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EFFECTS OF CLIMATE CHANGE ON THE TOXICITY OF SOILS POLLUTED BY METAL MINE WASTES TO *ENCHYTRAEUS CRYPTICUS*

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Abstract: The present study aimed to assess the effects of climate change on the toxicity of metal-polluted soils. Bioassays with *Enchytraeus crypticus* were performed in soils polluted by mine wastes (mine tailing, forest, and watercourse) and under different combinations of temperature (20 °C and 25 °C) and soil moisture content (50% and 30% of the soil water-holding capacity). Survival and reproduction were set as endpoints. No effect was observed on survival (average survival ≥ 80%). Reproduction was the most sensitive endpoint, and it was reduced between 65% and 98% compared with control after exposure to watercourse soil (lower pH, higher salinity, and higher available metal(loid) concentrations). In this soil, effective concentrations at 50% and 10% (EC50 and EC10) significantly decreased with decreasing soil moisture content. In general, the worst-case scenario was found in the driest soil, but the toxicity under a climate change scenario differed among soil types in relation to soil properties (e.g., pH, salinity) and available metal(loid) concentrations. *Environ Toxicol Chem* 2015;34:346–354. © 2014 SETAC

Keywords: Climate change Risk assessment Soil moisture Soil invertebrates Temperature

INTRODUCTION

According to the Intergovernmental Panel on Climate Change, in the coming decades we will face rising global temperatures and alterations in precipitation patterns, with an increasing frequency of extreme events. Temperature is expected to increase by 0.2 °C per decade under a range of probable greenhouse gas emission scenarios [1]. Forzieri et al. [2] have noted that climate warming is expected to alter the water balance across Europe, with more severe droughts in the southern parts (e.g., the Iberian Peninsula and the southernmost regions in France, Italy, and the Balkans).

Changes in climate conditions might alter physical, chemical, and biological properties of ecosystems, affecting organisms but also the behavior and distribution of chemicals, including pollutants [3,4]. Changes in environmental conditions could therefore markedly increase the toxicity risk of harmful substances present in the environment. Given this scenario of climate change, the impacts of human activities that negatively affect the sustainability of ecosystems will be intensified.

Mining is one of the most detrimental activities worldwide because of the large volumes of potentially toxic wastes generated. Mining wastes are often characterized by unfavorable conditions for the development of living organisms, such as a wide range of pH values (from acid to basic), high contents of salts, deficiency of organic matter and nutrients, high concentration of metals, and low water retention capacity [5]. Traditionally, the evaluation of metal-polluted sites has been performed through chemical analysis of soil for total and exchangeable metal fractions. However, the latter is not enough to evaluate the environmental risks of metal-polluted areas, and an integration of chemical and biological (toxicological) information is necessary to properly assess ecotoxicological risks [6,7].

Temperature and soil moisture content are key factors to be regulated during the performance of ecotoxicity tests. Previous studies have shown that in metal-polluted systems, the performance of organisms might be dependent on the environmental conditions to which they are exposed [8,9]. Thus bioassays might be used to simulate different scenarios of climate change and to evaluate the effects of metal pollution on the health and quality of soils and thus on the environment.

Soil invertebrates are often used in bioassays because of their role as bioindicators of both soil quality and health, as well as of the biological impact of pollutants present in the system. In addition, invertebrate bioassays are relatively fast and low-cost [10]. Among soil invertebrates, enchytraeids (class Oligochaeta, family Enchytraeidae) play a key role in terrestrial ecosystems. They are involved in organic matter decomposition, nutrient mobilization, soil bioturbation, and the improvement of soil structure [11]. Because they are soft-bodied organisms, enchytraeids live in close contact with soil solution and the contaminants present in the soil, and therefore are suitable bioindicators of stress conditions [12].

The aim of the present study was to assess the effects of climate change (temperature and soil moisture content) on the toxicity to *Enchytraeus crypticus* of soils polluted by metal mine wastes. To achieve this goal, bioassays with enchytraeids were performed in different soils polluted by metal mine wastes and under different combinations of temperature (20 °C and 25 °C) and soil moisture content (50% and 30% of the soil water-holding capacity [WHC]). Survival and reproduction were determined as endpoints. Our hypothesis was that an increase in ambient temperature and a decrease in soil moisture content affect the toxicity to *E. crypticus* of metal-polluted soils, enhancing the susceptibility of the organisms.

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MATERIALS AND METHODS

Study area

The Cartagena–La Unión mining district (0-392 m a.s.l; 50 km^2; 37°37′20 ″N, 0°50′55 ″W to 37°40′03 ″N, 0°48′12 ″W) is located in southeastern Spain (Figure1). The area is characterized by a semiarid Mediterranean climate (annual mean temperature ~18°C, annual mean precipitation ~250 mm–300 mm, and mean evapotranspiration rate ~856 mm yr^{-1}).

The area is among those most affected by the impact of mining activities in Europe [13]. Presently, there are more than 40 mine tailings at which the wastes have been piled up and that face erosion problems because no environmental restoration has been attempted. As a consequence, the short intensive rainfall, typical of this area, can relocate large volumes of polluted wastes with high concentrations of metals from these sites to the surrounding lowland areas [14].

Soil sampling and characterization

Three soils from different environments inside the mining district were selected: 1) soil from the slope of a mine tailing, 2) soil from a natural forest close to the mine tailing and with the presence of metal pollution, and 3) soil from an intermittent watercourse coming from the mining area and with the presence of metal-polluted sediments. Soil samples were taken from the top 20 cm by mixing 3 randomly distributed subsamples to constitute a composite sample per environment. Soil samples were air-dried, sieved through a 2-mm mesh, and homogenized.

