Ambient trace element background concentrations in soils and their use in risk assessment

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ABSTRACT

The definition of ambient background concentrations (ABCs) is used in this study to assess the potential environmental risk of trace elements in soils and parent materials from Granada, Spain. Two different layers of soil (0–20 and 20–40 cm) and parent material samples were collected at 93 sites. From cumulative frequency distribution curves, ABCs for As, Co, Cu, Cr, Ni, Pb and Zn were estimated at 3.5–20, 7–23, 13–25.6, 29–66, 7–20, 15–36, and 5.5–76 mg kg^{-1}, respectively. Tukey box-plots were used to discriminate different concentration classes and identify potentially contaminated sites. Weakly-weathered soils (Entisols) over carbonate materials showed the lowest background contents, the most developed soils (Alfisols) over metamorphic rocks the highest ones. Outliers were mainly found near a former iron mine where arsenic concentrations were by far exceeding the corresponding regional ABC. These soils were however, not toxic to *Escherichia coli* and *Vibrio fischeri*. The prediction of site-specific ABCs together with bioavailability and toxicity assessment is a valuable tool for giving further insight into the risk of trace elements in soils.

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1. Introduction

Risk assessment of trace elements in soils is a key to many environmental protection measures and requires prediction of site-specific ambient background concentrations (Reimann and Garrett, 2005). Ecological risk assessment (ERA) of contaminated soil covers four tiers gaining in detail and definition: simple screening (Tier I), refined screening (Tier II), detailed assessment (Tier III) and final assessment (Tier IV) (Jensen and Mesman, 2006).

Trace elements (TE) occur naturally in the terrestrial environment and, therefore, TE ambient background concentrations (ABCs) in soils of a region will be strongly related to the pedo-geochemical fraction, to the dynamics of the environment where the soils interact as open systems (Chen et al., 1991; Alloway, 1995; De Temmerman et al., 1984), but also to the contribution of human activities. The anthropogenic fraction consists mainly of moderate diffuse inputs into the soil (ISO, 2005).

Determination of a unique ABC for a large area (regional scale) does not take into account spatial variation of soil type and properties. In this way, many single ABCs have been defined for a country, which could give rise to either overestimation or underestimation of metal contamination and the associated risk for a particular soil. Moreover, in most cases soil guidelines are based on total concentrations, while the potential ecological risk of metals is more related to their (bio) available concentration.

In this study, we aimed at screening the site-specific risk of trace elements in soils in Granada province (Spain) based on the estimation of ambient background concentrations at a regional scale (Tier I). Secondly, this study aimed to assess the environmental risk of trace elements in one of these areas where outliers or potentially polluted soils were identified (Tier II).

2. Materials and methods

2.1. Study area and sampling procedure

2.1.1. Tier I: simple screening: Granada region

Granada province in southeast Spain occupies an area of 12,531 km^2 (Fig. 1) and has a Mediterranean type climate (Ferrer Rodríguez, 2002). Two large-scale geological units can be differentiated: the Betic Cordillera and the Neogene Basin (Bosque Maurel and Ferrer Rodríguez, 1999). The Betic Cordillera is divided between a younger External Zone (24% of the total area) with a predominance of carbonate materials, e.g., limestones, marbles and dolomites (hereafter "limestones"), and an older Internal Zone (20% of the total area), with a predominance of metamorphic rocks, e.g., micaschists, quartzites, shales and gneisses (hereafter "micaschists"). The Neogene Basin contains the following materials: a) Miocene silts and marls (14% of the total area), hereafter designated "marls"; b) continental and lacustrine deposits of diverse
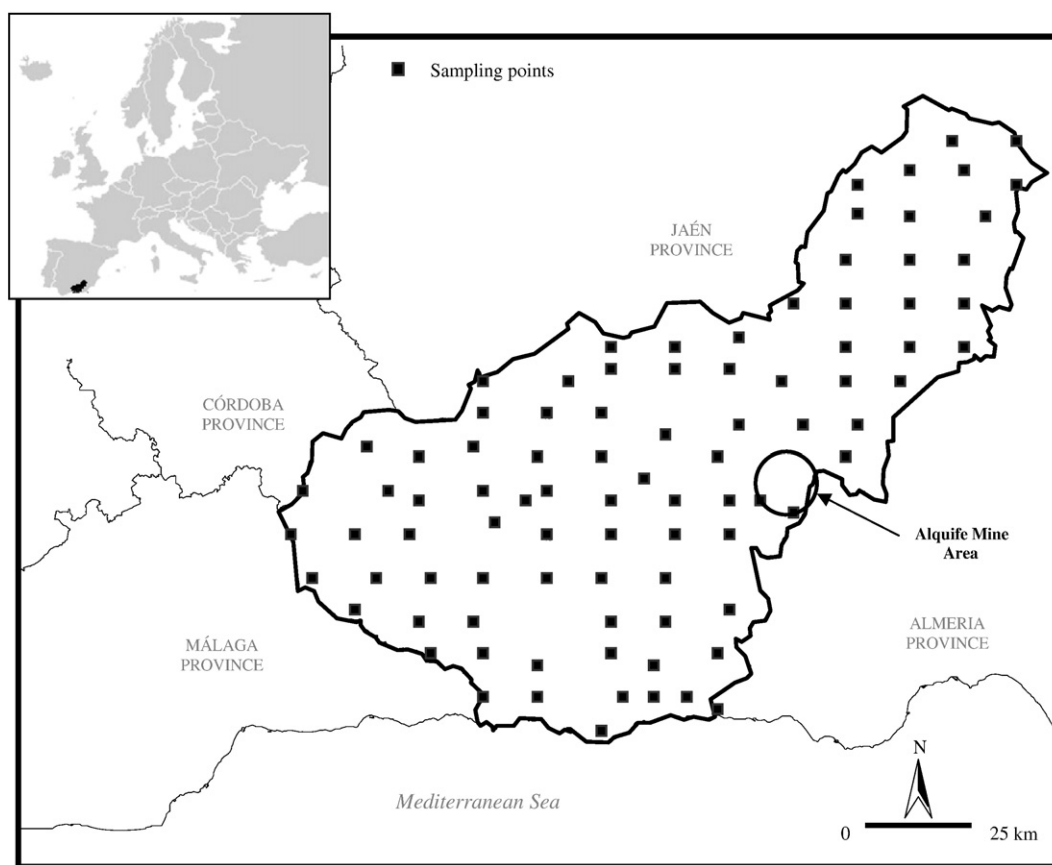
Fig. 1. Tier I screening of trace element concentrations: sampling points in Granada region (southeast of Spain). Ninety-three soil samples were collected at two different depths (0–20 and 20–40 cm) together with their corresponding parent materials. The location of Alquife mine area, sampled for further study (Tier II) is also indicated.

composition and grain size, divided between *consolidated sediments* (18% of the total area, dating from the late Miocene–Pliocene) and *unconsolidated sediments* (occupying 14% of the total area, dating from the Pleistocene); and c) sediments deposited at the bottom of brackish lakes, designated *evaporites* (10% of the total area).

The province was divided into 93 uniformly distributed sectors of 135 km^2 each, independent of soil type or land use. A square plot was laid out (10×10 m) and georeferenced by Global Positioning System (GPS) at the approximate centre of each sector. Samples were taken from the four corners and the centre of each plot at depths of 0–20 cm and 20–40 cm and from the parent material (PM; unaltered parent materials fragments from the C-horizon), and the 5 samples taken at each depth were pooled.

2.1.2. Tier II: refined screening: sampling in Alquife mine area

A more detailed study was carried out in the Alquife plain, the largest area where outliers for most elements were identified. Soils from this area are mainly affected by mining (i.e. iron extraction processes) and agricultural activities (olive and almond groves are the most important).

