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Bioaccumulation and potential ecotoxicological effects of trace metals along a management intensity gradient in volcanic pasturelands

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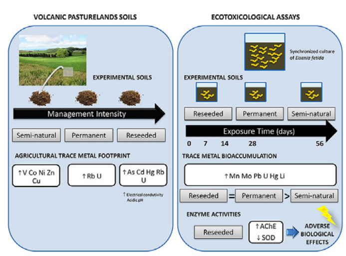
HIGHLIGHTS

- The intensity of pasture management impacts differentially soil health.
- Volcanic pasturelands are more susceptible to such the impacts.
- Management intensity ensues distinct TM footprints and ecotoxicological impacts.
- Ecotoxicological effects are more pronounced in reseeded pasturelands.

GRAPHICAL ABSTRACT



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ABSTRACT

The particularities of volcanic soils raise the need to better understand the link between soil agricultural management intensity and trace metal bioaccumulation. The Azores are a region characterized by volcanic soils, which were changed in different degrees according to the intensity of the agricultural practices. The main objective of this study was to assess the potential ecotoxicological effects of the trace metals present in volcanic pastureland soils along a gradient of management intensity (i.e., semi-natural, permanent and reseeded), using earthworms (*Eisenia fetida*) as biological indicators. For this purpose earthworms were exposed during 7, 14, 28 and 56 days to soils from the three types of pastures. At each exposure time, we quantified trace element bioaccumulation (As, Cd, Co, Cr, Cu, Hg, Li, Mn, Mo, Ni, Pb, Rb, U, V and Zn) and the activities of superoxide dismutase and acetylcholinesterase in earthworm tissues. Overall, the results showed that the type of pastureland management significantly increased the soil contents in trace metals: V, Co, Ni and Zn in semi-natural pasturelands; As, Cd and Hg in reseeded pasturelands; and, Rb and U in both permanent and reseeded pasturelands. The soil physicochemical properties observed in the reseeded pastureland systems (higher electric conductivity values associated with a moderately acid pH value) modulated the metal bioavailability, from soil to biota, leading to a greater Hg bioaccumulation in earthworm tissues. The long-term exposure (56 days) of earthworms to reseeded pastureland soil was associated with adverse biological effects (intensification of AChE activity

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and decrease of SOD activity), encompassing key processes such as neurotransmission and antioxidant defence mechanisms in resident soil biota (earthworms). This study point towards the increased importance of semi-natural and permanent pastureland management, over the intensive management (reseeded pasturelands), in favour of more sustainable ecosystems.

1. Introduction

Volcanic soils only cover 1–2% of the world's land surface; yet they support 10% of the world's population (Takahashi and Shoji, 2002). These soils are often among the most fertile and therefore are the foundations for some of the most densely populated areas of the world (Arnalds et al., 2007), although their fertility is affected by climate, elevation and use intensity (Abe et al., 2020; De Bauw et al., 2016). The specific andic properties of volcanic soils, particularly their high organic carbon content, variable charge characteristics, high phosphorus retention, low bulk density, great water retention capacity and the natural enrichment with trace metals (TM) (Doelsch et al., 2006; Marques et al., 2017, 2019; Parelho et al., 2014), place them as well suited for agricultural purposes. Nevertheless, volcanic soils are also vulnerable to management practices, since they can act simultaneously as source and sink of potentially toxic elements (Fabricio Neta et al., 2018; Ma et al., 2019; Memoli et al., 2018a). The presence of clay minerals, formed through the weathering of the tephra material (e.g. allophane and imogolite), coupled with variable surface charge characteristics and exposure of $(OH)Al(OH_2)$ groups at wall perforations (defects), explains the strong affinity of volcanic soils to TM, particularly metal cations (Harsh et al., 2002; Mazhari et al., 2017). Nevertheless, besides depending on the age and chemical composition of parent material, as well as on the plant cover, TM presence in volcanic soils might also be derived from human activities (Memoli et al., 2018b). Therefore, although the agricultural volcanic soils chemical derives mainly from the volcanic parent rock (natural TM background), it can be modulated by agricultural land use history (Abe et al., 2020; De Bauw et al., 2016; Parelho et al., 2014). In the case of agricultural volcanic soils under intensive management, the scenario is particularly aggravated, as the use of agrochemicals (metal-containing pesticides and fertilizers) can contribute to the accumulation of TM in the soil matrix (Reboredo et al., 2019; Rezapour et al., 2020; Rodríguez Martín et al., 2006). This may trigger negative effects on resident soil organisms, which may compromise the soil-based ecosystem services.