Soil pH in 0.01 M $CaCl_2$ and electrical conductivity in water were measured in 1: 5 (w/v) suspensions, after 2 h shaking at 200 rpm. Samples were left overnight to allow floating particles to settle. The pH and electrical conductivity were measured with a WTW pH 7110 meter and a WTW Multiline P4 meter, respectively. Available metal(loid)s (As, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn) were determined in the $CaCl_2$ suspensions, after filtration (0.45 μm) and acidification of the samples with concentrated HNO_3. The concentrations of Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn were measured by flame atomic absorption spectroscopy (AAS; Perkin-Elmer Analyst 100) and that of As by graphite furnace atomic absorption spectroscopy (Perkin-Elmer 5100 ZL). Organic matter content was determined as loss on ignition at 500 °C. Organic carbon content was calculated by dividing the organic matter content by the van Bemmelen factor 1.724 [15]. Cation exchange capacity (CEC) was determined by saturation of the soil exchange complex with 1 N CH_3COONa, pH 8.2 [16]. Then sodium was displaced with 1 N CH_3COONH_4, pH 7.0, and measured by flame atomic absorption spectroscopy (Perkin-Elmer Analyst 100). Water-holding capacity was determined by the sandbox method, after saturation of the soil with water for 3 h [17]. Particle size distribution was determined by laser grain size analysis with laser diffraction sensors (HELOS-QUIXEL) [18].

Total metal(loid)s (As, Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn were extracted by acid digestion with HNO_3, $HClO_4$ (4:1) in tightly Teflon-lined bombs at 140 °C for 7 h in a destruction oven and analyzed by flame and graphite furnace atomic absorption spectroscopy (Perkin-Elmer 5100 ZL). Quality control was checked using the certified reference soil ISE sample 989 from the International Soil-Analytical Exchange with recoveries of 107% for As, 113% for Cd, 121% for Co, 97% for Cu, 93% for Fe, 77% for Mn, 96% for Ni, 108% for Pb, and 121% for Zn.

Experimental setup

Test treatments. According to the standardized guidelines International Association for Standardization (ISO) 16387 [19] and Organisation for Economic Co-operation and Development (OECD) 220 [20], ecotoxicity tests with enchytraeids have to be performed at an ambient temperature of approximately 20 °C and a soil water content of approximately 50% of the WHC. Taking this into account, 2 temperatures (20 °C and 25 °C) and 2 soil water contents (50% and 30% of the soil WHC) were selected. Thus 4 climate conditions were tested: 1) 20°C + 50% WHC, 2) 20°C + 30% WHC, 3) 25°C + 50% WHC, and 4) 25°C + 30% WHC.

Soils preparation. A dilution approach was chosen to assess the overall toxicity of the study soils. The standard reference soil Lufa 2.2 (Speyer) was used for diluting the study soils. As shown in Table 1, Lufa 2.2 had lower pH (5.49) than the study soils. Because soil pH might influence metal availability, soil

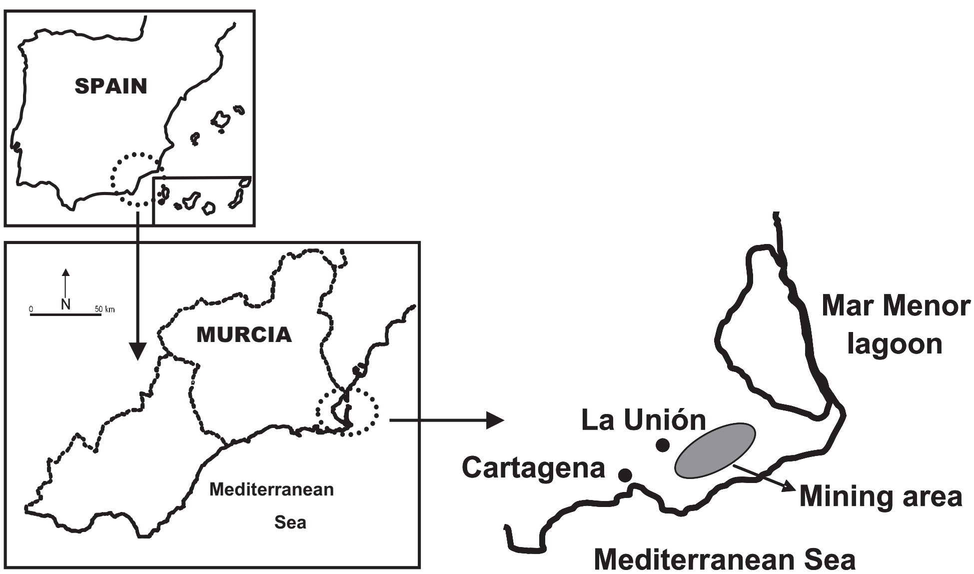

Figure 1. Location map of the sampling site (southeast Spain).

Table 1. Characterization of the study soils and the Lufa 2.2 control soil[a]

Parameter	Unit	Soil			
		Mine tailing	Forest	Watercourse	Lufa 2.2
pH 0.01 M $CaCl_2$		7.44	7.24	6.04	5.49
EC	mS cm^{-1}	2.22	0.39	2.43	0.14
OC	%	0.87	5.69	2.52	2.04
WHC	%	36.8	74.2	49.8	44.6
CEC	$cmol_c\ kg^{-1}$	10.0	28.2	13.1	9.10
Sand	%	76	37	46	75
Silt	%	13	35	33	13
Clay	%	11	28	21	12
Total metal(loid)s	mg kg^{-1}				
As		843	808	742	4.24
Cd		32.8	22.9	16.3	<DL
Co		18.5	16.9	12.4	1.30
Cu		118	66.4	177	3.43
Fe		218 316	192 741	147 763	4286
Mn		7423	7419	2791	127
Ni		19.0	21.6	16.5	3.85
Pb		6461	5783	5524	<DL
Zn		9813	8274	7990	20.7
Available metal(loid)s	μg kg^{-1}				
As		<DL	<DL	<DL	<DL
Cd		1976	45	678	89
Co		<DL	<DL	<DL	<DL
Cu		30	23	20	<DL
Fe		<DL	<DL	<DL	194
Mn		<DL	118	5533	8283
Ni		<DL	<DL	<DL	<DL
Pb		<DL	<DL	807	<DL
Zn		309	548	31 020	35