The area belongs to the Betic zone, the most meridional part of the Betic Cordillera in southeast Spain. Alquife mine area, also known as the Marquesado deposit, has being mined since 1895. This iron-open-cut mine produced about 3.3 million metric tons per year (MTn/y) in the mid-nineties. The main ore minerals in Alquife outcrop are hematite, goethite and siderite (Torres Ruiz, 1980).

The studied area is located in the south of the Guadix-Baza depression (an intramontane plain 1150 m above sea level), which is formed primarily on consolidated and unconsolidated Quaternary sediments: calcareous from Sierra de Baza (to the north) and metamorphic from Sierra Nevada (to the south) (Aldaya et al., 1979).

The area was divided into 57 uniformly distributed sectors (Fig. 2) with a sampling network established on a grid of 2 km×2 km. A square plot was laid out (10×10 m) and georeferenced by Global Positioning System (GPS) at the approximate centre of each sector. Samples were taken from the four corners and the centre of each plot at depths of 0–20 cm and 20–40 cm, and the 5 samples taken at each depth were pooled.

2.2. Chemistry tools

2.2.1. Soil analysis

The pH of all soils was measured potentiometrically in a 1:2.5 soil (fine earth)–water suspension using a CRISON Model Microph 2002 instrument. Soil and PM samples were air-dried and finely ground (<0.05 mm). The parent materials (PMs) were also identified in the field using a petrographic microscope. The CaCO$_3$ equivalent was analysed by manometric measurement of the CO$_2$ released after addition of hydrochloric acid (Williams, 1948).

Soil samples collected for Tier II in Alquife area were also analysed for some physical chemical properties. Particle-size distribution was determined by the pipette method after the elimination of organic matter with H$_2$O$_2$ and dispersion with sodium hexametaphosphate (Loveland and Whalley, 1991). Cation-exchange capacity (CEC) was determined with 1 M Na-acetate at pH 8.2, measuring sodium in a METEOR NAK-II flame-photometer. Exchange bases were determined with 1 M NH$_4$-acetate at pH 7.0, measuring sodium and potassium in a METEOR NAK-II flame-photometer and calcium and magnesium by atomic absorption spectrometry in a VARIAN SpectrAA 220FS. Free-iron oxides (Fe$_d$) were extracted with citrate dithionite (Holmgren, 1976) and poorly crystallized iron oxides (Fe$_o$) were extracted with ammonium oxalate (Schwertmann and Taylor, 1977), both measured
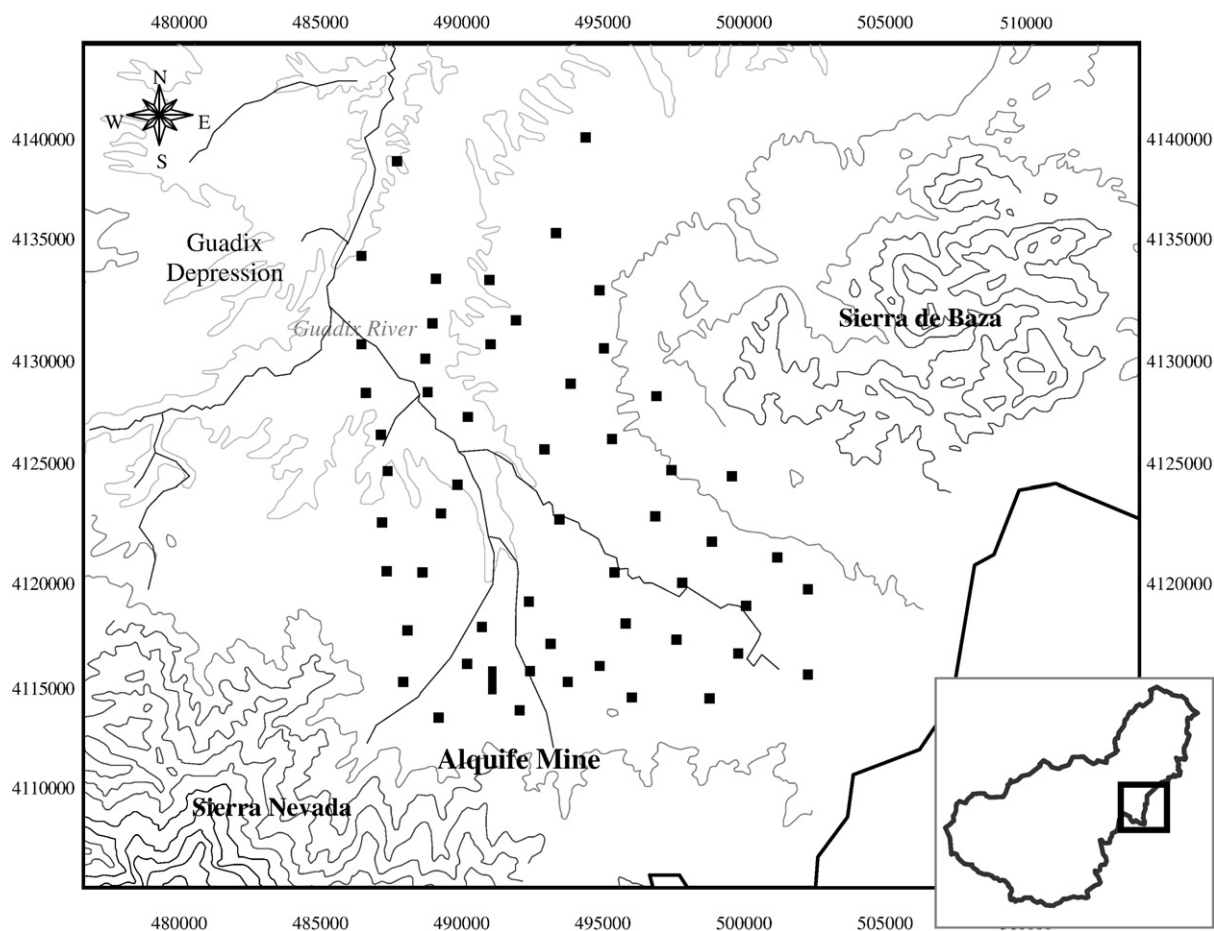
Fig. 2. Tier II refined screening of trace element concentrations: sampling points in the surroundings of Alquife mine area (north east of Granada province) where trace element concentrations were deviating from the regional ambient background concentration. Fifty seven soil samples were taken at two different depths (0–20 and 20–40 cm). Coordinates from the 1:50,000 map are shown.

by atomic absorption spectroscopy in a VARIAN SpectrAA 220FS instrument.

2.2.2. Trace element concentrations

For the Simple Tier I Screening, soil and PM samples were digested in strong acids ($HNO_3 + HF + HCl$), and total arsenic, cadmium, copper, cobalt, chromium, molybdenum, nickel, lead and zinc concentrations in each digested sample were measured by Inductively Coupled Plasma–Mass Spectrometry (ICP–MS) using a PE SCIEX ELAN-5000A spectrometer. The recovery of metals in standard reference material SRM2711 was in the range of 80–110% of the certified total concentrations (Table 1).

The chemistry tools for refined Tier II screening aimed at extracting a more ecotoxicologically relevant fraction of the "contamination" than the total concentration. Soil was extracted in 1:2 soil:water mixture by adding 40 ml of deionized water to 20 g dry weight of soils (Flynn et al., 2002). The mixture was then shaken overnight on an end-over-end shaker at 40 rpm, centrifuged (Coolspin 2RR/1061) for 60 min at 1050 ×g at 4 °C and then filtered and stored at 4 °C. This soil elutriate represented the water-extractable fraction of trace elements. A 10 ml aliquot of these elutriates was retained for bioassay testing.

NH_4NO_3-extractable (1 M) contents of arsenic, copper, chromium, cobalt, nickel, lead and zinc (1:2.5) were also measured following the extraction procedure proposed by the German national standard DIN 19730 (Prueb, 1997). Trace element concentrations in these extracts were measured by flame atomic absorption spectrophotometry (Perkin Elmer AAnalyst 100) following acidification with 2% HNO_3.