According to FAO data (FAO and ITPS, 2015), pasturelands represent 26% of the world land area and 70% of the agricultural area. Pasturelands are typically used for grazing-pastures or mechanical harvesting of grass meadows. The current European Common Agricultural Policy (CAP) and European Habitats Directive for 2014 to 2020 point towards an increased importance of permanent pastureland systems for feed and food production ('provision' function) (Hopkins and Holz, 2006), for the conservation of biodiversity above- and belowground ('support' function), and mitigation of climate change through enhanced sequestration of carbon and nutrients ('regulation' function) (FAO and ITPS, 2015; Fornara et al., 2016; Minns et al., 2001; Tilman et al., 2001; Wiesmeier et al., 2020). Furthermore, in many volcanic regions, particularly for those listed as outermost regions (Treaty on the Functioning of the European Union, article 349), the remoteness, insularity, small size, difficult topography, climate, and economic dependence on local agriculture, assigns to soil use management a pivotal role, for the provision of soil-based ecosystem services. The particularities of volcanic soils raise the need to better understand the link between soil management intensity, TM contamination and their joint effects on soil ecosystem functioning, to uphold sustainable environments supporting local, regional, and global environmental health. Several studies have focused on the concentration or distribution of TM in soils formed from volcanic parent material (Fabricio Neta et al., 2018; Ma et al., 2019; Memoli et al., 2018a,b; Mendoza-Grimón et al., 2014; Parelho et al., 2014) but fewer addressed their effects on resident soil biota (Memoli et al., 2019; Parelho et al., 2016a,b, 2018).

Earthworms are considered highly appropriate biological indicators to assess the bioavailability of metals in soils and their ecotoxicity to soil organisms (Asensio et al., 2013; Calisi et al., 2013; Šrut et al., 2019; Wang et al., 2020; Xiao et al., 2020). They are naturally in contact with the solid, aqueous and gaseous soil phases and, as a result, are directly exposed to soil contaminants (Schreck et al., 2012). Earthworms are key functional groups, important for soil formation and organic matter breakdown in most terrestrial environments, playing a key role in soil profile, influencing its physical, chemical and microbiological properties; thus, contributing soil fertility enhancement (Cunha et al., 2016; Fonte et al., 2019; Xiao et al., 2020). Since native earthworm populations are capable to adapt to heavily polluted soils (Spurgeon et al., 2000; Xiao et al., 2020), pollutant effects could be masked and lead to misleading conclusions when using native organisms for ecotoxicological assessment. Therefore, ecotoxicity tests using standard species, such as *Eisenia fetida* earthworms (OECD, 1984; OECD, 2016), are frequently used to evaluate traditional endpoints such as mortality, growth and reproduction. Other biomarkers, useful as early indicators of environmental risk, can be quantified in *E. fetida* to assess additional sublethal effects of pollutants. For instance, acetylcholinesterase enzyme activity (AChE), a biomarker of exposure to neurotoxic compounds, has been widely used as an earthworm biomarker for neurotoxic effects (Calisi et al., 2013), replying to the presence of several pollutants in soil such as pesticides, metals and polycyclic hydrocarbons (Lionetto et al., 2013). The activity of the superoxide dismutase (SOD), an essential enzyme in the antioxidant defence system of organisms, is consensually regarded as a fast and reliable biomarker of exposure to environmental pollutants and oxidative stress effects (Łaszczyca et al., 2004; Malqui et al., 2018). This enzyme plays an active role in scavenging the reactive oxygen species (ROS) produced during exposure to various environmental stressors and protecting cells from damage during biological oxidation (Lesser, 2006).

