPPE 1998), as can be seen in Table 1. In all the cases, the % RSD ranges between 5.7% and 16%.

The saturation extracts obtained from soil samples from the eastern area are characterized by the highest concentrations of As, whereas the saturation extracts from the western area contain in general terms the highest Cu-soluble and the lowest As-soluble concentrations.

Table 2 shows the percentage of metal(loid)s solubilized from soils to saturation extracts. It can be observed that in all cases the elements we studied are present in non-soluble form with values always below 0.50% (all the values are expressed as a percentage of the element concentration in the saturation extract (referred to soil sample) as a proportion of the element concentration in the soil sample). Particular attention must be paid to sample S-41 in which the highest percentage of solubilized elements has been found. This fact can be attributed to a "barrier effect" originated by the topography of the area, which acts as a sedimentary basin hosted in volcanic rocks.

3.6 *L. sativa* bioassay

The results obtained in these bioassays, plus the previously defined toxicity indices, are presented in Table 3. Figure 3 shows the Box plot diagram for the toxicity indices. We can see that the distributions for SG and RE are different, with RE the most discriminant index. RE shows a symmetrical distribution and great variability (more sensitivity to

Table 2 Percentages of solubilized metal(loids) from soils to saturation extracts

Samples	As	Cu	Mn	Pb	Zn
S-6	0.099[a] (±0.138)[B]	0.085 (±0.122)	0.015 (±0.125)	–	0.023 (±0.129)
S-10	0.056 (±0.169)	0.075 (±0.139)	–	0.011 (±0.093)	0.030 (±0.140)
S-16	0.083 (±0.106)	0.031 (±0.086)	0.010 (±0.102)	–	0.067 (±0.089)
S-20	0.154 (±0.165)	0.059 (±0.071)	0.035 (±0.075)	0.011 (±0.135)	0.155 (±0.120)
S-21	0.286 (±0.153)	0.036 (±0.078)	–	0.014 (±0.121)	0.057 (±0.089)
S-25	0.069 (±0.142)	0.032 (±0.080)	0.028 (±0.102)	–	0.071 (±0.139)
S-26	0.045 (±0.130)	0.028 (±0.081)	0.006 (±0.112)	–	0.057 (±0.127)
S-32	0.157 (±0.166)	0.124 (±0.085)	0.010 (±0.142)	–	0.018 (±0.134)
S-34	0.089 (±0.152)	0.031 (±0.080)	–	–	0.023 (±0.073)
S-35	0.161 (±0.136)	0.106 (±0.082)	0.012 (±0.131)	–	0.036 (±0.079)
S-36	0.123 (±0.166)	0.066 (±0.084)	–	0.025 (±0.128)	0.148 (±0.108)
S-44	0.047 (±0.151)	0.142 (±0.080)	–	–	0.016 (±0.091)
S-41	0.240 (±0.152)	0.458 (±0.102)	0.119 (±0.090)	0.141 (±0.131)	0.323 (±0.054)
S-52	0.124 (±0.156)	0.351 (±0.098)	0.044 (±0.095)	–	0.075 (±0.120)
S-55	0.016 (±0.103)	0.115 (±0.115)	–	–	0.050 (±0.089)
S-56	0.091 (±0.126)	0.374 (±0.127)	0.037 (±0.099)	–	0.028 (±0.104)
S-58	0.086 (±0.117)	0.328 (±0.144)	0.059 (±0.130)	–	0.050 (±0.094)
S-59	0.069 (±0.117)	0.136 (±0.147)	0.024 (±0.141)	0.042 (±0.101)	0.090 (±0.157)
S-60	0.103 (±0.135)	0.124 (±0.068)	0.013 (±0.122)	–	0.101 (±0.134)

[a] Mean values

[b] Interval of confidence ($p=0.95$, $n=3$)

changes in the composition of the saturation extract). The values obtained for samples S-41, S-58 and S-60, out of the boxes in both cases, can be explained by taking into account the "barrier effect" mentioned above, which acts by accumulating soluble metal(loid) forms through the downstream transport processes.

