Soil moisture influences the avoidance behavior of invertebrate species in anthropogenic metal(loid)-contaminated soils

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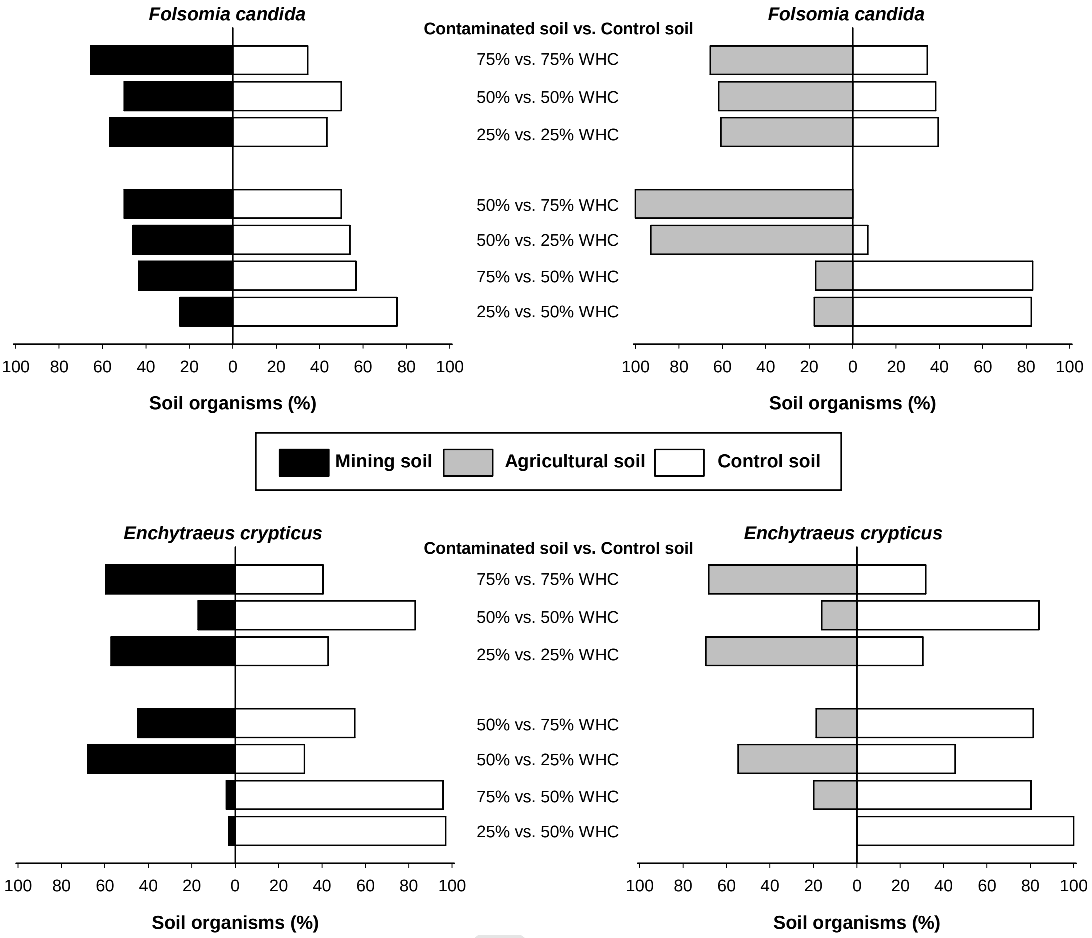
Folsomia candida
Contaminated soil vs. Control soil
Folsomia candida
75% vs. 75% WHC
50% vs. 50% WHC
25% vs. 25% WHC
50% vs. 75% WHC
50% vs. 25% WHC
75% vs. 50% WHC
25% vs. 50% WHC
100 80 60 40 20 0 20 40 60 80 100
Soil organisms (%)
■ Mining soil
▨ Agricultural soil
☐ Control soil
Enchytraeus crypticus
Contaminated soil vs. Control soil
Enchytraeus crypticus
75% vs. 75% WHC
50% vs. 50% WHC
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100 80 60 40 20 0 20 40 60 80 100
Soil organisms (%)

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Abstract

Water availability is paramount in the response of soil invertebrates towards stress situations. This study aimed to evaluate the effects of forecasted soil moisture scenarios on the avoidance behavior of two invertebrate species (the arthropod *Folsomia candida* and the soft-bodied oligochaete *Enchytraeus crypticus*) in soils degraded by different types of anthropogenic metal(loid) contamination (mining soil and agricultural soil affected by industrial chemical wastes). Different soil moisture contents (expressed as % of the soil water holding capacity, WHC) were evaluated: 50% (standard soil moisture conditions for soil invertebrates' tests); 75% (to simulate increasing soil water availability after intense rainfalls and/or floods); 40%, 30%, 25% and 20% (to simulate decreasing soil water availability during droughts). Invertebrates' avoidance behavior and changes in soil porewater major ions and metal(loid)s were assessed after 48 h exposure. Soil incubations induced a general solubilization/mobilization of porewater major ions, while higher soil acidity favored the solubilization/mobilization of porewater metal(loid)s, especially at 75% WHC. *Folsomia candida* preferred soils moistened at 50% WHC, regardless the soils were contaminated or not and the changing soil porewater characteristics. *Enchytraeus crypticus* avoided metal(loid) contamination, but this depended on the soil moisture conditions and the corresponding changes in porewater characteristics: enchytraeids lost their capacity to avoid contaminated soils under water stress situations (75% and 20-25% WHC), but also when contaminated soils had greater water availability than control soils. Therefore, forecasted soil moisture scenarios induced by global warming changed soil porewater composition and invertebrates capacity to avoid metal(loid)-contaminated soils.

Capsule: Forecasted soil moisture scenarios altered invertebrates' avoidance behavior towards field metal(loid) contamination

Keywords: Global warming; Multiple stressors; Metal(loid) availability; *Folsomia candida; Enchytraeus crypticus*

1. Introduction

Global warming is changing Earth's hydrological cycle. Among others, we are facing important alterations in precipitation patterns (e.g., increasing frequency and intensity of extreme events), higher evapotranspiration rates and changes in soil runoff and moisture (Bates et al., 2008; Sheffield et al., 2012; IPCC, 2013, 2014; Forzieri et al., 2014). Future climate projections for Europe predict increasing risk of flash flood events throughout the continent, while southern Europe will be also susceptible to more severe and prolonged dry spells (Bates et al., 2008; IPCC, 2013, 2014; Forzieri et al., 2014). The Mediterranean region is among those more prone to suffer water stress and continued drought intensification; the IPCC predicts ~10-20% of soil moisture decrease by the end of the 21st century (Bates et al., 2008).

Water availability is a key factor in terrestrial ecosystems; their response against water stress often involves complex interactions of biotic and abiotic processes. This situation might be compromised in degraded ecosystems, such as those affected by anthropogenic metal(loid) contamination, where soil living organisms have to deal with already unfavorable conditions (i.e., multi-stressed environments). In such degraded areas soil moisture alterations might modify the response of organisms, directly influencing their performance and/or indirectly modifying key soil parameters (e.g., metal(loid) availability and salinity; Peijnenburg and Jager, 2003; Holsmtrup et al., 2010; Karmakar et al., 2016). Among the organisms that may be more affected are soil invertebrates since they live in close contact with pore water, being highly dependent on the surrounding available water and the substances dissolved (e.g. metal(loid)s and salts).

In the last years, the evaluation of invertebrates' avoidance behavior has become a usual first-screening tool to assess the adverse effects of contaminants as well as the habitat potential of natural, uncontaminated or contaminated soils (e.g., Hund-Rinke et al., 2003; Natal-da-Luz et al., 2004; Loureiro et al., 2005; Sousa et al., 2008; Owojori et al., 2014). This is because avoidance tests are sensitive, short-time consuming (few days), cost-effective and ecological relevant (ISO, 2008, 2011). In these tests the avoidance or preference response of organisms towards a stress situation is measured. Soil invertebrates have chemical and mechanical sensory organs allowing them to escape from harmful conditions and/or move to more favorable places (Slifer and Sekhon, 1978; Edwards and Bohlen, 1992; Lukkari et al., 2005; Curry and Schmidt, 2007). Despite the importance of water availability in the response of soil invertebrates towards stress situations, avoidance behavior tests are normally performed under optimal soil moisture conditions (ISO, 2008, 2011). However, no studies have evaluated how invertebrates' avoidance behavior may be affected by soil moisture alterations induced by global warming.