Given the physicochemical particularities of volcanic soils, we hypothesize that soil management intensity in volcanic pasturelands entails a distinct TM footprint, being the bioaccumulation and ecotoxicological effects on resident soil organisms more pronounced in pasturelands under intensive management (reseeded pasturelands), compromising the local soil-based ecosystem services. Therefore, this study aims to assess the toxicity of volcanic pasture soils, subjected to a gradient of management intensity (semi-natural, permanent and reseeded pasturelands) to soil organisms, by integrating chemical and ecotoxicological approaches. The measured TM (As, Cd, Co, Cr, Cu, Hg, Li, Mn, Mo, Ni, Pb, Rb, U, V and Zn) were chosen among the main pollutants present in agricultural soils due to both natural and agricultural sources (Parelho et al., 2014). To evaluate soil toxicity, earthworms (*Eisenia fetida*) were exposed for 7, 14, 28 and 56 days to soil samples from pasturelands with different management intensity levels to assess TM bioaccumulation and the effects on validated earthworm endpoints (AChE, SOD). Finally, the relationships among soil physicochemical properties, soil TM concentrations, sources of contamination (given by the enrichment factor), bioaccumulation and biological effects in *E. fetida*, are discussed towards the increased importance of semi-natural and permanent pasturelands to sustain ecosystem health in volcanic regions.

2. Materials and methods

2.1. Study sites description

The study area was located on São Miguel island, the largest (744.6 km^2 and about 140.000 inhabitants) of the Azores archipelago (Portugal). According to the latest regional land use survey, published in 2018, mostly based on orbital data by Satellite Pour l'Observation de la Terre [SPOT], pastureland represents 47.07% of the total land use of São Miguel Island (COS.A, 2018), typically used as pastureland systems for dairy cattle grazing, with management ranging from low (semi-natural pastures) to high (reseeded) intensity. In the study area soil management depends mainly on altitude; at low altitude (up to 250–300 m) soil pastures are managed very intensively (herein designated as reseeded), while at higher altitudes (up to 900 m) pastures are typically restricted by permanent or semi-natural soil management.

The selected study sites are representative of each pasture management system (semi-natural, permanent and reseeded) and are located in the same geological unit (Fogo volcano), ensuring similar bedrock and pedological conditions. One pastureland per each level of management intensity was selected for this study. At the local context, semi-natural pastures (**SN**) represent the less disturbed systems, used for animal grazing during two to three times per year, with the dominance of native flora, and where no chemical fertilizers are applied. Permanent pastures (**PE**) refers to an intermediate management intensity, where pastures are not reseeded for at least 10 years, have low inputs of fertilizers (farmyard manure, slurry, chemical fertilizers) and have a low/medium utilization frequency (short grazing times, with long periods of non-grazing). Reseeded pastures (**RS**) represent the most disturbed systems, reseeded every one to two years, with high inputs of chemical fertilizers (farmyard manure, slurry, chemical fertilizers) and are frequently grazed (regular and long grazing times and/or intensive continuous grazing). A more detailed description of the study sites is presented in Table 1 Supplementary Material (SM).

2.2. Soil collection

At each study (**SN**, **PE** and **RS**) and reference (**RF**) site, soil samples were collected from topsoil (0–20 cm in depth) using a stainless steel auger soil sampler. Soil samples were collected during the Autumn season of 2017. At each site, a total of 16 subsamples were pooled together to form four composite samples. A detailed description of the soil sampling scheme is provided in Fig. 1 SM.

2.3. Soil physicochemical properties and trace element concentrations

Soil physicochemical properties, including particle-size fractions, soil organic matter, pH (H_2O) and electric conductivity, were analyzed in quadruplicates for each site, following the Portuguese recommended procedures (LNEC, 1967a, 1967b).

Soil TM (As, Cd, Co, Cr, Cu, Hg, Li, Mn, Mo, Ni, Pb, Rb, U, V and Zn) concentrations were determined by inductively coupled plasma mass spectrometry (ICP/MS) and inductively coupled plasma optical emission spectrometry (ICP/OES; Activation Laboratories Ltd., Canada). Quality control was assured by the analysis of duplicate samples, blanks and reference materials (GXR-1, GXR-4, GXR-6 and USGS SAR-M).