By establishing the relationship between these indices and the phytotoxicity of the set, we can conclude that, while for SG the soils of the eastern area show a low toxicity, the samples present a moderate phytotoxicity for RE. This fact leads us to conclude that for the assessment of toxicity in soils with moderate/low phytotoxicity, the final conclusions are more effective and accurate if they are based on measurements of the elongation of the germinated root rather than on the number of germinated seeds.

3.7 Assessment of the toxicity in the area using the *L. sativa* L. bioassay

As can be seen in Table 3, samples from the eastern area present a moderate or low toxicity for RE (values below −0.5 or −0.25, respectively). In the western area, this index varies from 0 (no phytotoxicity) to −0.97. As has been observed previously, the highest values are for those located furthest away ("barrier effect") from the abandoned pile, probably due to the fact that the washing used in the heap leaching process produced soluble salts of Zn and Cu that could easily be spread downstream, contributing to the electrical conductivity of the soil solution, i.e. the solubilization of metal(loid) species present in soils.

PCA was applied to the normalized data set (WAs, WCu, WMn, WPb, WZn and WRE) in order to compare the composition patterns within the studied samples and to identify the factors that influence each one. On the basis of eigenvalues >1 criterion, three principal components were found explaining 86.72% of total accumulative variance. This facilitated the explanation of all the samples, with the original six variables being reduced to a matrix made up of a set of three calculated principal components (PCs).

As can be observed in Table 4, PC1, which explained 38.09% of variance, is mainly positively influenced by the loadings of Cu, Zn and Mn concentrations and negatively by RE and As concentration. PC2 (28.04% of explained variance) is influenced positively by loadings of As, Zn and Pb concentration. The last component, PC3, explains 20.59% of total variance.

Taking into account PC1 (Fig. 4), a good separation of samples was obtained. Two groups of samples can be seen which are characterized by their RE values. This allows us to determine the level of phytotoxicity of the samples, as the negative PC1 values indicate a high level of phytotoxicity and the positive values a low level. In addition, samples which present a high concentration of As and Pb induce RE values of approximately −1, while samples which contain a high concentration of Mn, Zn and Cu do not induce an RE with toxic values. On the basis of these analyses, it can be argued that there is a relationship between the metal(oid) concentration and the phytotoxicity of samples. The soluble species of As and Pb induced the highest toxicity levels, while concentrations of Mn, Zn and

Table 3 Parameters measured and toxicity indices for *L. sativa* L. bioassay

| Samples | *Lactuca sativa* L. | | | |
	Germ (%)	Length (cm)	SG	RE
S-6	100.0	4.9[a] (0.05)[b]	0	−0.12
S-10	93.0	4.6 (0.05)	−0.07	−0.17
S-16	97.0	3.7 (0.03)	−0.03	−0.34
S-20	93.0	3.2 (0.03)	−0.07	−0.42
S-21	93.0	2.8 (0.05)	−0.07	−0.49
S-25	93.0	3.7 (0.02)	−0.07	−0.33
S-26	87.0	4 (0.05)	−0.13	−0.27
S-32	90.0	3.3 (0.02)	−0.1	−0.4
S-34	97.0	4.3 (0.02)	−0.03	−0.22
S-35	80.0	3.4 (0.02)	−0.2	−0.38
S-36	97.0	4.3 (0.03)	−0.03	−0.22
S-44	93.0	4.4 (0.03)	−0.07	−0.2
S-41	27.0	0.7 (0.03)	−0.88	−0.73
S-52	97.0	4.7 (0.05)	−0.15	−0.03
S-55	100.0	5.3 (0.04)	−0.03	0
S-56	97.0	3.9 (0.07)	−0.30	−0.03
S-58	3.0	0.0 (0.05)	−0.99	−0.97
S-59	97.0	5.4 (0.05)	−0.01	−0.03
S-60	13.0	0.3 (0.05)	−0.94	−0.87