2.3. Toxicological tools

Potentially contaminated soil samples were screened for the presence of toxic compounds by means of two different bioassays (acute luminescence bacteria tests).

2.3.1. Escherichia coli test

Bioassays were carried out on 22 randomly selected pore water samples using a genetically modified lux-based biosensor. Luminescence of the biosensor reflects metabolic activity, which is reduced by contaminants. The test organism was *E. coli* HB101 pCD607 (Rattray et al., 1990). The biosensor contains the luxCDABE genes isolated from

Table 1
Analysis of the standard reference material 2711 (soil with moderately high trace element concentrations).

Element	Certified		Experimental	
	Mean	S.D.	Mean	S.D.
As	105	8.0	102	1.1
Cd	41.7	0.25	40.8	0.27
Cu	114	2.0	115	1.6
Co	10.0	–	12.2	0.37
Cr	47.0	–	51.4	1.3
Mo	1.60	–	1.43	0.11
Ni	20.6	1.1	18.7	1.6
Pb	1162	31.0	1138	11.0
Zn	350	4.8	350	3.4

–, non provided values. All values are in mg kg^{-1} dry soil.

Table 2
Total concentrations (mg kg^{-1} dry soil) of trace elements in parent material and pooled soil samples ($n = 279$) from Granada with associated statistical parameters.

Statistical parameters	As	Co	Cr	Cu	Ni	Pb	Zn
Median	7.90	9.00	44.0	20.4	19.8	17.1	37.1
MAD	2.04	2.04	27.0	7.58	1.70	1.59	1.82
Min.	0.50	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.
Max.	116	43.0	145	65.1	145	822	529
Skewness[a]	3.49	1.26	0.52	0.62	0.93	8.83	4.42
Skewness[b]	−0.01	−0.75	–	–	−0.69	0.94	−0.61
n	279	279	279	279	279	279	279

MAD = 2 median absolute values.
<d.l.: below the detection limit.
[a] Untransformed data.
[b] Log-transformed data.

Vibrio fischeri as a multicopy plasmid. Previously freeze-dried metabolic biosensor cells were resuscitated for 1 h in 10 ml of 0.1 M KCl at 25 °C on an orbital shaker at 200 rpm/min and used immediately. In each luminometer cuvette, a 100 µl aliquot of the resuscitated cells was added to 900 µl of pore water test solutions and mixed at 20-s intervals. The luminescence of the samples was measured after 15 min of exposure on a portable Jade luminometer (Labtech International) at 20-s intervals at ambient temperature. The assays were performed as three independent replicates and luminescence expressed as percentage of the luminescence of the control (deionized water).

2.3.2. MICROTOX bioassay — basic test for aqueous extracts

The inhibitory effects of potentially contaminated soil elutriates were measured using the marine bacteria *V. fischeri*. The bacteria produce light as a by-product of metabolism and this is measured using a Microtox photometer analyser. In this bioassay, 22 selected soil elutriates were evaluated following the procedure proposed by the MICROTOX® with some adaptations (Microbics Corporation, 1992).

The freeze-dried bioluminescence bacteria *V. fischeri* were activated prior to use by a reconstitution solution. Since *V. fischeri* is a marine organism, the osmotic pressure of the samples was adjusted to 2% salinity, using a solution of 3.5% NaCl in deionized water. Five and fifteen minutes after transferring the bacteria to elutriate vials, the light emission was measured using the Microtox 500 analyzer from Microbics Corporation. The analyzer was equipped with a 30-well temperature-controlled incubator chamber regulated at 15 °C. A small compartment held at 5 °C was used to store the bacteria suspension before dilution. The assays were carried out as three independent replicates. Inhibition values defined as $I = (I_0 - I_t)/I_t$ were I_0 is the luminescence of the cell in contact with concentrated salt solution at time 0 and I_t is the luminescence of the sample at $t = 5$ ($I_{5'}$) and 15 min ($I_{15'}$).

A standard solution (ZnSO$_4$.7H$_2$O) of known toxicity was run as a control at least twice for every bacterial lot to ensure that the variability among organisms was not a contributing factor to apparent toxicity. Results from the current study (15-min EC50 ± SE = 1.38 ±
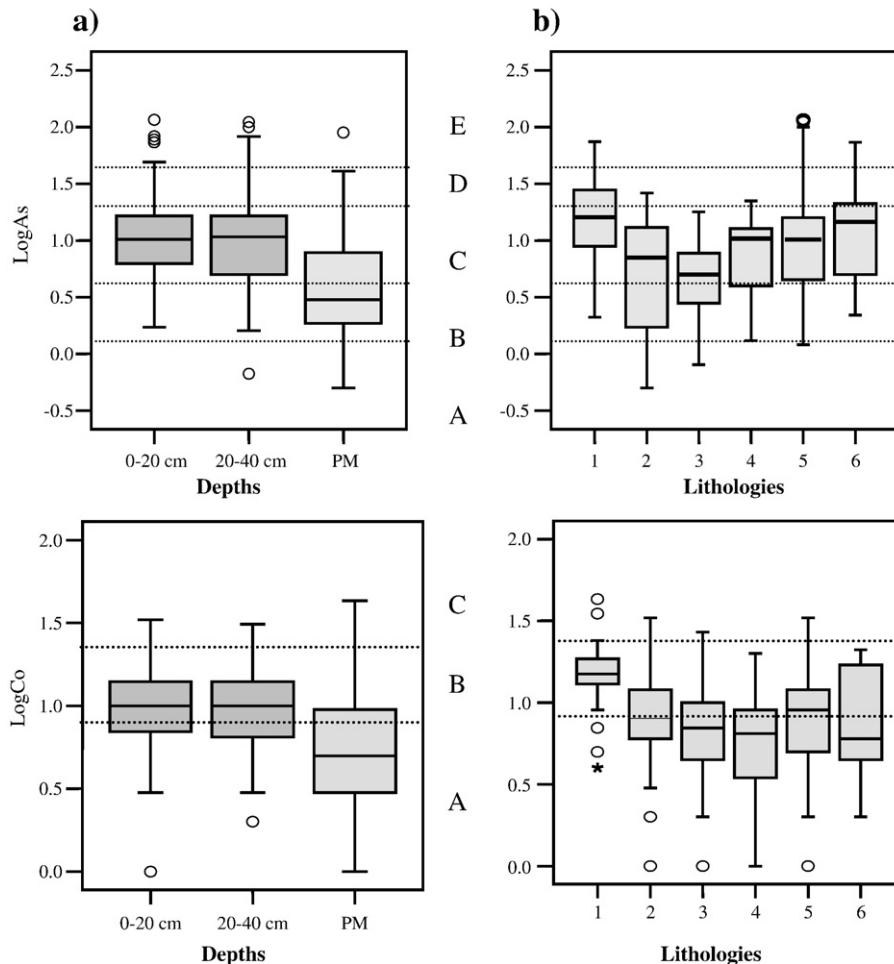