2.4. Enrichment factor

A pristine area (reference site), also located in the Fogo geological unit, was selected to assess the local soil TM background values to calculate the enrichment factors in the experimental soils. To evaluate the anthropogenic contribution to the TM contents in the volcanic pasture soils, the enrichment factor index (EF), for each system (**SN**, **PE**, **RS**) and TM, was calculated. The EF proposed by Sutherland (2000) is a geochemical index based on the normalization of a tested element to a reference one, which is an element with low occurrence variability. The local background values of aluminium (Al) from the **RF** site were used as the reference element. The EF is defined as follows:

$$EF = (X/Y)sample \diagup (X/Y)background$$

where X is the average concentration of the considered trace element, Y is the average concentration of the reference element (Al), for the sample and for the background soil used as reference (**RF**).

The EF method considers that: (1) X and Y have the same behaviour in the soil column; (2) there are no anthropogenic sources of Y and, (3) the reference soil is not contaminated by X. According to Hernandez et al. (2003), an EF = 2 defines the limit above which the studied sample is considered to be significantly enriched by an anthropogenic TM contamination, compared with the local background soil. Enrichment factors were ranked according to Sutherland (2000) criteria.

2.5. Ecotoxicological assays

2.5.1. Test organism

A synchronized culture of 819 *Eisenia fetida* adults (4 months old, with clitellum and individual weight of 300–600 mg) was maintained under controlled laboratory conditions [temperature (20 ± 1 °C), photoperiod (16 h^L: 8 h^D), moist substrate (40%)] according to OECD guideline 222 (OECD, 2016). The substrate used to maintain the earthworm cultures was composed of a mixture of peat moss, horse manure and $CaCO_3$ (to adjust the pH between 6 and 7). This substrate was weekly moisturized and monitored for pH and corrected as necessary. At every 7 days, sterilized horse manure was added as a food supply. Prior to the start of the laboratory exposure to the experimental soils, earthworms were allowed to acclimatize to uncontaminated natural LUFA 2.2 (Speyer) standard soil (loamy sand soil; 1.93% organic carbon, pH-$CaCl_2$ 5.5), under the experimental conditions (20 ± 1 °C, 16 h^L: 8 h^D), during one week.

2.5.2. Earthworms exposure to pastureland soils

Earthworms were removed from the acclimation containers, randomly allocated in groups (n = 13) and exposed to 1000 g of recently (24 h) field-collected soils (**SN**, **PE**, **RS**) inside plastic chambers (19,5 cm × 15 cm x 10 cm) covered with 300 μm nylon mesh. The ecotoxicological assays (sections 2.5.3 and 2.5.4) were also performed on LUFA 2.2, used as a procedure control. Four chambers (replicates) were used for **SN**, **PE** and **RS** soils and, three for the LUFA 2.2 soil. Each of the replicates corresponds to one of the four composite soil samples taken from each pasture system (as described in section 2.2). Four exposure periods were considered (7, 14, 28 and 56 days), making a total of 60 chambers. After each period of exposure, earthworms were removed from each chamber and kept on moist filter paper during 24 h for gut clearance before further analyses. In this study, *per* each chamber, four earthworms were pooled together for trace metal analysis (section 2.5.3) and three earthworms were individually processed for enzyme activities (SOD and AChE; section 2.5.4).

The experiment was carried at 20 ± 1 °C, with 16 h^L: 8 h^D photoperiod. During the experiment, earthworms were weekly fed with 5 g of sterilized horse manure *per* chamber and, each chamber monitored for moisture loss (correction was made with distilled water).

2.5.3. Trace metal bioaccumulation

The earthworms body metal concentrations (As, Cd, Co, Cr, Cu, Hg, Li, Mn, Mo, Ni, Pb, Rb, U, V and Zn), after the exposure to LUFA and to each experimental soil (SN, PE, RS), were determined by mass spectrometry with inductively coupled plasma (ICP/MS, Activation Laboratories Ltd., Canada) at the begin of the experiment (T0) and after 7,14, 28 and 56 days of exposure (a pool of four earthworms per experimental group). Quality control was assured by analysis of duplicate samples, blanks and reference materials (DOLT-3 and DORM-2). For statistical purposes, the values below the detection limit were assigned as equal to its lower detection limit.