The aim of the present study was to assess the effects of forecasted soil moisture scenarios on the avoidance behavior response of two model soil invertebrate species (the arthropod *Folsomia candida* and the soft-bodied oligochaete *Enchytraeus crypticus*) in natural soils affected by anthropogenic metal(loid)-contamination. To achieve this goal avoidance behavior tests were performed at different soil moisture contents simulating changes in soil water availability due to intense rainfalls/floods and drought situations in two terrestrial ecosystems degraded by different types of anthropogenic activities. Moreover, invertebrates' behavior under different soil moisture conditions was related to changes in soil porewater salinity (electrical conductivity and major ions) and metal(loid)s. We hypothesized that changing soil moisture content and the corresponding changes in soil porewater characteristics would affect the behavior of soil invertebrates, and that this would depend on the invertebrate species.

2. Material and methods

2.1. Test soils

Two soils affected by anthropogenic activities were selected in central-northern Portugal (Figure S1, Supplementary material): a soil from Braçal mining district (hereafter mining soil), and a soil from an agricultural field close to the Estarreja Chemical Complex (hereafter agricultural soil). The mining district of Braçal (Sever do Vouga, Northeast of Aveiro) was mainly exploited for Pb during the 19th and 20th centuries, and its activity ceased in 1958 (Allan, 1965; Cerveira, 1966). Several waste dumps containing high metal(loid) concentrations remain in the area affecting the surrounding ecosystems (Anjos et al., 2012; Vidal et al., 2012). The municipality of Estarreja (Northeast of Aveiro) is a clear example of an area affected by intense industrial contamination. From the middle of the 20th century the Estarreja Chemical Complex has produced several tons of solid and liquid wastes containing high metal(loid) concentrations (e.g., As, Hg, Pb and Zn) that have reached the nearby ecosystems (Costa and Jesus-Rydin, 2001; Inácio et al., 2014), including the surrounding agricultural fields (Rodrigues et al., 2012).

A composite soil sample per location was taken by mixing three randomly distributed subsamples (top 20 cm). Samples were air-dried, sieved (2 mm mesh) and homogenized before being completely characterized (n = 3). Soil pH (in water and 0.01M $CaCl_2$) and electrical conductivity (EC) in water were determined in 1:5 w:v suspensions after shaking for 2 h at 200 rpm. The pH was measured with a WTW-pH 330i/set meter and the EC with a WTW 3110/set meter. The water extracts were filtered through nylon membrane syringe filters (0.45 μm pore diameter; Albet-JNY) and analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), major ions (Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, Ca^{2+} and Mg^{2+}) and metal(loid)s (Al, As, Cd, Cu, Fe, Mn, Ni, Pb, Sb and Zn; samples were acidified with a drop of concentrated HNO_3). DOC and TDN concentrations were determined with an automatic TOC analyzer (TOC-VCSH Shimadzu), major ions with an ion chromatographer (Metrohm 861) and metal(loid)s by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7900). The 0.01M $CaCl_2$ extracts were also filtered (0.45 μm pore diameter), acidified (one drop of concentrated HNO_3) and analyzed for metal(loid)s (Al, As, Cd, Cu, Fe, Mn, Ni, Pb, Sb and Zn; ICP-MS). Water and 0.01M $CaCl_2$ extractable metal(loid) concentrations were used to evaluate soil metal(loid) availability (Ernst, 1996; Houba et al., 2000; Menzies et al., 2007). Cation exchange capacity (CEC) was determined by saturation of the soil exchange complex with 1N CH_3COONH_4 pH 7.0 and displacement of the adsorbed NH_4^+ with 10% NaCl (Chapman, 1965). Ammonium concentration was measured with a Lambda 25 UV/VIS spectrometer (Perkin Elmer) at λ=670 nm (NEIKER, 2005). Water holding capacity (WHC) was determined in porous base glass cylinders after soil saturation with water for 3 h followed by 2 h of water excess removal (ISO, 1998). Particle size distribution was determined by the Bouyouco's densimeter method (Gee and Bauder, 1986). Soil aliquots were ground in an Agatha mortar for the determination of total organic carbon (TOC), total nitrogen (TN), total metal(loi)s (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb and Zn) and mineralogical composition. TOC and TN concentrations were determined with an automatic TOC analyzer (TOC-VCSH Shimadzu) and total metal(loid)s by X-ray fluorescence (Bruker S4 Pioneer). Soil mineralogical composition was determined by semi-quantitative estimation of the crystalline fraction by power X-ray diffraction (Bruker-AXS D8-Advance diffractometer).

Based on total metal(loid) concentrations, the Soil Quality Index (SoQI) developed by the Canadian Council of Ministers of the Environment (CCME) for screening contaminated soils and their relative hazard was calculated for each test soil (CCME, 2007b). Its calculation was based on the comparison of the total metal(loid) concentrations measured with the Canadian Soil Quality Guidelines that establishes threshold values for total metal(loid) concentrations according to the use of the territory (agricultural, residential/parkland, commercial and industrial) and the necessary level of protection (CCME, 2007a). Calculation details are available in the Supplementary material.

2.2. Soil invertebrate test species

The model test species *Folsomia candida* Willem 1902 (phylum Uniramia, class Collembola, family Isotomidae) and *Enchytraeus crypticus* Westheide and Graefe 1992 (phylum Annelida, class Oligochaeta, family Enchytraeidae) were selected to perform the avoidance behavior tests. Both invertebrate species play a key role in terrestrial ecosystems by participating in the biogeochemical cycling of organic matter and nutrients, soil bioturbation and soil structure improvement (Didden, 1993; Didden and Römbke, 2001; Fountain and Hopkin, 2005). They are suitable bioindicators of stress conditions.

Soil invertebrates were cultured at the University of Aveiro (Aveiro, Portugal) under laboratory conditions. *Folsomia candida* cultures were maintained on moist plaster of Paris mixed with activated charcoal (9:1 w:w) at 20 ± 2 °C and 16:8 h light:dark photoperiod (ISO, 1999). Once a week cultures were fed with granulated dry baker’s yeast (*Saccharomyces cerevisae*) and substrate was remoistened. Age-synchronized organisms were used for avoidance behavior tests (ISO, 1999). For this *F. candida* adults were separated from the main culture, placed in new containers and allowed to lay eggs for 3 d. Adults were then removed, eggs were allowed to hatch and 10-12 d old juveniles were used for testing. *Enchytraeus crypticus* cultures were kept in agar medium prepared with aqueous soil extracts at 20 ± 2 °C and complete darkness. Cultures were fed once a week with a mixture of oatmeal, dry yeast, yolk powder, fish oil and milk. Sexually mature organisms (clearly visible clitellum) and of approximately 1 cm length were used for the tests.

2.3. Avoidance behavior tests

Avoidance behavior of *F. candida* and *E. crypticus* was evaluated according to the standardized ISO 17512 guidelines (ISO, 2008, 2011). Avoidance tests were performed in two-section vessels consisting on cylindrical plastic containers (8 cm diameter x 6 cm height) divided into two equal sections by a removable plastic split (~1 mm thickness). Each section was filled with 25 g of moistened soil, one with the test soil and the other one with a clean soil (control soil). Dual controls (both sections filled with control soil) were also performed. Lufa 2.2 soil (sandy loam texture, pH in H_2O ~5.8, pH in 0.01M $CaCl_2$ ~5.3, EC ~0.1 dS m^{-1} and WHC of ~45%; Speyer, Germany) was used as control soil. Its selection as control soil was based on the fact of presenting similar characteristics in terms of texture, pH and EC than the test soils.

Avoidance tests were performed at different soil moisture contents (expressed as % of the soil WHC): 50% (standard soil moisture content recommended by ISO guidelines); 75% (to simulate increasing soil water availability after intense rainfalls and/or floods); 40%, 30%, 25% and 20% (to simulate decreasing soil water availability during droughts). Two types of avoidance tests were performed: i) test soil vs. control soil both at the same soil moisture content; ii) test soil vs. control soil both at different soil moisture contents. The first type of tests allowed checking whether invertebrates' avoidance behavior towards metal(loid)-contaminated soils could change under water stress situations (intense rainfalls/floods and droughts), while the second one allowed inferring which factor (anthropogenic contamination and/or soil moisture content) was the main responsible for invertebrates' behavior under water stress situations. Ten soil moisture content combinations were performed in total (test soil WHC vs. control soil WHC): 1) 75% vs. 75%, 2) 50% vs. 50% (standard soil moisture conditions), 3) 40% vs. 40%, 4) 30% vs. 30%, 5) 25% vs. 25%, 6) 20% vs. 20%, 7) 50% vs. 75%, 8) 50% vs. 25%, 9) 75% vs. 50%, and 10) 25% vs. 50%; (5 replicates per test soil/invertebrate species/moisture content combination were used). Soil moisture content combinations 7, 8, 9 and 10 were selected based on the results of the previous ones. Dual control tests (control soil vs. control soil) were also performed at the different soil moisture content combinations established (5 and 10 replicates were used per invertebrate species for soil moisture content combinations 1 to 6 and 7 to 10, respectively).