Fig. 3. Tier I (simple screening): Tukey box-plots of (log-transformed) trace element concentrations in Granada soils (mg kg^{-1} dry soil) against: a) depth of samples (soil samples at 0–20, 20–40 cm and parent material (PM)), and b) lithology (1 = micaschists, 2 = limestones, 3 = marls, 4 = evaporites, 5 = unconsolidated sediments, 6 = consolidated sediments). Relationship of box-plots with classes (A, B, C, D and E) identified by cumulative frequency distribution curves (CFDCs).
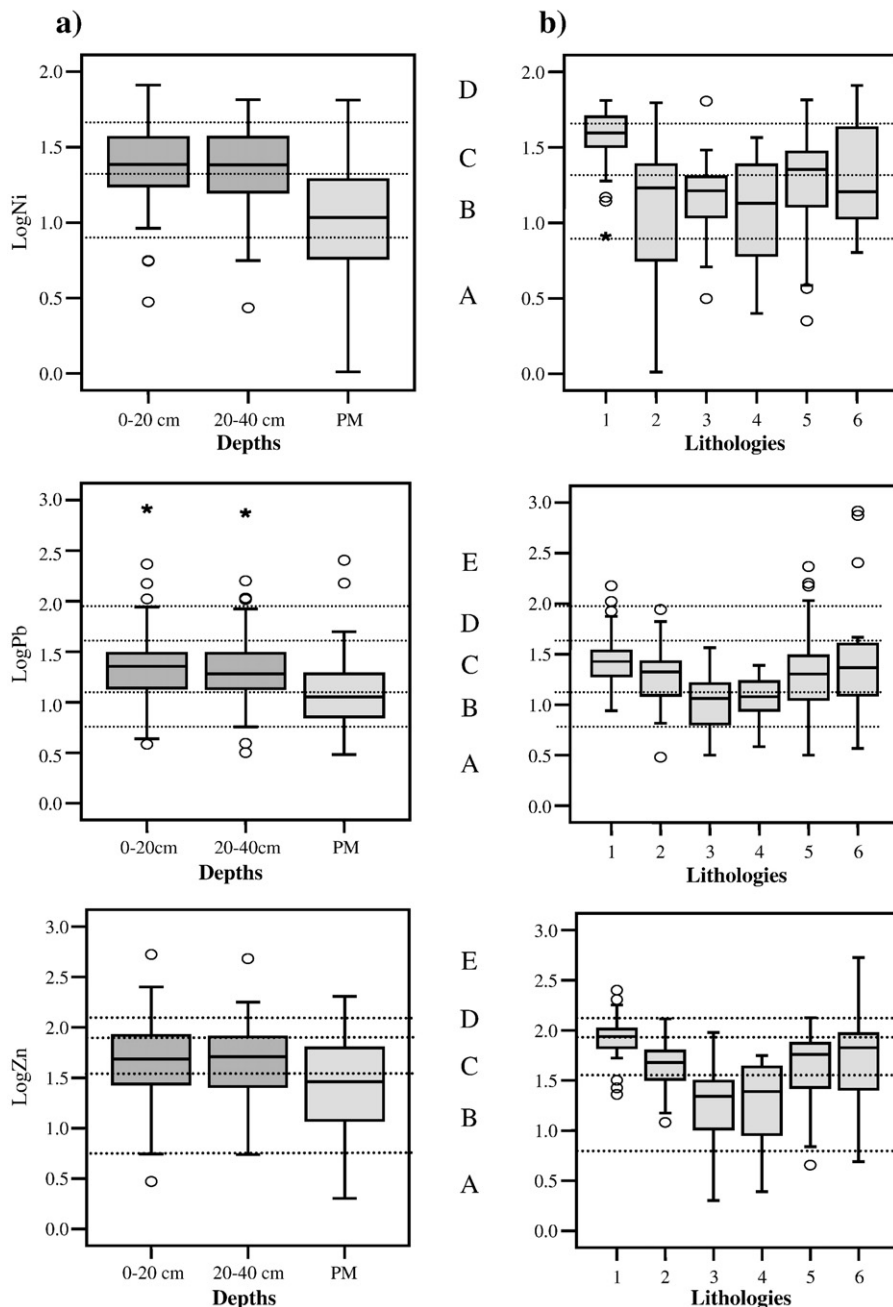
Fig. 3 (*continued*).

0.12 mg Zn l^{-1}) were similar to published EC50 values for this compound (0.6–2.2 mg Zn l^{-1}; Mowat and Bundy, 2002).

2.4. Data processing

Trace elements data distribution was graphically displayed in a box-plot constructed by using SPSS v.12.0 software package, allowing identifying outliers and determining the median, 25th and 75th percentiles. The median ± 2 median absolute deviations (MADs) was also calculated, which Reimann et al. (2005) proposed as a robust tool for geochemical prospection. Box-plots and whisker box-plots were also displayed to analyse trace element concentrations at differences depths (0–20, 20–40 cm and PM) and parent materials (6 lithologies: limestones, marls, evaporites, micaschists and unconsolidated and consolidated sediments). Correlation analysis was performed to

provide an overview on the relationships between trace element concentrations and soil properties.

2.4.1. Background determination and outlier detection

In order to determine background trace element concentrations and elucidate the existence of outliers, a graphical approach was carried out by constructing the cumulative frequency distribution curves (CFDCs) using the paired metal concentration (cumulative percentage data). The procedure for plotting implies the representation of metal concentrations on the *y*-axis, and the cumulative percentage that corresponds to each data point on the *x*-axis. Log-transformation was applied to most data as it was found appropriate for most situations encountered during distribution analysis.

In regional studies across several ecosystems with different soil-forming factors, natural and human-induced processes lead not only to a
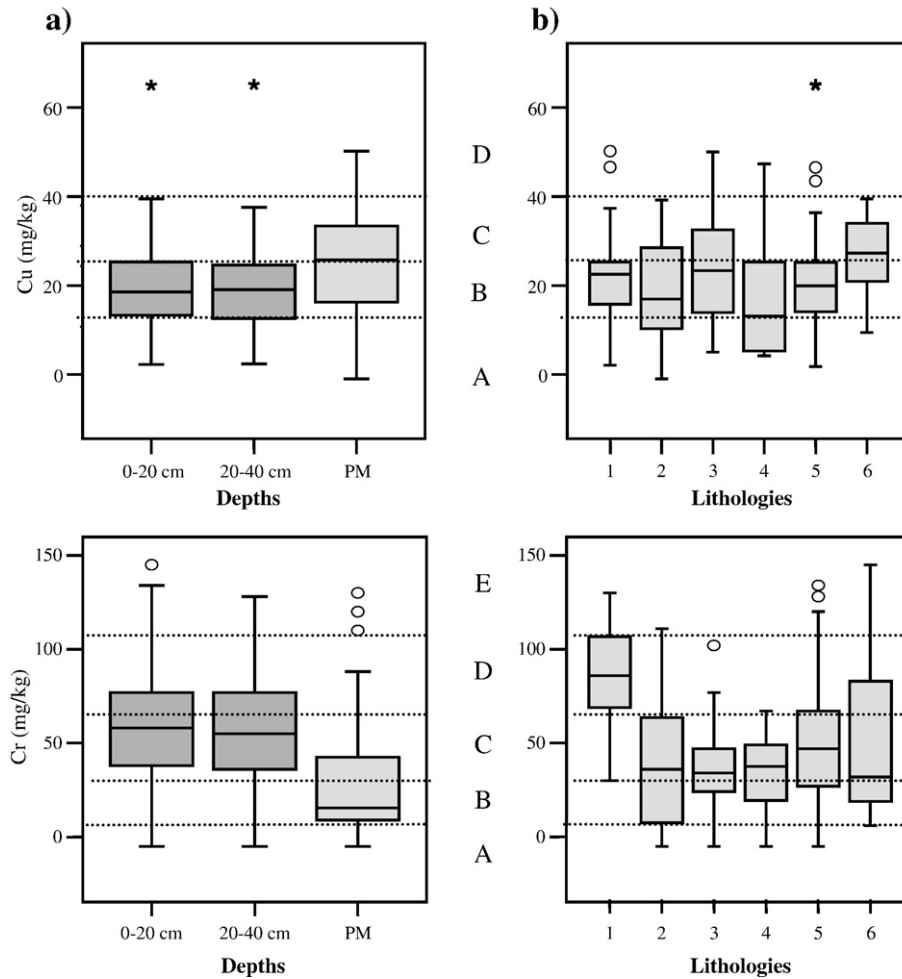
Fig. 3 (continued).

broader data range but also to a multimodal distribution in which each mode or class has a specific background range and represents a particular combination of factors and processes (Matschullat et al., 2000).