[a]Values are average of 2 replicates (variation between replicates less than 10%). Total metal(loid)s DL (in mg kg^{-1}): As (0.31), Cd (0.23), Co (0.46), Cu (0.23), Fe (1.6), Mn (0.69), Ni (0.69), Pb (2.1), and Zn (0.23). Available metal(loid)s DL (in μg kg^{-1}): As (20), Cd (15), Co (30), Cu (15), Fe (105), Mn (45), Ni (45), Pb (135), and Zn (15).

EC = electrical conductivity; OC = organic carbon; WHC = water-holding capacity; CEC = cation exchange capacity; DL = detection limit.

dilutions were prepared with Lufa 2.2 in which the pH was adjusted to approximately 7 (4 mg $CaCO_3$ g^{-1} dry soil) or 6 (0.5 mg $CaCO_3$ g^{-1} dry soil), depending on the pH of the study soils. Lufa 2.2 soil was also used as a control to check for the performance of enchytraeids under the different climate conditions tested (20 replicates).

Based on previous range-finding tests, the dilutions used for the soil from the mine tailing and the forest were (in percentage of polluted soil): 100% (original soil), 50%, 10%, 5%, and 0% (Lufa 2.2). For the watercourse soil the dilutions were 100%, 40%, 20%, 10%, 5%, and 0%. The WHC of all the dilutions was determined, and just before starting the bioassays soil samples were moistened at 50% or 30% of their WHC.

Toxicity tests. Enchytraeus crypticus were cultured at VU University (Amsterdam, The Netherlands) for several years in agar medium prepared with aqueous soil extracts. The cultures were maintained at 16 °C, 75% relative humidity, in complete darkness, and fed once a week with a mixture of oatmeal, dried yeast, yolk powder, and fish oil. Sexually mature animals (with clearly visible clitella) and of approximately the same size were used in the experiment. Toxicity tests were performed according to ISO guideline 16387 [19] and OECD guideline 220 [20]. Ten adult enchytraeids were introduced into 100-mL glass jars containing 20 g of soil previously moistened to the moisture content to be tested (5 replicates). Then 2 mg of oatmeal was supplied for food, and the jars were covered with perforated aluminum foil. The containers were incubated for 21 d (3 wk) at 20 °C or 25 °C, 75% relative humidity, and 12:12-h light:dark photoperiod. Soil moisture content was checked twice a week, by weighing the test jars, and replenishing them with water when necessary to keep soil moisture contents constant during the incubation period. Additional food was provided weekly if needed (only when no food was left in the test jar, to avoid fungal growth).

After 3 wk, the numbers of surviving adults and juveniles produced were determined in each test jar as follows. All samples were fixated by adding 10 mL of 96% ethanol. After 2 min, 100 mL of water was used to transfer the samples into plastic containers where they were stained with 200 μL of Bengal rose solution (1% in ethanol). The containers were tightly closed, agitated vigorously, and incubated overnight at 4 °C to achieve optimal staining conditions of enchytraeids. Then the samples were sieved over 160 μm to separate enchytraeids from most of the soil particles and transferred into white trays (80 × 50 cm^2) divided into fractions to facilitate counting under a magnifying glass (×2.5 power).

Statistical analyses

Statistical analyses were performed with SPSS Statistics 21. One-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test, was performed to check differences in the control performance of *E. crypticus* among the different climate conditions tested. Differences were considered significant at $p < 0.05$.

For each study soil and climate condition tested, survival and reproduction were obtained as endpoints. One-way ANOVA, followed by Dunnett's post hoc test, was carried out to determine the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC) values. The NOEC was defined as the highest percentage of polluted soil with no significant effect compared with control. The LOEC was defined as the lowest percentage of polluted soil with a significant effect compared with control. To estimate the effect of dilutions (percentages of polluted soil) causing *x*% reduction in survival (LC*x*) and reproduction (EC*x*), the datasets were fitted to a 3-parameter logistic dose–response model according to Haanstra et al. [21].

For LC*x* and EC*x* values, 95% confidence intervals were calculated by nonlinear regressions. A generalized likelihood ratio test [22] was applied to compare differences between the climate conditions tested in each soil.

RESULTS

Soil characterization

Soil properties are shown in Table 1. The pH in 0.01 M $CaCl_2$ was approximately 7 in the soils from the mine tailing and the forest, whereas the watercourse soil had a pH of 6.04. The electrical conductivity values were approximately 2 mS cm^{-1} except for the forest soil, which had an electrical conductivity value of 0.39 mS cm^{-1}, similar to Lufa 2.2 soil (0.14 mS cm^{-1}). The forest soil had the highest organic carbon content (5.69%), WHC (74.2%), and CEC (28.2 $cmol_c$ kg^{-1}), whereas the soil from the mine tailing had the lowest (organic carbon < 1%, WHC 36.8%, and CEC 10.0 $cmol_c$ kg^{-1}). The watercourse soil showed intermediate values of these parameters, closer to those of Lufa 2.2 soil, except for the CEC. The soil from the mine tailing had a sandy loam texture, similar to Lufa 2.2 soil (sand content > 70%), whereas the soils from the forest and the watercourse had a clay loam and loam texture, respectively.