Once prepared the two-section vessels the plastic split was carefully removed, the base of the test containers was gently tapped to avoid a physical gap between both sections and to ensure direct contact between soils, and organisms were placed in the soil midline (20 individuals for *F. candida* and 10 individuals for *E. crypticus*). The experimental containers were covered with perforated plastic lids and kept for 48 h at 20 ± 2 °C and 16:8 h light:dark photoperiod. After 48 h the plastic split was carefully reintroduced and the number of surviving organisms in each section was recorded. In the case of *F. candida*, both sections were filled with water at the same time and the soil was gently stirred to enable organisms to float and be counted by eye. In the case of *E.*

crypticus, the soil of each section was transferred to a 250 µm sieve, water was added to remove most soil particles and organisms were counted by eye. Organisms in the midline were counted as 0.5 for each vessel section.

Avoidance behavior was calculated using the following equation:

$$A=(C-T)/N*100 \qquad \text{(Eq. 1)}$$

where A is avoidance (%), C is the number of organisms in the control soil, T is the number of organisms in the test soil, and N is the total number of surviving organisms. Positive values indicate that organisms avoided the test soil (avoidance response), negative values that organisms preferred the test soil (preference response), and zeros that organisms were equally distributed in both sections (neutral response).

2.4. Soil porewater analyses

In parallel to avoidance behavior tests performed at different moisture contents both in test and control soils (moisture content combinations 7, 8, 9 and 10), test soil samples without organisms were incubated at 75%, 50% and 25% WHC for 48 h at 20 ± 2 °C and 16:8 h light:dark photoperiod to assess the influence of these three moisture conditions on soil porewater characteristics and their relation with invertebrates' behavior (4 replicates per test soil/moisture content were used). After the 48 h incubation period soils were saturated with water until reaching 100% WHC, immediately centrifuged for 5 min at 5000 rpm and the supernatant filtered through nylon membrane syringe filters (0.45 µm pore diameter; Albet-JNY). Porewater pH and EC were measured with a WTW-pH 330i/set meter and a WTW 3110/set meter, respectively. The concentration of major ions (Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, Ca^{2+} and Mg^{2+}) and metal(loid) (As, Cd, Cu, Fe, Mn, Ni, Pb and Zn; samples were acidified with a drop of concentrated HNO_3) was analyzed by ion chromatography (Metrohm 861) and ICP-

MS (Agilent 7900), respectively. To discard elements solubilized/mobilized due to the porewater extraction process, initial dry soil samples (non-incubated soil samples) were directly saturated with water at 100% WHC, centrifuged for 5 min at 5000 rpm and filtered supernatants analyzed for pH, EC, major ions and metal(loid) as described above (hereafter blanks).

2.5. Statistical analyses

The avoidance behavior response of both invertebrate species towards each test soil in each soil moisture content combination tested was evaluated by Fisher's exact test, comparing the observed organism distribution with an expected distribution where avoidance response was not present (null hypothesis) (Natal da Luz et al., 2004). One-tailed tests were used for avoidance response towards test soils (the null hypothesis considered that half of the organisms stayed in the test soil) and two-tailed tests for dual control tests (the null hypothesis considered an equal organism distribution between both vessel sections). Statistical analyses were performed with GraphPad Software; significant differences at $p<0.05$.

For each test soil, one-way ANOVA followed by Bonferroni post hoc test was used to check for differences in porewater parameters (pH, EC, major ions and metal(loid)s) among soil moisture contents (75% WHC, 50% WHC, 25% WHC and blanks). The relationships among porewater parameters were evaluated through Pearson' correlations. Statistical analyses were performed with IBM SPSS Statistics 22 and differences were considered significant at $p<0.05$. Data were log-transformed when they failed to pass the Leven's test for the homogeneity of variances. When soil samples incubated at 75%, 50% and 25% WHC had significant higher concentrations of porewater major ions/metal(loid)s than blanks we assumed that elements were more easily solubilized/mobilized during soil incubations. However, in the opposite situation, we assumed that porewater major ions/metal(loid)s were in less solubilized/mobilized forms during soil incubations. This could be an estimation of the porewater conditions of test soils to which invertebrates would have been exposed during avoidance behavior tests at 75%, 50% and 25% soil WHC.

3. Results

3.1. Test soils characterization

General soil characterization data are shown in Table S1 (Supplementary material). Both test soils had loamy sand texture (~77-86% sand, ~8-16% silt and ~6-7% clay) and acidic pH (mining soil ~6.1 in water and ~5.9 in 0.01M $CaCl_2$; agricultural soil ~5.6 in water and ~4.8 in 0.01M $CaCl_2$). They were considered non saline soils (EC ~0.1-0.3 dS m^{-1}), with the mining soil showing higher EC values than the agricultural soil due to the greater concentration of some major ions (11-13 fold higher for SO_4^{2-}, Ca^{2+} and Mg^{2+}). The content of TOC was higher in the mining soil (~46 mg kg^{-1} mining soil vs. ~26 mg kg^{-1} agricultural soil), while both soils showed similar TN levels (~2 mg kg^{-1}). However, the agricultural soil presented greater DOC and TDN concentrations than the mining soil (3 and 2 fold higher, respectively). Both test soils showed low CEC values (~8-10 $cmol_c$ kg^{-1}) and a WHC of ~37-40%.

Total metal(loid) concentrations were considered as high in both test soils (Table 1), with the mining soil showing the highest levels for Co, Cr, Fe, Mn, Ni, Pb, Sb and Zn, while the agricultural soil for As and Hg. Considering the total metal(loid) concentrations analyzed and the Canadian Soil Quality Guidelines (CCME, 2007a), the resulting SoQI was 17.1 for the mining soil and 18.7 for the agricultural soil (Supplementary material). Concerning metal(loid)s extracted with water and 0.01M

$CaCl_2$, there were differences between both test soils depending on the element considered and the extractant used (Table 1). When extracted with water, the mining soil showed the highest concentrations of Cd, Ni, Pb and Zn, while the agricultural soil of Al, As, Cu, Fe and Sb. For 0.01M $CaCl_2$ extractions, the mining soil showed the highest concentrations of Cd, Ni and Pb and the agricultural soil of Al, As, Cu, Fe, Mn, Sb and Zn.

The major minerals identified in the test soils were (data not shown): mining soil, ~60% quartz, ~27% muscovite-2M1, ~9% albite, ~4% chlorite-serpentine, ~1% calcite and ~0.5% dolomite; agricultural soil, ~67% muscovite-2M1, ~22% chlorite-serpentine, ~7% quartz, 3% albite, ~1% maricopaite and <0.5% of calcite and plattnerite.

3.2. Changes in soil porewater pH, EC, major ions and metal(loid)s

3.2.1. Mining soil

No differences were found in porewater pH among soil moisture contents (75%, 50% and 25% WHC), including blanks (~6.4-6.5; Table S2, Supplementary material). Porewater EC significantly ($p<0.05$) increased with increasing soil moisture content in samples incubated for 48 h (from ~0.8 dS m^{-1} at 25% WHC to ~1.4 dS m^{-1} at 75% WHC), the blanks showing similar EC values to 50% and 25% WHC treatments (Table S2, Supplementary material). The EC values were significantly correlated with porewater SO_4^{2-}, K^+, Ca^{2+} and Mg^{2+} concentrations ($r\geq0.560$, $p<0.05$; Table S3, Supplementary material), with the samples incubated at 75% WHC showing significant ($p<0.05$) higher concentrations (Table S2, Supplementary material). On the contrary, soil incubation at 25% WHC led to significant ($p<0.05$) lower porewater SO_4^{2-}, Ca^{2+} and Mg^{2+} concentrations (Table S2, Supplementary material).

Unlike most major ions, soil samples incubated for 48 h at different moisture contents (75%, 50% and 25% WHC) showed lower porewater concentrations of some metal(loid)s compared to blanks (significant differences for As, Cu and Pb in samples incubated at 75%, 50% and 25% WHC and for Cd, Ni and Zn in those incubated at 50% and 25% WHC, $p<0.05$; Table S2, Supplementary material). Significant positive correlations were found among the concentrations of these metal(loid)s ($r\geq0.535$, $p<0.05$; Table S3, Supplementary material). On the contrary, porewater Mn concentrations significantly ($p<0.05$) increased with soil incubation at 75% WHC (Table S2, Supplementary material), Mn being significantly correlated with EC values ($r=0.905$, $p<0.05$) and Cl^-, SO_4^{2-}, K^+, Ca^{2+} and Mg^{2+} concentrations ($r\geq0.552$, $p<0.05$) (Table S3, Supplementary material).