The biota-soil accumulation factor (BSAF) was calculated *per* earthworm, for each *TM*, soil type exposure and exposure time, as follows:

$$BSAF = \frac{TML}{TMS}$$

where *TML* is the TM concentration in earthworm tissues (mg^{-1} kg^{-1}) and *TMS* is the TM concentration in soil (mg^{-1} kg^{-1}).

2.5.4. Enzyme activities

For each experimental group and exposure time (7,14, 28 and 56 days), three gut-cleaned specimens were placed into a pre-chilled mortar and ground under ice-cold conditions in phosphate buffer (1:5 w/v, 100 mM, pH 7.2). The homogenate was centrifuged at 10,400 *g* for 30 min at 4 °C to obtain the post-mitochondrial fraction (supernatant: S9). The sample (S9) was divided into several aliquots and stored at −80 °C for further enzyme activity assays. The acetylcholinesterase (AChE: EC 3.1.1.7) activity was determined according to the method of Ellman et al. (1961), adapted for 96-well microtiter plate. The reaction medium (200 µL, final volume) included sodium phosphate buffer (0.1 M, pH 7.2), DTNB (0.67 mM), acetylthiocholine iodide (1 mM) and sample (S9). Kinetics were recorded on iMark™ Microplate Absorbance Reader (Bio-Rad, Hercules, CA, USA), at 412 nm for 6 min at 25 °C. The enzyme activity was calculated subtracting the absorbance increase, due to thiols naturally present in each extract, quantified in a similar assay without acetylthiocholine iodide. The enzymatic activity was expressed as nmol of acetylthiocholine hydrolyzed per min per mg of protein and, for calculations, the absorption coefficient of 13.6×10^3 M^{-1} cm^{-1} was used.

The superoxide dismutase (SOD, EC 1.15.1.1) activity was determined as described by Misra and Fridovich (1972), also adapted for 96-well microtiter plates. The reaction medium (200 µL, final volume) included carbonate buffer (0.05 M, pH 10.2), EDTA (0.1 mM), adrenaline (0.6 mM), and sample (S9). The rate of adrenaline autooxidation at 30 °C was monitored at 490 nm for 3 min. One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the oxidation of the epinephrine (SOD_{50}), and the result was expressed as U mg^{-1} of protein.

The relative AChE and SOD activities were calculated as the relative percentage between the enzyme activity of earthworms exposed to each experimental soil (**SN**, **PE**, **RS**) and the enzyme activity of the control group (earthworms exposed to LUFA 2.2 soil) for each exposure time (7, 14, 28 and 56 days).

2.6. Statistical analysis

Normality and homogeneity of the variances of the data sets were tested using the Shapiro-Wilk and Levene tests, respectively, and, wherever appropriate data were transformed with a decimal log operator to meet normality criteria. Analysis of variance (ANOVA) was carried out to evaluate the significant differences in the soil physicochemical properties, TM concentrations and enrichment factors (EF), between the **SN**, **PE**, **RS** and **RF** sites. For the earthworm's body TM concentrations at each exposure time (7, 14, 28 and 56 days) generalized linear models (exposure time and soil type), discriminant analyses and Sidak tests were applied. For AChE and SOD activities, the effect of soil type (LUFA, **SN**, **PE**, **RS**) was compared through ANOVA followed by a Tukey HSD test. All statistical analyses were conducted using IBM SPSS 25.0 (IBM Corp, 2017) for Windows and R (R Core Team, 2017).

3. Results

3.1. Soil physicochemical properties

The soil properties for each study site are reported in Table 1. Soils of the study area are Andosols; although they have a sandy texture with small amounts of clay, according to Fontes et al. (2004) these soils can be classified as allophonic volcanic ash soils.These soils area were moderately acidic, with pH values ranging from 5.5 (**PE**) to 5.9 (**RS**). Soil from the intensively managed pasture (**RS**) displayed, on average, a significantly higher electrical conductivity (13.2 µS cm^{-1}). No statistical differences were found between the soils for the remaining parameters (Table 1).