As shown in Table 1, all the study soils had high concentrations of total metal(loid) s as a result of the pollution by metal mine wastes (742 mg kg^{-1}–843 mg kg^{-1} As, 16.3 mg kg^{-1}–32.8 mg kg^{-1} Cd, 12.4 mg kg^{-1}–18.5 mg kg^{-1} Co, 66.4 mg kg^{-1}–177 mg kg^{-1} Cu, 148–218 g kg^{-1} Fe, 2.79–7.42 g kg^{-1} Mn, 16.5 mg kg^{-1}–21.6 mg kg^{-1} Ni, 5.52–6.46 g kg^{-1} Pb, and 7.99–9.81 g kg^{-1} Zn). The highest available concentrations of Cd (1976 μg kg^{-1}) and Cu (30 μg kg^{-1}) were found in the soil from the mine tailing, whereas the watercourse soil had the highest concentration of available Pb (807 μg kg^{-1}) and Zn (31 020 μg kg^{-1}). Lufa 2.2 soil had the highest concentration of available Mn (8283 μg kg^{-1}).

Control performance

The control performance of *E. crypticus* in Lufa 2.2 soil and under the different climate conditions tested was evaluated according to the following validity criteria: adult survival $\geq$ 80%, number of juveniles per replicate $\geq$ 50, and coefficient of variation for reproduction $\leq$ 50% within replicates [19,20]. As shown in Table 2, the validity criteria were met in all the climate conditions tested: adult survival between 90% and 98%, number of juveniles between 576 and 1019, and coefficient of variation for reproduction between 10% and 27%.

Adult survival was significantly different between both temperatures at 30% of the soil WHC ($p = 0.035$), with the highest survivals at 25 °C and 30% of the soil WHC (Table 2). Reproduction was affected by the climate conditions tested, with the highest number of juveniles at 25 °C and 50% of the soil WHC. The number of juveniles significantly decreased when soil moisture content was decreased (20%–22% reduction, $p \leq 0.04$) and significantly increased when the temperature was increased (38%–41% increase, $p = 0.000$; Table 2).

Effects of climate conditions on the toxicity of metal-polluted soils

Adult survival was not affected after 21-d exposure to the different climate conditions in the 3 metal-polluted soils (average survival between 80% and 100%). In contrast, reproduction was affected both by metal pollution and the climate conditions tested, but in a different way depending on the soil type. In the soil from the mine tailing (Figure 2) the number of juveniles decreased with an increasing percentage of polluted soil (from 0% dilution [Lufa 2.2] to 100% dilution [original soil]) as follows: 1) from 917 ± 54 to 681 ± 41 at 20 °C and 50% of the soil WHC; 2) from 766 ± 74 to 556 ± 73 at 20 °C and 30% of the soil WHC; 3) from 1017 ± 132 to 716 ± 48 at 25 °C and 50% of the soil WHC; and 4) from 698 ± 168 to 516 ± 103 at 25 °C and 30% of the soil WHC. Reductions in reproduction were between 26% and 30%, compared with the Lufa 2.2 control soil, and they were significant ($p \leq 0.006$) for all the climate conditions except for 25 °C and 30% of the soil WHC.

In the forest soil (Figure 3), for both temperatures at 30% of the soil WHC, reproduction values were similar to those of mine tailing soil (Figure 3B and D). The number of juveniles decreased with an increasing percentage of polluted soil, with significant ($p \leq 0.035$) reductions of 35% and 19% compared with the Lufa 2.2 control soil at 20 °C and 25 °C, respectively. However, at 50% of the soil WHC, the behavior was different. At 20 °C, the number of juveniles was similar between the control (658 ± 106, 0% dilution) and the original soil (624 ± 187, 100% dilution), with higher numbers of juveniles in the intermediate dilutions (768–846; Figure 3A). When the temperature increased to 25 °C, the reproduction was more or less similar among the different dilutions (971–1199 juveniles; Figure 3C).

In the soil from the mine tailing, NOEC values decreased with decreasing soil moisture content (from 50% to 10% of polluted soil) at 20 °C, although it increased (from 50% to 100% of polluted soil) at 25 °C. In the forest soil, at both temperatures, NOEC values decreased with decreasing soil moisture content (from 100% to 50% of polluted soil). In most cases, LOEC values were $\geq$100% of polluted soil, except for the soil from the mine tailing at 20 °C and 30% of the soil WHC, in which the LOEC value was 50% of polluted soil.

Table 2. Control performance of *Enchytraeus crypticus* in Lufa 2.2 soil after 21-d exposure to the different climate conditions tested[a]

Climate condition	Adult survival (%)	No. of juveniles	CV for reproduction (%)
20° + 50% WHC	94 ± 9 AB	737 ± 128 B	17
20° + 30% WHC	90 ± 12 A	576 ± 154 A	27
25° + 50% WHC	97 ± 8 AB	1019 ± 98 C	10
25° + 30% WHC	98 ± 5 B	817 ± 175 B	21

[a]Values are average ± standard deviation (SD) ($n = 20$ replicates). For adult survival and number of juveniles, different letters indicate significant differences among climate conditions (one-way analysis of variance with Bonferroni's post hoc test, $p < 0.05$).
WHC = water-holding capacity; CV = coefficient of variation.