3.2.2. Agricultural soil

Significant ($p<0.05$) higher pH values in porewater were found in soil samples incubated for 48 h at different moisture contents (75%, 50% and 25% WHC) compared to blanks (~5.4 for blanks vs. ~5.9-6.0 for incubated soil samples; Table S4, Supplementary material). No differences were found in porewater EC among soil moisture contents, including blanks (~0.1-0.2 dS m^{-1}; Table S4, Supplementary material), and no significant correlations were found between EC and porewater major ions (Table S5, Supplementary material). Nevertheless, soil incubation at different moisture contents (75%, 50% and 25% WHC) resulted in greater porewater concentrations of Cl^-, SO_4^{2-}, Na^+ and K^+ compared to blanks (significant for Cl^-, SO_4^{2-} and K^+ in samples incubated at 75%, 50% and 25% WHC and for Na^+ at 50% WHC, $p<0.05$; Table S4, Supplementary material). A similar result was found for porewater NO_3^- concentrations in soil samples incubated at 50% and 25% WHC (significant higher compared to blanks; $p<0.05$), while soil incubation at 75% WHC led to significantly lower NO_3^- concentrations (Table S4, Supplementary material).

Soil samples incubated for 48 h at 75% WHC showed significant ($p<0.05$) higher porewater concentrations of As, Cu, Fe, Mn and Pb compared to the other moisture content treatments, including blanks (Table S4, Supplementary material). Significant positive correlations were found among the concentrations of these metal(loid)s ($r\geq0.527$, $p<0.05$; Table S5, Supplementary material). For Ni, soil incubation, regardless of the moisture content (75%, 50% and 25% WHC), resulted in significant higher porewater concentrations compared to blanks, as well as for Mn at 50% WHC (Table S4, Supplementary material).

3.3. Soil invertebrates' avoidance behavior

3.3.1. Validity of avoidance behavior tests

The validity of the avoidance behavior tests was checked according to the following criteria established by the ISO guidelines (ISO, 2008, 2011): survival >80% for *F. candida* and >90% for *E. crypticus*, and homogeneous distribution of organisms in dual control tests (40-60% of surviving organisms in each section of the test vessel). In all the avoidance tests performed, the survival of *F. candid*a and *E. crypticus* was ≥90% and ≥86%, respectively (Tables S6, S7 and S8, Supplementary material). In dual control tests (only Lufa 2.2 soil), both invertebrate species showed a homogeneous distribution when both vessel sections were moistened at the same WHC (Tables S6 and S7, Supplementary material), with the only exception of *E. crypticus* at 20% soil WHC (significant higher number of organisms in one of the sections of the test vessel, $p<0.05$). However, this was not the case of dual control tests performed with different moisture contents in each vessel section. For *F. candida*, ~68-78% of individuals had a significant ($p<0.05$) preference for the section moistened at 50% WHC (Table S6, Supplementary material). On the contrary, *E. crypticus* had a variable response: organisms showed a homogeneous distribution between sections when facing 50% WHC vs. 75% WHC, while ~69% of organisms significantly ($p<0.05$) preferred the section moistened at 50% WHC when facing 50% WHC vs. 25% WHC (Table S7, Supplementary material).

3.3.2. Avoidance behavior tests with similar moisture content in test and control soils

When avoidance tests were performed at the same moisture content both in test and control soils, *F. candida* did not avoid the mining and agricultural soils in most of the cases (a higher number of organisms was found on the vessel section containing the test soils; Figure 1). This response was more pronounced in the presence of the agricultural soil, with *F. candida* preferring significantly ($p<0.05$) the test soil over the control in most of the moisture contents tested (~23-81% of preference; Figure 1). When the mining soil was present, *F. candida* avoided it at 30% WHC (~26% of avoidance; significant, $p<0.05$), while organisms showed a neutral or preference response for the test soil at the rest of the moisture contents tested (significant preference at 75% and 20% soil WHC, $p<0.05$; Figure 1).

In the case of *E. crypticus*, its response was highly dependent on the soil moisture content tested (Figure 1). Organisms significantly ($p<0.05$) avoided the mining soil when moistened at 50%, 40% and 30% WHC (~27-66% of avoidance) and the agricultural soil at 50% and 40% WHC (~32-68% of avoidance) (Figure 1). However, this was not the case of *E. crypticus* exposed to higher (75% WHC) or lower (20-25% WHC) soil moisture contents (Figure 1), with organisms showing a preference for the vessel section containing the test soil (significant in the agricultural soil at 75% and 25% WHC, $p<0.05$; ~36-39% of preference).

3.3.3. Avoidance behavior tests with different moisture contents in test and control soils

When avoidance tests were performed at different moisture contents both in test and control soils *F. candid*a showed a preference for the vessel section moistened at 50% WHC (Figure 2). This behavior was significant ($p<0.05$) in all the moisture content combinations tested with the agricultural soil (~65-100% of preference for 50% soil WHC; Figure 2). In the case of the mining soil this preference was only significant ($p<0.05$) in the moisture content combination test soil at 25% WHC vs. control soil at 50% WHC (~51% of preference for 50% soil WHC; Figure 2).

Enchytraeus crypticus avoided both the mining and agricultural soils when they were moistened at 50% WHC and the control soil was at 75% WHC (~10% and ~63% of avoidance in the mining and agricultural soil, respectively; significant for the agricultural soil, $p<0.05$), but the contrary happened when the control soil was at 25% WHC (~36% and ~9% of preference in the mining and agricultural soil, respectively; significant for the mining soil, $p<0.05$) (Figure 2). However, when both test soils were moistened at 75% or 25% WHC and the control soil at 50% WHC *E. crypticus* significantly ($p<0.05$) avoided the test soils (~60-100% of avoidance; Figure 2).

4. Discussion

4.1. Contamination status of the test soils

Both test soils presented high total metal(loid) concentrations (Table 1), especially when comparing with background levels reported for natural Portuguese soils not affected by anthropogenic contamination (Table S9, Supplementary material; Inácio et al., 2008). The mining soil greatly exceeded these levels in particular for Al (~3-fold), As (~10-fold), Cu (~14-fold), Ni (~3-fold), Pb (~700-fold) and Zn (~24-fold). In the case of the agricultural soil total Al, As, Cu, Hg, Pb and Zn concentrations also surpassed the background levels reported by Inácio et al. (2008), especially for As (~64-fold), Hg (~1600-fold) and Pb (~33-fold).

No national policy exists in Portugal concerning the regulation and management of contaminated soils; the Portuguese Government recommends the use of the Canadian Soil Quality Guidelines (Table S9, Supplementary material; CCME, 2007a). Following these guidelines, both test soils were highly contaminated by several metal(loid)s: mining soil (As, Cr, Cu, Ni, Pb, Sb and Zn); agricultural soil (As, Cu, Hg, Pb and Zn). Moreover, according to the SoQI calculated (~17-19) both test soil presented very high level of concern due to metal(loid) contamination, posing a high environmental risk.

Despite the fact that both test soils presented high total metal(loid) concentrations, metal(loid) availability (evaluated through water and 0.01M $CaCl_2$ extractions) depends on other soil properties such as pH, salinity, organic matter and texture (Allen, 2002; Lanno et al., 2004). Water and/or 0.01M $CaCl_2$ extractable metal(loid)s were relatively high in both test soils (Table 1), although they accounted for less than 1% of the total element concentration. This was not the case of Mn and Zn extracted with 0.01M $CaCl_2$ in the agricultural soil (~2.5% and ~3.4%, respectively). When comparing the percentages of metal(loid)s extracted with water and 0.01M $CaCl_2$ respect to the total concentrations, greater availability was shown by the agricultural soil (~1.2-20.1 and ~2.2-21.5 fold higher with water and 0.01M $CaCl_2$, respectively) in relation to its higher acidity (Table S1, Supplementary material).

4.2. Effects of soil moisture content on soil porewater composition

Soil incubation for 48 h induced changes in the porewater composition of both test soils (Tables S2 and S4, Supplementary material). These changes depended on the moisture content tested (75%, 50% and 25% WHC) and were different depending on the soil type.

For the mining soil, its incubation at 75% WHC favored higher salt solubilization/mobilization, compared with non-incubated soil samples (Figure 3), as shown by the higher porewater concentrations of some major ions (Cl^-, SO_4^{2-}, K^+, Ca^{2+} and Mg^{2+}) and therefore higher EC (Table S2, Supplementary material). When incubated at lower soil moisture contents (50% and 25% WHC) no differences were observed for major ions, except in the case of SO_4^{2-}, Ca^{2+} and Mg^{2+} that showed lower solubilization/mobilization in porewater at 25% WHC compared to non-incubated soil samples (Figure 3). On the contrary, most metal(loid)s (As, Cd, Cu, Ni, Pb and Zn) showed lower solubilization/mobilization in porewater when soil samples were incubated, regardless of the soil moisture content (Figure 3). This is in line with the findings of González-Alcaraz and van Gestel (2016) who found decreasing Cd and Zn concentrations in the porewater of a slightly acidic mining soil incubated for 21 d under controlled conditions of soil moisture content and air temperature. Among others, the authors related these changes to the precipitation/co-precipitation of metals with carbonates (Simón et al., 2005) and/or their immobilization due to the disruption and reformation of organo-mineral complexes after initial soil dry samples being rewetted (Haynes and Swift, 1991). This was not the case of Mn, the samples incubated at 75% WHC showing greater solubilization/mobilization than non-incubated soil samples (Figure 3) in relation to the great mobility of this element (Reddy and DeLaune, 2008).