The presence of multiple classes within the data sets can be assessed by using CFDCs (Reimann et al., 2005). Inflexion points formed by the overlapping of two classes were identified as the end data points for which the resulting population showed a skewness closest to 0 (Fleischhauer and Korte, 1990).

Tukey (1977) box-plots according to known factors in the region (like parent materials composition and depth of sampling) and trace elements distribution maps were displayed to interpret the different classes identified by CFDCs.

Regional trace element distribution maps were created. The different classes identified by CFDC were used to delineate different shading categories using ArcView GIS 3.2; spatial interpolation of inverse distance weight (IDW) was applied with 12 neighbouring samples for estimation of each grid point.

2.4.2. Identification of outliers

Outliers for all trace elements are represented by the classes located at both ends of the CFDC (Bauer and Bor, 1995), which delineate the background ranges that characterize most of the soils in the region of study and would include the total trace element concentrations in soils without human influence (Reimann et al., 2005).

According to the EU Technical Guidance Document (TGD) for risk assessment (European Commission, 2003), the upper whisker of the $\log_{10}$-tranformed data can be taken as the cut-off level for the upper outliers, if the dataset is sufficiently large. The upper outliers are defined

as: $\log_{10}(xi) > \log_{10}(p75) + 1.5 (\log_{10} p(75) - \log_{10} p(25))$ where xi is the outlier cut-off value, $p25$ and $p75$ the 25th and 75th percentile of the distribution.

3. Results and discussion

3.1. Tier I: simple screening

3.1.1. Soil properties/soil survey data

The most representative soils in the area, in decreasing order of abundance, were Entisols, Inceptisols, Mollisols, Aridisols and Alfisols (Soil Survey Staff, 1999). They occur in a great variety of parent materials: carbonate rocks (limestones, marbles and dolomites); metamorphic rocks (schists, quartzites, gneiss and serpentinites); marls; consolidated and unconsolidated sediments and evaporitic materials (mainly gypsiferous).

Soil pH_{H_2O} ranged from 5.80 to 8.92 (mean ± SE: 8.22 ± 0.49), and 50% of the samples had a pH > 8.37. The $CaCO_3$ content ranged from 0 to 88.5% (mean 35.3 ± 27.3), and 50% of samples had a content > 36.3%. No statistically significant differences were found for pH values and $CaCO_3$ content between soil samples taken from 0 to 20 cm and from 20 to 40 cm. The most developed soils, those with Bw and Bt horizons, showed partial or complete decarbonation of the soil material (at least in the uppermost 40 cm).

3.1.2. Total trace element concentrations

Median values and their variation (median absolute deviation, MAD) of trace elements are given in Table 2. The median concentrations of
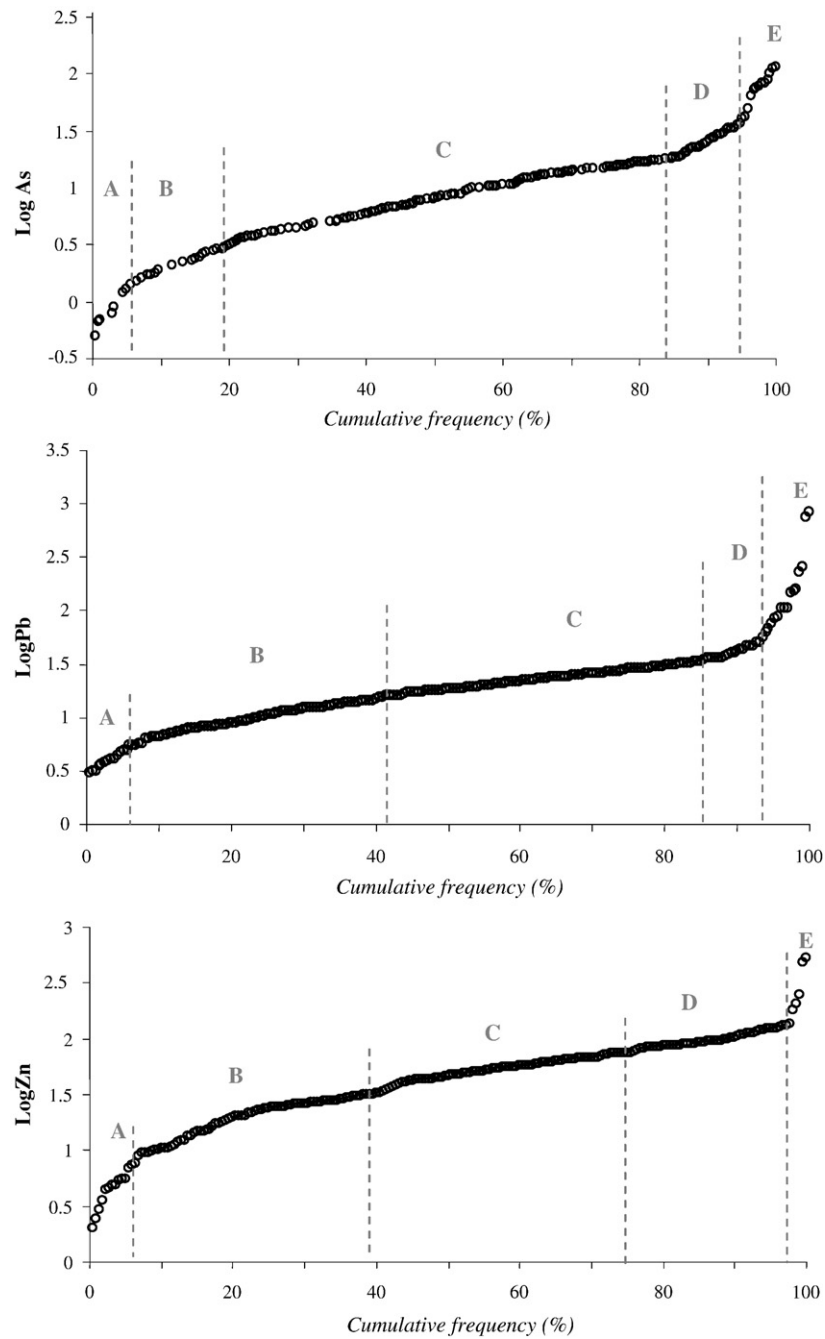
Fig. 4. Tier I (simple screening): cumulative frequency distribution curve of log-transformed data for arsenic, lead and zinc concentrations in Granada soils indicating the inflexion points that separate the different classes in the dataset (A, B, C, D and E). Dashed lines indicate the inflexion points.

trace elements varied greatly: As and Co around 10 mg kg^{-1}; Cu, Ni, and Pb roughly 20 mg kg^{-1}; Cr and Zn about 40 mg kg^{-1}; Cd and Mo concentrations were below the detection limit with 50% of the soils showing concentrations lower than 0.6 mg kg^{-1}.

Cu and Cr frequency data displayed a normal distribution curve whereas for As, Co, Ni, Pb and Zn the distribution curve of the log-transformed concentrations adjusted better to a normal curve, with lower skewness (Table 2).

The box and whisker plots of the data distribution showed no difference in contents of any of these elements between soil samples from 0 to 20 and 20 to 40 cm (Fig. 3a). There was no significant difference in trace element contents between 0–20 and 20–40 cm, this uniformly could be explained by mixing of soil layers during cultivation. However, significant differences were observed between

soils and PM samples ($P < 0.05$), with higher concentrations in soil than in PM for all the studied elements. Only exception was copper for which a slightly higher content was measured in parent materials. This shows that decarbonation (decalcification) and evolution of soil horizons are largely responsible for the enrichment of trace elements, with the development of partially or completely decarbonated Bw and Bt horizons with increasing element concentrations. TE concentrations significantly differed among parent materials, with lowest values in limestones and marls and highest in micaschists for most elements (Fig. 3b).