Table 1
Mean values (±SD) of soil physicochemical properties of semi-natural (SN), permanent (PE) and reseeded (RS) pastures. Means within each line followed by different letters are significantly different at $p < 0.05$ (Tukey test).

	Pasture management		
	Semi-natural [SN]	Permanent [PE]	Reseeded [RS]
pH	5.6 ± 0.2	5.5 ± 0.2	5.9 ± 0.3
Electric conductivity (µS cm^{-1})	8.0 ± 1.2 a	9.8 ± 2.0 a	13.2 ± 1.3 b
Organic matter (%)	4.3 ± 2.4	5.4 ± 1.9	6.1 ± 2.5
Clay-Silt (%)	2.4 ± 1.1	1.9 ± 0.3	1.9 ± 1.4

3.2. Trace metal

3.2.1. Trace metal soil contents

Soil TM concentrations for **SN**, **PE** and **RS** pasturelands are presented in Fig. 1 (see also Table 2 SM). Results showed that the degree of management intensity entailed a different metal footprint for each pastureland system (Fig. 1). The **SN** pasture soils presented significantly higher concentrations of V (27.5 ± 4.12 mg kg^{-1}), Co (3.18 ± 1.18 mg kg^{-1}), Ni (7.80 ± 3.03 mg kg^{-1}) and Zn (66.95 ± 13.56 mg kg^{-1}) than **RS** and **PE**. Soils **RS** presented significantly higher concentrations of As (7.90 ± 1.53 mg kg^{-1}), Cd (0.32 ± 0.02 mg $kg^{-1)}$ and Hg (0.10 ± 0.01 mg kg^{-1}) than **SN** and **PE**. Both **PE** and **RS** soils displayed similar concentrations for Rb and U: mean Rb concentrations were significantly higher (Rb = 23.68 ± 1.24 mg kg^{-1}) than those observed in the **SN** pasture and reference soils, while U concentration (U = 3.38 ± 0.19 mg kg^{-1}) only differed between **RS** on one hand, and **SN** and **RS** on the other, with intermediate values for **PE**. Similar significantly higher soil concentrations of Li and Cr were observed for all pastureland systems when compared to the background values. No significant differences were found between soils for Mn and Mo. There was a tendency towards a higher value of Cu in **SN** in comparison with **RS** and a slightly lower value of Pb at **PE** (Fig. 1).

3.2.2. Source of soil trace metals

Trace metal enrichment factors (Table 2) were calculated separately for each pasture system (**SN**, **PE** and **RS**) to evaluate the contribution of the agricultural practices to the TM soil contents. Results revealed that all studied pastureland soils presented a major enrichment with Cr, whose source is associated with the local agricultural practices (EF 5–20, Table 2). The EF values calculated for Mn, Zn, Mo, Pb, U and Li were all below 2, regardless of the pasture management intensity, indicating that the observed soil TM concentrations were mainly associated with geogenic factors. A major enrichment with Ni was also observed in the **SN**