In the case of the agricultural soil, its incubation did not induce changes in the salinity of the porewater (Table S4, Supplementary material) although some major ions

(Cl^-, SO_4^{2-} and K^+) showed greater solubilization/mobilization, compared to non-incubated soil samples, regardless of the soil moisture content (Figure 3). This was not the case of NO_3^- which concentration in porewater decreased when the agricultural soil was incubated at 75% WHC while it increased at 50% and 25% WHC (Figure 3; Table S4, Supplementary material). The high organic matter content of this soil could have stimulated microbial activity during soil incubation, favoring oxygen depletion due to the high soil moisture content (75% WHC) and the loss of NO_3^- via denitrification (Vepraskas and Faulkner, 2001; Reddy and DeLaune, 2008). Similar to some major ions, and opposite to what happened in the mining soil, the incubation of the agricultural soil resulted in higher solubilization/mobilization of most metal(loid)s in porewater, compared to non-incubated soil samples, especially at 75% WHC (As, Cu, Fe, Mn, Ni and Pb; Figure 3). The dissolution effect observed for Mn in the mining soil at 75% WHC could be increased in the agricultural soil. Oxygen depletion during soil incubation at 75% WHC could lead to the dissolution of Fe and Mn oxy-hydroxides (Vepraskas and Faulkner, 2001; Reddy and DeLaune, 2008), as shown by the high concentration of these metals in the pore water of the agricultural soil (Table S4, Supplementary material). The mobilization of As, Cu, Ni and Pb could be favored by this phenomenon since Fe and Mn oxy-hydroxides control the retention of other metal(loid)s in soil (Tack et al., 2006; Du Laing et al., 2007). Unlike the mining soil, the higher acidity of the agricultural soil could contribute to keep these metal(loid)s in solution.

4.3. Effects of soil moisture content on invertebrates' avoidance behavior

Avoidance behavior towards contaminated test soils differed between invertebrate species, and was related to the soil type and the moisture content tested (Figures 1 and 2).

In general, when avoidance tests were performed at the same moisture content both in test and control soils, the arthropod *F. candida* preferred the contaminated test soils (Figure 1), regardless their high total and available metal(loid) concentrations (Table 1). Our results agree with previous studies (Natal-da-Luz et al., 2009; Bori et al., 2016, 2017) and could be related to the relatively resistance of collembolans to metal(loid) contamination (Fountain and Hopkin, 2001, 2005). The preference response of *F. candida* was more pronounced for the agricultural soil, in relation to the greater concentrations of DOC and TDN shown by this soil (Table S1, Supplementary material). Natal-da-Luz et al. (2008, 2009) already found a preference of *F. candida* towards soils with high content of organic matter. Moreover, in this type of tests, soil moisture content and the corresponding changes in porewater major ions and metal(loid)s (Tables S2 and S4, Supplementary material) did not seem to have any influence on the behavior of *F. candida* since its preference response towards contaminated test soils occurred at different moisture levels (Figure 1). However, when avoidance tests were performed at different soil moisture contents in both vessel sections *F. candida* behaved differently. In such tests *F. candida* showed a preference for the soil moistened at 50% WHC (dual control tests and tests involving contaminated test soils; Figure 2 and Table S6, Supplementary material). Therefore soil moisture content was the main factor controlling the behavior of *F. candida* when having the option to choose between different moisture levels. Organisms clearly avoided flood and drought situations, regardless whether the soil was contaminated or not. This agrees with Domene et al. (2011) who found soil moisture content as the only factor contributing to explain *F. candida* avoidance behavior when comparing soils with different properties.

Unlike collembolan, *E. crypticus* was more sensitive to metal(loid)s and avoided both contaminated test soils but only at certain moisture contents. When avoidance tests were performed at the same moisture content both in test and control soils *E. crypticus* significantly avoided metal(loid) contamination in the range of 50% to 30% WHC (~27-68% of avoidance; Figure 1). The capacity of different enchytraeid species (*E. crypticus* and *Enchytraeus albidus*) to avoid metal(loid)s has been previously shown by other authors, both in Lufa 2.2 soil spiked with single/multiple metal salts (e.g., $CuCl_2$, $ZnCl_2$, $CdCl_2$; Amorim et al., 2008a,b; Loureiro et al., 2009) and in mixtures of Lufa 2.2 soil with metal(loid)-enriched wastes (Kobetičova et a., 2010). All these studies were performed at 40-60% WHC, similar to the moisture range in which we found an avoidance response of *E. crypticus* towards the contaminated test soils. However, when *E. crypticus* was exposed to higher (75% WHC) or lower (20-25% WHC) soil moisture conditions it was not able to avoid metal(loid) contamination, organisms showing both preference or neutral response towards the contaminated test soils (Figure 1). This response could be related to the high vulnerability of enchytraeid species to water stress conditions because of their highly permeable skin (Lindberg et al., 2002; Maraldo et al., 2008). In fact, organisms exposed to 75% or 20-25% WHC had a different appearance to those at 50% to 30% WHC (author’s visual observations). At 20-25% WHC enchytraeids appeared rolled-up on themselves, most of the times on the soil surface. This behavior could have been a strategy to diminish body dehydration, as it has been reported for earthworm species (a closely related group) under soil desiccation situations (Jiménez et al., 2000; Blume et al., 2016). The severe stress induced by the intense drought conditions at 20-25% WHC could have led enchytraeids to lose their capacity to avoid the contaminated test soils. On the contrary, at 75% WHC, enchytraeids had a bloated translucent appearance, possibly indicating a disruption of the organism's water balance. Moreover, the changes observed in the soil porewater composition in terms of major ions and/or metal(loid)s could have interfered with the behavior of *E. crypticus* towards the contaminated test soils, especially when increasing the soil moisture content (75% WHC). In the case of the mining soil, the highest solubilization/mobilization of porewater major ions occurring at 75% WHC could favor the disruption of the organism osmotic homeostasis, enchytraeids losing their capacity to avoid metal(loid) contamination. In the case of the agricultural soil, the highest concentration of porewater metal(loid)s solubilized/mobilized at 75% WHC could induce greater toxic effects on enchytraeids and, consequently, an alteration of their avoidance capacity towards metal(loid)-contaminated soils.

The importance of soil water availability on enchytraeid performance was demonstrated in those tests performed at different soil moisture contents both in contaminated and control soils (Figure 2). In this case *E. crypticus* avoided both contaminated test soils in all the moisture combinations tested (>10% of avoidance), except when the control soil had lower soil moisture content (contaminated soil at 50% WHC vs. control soil at 25% WHC; ~9-36% of preference). Therefore, *E. crypticus* could avoid metal(loid) contamination when having the option to choose for a clean soil with adequate moisture content, but not when the control soil showed higher water deficiency than the contaminated soil. This result pointed out that soil moisture content was the main factor controlling *E. crypticus* avoidance behavior towards metal(loid)-contaminated soils under water stress situations.

5. Conclusions

Soil incubation under controlled soil moisture and air temperature conditions induced changes in porewater salinity (major ions and therefore EC) and metal(loid)s.

These changes differed between soil types and depended on the moisture content at which the soils were incubated. A general solubilization/mobilization of porewater major ions was observed compared to non-incubated soil samples, while higher soil acidity favored the solubilization/mobilization of metal(loid)s in porewater, especially at higher soil moisture levels (75% soil WHC). The model soil invertebrate species selected (*Folsomia candida* and *Enchytraeus crypticus*) differed in their avoidance response towards anthropogenic metal(loid)-contaminated soils. In general *F. candida* preferred soils moistened at 50% WHC (standard soil moisture conditions), regardless whether the soil was contaminated or not and the changing soil porewater composition. On the contrary, *E. crypticus* avoided soil contamination, but its capacity was highly dependent on the soil moisture conditions and presumably the corresponding porewater changes. Enchytraeids lost their capacity to avoid metal(loid)-contaminated soils under extreme water stress situations (intense rainfalls/floods and drought conditions simulated by 75% and 20-25% soil WHC, respectively), but also when contaminated soils had greater water content than control soils. Therefore, the present study shows that the forecasted soil moisture alterations induced by global warming may change soil porewater salinity and metal(loid) concentrations and the capacity of soil invertebrates to avoid metal(loid)-contaminated soils. This issue is of major concern considering both the toxicity risks of contaminated soils and the environmental implications that the observed invertebrate behavioral response would have on the functionality of anthropogenic-degraded ecosystems.

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Conflict of interest

There is no conflict of interest.

References

Allan, J.C. (1965). A mineração em Portugal na Antiguidade. *Boletim de Minas*, 2, 139-175.