3.1.3. Ambient background concentration (ABC)

In this study, ABCs were estimated using cumulative frequency distribution curves (CFDCs). CFDCs were displayed for all trace

elements studied, in Fig. 4 CFDCs for arsenic, lead and zinc are shown (Fig. 4). Classes located at both ends of the CFDC represent the outliers. This allows for the identification of background ranges that characterize most of the soils in the region of study (Fig. 4 upper and lower limits of classes A and E, respectively). The occurrence of different classes (B, C and D) in the dataset indicates that a single background value would be insufficient in detailed studies (Von Steiger et al., 1996; Reimann et al., 2005).

Tukey box-plots for different depths of sampling and lithologies (Fig. 3) together with the presentation of data in the regional map facilitated the interpretation of the classes identified by the CFDC (Table 3).

For all trace elements the upper limited defined by the CFDC approach resulted to be more conservative than those defined as recommended by the TGD (European Commission, 2003). Thus, use of the CFDC approach may be more suitable for deriving regional ABCs (Table 3).

ABCs can be used as references for determining whether or not a soil might be contaminated (*outliers*). Such an indication of contamination only implies that the total contents are higher than would normally be expected in unpolluted areas.

To assess the risk associated with such outlier concentrations, a higher tier refined screening was performed. Outliers for high As, Zn and Pb concentrations were mainly located close to a former iron mine, Alquife, in the region, where anomalous concentrations may originate both from heavy road traffic and mining activities (Fig. 5).

3.2. Tier II: refined screening

The aim of this tier was to gain further insight into the toxicological and ecological properties of the potentially contaminated soils. Moving away from total concentration to (rough) estimated (bio) available concentrations, the risk assessment is likely to become more realistic and less conservative.

3.2.1. Soil properties

Soils in the surroundings mine area were mainly formed on consolidated and unconsolidated sediments derived from *Sierra de Baza*, when they were calcareous, and *Sierra Nevada*, when they had metamorphic features (Fig. 2). Soil properties in this area were found to be significantly different ($P<0.05$) and influenced by the composition of the parent materials. Soils from Sierra Nevada were neutral (pH = 7.68) with a mean calcium carbonate content of 1.21% and contained 5.37%, 1.50% and 0.12% of total, dithionite-extractable and oxalate-extractable iron, respectively. Soils with their parent materials coming from Sierra de Baza showed a basic pH (pH = 8.38), high calcium carbonate content (29.6%) and low iron content ($Fe_t = 3.48\%$, $Fe_d = 0.96\%$ and $Fe_o = 0.05\%$).

Table 3
Ambient background concentrations (ABCs) of trace elements in Granada soils (mg kg^{-1} dry soil) derived using the cumulative frequency distribution curve (CFDC) approach.

Trace elements	ABC — most bed rock and soil types	Weakly-weathered soils (Entisols) over highly carbonate rocks	Most developed soils (Alfisols) over metamorphics rocks	Upper limit of ABC (TGD)
As	3.5–20	<3.5	20–34	112
Co	7–23	<7.0	7–23	53.1
Cu	13–26	<13	25.6–40	51.0
Cr	29–66	<29	66–110	137
Ni	7–20	<7.5	20–56	153
Pb	15–36	<36	36–50	120
Zn	5.5–76	<76	76–135	404

Upper limits of ABC calculated as recommended by Technical Guidance Document on Risk Assessment (TGD; European Commission, 2003) are also shown.
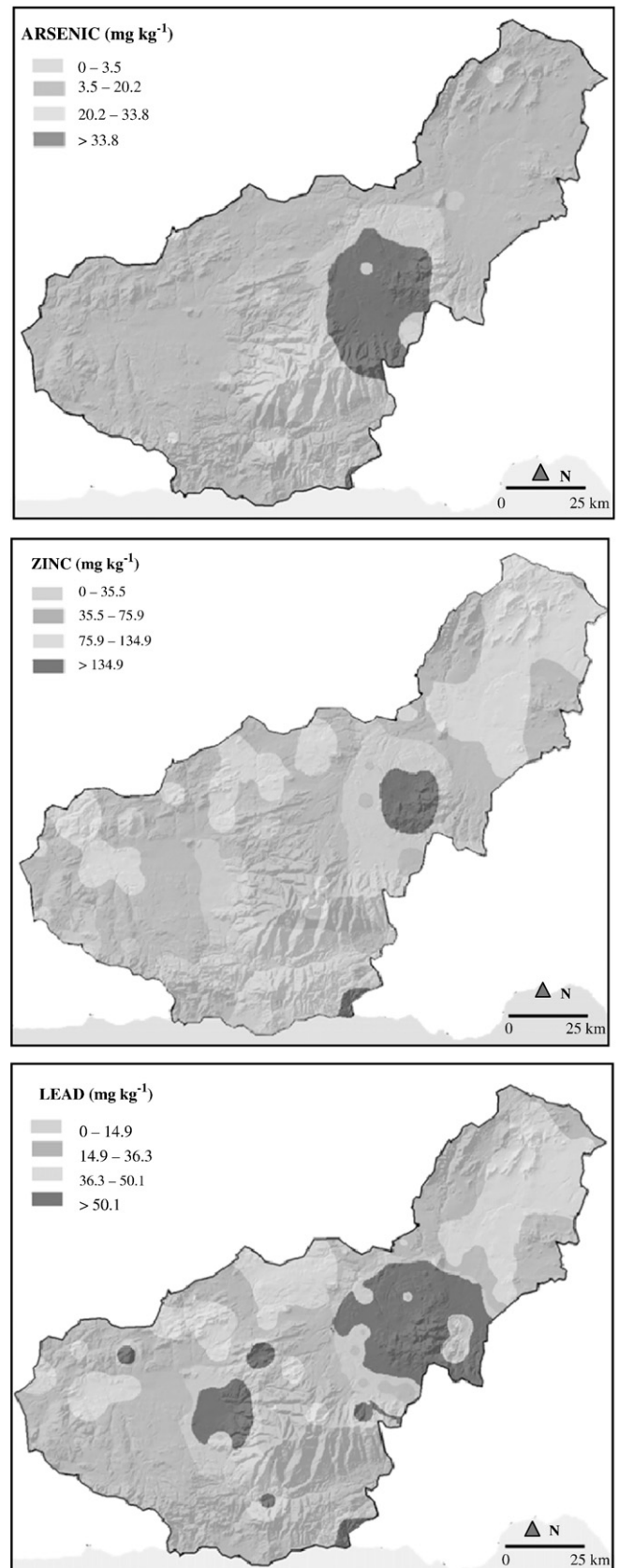

Fig. 5. Spatial distribution of different As, Pb and Zn concentrations in soils (0–20 and 20–40 cm) and parent materials of Granada province, Spain.
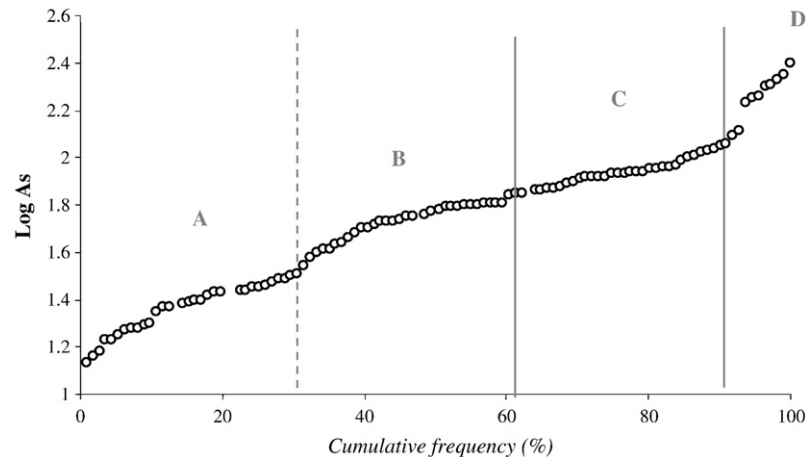
Fig. 6. Tier II (refined screening): relative cumulative frequency curve of log-transformed arsenic concentrations in Alquife soils, Granada, Spain. Dashed line separates samples showing contents higher than the regional ambient background concentration for As (----) and solid lines correspond to inflexion points delimiting different classes (—). As concentrations in mg kg^{-1} dry soil.