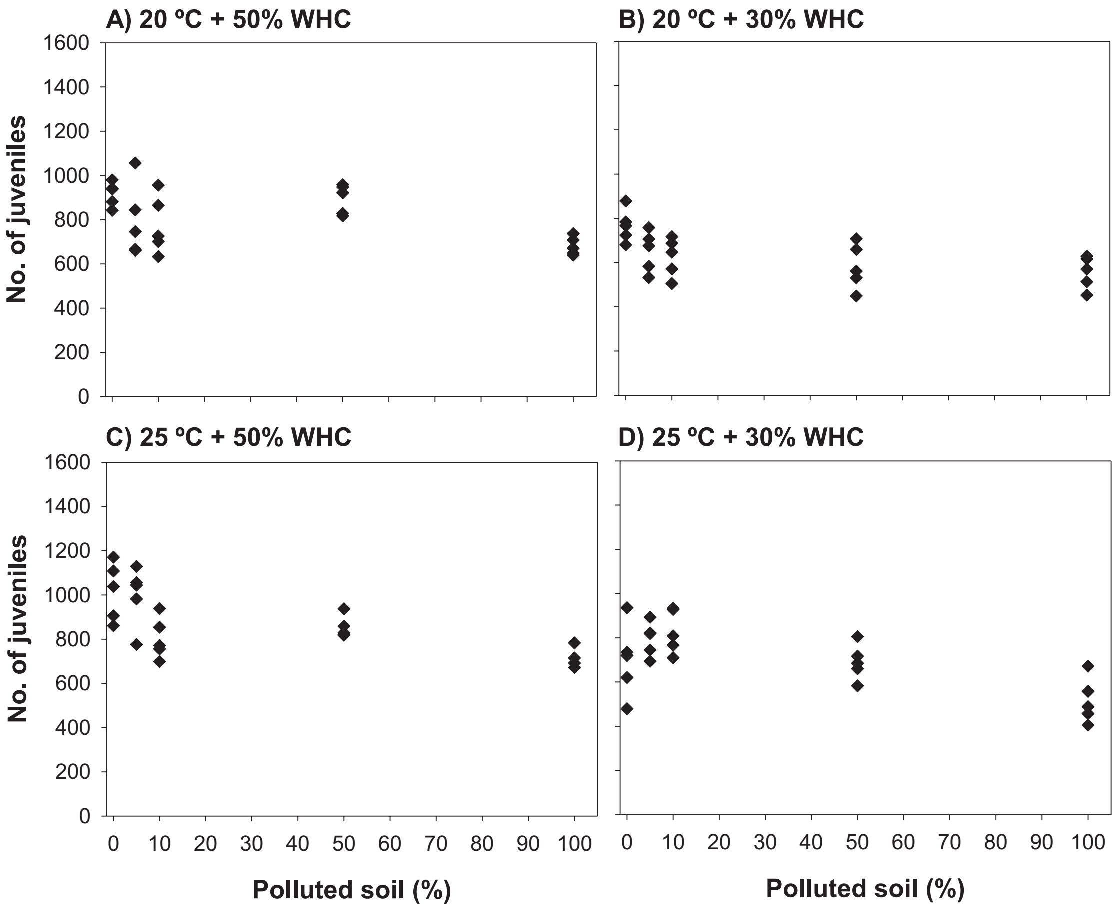

Figure 2. Reproduction of *Enchytraeus crypticus* after 21 d of exposure to different dilutions of mine tailing soil under the different climate conditions tested. (**A**) 20 °C and 50% of the soil WHC. (**B**) 20 °C and 30% of the soil WHC. (**C**) 25 °C and 50% of the soil WHC. (**D**) 25 °C and 30% of the soil WHC. X-axes show the percentage of polluted soil in relation to Lufa 2.2 soil and Y-axes the number of juveniles ($n = 5$). WHC = water holding capacity.

The watercourse soil had a different behavior (Figure 4). The number of juveniles decreased when the percentage of polluted soil was increased, but in a dose–response manner, with significant ($p = 0.000$) reductions in reproduction up to between 65% and 98% compared with the Lufa 2.2 control soil. At both temperatures, a decrease in soil moisture content significantly ($X^2_{df=1} > 10.8$; $p = 0.01$) decreased EC50 and EC10 values, with no differences at 30% of the soil WHC (Table 3). When the soil was moistened to 50% of its WHC, an increase in temperature significantly decreased the EC50 value ($X^2_{df=1} > 3.84$; $p = 0.05$; Table 3).

DISCUSSION

Assessment of metal pollution levels in the study soils

All the soils collected for ecotoxicity bioassays had high concentrations of total metal(loid)s (Table 1). Our data were in the same range as those shown by other studies carried out in the mining district of La Unión–Sierra de Cartagena. Conesa et al. [23] found total concentrations of 94 mg kg^{-1} to 530 mg kg^{-1} Cu, 4900 mg kg^{-1} to 7900 mg kg^{-1} Pb, and 7670 mg kg^{-1} to 12 300 mg kg^{-1} Zn in different tailings. Párraga-Aguado et al. [14] studied the spread of metal pollution from different tailings to the surrounding forest area. They found total concentrations of 320 mg kg^{-1} to 657 mg kg^{-1} As, 27 mg kg^{-1} to 47 mg kg^{-1} Cd, 94 mg kg^{-1} to 112 mg kg^{-1} Cu, 6288 mg kg^{-1} to 11 854 mg kg^{-1} Pb, and 8653 mg kg^{-1} to 10 715 mg kg^{-1} Zn in a radius of 100 m from the tailings. García-García [24] analyzed sediments polluted by metal mine wastes from different watercourses coming from the mining district, with total concentrations of 15 mg kg^{-1} to 24 mg kg^{-1} Cd, 30 mg kg^{-1} to 42 mg kg^{-1} Cu, 950 mg kg^{-1} to 3690 mg kg^{-1} Pb, and 650 mg kg^{-1} to 3500 mg kg^{-1} Zn.