Allen, H.E. (2002). *Bioavailability of metals in terrestrial ecosystems: Importance of partitioning for bioavailability to invertebrates, microbes, and plants*. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, Florida.

Amorim, M.J.B., Novais, S., Römbke, J., & Soares, A.M.V.M. (2008a). *Enchytraeus albidus* (Enchytraeidae): A test organism in a standardized avoidance test? Effects of different chemical substances. *Environment International*, 34(3), 363-371.

Amorim, M.J.B., Novais, S., Römbke, J., & Soares, A.M.V.M. (2008b). Avoidance test with *Enchytraeus albidus* (Enchytraeidae): Effects of different exposure time and soil properties. *Environmental Pollution*, 155(1), 112-116.

Anjos, C., Magalhães, M.C., & Abreu, M.M. (2012). Metal (Al, Mn, Pb and Zn) soils extractable reagents for available fraction assessment: Comparison using plants, and dry and moist soils from the Braçal abandoned lead mine area, Portugal. *Journal of Geochemical Exploration*, 113, 45-55.

Bates, B.C., Kundzewicz, Z.W., Wu, S., & Palutikof, J.P. (2008). *Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change*. IPCC Secretariat, Geneva, Switzerland.

Blume, H.P., Brümmer, G.W., Fleige, H., Horn, R., Kandeler, E., Kögel-Knabner, I., Kretzschmar, R., Stahr, K., & Wilke, B.M. (2016). *Soil organisms and their habitat.* In: Scheffer/Schachtschabel Soil Science. Springer, Berlin, Heidelberg. pp. 87-122.

Bori, J., Vallés, B., Navarro, A., & Riva, M.C. (2016). Geochemistry and environmental threats of soils surrounding an abandoned mercury mine. *Environmental Science and Pollution Research*, 23(13), 12941-12953.

Bori, J., Vallés, B., Navarro, A., & Riva, M.C. (2017). Ecotoxicological risks of the abandoned F–Ba–Pb–Zn mining area of Osor (Spain). *Environmental Geochemistry and Health*, 39(3), 665-679.

CCME. (2007a). *Canadian soil quality guidelines for the protection of environmental and human health: Summary tables. Updated September, 2007*. In: Canadian environmental quality guidelines, 1999, CCME (Canadian Council of Ministers of the Environment), Winnipeg.

CCME. (2007b). *CCME Soil Quality Index 1.0: Technical Report*. In: Canadian environmental quality guidelines, 1999, CCME (Canadian Council of Ministers of the Environment), Winnipeg.

Cerveira, A.M. (1966). O aproveitamento dos valores minerais de Portugal Continental. *Boletim de Minas*, 3, 107-118.

Chapman, H.D. (1965). *Cation-exchange capacity*. In: Black, C.A. (Ed.), Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. American Society of Agronomy, Madison, pp. 891-900.

Costa, C., & Jesus-Rydin, C. (2001). Site investigation on heavy metals contaminated ground in Estarreja–Portugal. *Engineering Geology*, 60(1-4), 39-47.

Curry, J.P., & Schmidt, O. (2007). The feeding ecology of earthworms - a review. *Pedobiologia*, 50(6), 463-477.

Didden, W.A.M. (1993). Ecology of terrestrial Enchytraeidae. *Pedobiologia*, 37(1), 2-29.

Didden, W.A.M., & Römbke, J. (2001). Enchytraeids as indicator organisms for chemical stress in terrestrial ecosystems. *Ecotoxicology and Environmental Safety*, 50(1), 25-43.

Domene, X., Chelino, S., Campana, P., Natal-da-Luz, T., Alcañiz, J.M., Andrés, P., Römbke, J., & Sousa, P. (2011). Influence of soil properties on the performance of *Folsomia candida*: implications for its use in soil ecotoxicology testing. *Environmental Toxicology and Chemistry*, 30(7), 1497-1505.

Du Laing, G., Vanthuyne, D.R.J., Vandecasteele, B., Tack, F.M.G., & Vaerloo, M.G. (2007). Influence of hydrological regime on pore water metal concentrations in a contaminated sediment-derived soil. *Environmental Pollution*, 147(3), 615-625.

Edwards, C.A., & Bohlen, P.J. (1992). The effects of toxic chemicals to earthworms. *Reviews of Environmental Contamination and Toxicology*, 125, 23-99.

Ernst, W.H.O. (1996). Bioavailability of heavy metals and decontamination of soils by plants. *Applied Geochemistry*, 11(1-2), 163-167.

Forzieri, G., Feyen, L., Rojas, R., Flörke, M., Wimmer, F., & Biamchi, A. (2014). Ensemble projections of future streamflow droughts in Europe. *Hydrology and Earth System Sciences*, 18, 85-108.

Fountain, M.T., & Hopkin, S.P. (2001). Continuous monitoring of *Folsomia candida* (Insecta: Collembola) in a metal exposure test. *Ecotoxicology and Environmental Safety* 48(3), 275-286.

Fountain, M.T., & Hopkin, S.P. (2005). *Folsomia candida* (Collembola): a ‘‘standard’’ soil arthropod. *Annual Review of Entomology*, 50, 201-222.

Gee, G.W. & Bauder, J.W. (1986). Particle Size Analysis. In: Methods of Soil Analysis, Part A. Klute (ed.). 2 Ed., Vol. 9 nd. Am. Soc. Agron., Madison, WI, pp: 383-411.

González-Alcaraz, M.N., & van Gestel, C.A.M. (2016). Metal/metalloid (As, Cd and Zn) bioaccumulation in the earthworm *Eisenia andrei* under different scenarios of climate change. *Environmental Pollution*, 215, 178-186.

Haynes, R.J., & Swift, R.S. (1991). Concentrations of extractable Cu, Zn, Fe and Mn in a group of soils as influenced by air- and oven-drying and rewetting. *Geoderma*, 49(3-4), 319-333.

Holmstrup, M., Bindesbøl, A.M., Oostingh, G.J., Duschl, A., Scheil, V., Köhler, H.R., Loureiro, S., Soares, A.M.V.M., Ferreira, A.L.G., Kienle, C., Gerhardt, A., Laskowski, R., Kramarz, P.E., Bayley, M., Svendsen, C., & Spurgeon, D.J. (2010). Interactions between effects of environmental chemicals and natural stressors: A review. *Science of the Total Environment*, 408(18), 3746-3762.

Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., & vanVark, W. (2000). Soil analysis procedures using 0.01M calcium chloride as extraction reagent. *Communications in Soil Science and Plant Analysis*, 31(9-10), 1299-1396.

Hund-Rinke, K., Achazi, R., Römbke, J., & Warnecke, D. (2003). Avoidance test with Eisenia fetida as indicator for the habitat function of soils: results of a laboratory comparison test. *Journal of Soils and Sediments*, 3(1), 7-12.

Inácio, M., Neves, O., Pereira, V., & Ferreira da Silva, E. (2014). Levels of selected potential harmful elements (PHEs) in soils and vegetables used in diet of the population living in the surroundings of the Estarreja Chemical Complex (Portugal). *Applied Geochemistry*, 44, 38-44.

Inácio, M., Pereira, V., & Pinto, M. (2008). The Soil Geochemical Atlas of Portugal: Overview and applications. *Journal of Geochemical Exploration*, 98(1-2), 22-33.

IPCC. (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

IPCC. (2014). *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, and L.L. White (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1132 pp.

ISO. (1998). *ISO 11274: Soil Quality - Determination of the water-retention characteristic*. ISO (International Organization for Standardization), Geneva, Switzerland.

ISO. (1999). *ISO 11267: Soil Quality – Inhibition of reproduction of collembolan (Folsomia candida) by soil pollutants*. ISO (International Organization for Standardization), Geneva, Switzerland.

ISO. (2008). *ISO 17512: Soil Quality - Avoidance test for determining the quality of soils and effects of chemicals on behavior - Part 1: Test with earthworms (Eisenia fetida and Eisenia andrei)*. Geneva, Switzerland.

ISO. (2011). *ISO 17512: Soil Quality - Avoidance test for determining the quality of soils and effects of chemicals on behavior - Part 2: Test with collembolans (Folsomia candida)*. Geneva, Switzerland.

Jiménez, J.J., Brown, G.G., Decaëns, T., Feijoo, A., & Lavelle, P. (2000). Differences in the timing of diapause and patterns of aestivation in tropical earthworms. *Pedobiologia*, 44(6), 677-694.

Karmakar, R., Das, I., Dutta, D., & Rakshit, A. (2016). Potential Effects of Climate Change on Soil Properties: A Review. *Science International*, 4(2), 51-73.

Kobetičová, K., Hofman, J., & Holoubek, I. (2010). Ecotoxicity of wastes in avoidance tests with *Enchytraeus albidus, Enchytraeus crypticus* and *Eisenia fetida* (Oligochaeta). *Waste Management*, 30(4), 558-564.