3.2.2. Total trace element concentrations and correlation with soil parameters

Soils in the Alquife area contained mean total concentrations higher than 65 mg kg^{-1} dry soil for arsenic, chromium and zinc; between 25 and 40 mg kg^{-1} for nickel, lead, and copper; and 14 mg kg^{-1} for cobalt. Compared with the regional ABC (Table 3), 69% of the samples in Alquife area exceeded the upper limit for arsenic, <10% for Cu, and only less than 5% for the others elements. So, the TE of most concern was arsenic, which therefore was studied in more detail.

3.2.2.1. Different arsenic concentrations with depths and geology.
No significant differences in total arsenic concentration were found with depth, which could be related to the homogenization of the top soil layer by plowing or agricultural activities or due to bioturbation mediated by earthworms or small mammals (Zorn et al., 2008). Significant differences were found between the soils developed over the metamorphic sediments coming from Sierra Nevada (mean arsenic 89.9 mg kg^{-1} dry soil) and the carbonate materials coming from Sierra de Baza (mean arsenic 29.5 mg kg^{-1}), indicating a strong influence of the lithology on the arsenic concentration in soils.

3.2.2.2. Arsenic distribution derived from the cumulative frequency distribution curve approach.
In the CFDC, four different classes (A–D in Fig. 6) were identified for arsenic concentrations in the Alquife area, each one explained by Tukey box-plots in Fig. 7. The first class (A) corresponds to concentrations below the regional ABC range (33.8 mg kg^{-1} dry soil): however, a more detailed analysis of the dataset allowed to identify these samples as carbonate soils, characterized by an ABC below 3.5 mg kg^{-1} at the regional scale (see Table 3). These soils are potentially contaminated.

The other classes identified by CFDC corresponded to samples exceeding the regional arsenic ABC. Class C, with concentrations between 64.6 and 123 mg As kg^{-1} dry soil, was identified as most developed soils over metamorphic materials. Class B, with concentrations between 32.4 and 64.6 mg As kg^{-1} dry soil, corresponded to soils over consolidated and unconsolidated carbonate and metamorphic materials. Finally, soils with concentrations higher than 123 mg kg^{-1} dry soil (class D) would be most polluted, being located at the East of the mine and related to an area of ore residue accumulation (Fig. 8).

3.2.2.3. Association between arsenic content and soil parameters.
Regression analysis between Alquife soil properties and total arsenic concentration indicated a strong positive association ($P < 0.001$) with the different iron forms, mainly total and dithionite-extractable iron

forms (AsT (mg kg^{-1}) = 53.950 Fe$_d$ (%), $r^2 = 0.761$), as well as a negative association between total arsenic concentration and the calcium carbonate content in Alquife soils (As (mg kg^{-1}) = 87.70 − 1.689 CaCO$_3$ (%)). These relations may indicate that arsenic is distributed in the landscape related to iron; it suggests it may be adsorbed onto Fe oxyhydroxides and oxide minerals and so displayed in a similar distribution pattern in the mine area. Decarbonising and desaturation processes probably led to concentrating arsenic content in these soils.

3.2.3. Water and NH$_4$NO$_3$ extractable TE concentrations

Mean water-extractable and 1 M NH$_4$NO$_3$-extractable concentrations for all trace elements analysed are shown in Table 4.

In general, zinc was the TE with the highest water and NH$_4$NO$_3$-extractable concentration, with a mean concentration of 0.121 and 0.153 mg Zn kg^{-1}, respectively. Mean water-extractable concentrations exceeded the action level for As, Co and Cr, whereas the intervention levels based on 1 M NH$_4$NO$_3$ extractable concentrations (DIN v 19730) were not exceeded for any of the TE.
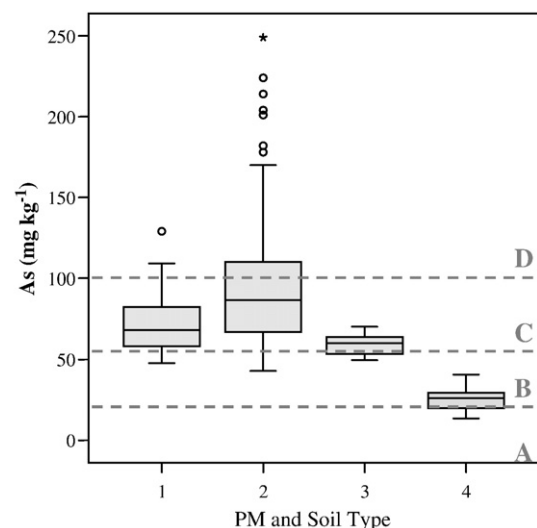

Fig. 7. Tier II (refined screening): box plot of arsenic concentrations in different types of parent materials (PMs) in Alquife soils, Granada, Spain (1 = Chromic Luvisols and Calcic Luvisols over consolidated and unconsolidated materials coming from Sierra Nevada and Sierra de Baza, 2 = Eutric Cambisol and Eutric Regosols over metamorphic materials, 3 = Fluvisol over unconsolidated sediments; 4 = Calcisols and Calcaric Regosols over carbonate materials).
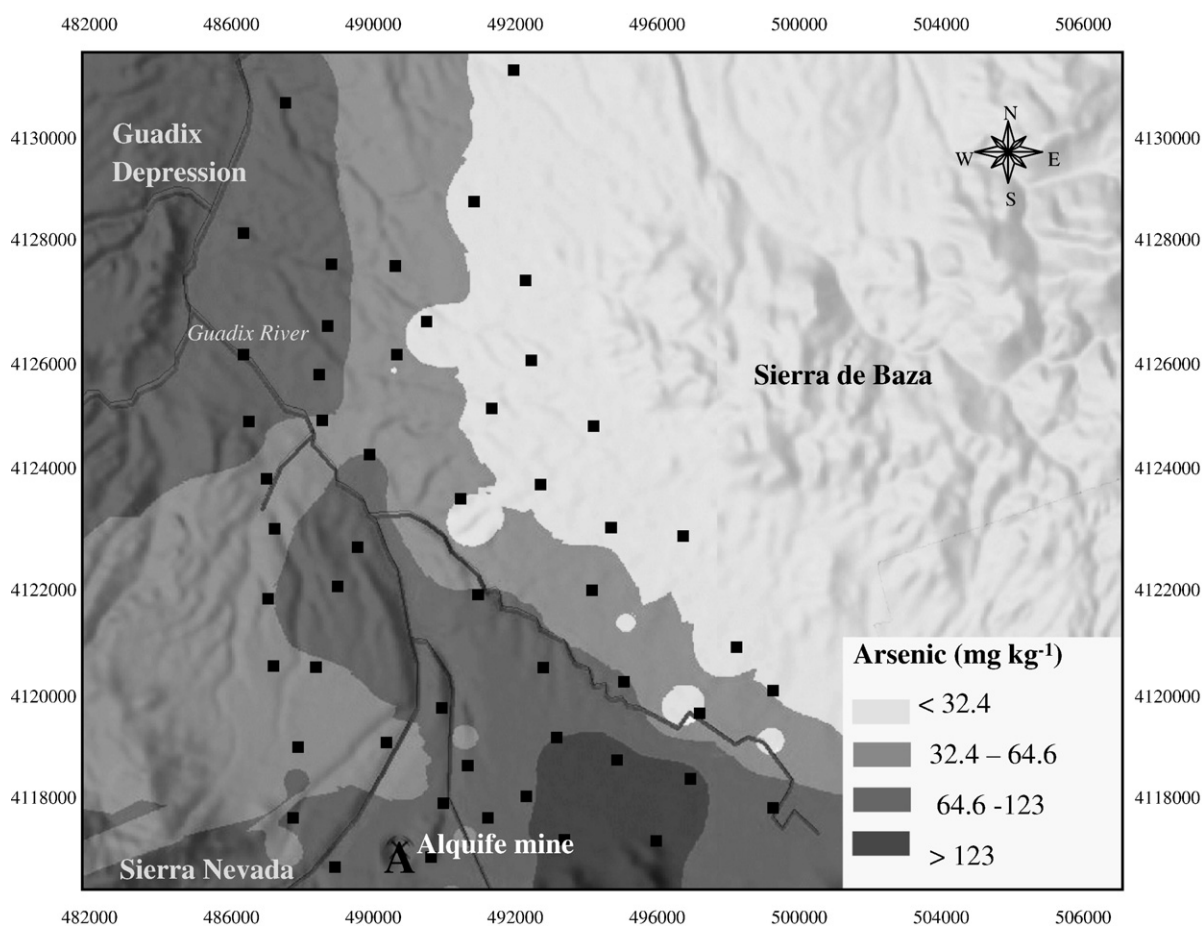
Fig. 8. Tier II (refined screening): map of arsenic concentrations in Alquife mine soils (Granada, Spain) with the different classes identified by CFDC (Fig. 6). Alquife mine and the main geology formations in the area (Sierra de Baza and Sierra Nevada) are also pointed out on the map. Coordinates from the 1:50,000 map are shown.