[a] Mean values

[b] Standard deviation

Cu did not have phytotoxic effects on the samples we analysed. Finally, it can be observed that the samples are grouped together according to the two areas (eastern and western) and are therefore closely related to the extraction procedure used in each area for the recovery of gold.
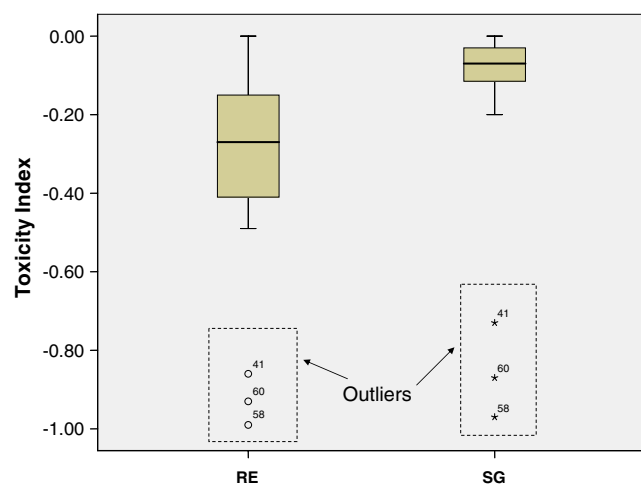
Fig. 3 Selection of the toxicity index that provides the best explanation of phytotoxicity using the Box plot diagram for the samples analysed in *L. sativa* L. bioassays

Table 4 Loadings for the components obtained in the PCA

	Component 1	Component 2	Component 3
As	−0.14	0.59	−0.43
Cu	0.52	−0.27	−0.07
Pb	0.11	0.60	0.48
Zn	0.48	0.43	0.10
Mn	0.60	−0.14	0.19
RE	−0.33	−0.10	0.73

4 Conclusions

The comparison of the two indices (SG and RE) defined using the *L. sativa* L. bioassay indicates that for areas with a low degree of contamination, the most sensitive toxicity index is RE, whereas in highly contaminated areas, both RE and SG are good estimators of soil phytotoxicity. Unsupervised pattern recognition methods such as HCA and PCA enable us to conclude that the low/moderate phytotoxicity of the soils is related to the extraction process used for the recovery of gold (mainly dynamic cyanidation in tanks located in the eastern area). In the samples of soil contaminated by the spread of pollution from the tailing pond (dynamic cyanidation process), the phytotoxicity in the lettuce seeds was directly related to the water-soluble As and Pb concentrations in the soils, whereas in the samples from the abandoned pile (heap leaching process), the toxicity in the lettuce seeds was related to the water-soluble Zn and Cu concentrations of the soils.
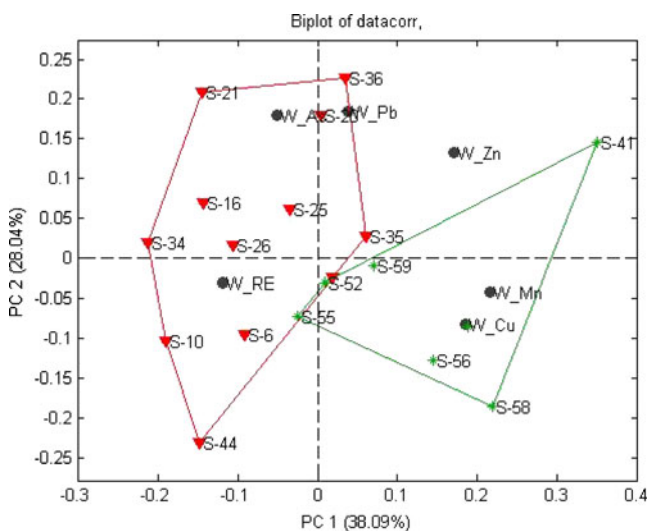
Fig. 4 Scatter plot of the principal scores (PC1 and PC2) for *L. sativa* L. bioassay, grouping the samples together according to the phytotoxicity of the samples and its relation with the extraction procedure used for the recovery of gold; *polyhedron*: dynamic cyanidation in tanks, *triangle*: heap leaching process

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