Lanno, R., Wells, J., Conder, J., Bradham, K., & Basta, N. (2004). The bioavailability of chemicals in soil for earthworms. *Ecotoxicology and Environmental Safety*, 57(1), 39-47.

Loureiro, S., Amorim, M.J.B., Campos, B., Rodrigues, S.M.G., & Soares, A.M.V.M. (2009). Assessing joint toxicity of chemicals in *Enchytraeus albidus* (Enchytraeidae) and *Porcellionides pruinosus* (Isopoda) using avoidance behaviour as an endpoint. *Environmental Pollution*, 157(2), 625-636.

Loureiro, S., Soares, A.M.V.M., & Nogueira, A.J.A. (2005). Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. *Environmental Pollution*, 138(1), 121-131.

Lukkari, T., Aatsinki, M., Väisänen, A., & Haimi, J. (2005). Toxicity of copper and zinc assessed with three different earthworm tests. *Applied Soil Ecology*, 30(2), 133-146.

Menzies, N.W., Donn, M.J., & Kopittke, P.M. (2007). Evaluation of extractants for estimation of the phytoavailable trace metals in soils. *Environmental Pollution*, 145(1), 121-130.

Natal da Luz, T., Ribeiro, R., & Sousa, J.P. (2004). Avoidance tests with collembola and earthworms as early screening tools for site-specific assessment of polluted soils. *Environmental Toxicology and Chemistry*, 23(9), 2188-2193.

Natal-da-Luz, T., Römbke, J., & Sousa, J.P. (2008). Avoidance tests in site-specific risk assessment—influence of soil properties on the avoidance response of collembola and earthworms. *Environmental Toxicology and Chemistry*, 27(5), 1112-1117.

Natal-da-Luz, T., Tidona, S., Jesus, B., Morais, P.V., & Sousa, J.P. (2009). The use of sewage sludge as soil amendment. The need for an ecotoxicological evaluation. *Journal of Soils and Sediments*, 9, 246-260.

NEIKER. (2005). *Determinación del contenido de amonio en aguas, lixiviados y extractos* (PEC/EN/A-059).

Owojori, O.J., Waskax, K., & Roembke, J. (2014). Avoidance and reproduction tests with the predatory mite *Hypoaspis aculeifer*: effects of different chemical substances. *Environmental Toxicology and Chemistry*, 33(1), 230-237.

Peijnenburg,W.J.G.M., & Jager,T. (2003). Monitoring approaches to assess bioaccessibility and bioavailability of metals: Matrix issues. *Ecotoxicology and Environmental Safety*, 56(1), 63-77.

Reddy, K.R., & DeLaune, R.D. (2008). *Biogeochemistry of Wetlands: Science and Applications*. CRC Press, Boca Raton, Florida.

Rodrigues, S.M., Pereira, E., Duarte, A.C., & Römkens, P.F.A.M. (2012). Derivation of soil to plant transfer functions for metals and metalloids: impact of contaminant's availability. *Plant and Soil*, 361(1-2), 329-341.

Sheffield, J., Wood, E.F., & Roderick, M.L. (2012). Little change in global drought over the past 60 years. *Nature,* 491, 435-38.

Simón, M., Martín, F., García, I., Bouza, P., Dorronsoro, C., & Aguilar, J. (2005). Interaction of limestone grains and acidic solutions from the oxidation of pyrite tailings. *Environmental Pollution*, 135(1), 65-72.

Slifer, E.H., & Sekhon, S.S. (1978). Sense organs on the antennae of two species of collembola (insecta). *Journal of Morphology*, 157, 1-19.

Sousa, A., Pereira, R., Antunes, S.C., Cachada, A., Pereira, E., Duarte, A.C., & Gonçalves, F. (2008). Validation of avoidance assays for the screening assessment of soils under different anthropogenic disturbances. *Ecotoxicology and Environmental Safety*, 71(3), 661-670.

Tack, F.M.G., Van Ranst, E., Lievens, C., & Vandenberghe, R.E. (2006). Soil solution Cd, Cu and Zn concentrations as affected by short-time drying or wetting: The role of hydrous oxides of Fe and Mn. *Geoderma*, 137(1-2), 83-89.

Vepraskas, M.J., & Faulkner, S.P. (2001). *Redox chemistry of hydric soils*. In: Richardson, J.L., Vepraskas, M.J. (Eds.), Wetland Soils. Genesis, Hydrology, Landscape and Classification. Lewis Publishers, Florida, pp. 85-106.

Vidal, T., Pereira, J.L., Abrantes, N., Soares, A.M.V.M., & Gonçalves, F. (2012). Ecotoxicological assessment of contaminated river sites as a proxy for the water framework directive: an acid mine drainage case study. *Water, Air and Soil Pollution*, 223(9), 6009-6023.

Figure captions

Figure 1. Avoidance behavior of *Folsomia candida* and *Enchytraeus crypticus* in tests performed for 48 h at the same soil moisture content (expressed as % of the soil water holding capacity, WHC) both in test and control soils (test soil WHC vs. control soil WHC). Lufa 2.2 soil was used as control soil. Data are average ± SD (n = 5). Positive values indicate test soil avoidance, negative values test soil preference, and zeros neutral response. Asterisks (*) indicate significant differences in test and control soils (Fisher's one-tailed exact test, $p<0.05$).

Figure 2. Avoidance behavior of *Folsomia candida* and *Enchytraeus crypticus* in tests performed for 48 h at different soil moisture contents (expressed as % of the soil water holding capacity, WHC) both in test and control soils (test soil WHC v. control soil WHC). Lufa 2.2 soil was used as control soil. Data are average ± SD (n = 5). Positive values indicate test soil avoidance, negative values test soil preference, and zeros neutral response. Asterisks (*) indicate significant differences in test and control soils (Fisher's one-tailed exact test, $p<0.05$).

Figure 3. Porewater major ions and metal(loid)s in test soils incubated for 48 h at 75%, 50% and 25% water holding capacity (WHC). Dark grey color represents that incubated soil samples showed significant higher porewater concentrations than non-incubated soil samples (blanks), light grey color that incubated soil samples showed significant lower concentrations than non-incubated soil samples, and white color that there were no differences between incubated and non-incubated soil samples. Significant differences tested by one-way ANOVA with Bonferroni posthoc test ($p<0.05$).

Table 1. Metal(loid) concentrations (total, water extractable and 0.01M $CaCl_2$ extractable) in the test soils from central-northern Portugal (average ± SD; n = 3). d.l. (detection limit). Other details from the soils are available in the text.

Soil parameter	**Mining soil**	**Agricultural soil**
Total metal(loid)s – mg kg^{-1}		
Al	90,297 ± 497	70,603 ± 7490
As	473 ± 47	3087 ± 968
Cd	<d.l.	<d.l.
Co	42.2 ± 17.8	14.0 ± 2.8
Cr	137 ± 8	72.0 ± 8.2
Cu	640 ± 46	753 ± 204
Fe	95,990 ± 3096	34,613 ± 8035
Hg	<d.l.	127 ± 35
Mn	847 ± 30	375 ± 87
Ni	174 ± 4	35.3 ± 6.1
Pb	32,067 ± 2401	1480 ± 419
Sb	167 ± 15	<d.l.
Zn	2551 ± 129	977 ± 287
Water extractable metal(loid)s (1:5 w:v) – µg kg^{-1}		
Al	<d.l. (2.50)	1570 ± 269
As	<d.l. (1.25)	6228 ± 146
Cd	26.1 ± 4.1	<d.l. (1.25)
Cu	23.2 ± 9.1	548 ± 16
Fe	314 ± 147	1508 ± 466
Mn	867 ± 24	633 ± 122
Ni	159 ± 7	70.0 ± 5.2
Pb	1264 ± 424	150 ± 59
Sb	52.1 ± 3.5	139 ± 2
Zn	3324 ± 73	1517 ± 46
0.01M $CaCl_2$ extractable metal(loid)s (1:5 w:v) – µg kg^{-1}		
Al	<d.l. (2.50)	2396 ± 50
As	<d.l. (1.25)	1782 ± 82
Cd	260 ± 8	152 ± 1
Cu	25.1 ± 7.8	635 ± 51
Fe	195 ± 45	500 ± 136
Mn	3038 ± 26	9275 ± 477
Ni	693 ± 18	306 ± 8
Pb	14,221 ± 469	350 ± 20
Sb	37.3 ± 2.8	88.6 ± 7.5
Zn	16,366 ± 94	32,855 ± 1137