Thus, besides the high total arsenic content analysed in Alquife soils, this element was found in relatively soluble forms reaching values that could pose a risk for the surrounding ecosystems.

3.2.4. Soil toxicity

Although chemical analysis provides data about environmental pollutant concentrations, it gives little information about toxicity at the site. Therefore, biological analyses combined with chemical analyses are essential to infer probable adverse biological effects (Chapman et al., 2002).

Water extracts of 22 selected soils were investigated with two different bioassays (Table 5). A significant reduction in light output of *E. coli* was observed in just one sample (sample A1), with the rest of luminescence values ranging from 38% to 110%. In the Microtox test, most of the samples showed light inhibition values (I) ranging from 1.11 to 2.65 after 5 minutes exposure and from 1.25 to 5.41 after 15 minutes exposure. *V. fischeri* is a marine luminescent bacterium, which requires optimal conditions of pH and salinity, and values of I

close to 1 may also be measured in distilled water. Therefore, *V. fischeri* toxicity data were interpreted as relative toxicity between samples. Samples 1, 9 and 21 showed the highest reduction in the light output of this bacterium.

Both bioassays indicate that solutions of the potentially contaminated soils showed low toxicity to *E. coli* and *V. fischeri*, with just one soil sample being toxic to both organisms (sample 1). In this sample, only the low pH value (<5) could explain the higher toxicity to these organisms. Indeed, effects of pH, medium composition or salinity were shown to be important as these factors change the chemical speciation what, in turn, may modify toxicity (Villaescusa et al., 1997).

4. Conclusions

For regional and local studies covering soil samples belonging to both uncontaminated and contaminated populations, the CFDC has shown to be a powerful tool to discriminate different populations of samples and define their background TE levels.

Table 4

Mean water (E_w) and 1 M NH_4NO_3 extractable ($E_{NH_4NO_3}$) concentrations of trace elements in soils from Alquife mine area in Granada, Spain (minimum and maximum values in brackets).

TE (mg kg^{-1})	As	Co	Cr	Cu	Ni	Pb	Zn
E_W	0.11 (0.01–0.32)	0.03 (0.01–0.15)	0.01 (0.00–0.07)	0.08 (0.00–0.31)	0.03 (0.01–0.16)	0.08 (0.00–0.37)	0.12 (0.00–0.50)
$E_{NH_4NO_3}$	0.012 (0.00–0.11)	0.04 (0.00–1.00)	0.02 (0.00–0.05)	0.01 (0.00–0.04)	0.07 (0.00–1.91)	0.01 (0.00–0.21)	0.15 (0.00–0.87)
AV_W	0.04	0.01	0.01	1.00	0.20	1.00	1.00
$AV_{NH_4NO_3}$	0.10	0.50	0.10	1.00	1.00	3.00	5.00

Action values (AVs) for trace elements in soil solution (AV_w, mg kg^{-1}) (Bohn et al., 2001; Ewers, 1991) and for mobile trace elements ($AV_{NH_4NO_3}$) in soils based on the German national standard DIN 19730 (Prueb, 1997) are also shown.

Table 5
Bioluminescence levels expressed by the two biosensors (*E. coli* and *V. fischeri*) exposed to Alquife soils' elutriates (samples labelled from A1 to A22) for the soil samples.

Sample	$I_{E.\ coli}$	$I_{5'}$	$I_{15'}$
Al	38.7	2.30	5.41
A2	83.4	1.72	2.12
A3	91.5	1.30	1.73
A4	91.4	1.37	1.70
A5	89.1	1.22	1.72
A6	76.7	1.21	1.37
A7	69.8	1.13	1.55
A8	83.0	1.16	1.44
A9	78.5	2.24	2.69
A10	76.6	1.23	1.40
A11	74.3	1.24	1.63
A12	78.3	1.38	1.74
A13	85.6	1.53	1.71
A14	84.1	1.26	1.48
A15	78.3	1.12	1.31
A16	84.6	1.37	1.58
A17	79.8	1.54	1.69
A18	81.1	1.42	1.70
A19	81.1	1.64	1.85
A20	104.4	1.11	1.26
A21	110.2	2.66	2.89
A22	103.3	1.28	1.48

E. coli luminescence values ($I_{E.\ coli}$) were recorded as mean of three replicated values and expressed as a percentage in relation to the control (deionized water). *V. Fischeri* inhibition values were recorded as Inhibition $= I_0 - I_t / I_t$ were I_0 is the luminescence of the cell in time 0 and It is the luminescence of the sample in $t = 5$ (I5′) and 15 min (I15′).

In this study conducted in the Granada region, the degree of soil development and parent material composition were the two main parameters that influenced the trace element concentrations in samples and the segregation of the dataset into different classes. The more detailed analysis showed that most parent materials (PMs) and soils of the region were defined by the following ABC: 3.5–20 mg As kg^{-1}, 7–23 mg Co kg^{-1}, 13–25.6 mg Cu kg^{-1}, 29–66 mg Cr kg^{-1}, 7–20 mg Ni kg^{-1}, 15–36 mg Pb kg^{-1} and 5.5–76 mg Zn kg^{-1}. Highly carbonated materials and very weakly-weathered soils developed on them (Entisols) were characterized by low TE contents: As < 3.5 mg kg^{-1}, Co < 7 mg kg^{-1}, Cu < 13 mg kg^{-1}, Cr < 29 mg kg^{-1}, Ni < 7.5 mg kg^{-1}, Pb < 36 mg kg^{-1} and Zn < 76 mg kg^{-1}. Most developed soils (Alfisols) and those developed over metamorphic rock presented the following ranges of trace element contents: 20–33.8 mg As kg^{-1}, 25.5–40 mg Cu kg^{-1}, 66–110 mg Cr kg^{-1}, 20–55.9 mg Ni kg^{-1}, 36–50 mg Pb kg^{-1} and 75.9–135 mg Zn kg^{-1}.

In Granada province, outliers for arsenic, lead and zinc concentrations were identified in the surroundings of an iron mine, Alquife. The high iron content of the soils and the stability of oxides and hydroxides in this climate may increase arsenic retention. The high carbonate content may also be responsible for the precipitation and immobilization of other TE by the formation of stable complexes that retain TE in relatively stable forms, reducing harmful effects on the environment.

This study shows that the use of total concentration of trace elements for regulatory purposes could give rise to an overestimation of the risk of historically contaminated soils. After identification of potentially contaminated sites, the use of measures of bioavailability and ecotoxicity in ERA will improve the realism of site-specific ecological risk assessment.

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