For the present study area there is no legislation to declare a soil polluted by metal(loid)s. However, the total concentrations of As, Pb, and Zn greatly surpassed the intervention values established in Andalusia, a Spanish province nearby (As > 300 mg kg^{-1}, Pb > 2000 mg kg^{-1}, and Zn > 3000 mg kg^{-1}; [25]), and those of Cd were close to proposed levels (Cd > 30 mg kg^{-1}; [25]). The total metal(loid) concentrations also surpassed the intervention values proposed by other European countries: The Netherlands (As > 76 mg kg^{-1}, Cd > 13 mg kg^{-1}, Pb > 530 mg kg^{-1}, and Zn > 720 mg kg^{-1}; [26]) and Denmark (As > 20 mg kg^{-1}, Cd > 5 mg kg^{-1}, Pb > 400 mg kg^{-1}, and Zn > 1000 mg kg^{-1}; [27]).

The total metal(loid) concentrations measured in the study soils were in many cases higher than those found in soils polluted by different metal toxic spills. Simón et al. [28] reported total concentrations of 46.3 mg kg^{-1} As, 2.2 mg kg^{-1} Cd, 15.5 mg kg^{-1} Co, 116 mg kg^{-1} Cu, 674 mg kg^{-1} Mn, 28.0 mg kg^{-1} Ni, 135 mg kg^{-1} Pb, and 634 mg kg^{-1} Zn in the top 10 cm of soils polluted by the Aznalcóllar accident (Spain, 1998), one of the worst ecological disasters in Europe. Renforth et al. [29] found total concentrations of 14.9 mg kg^{-1} to 78.5 mg kg^{-1} As, <1 mg kg^{-1} to 4.0 mg kg^{-1} Cd, 9.1 mg kg^{-1} to 97.1 mg kg^{-1} Co, 21.9 mg kg^{-1} to 60.3 mg kg^{-1} Cu,

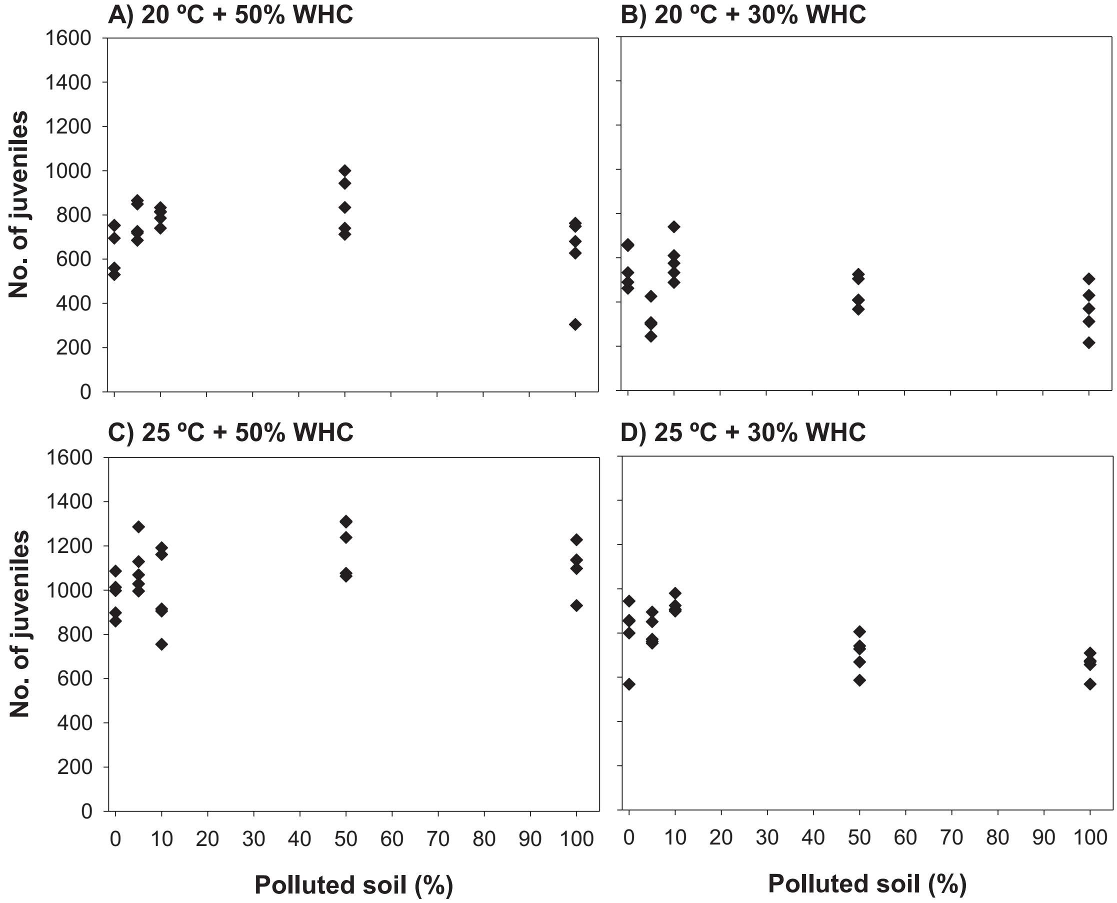

Figure 3. Reproduction of *Enchytraeus crypticus* after 21 d of exposure to different dilutions of the forest soil under the different climate conditions tested. (**A**) 20 °C and 50% of the soil WHC. (**B**) 20 °C and 30% of the soil WHC. (**C**) 25 °C and 50% of the soil WHC. (**D**) 25 °C and 30% of the soil WHC. X-axes show the percentage of polluted soil in relation to Lufa 2.2 soil and Y-axes the number of juveniles ($n = 5$). WHC = water holding capacity.

16.4 g kg^{-1} to 210 g kg^{-1} Fe, 1.3 g kg^{-1} to 2.6 g kg^{-1} Mn, 4.9 mg kg^{-1} to 79.8 mg kg^{-1} Pb, and 44.4 mg kg^{-1} to 173 mg kg^{-1} Zn in soil samples taken 1 mo after the Ajka red mud spill (Hungary, 2010) at a distance of 0 km to 86 km from the dike failure.