Figure 1

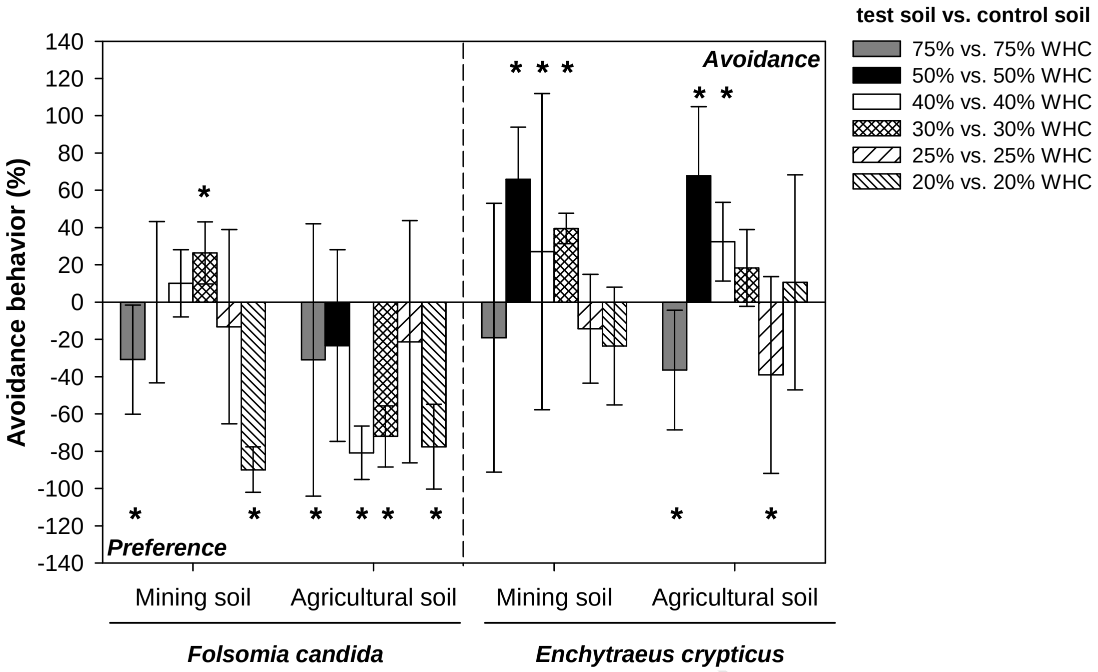

test soil vs. control soil
75% vs. 75% WHC
50% vs. 50% WHC
40% vs. 40% WHC
30% vs. 30% WHC
25% vs. 25% WHC
20% vs. 20% WHC
Avoidance behavior (%)
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
Avoidance
Preference
Mining soil
Agricultural soil
Mining soil
Agricultural soil
Folsomia candida
Enchytraeus crypticus

Figure 2

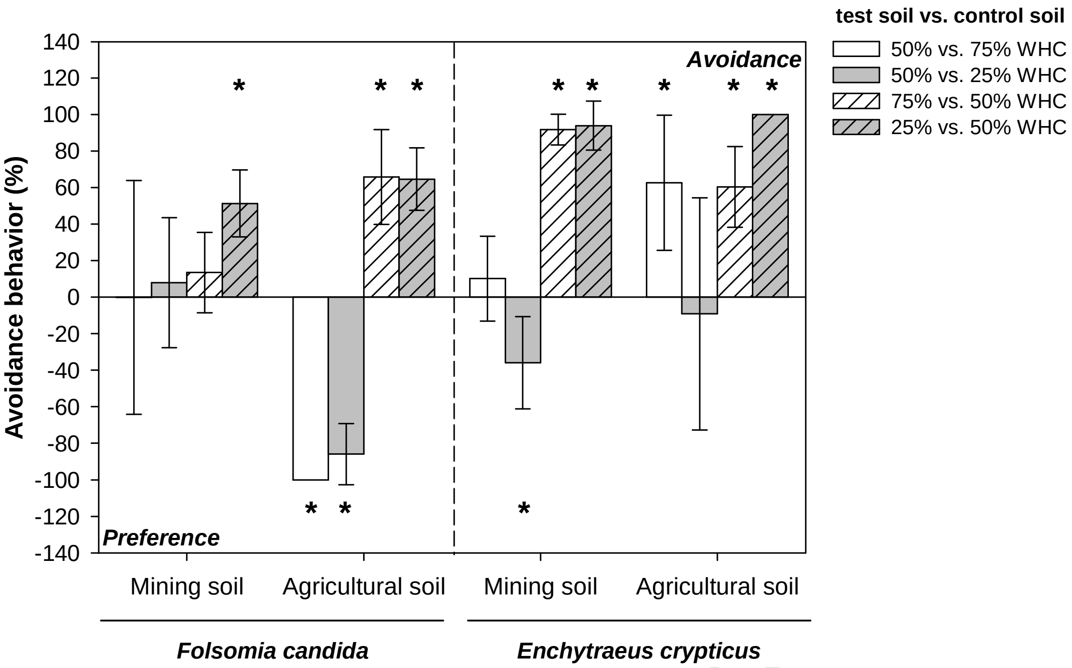
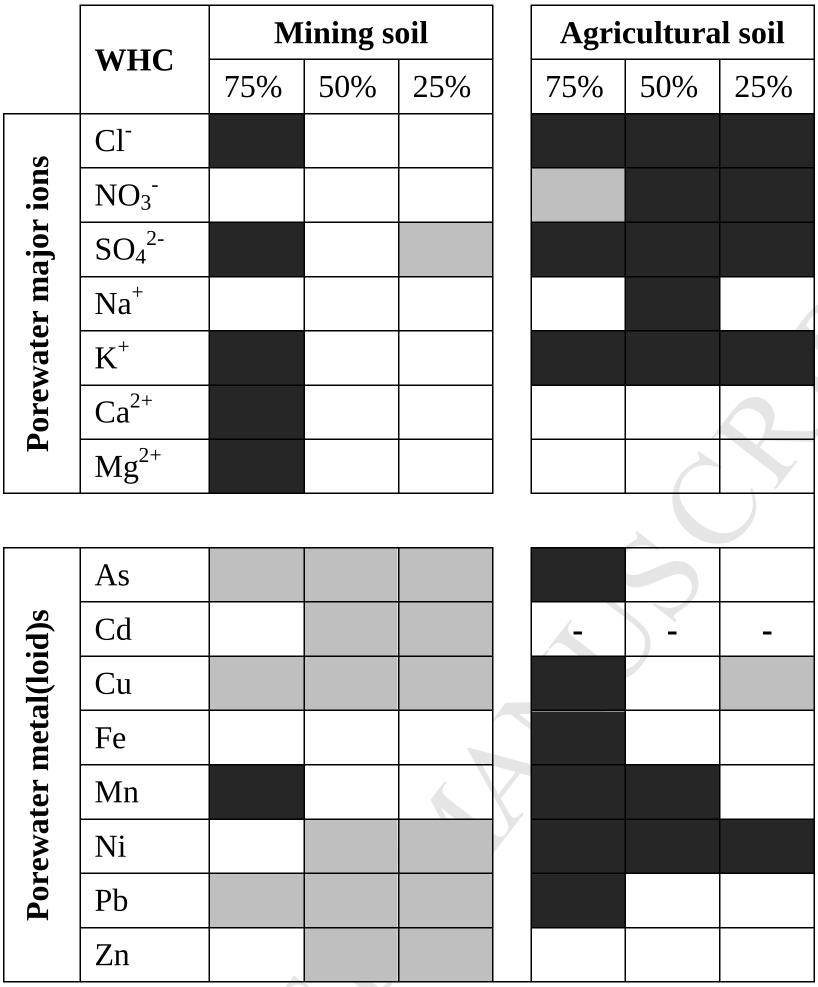

test soil vs. control soil
50% vs. 75% WHC
50% vs. 25% WHC
75% vs. 50% WHC
25% vs. 50% WHC
Avoidance behavior (%)
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
Avoidance
Preference
Mining soil
Agricultural soil
Mining soil
Agricultural soil
Folsomia candida
Enchytraeus crypticus
*

Figure 3



	WHC	**Mining soil**			**Agricultural soil**		
		75%	50%	25%	75%	50%	25%
Porewater major ions	Cl^-	■			■	■	■
	NO_3^-				▨	■	■
	SO_4^{2-}	■		▨	■	■	■
	Na^+					■	
	K^+	■			■	■	■
	Ca^{2+}	■					
	Mg^{2+}	■					
Porewater metal(loid)s	As	▨	▨	▨	■		
	Cd		▨	▨	-	-	-
	Cu	▨	▨	▨	■		▨
	Fe				■		
	Mn	■			■	■	
	Ni		▨	▨	■	■	■
	Pb	▨	▨	▨	■		
	Zn		▨	▨			

Highlights

1. Forecasted soil moisture scenarios changed soil porewater major ions and metal(loid)s

2. Forecasted soil moisture scenarios altered soil invertebrates' avoidance behavior

3. *F. candida* preferred soils moistened at 50% WHC regardless metal(loid) contamination

4. *E. crypticus* avoided metal(loid) contamination depending on soil moisture conditions

5. *E. crypticus* did not avoid metal(loid) contamination under water stress situations