Despite the high concentrations of total metal(loid)s in the study soils, the concentrations in 0.01 M $CaCl_2$ extracts were fairly low in most cases. This may suggest that bioavailability was low, although some authors [30,31] have shown that low porewater and/or 0.01 M $CaCl_2$ extractable metal(loid) concentrations do not guarantee low bioavailability.

Hence, the high level of metal pollution present in the soils from different environments in the mining district of La Unión–Sierra de Cartagena needs further investigation, (e.g., ecotoxicological studies), particularly taking into account future climate change predictions in semiarid areas.

Effects of climate conditions on the toxicity of metal-polluted soils

Survival of *E. crypticus* was not affected by the climate conditions tested (Table 2), the presence of metal pollution, or the combination of both. In all cases, adult survival was above 80%. However, reproduction was shown to be a more sensitive endpoint [10,32]. As shown by the control performance in Lufa 2.2 soil (Table 2), a decrease in soil moisture content negatively affected reproduction. Our data are in agreement with other studies noting that enchytraeid performance depends on the availability of water, and the animals being vulnerable to drought stress [32,33] because of their highly permeable skin [34]. In contrast, enchytraeids are rather tolerant of temperature ranges between 5 °C and 28 °C [34]. In the control group in the present study, reproduction was significantly higher with increasing temperatures (Table 2). This positive effect of temperature has also been shown by other studies [35,36].

When enchytraeids were exposed to changing climate conditions (temperature and soil moisture content) in the 3 metal-polluted study soils, their sensitivity differed among soils. The watercourse soil was the only one in which a reduction in reproduction of more than 50% was seen, under all the climate conditions tested. This soil was more toxic for *E. crypticus* than the soils from the mine tailing and the forest. In the soil from the watercourse (Figure 4), at both temperatures, a decrease in soil moisture content significantly decreased reproduction, with less than 30 juveniles in the original soil (100% dilution). Consequently, the worst-case scenario proved to be the driest soil (30% of the soil WHC), for which the lowest EC50 and EC10 values were observed (Table 3). This finding could be related both directly to a biological effect of drought stress, as shown by the control performance (Table 2), and indirectly to higher available metal concentrations at lower soil moisture contents.

In the watercourse soil, at 50% of the soil WHC, an increase in temperature of 5 °C significantly decreased the EC50 from 60.4% to 40.7% of polluted soil (Table 3), even though the controls showed a higher number of juveniles at higher temperature. This trend was not found at the lower soil water content. Our data agree with other studies showing increasing

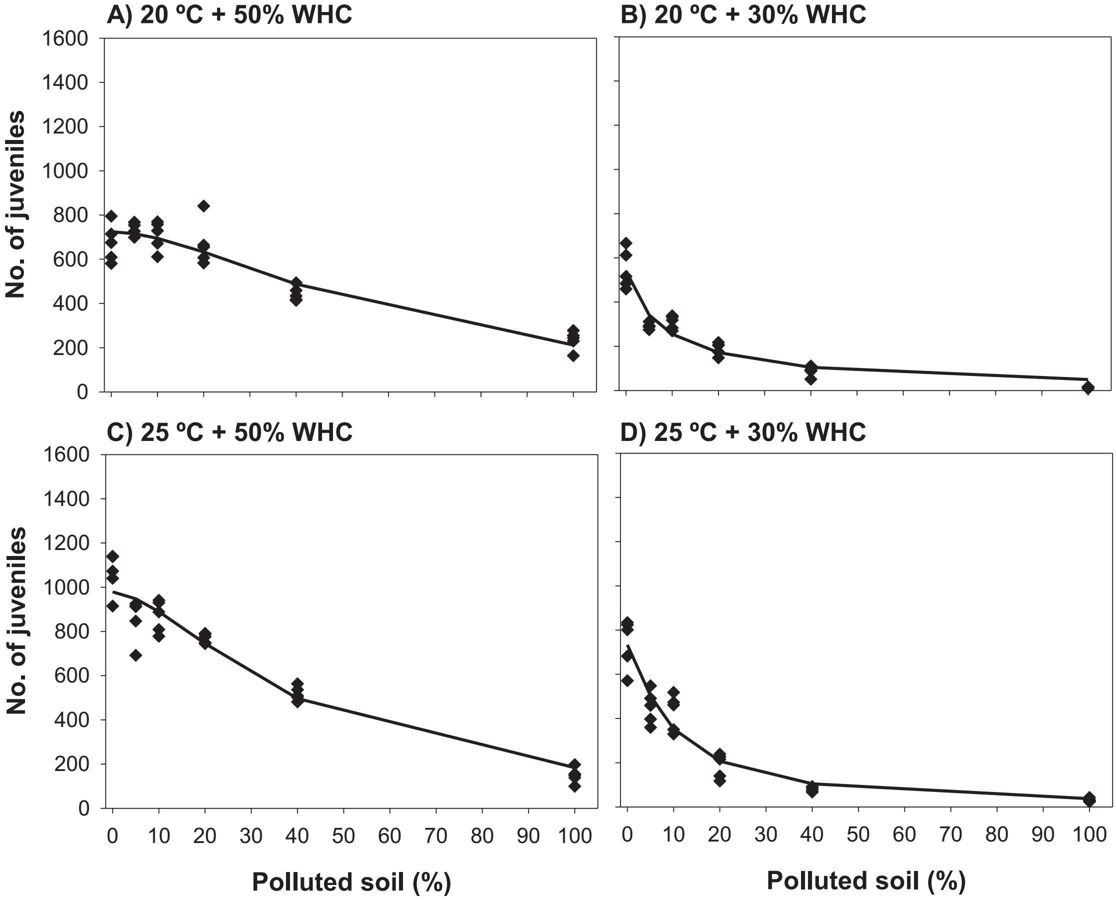

Figure 4. Reproduction of *Enchytraeus crypticus* after 21 d of exposure to different dilutions of the watercourse soil under the different climate conditions tested. (**A**) 20 °C and 50% of the soil WHC. (**B**) 20 °C and 30% of the soil WHC. (**C**) 25 °C and 50% of the soil WHC. (**D**) 25 °C and 30% of the soil WHC. X-axes show the percentage of polluted soil in relation to Lufa 2.2 soil and Y-axes the number of juveniles ($n = 5$). Lines show fit obtained with a 3-parameter logistic dose–response model. WHC = water holding capacity.

metal toxicity at higher temperatures in different organisms [37,38]. Spurgeon et al. [37] found greater Zn toxicity to the earthworm *Eisenia fetida* with increasing temperature after 21-d exposure at 15 °C, 20°C, and 25 °C. This trend was also shown by Otomo et al. [38] when *Enchytraeus doerjesi* was exposed to different Cd concentrations at 15 °C, 20 °C, and 25 °C. This behavior might be related to a faster metabolism of poikilothermic organisms at higher temperatures [39], resulting in increasing metal uptake rates. In addition, metal speciation and mobility might change if temperature changes [4]. In contrast, Cedergreen et al. [9] found an opposite behavior, with low temperatures enhancing the toxicity of Cd and Cu to *E. crypticus* in Lufa 2.2 soil.

Unlike the watercourse soil, the soils from the mine tailing and the forest did not have a toxic effect on *E. crypticus* under the different climate conditions tested. These differences in toxicity could be related to soil properties such as pH, salinity, organic matter content, and CEC [40] as well as to the different available metal(loid) concentrations. The soil from the watercourse had the highest salinity (2.43 mS cm^{-1}) and the lowest pH (6.04) among the 3 study soils (Table 1). Low pH, together with the moderate CEC value (13.1 $cmol_c\ kg^{-1}$) shown by watercourse soil, could be responsible for the highest available concentrations of Mn, Pb, and Zn (Table 1) and consequently the greater toxicicity to *E. crypticus*. In fact, the watercourses of this area receive lixiviates coming from higher

Table 3. No-observed-effect concentration (NOEC), lowest-observed effect concentration (LOEC), effect concentration causing 10% reduction (EC10), and effect concentration causing 50% reduction (EC50) for the effect of the watercourse soil on the reproduction of *Enchytraeus crypticus* after 21-d exposure to the different climate conditions tested[a]

Climate condition	NOEC	LOEC	EC10	EC50
20° + 50% WHC	20	40	17.2 (9.3–23.2) B	60.4 (48.9–71.8) C
20° + 30% WHC	<5	5	0.9 (0.1–1.6) A	8.9 (6.1–11.6) A
25° + 50% WHC	<5	5	10.6 (6.2–15.0) B	40.7 (33.7–47.7) B
25° + 30% WHC	<5	5	1.6 (0.6–2.6) A	9.4 (6.9–11.9) A

[a]Values are expressed as percentage of polluted soil in relation to Lufa 2.2 control soil. Values in parentheses are 95% confidence intervals. For EC10 and EC50, different letters indicate significant differences among climate conditions (likelihood ratio test, $p < 0.05$).
WHC = water-holding capacity.

topographic positions with high concentrations of mobile elements such as Zn, as shown by Conesa et al. [23]. In contrast, the forest soil had a higher pH (7.24), lower salinity (0.39 mS cm^{-1}), higher CEC (28.2 $cmol_c$ kg^{-1}) because of the greater content of organic matter and clay particles, and lower available concentrations of metal(loid)s (Table 1). Thus, this soil could have provided a more favorable environment for the performance of enchytraeids, even under changing climate conditions. In fact, in the forest soil, the number of juveniles was similar or even higher than in the Lufa 2.2 control soil at 50% of the soil WHC (Figure 3). Probably, when the metal pollution level was reduced by diluting the forest soil with Lufa 2.2, the higher organic matter content of the forest soil compared with the control soil could have favored a higher reproduction rate of the enchytraeids. The soil from the mine tailing, with a pH similar to the forest soil (7.44), low concentrations of available metal(loid)s except for Cd, but electrical conductivity and CEC values (2.22 mS cm^{-1} and 10.0 $cmol_c$ kg^{-1}, respectively) closer to those of the watercourse soil (Table 1), was intermediate in terms of toxicity, with a maximum reduction in reproduction of 30% compared with the Lufa 2.2 control soil. This variability between bioassays and the influence of soil properties has been studied extensively in different soil invertebrates, but only rarely from a climate change perspective. More studies on the effects of climate change on the toxicity of metal-polluted soils are necessary to improve our knowledge of the possible consequences of global warming in contaminated areas and to properly assess the ecotoxicological risks.

CONCLUSIONS

Survival of *E. crypticus* was not affected by either climate conditions (temperature and soil moisture content) or metal pollution in soils polluted by metal mine wastes from different environments inside a former mining district (mine tailing, forest, and watercourse). In contrast, reproduction was affected, but in a different way depending on soil characteristics. The soil from the watercourse (lower pH, higher salinity, and available metal(loid) concentrations) was the most toxic for *E. crypticus*, with reductions in reproduction between 65% and 98%, whereas in the mine tailing and forest soils the reductions in reproduction were less than 50%. In general, a decrease in soil moisture content negatively affected enchytraeid reproduction. In the soil from the watercourse, at 50% of the soil WHC, the toxicity was higher when the temperature increased.

Hence, the present study shows that the reproduction of *E. crypticus* was the most sensitive endpoint, the worst-case scenario was the driest soil, and the toxicity of different soils polluted by metal mine wastes under a climate change scenario will depend on soil properties (e.g., pH, salinity) and available metal(loid) concentrations.

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