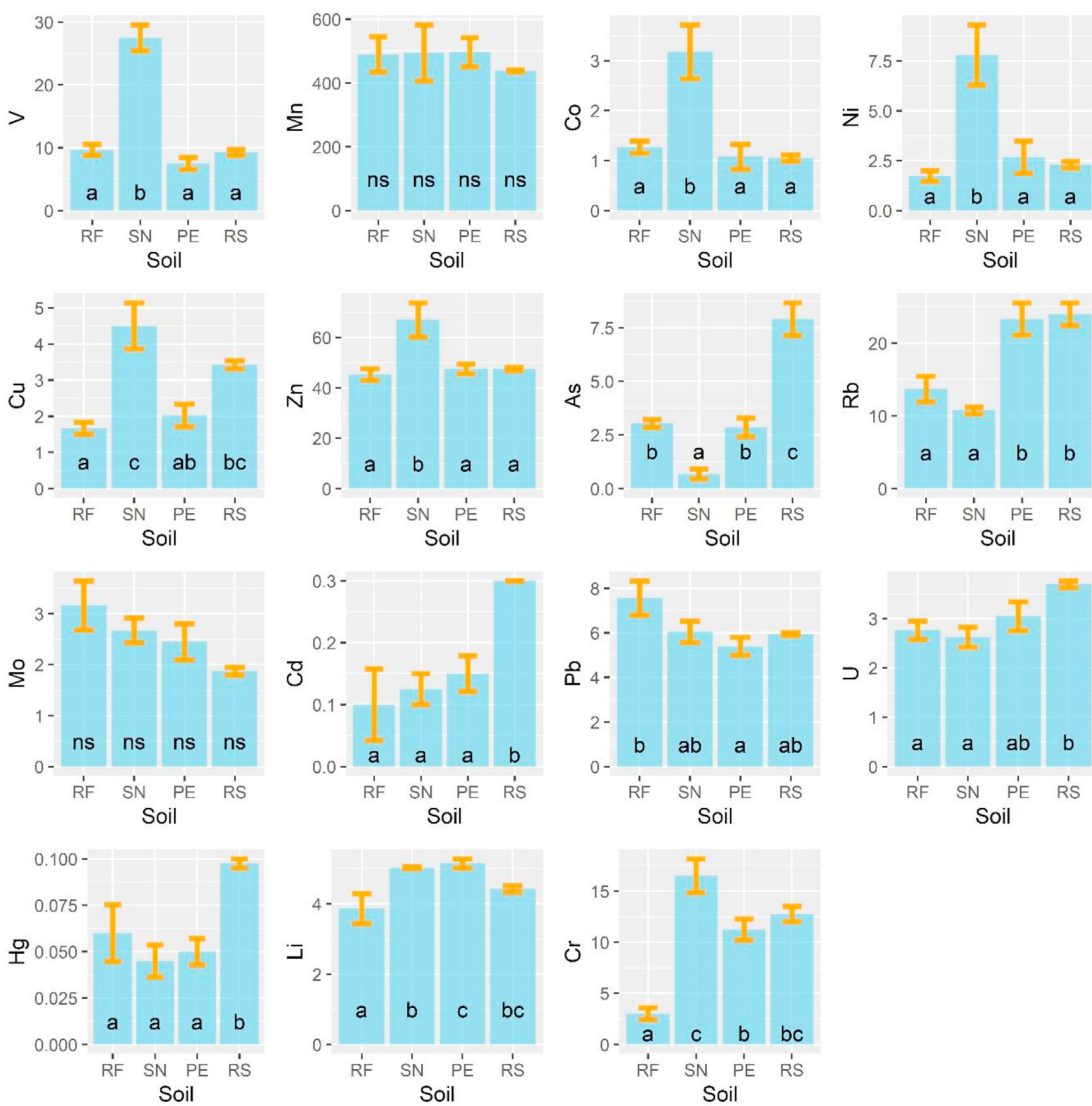

Fig. 1. Mean (±SD) concentration of trace metals (mg kg^{-1}, d.w.) in soil samples of semi-natural (SN), permanent (PE) and reseeded (RS) pastures. The natural background (reference site) values, is also shown. Bars with different letters indicate significant differences ($p < 0.05$, Tukey test) for each trace metal between soil types.

Table 2
Mean (±SD) enrichment factor (EF) of trace metals in soils from semi-natural (SN), permanent (PE) and reseeded (RS) pasture systems. To facilitate table interpretation, three different grey shadings are used to classify the EF (Sutherland, 2000): dark grey, major enrichment (EF 5–20); light grey, moderate enrichment (EF 2–5); and no shade, no enrichment (EF < 2). Means within each column followed by different letters are significantly different at p < 0.05 (Tukey test).

	As	Cd	Co	Cr	Cu	Hg	Li	Mn	Mo	Ni	Pb	Rb	U	V	Zn
Semi-natural [SN]	0.23 ±0.15 a	1.35 ±0.70 a	2.64 ±0.95 a	5.82 ± 1.84	2.85 ±0.94	0.79 ±0.31 a	1.36 ±0.17 a	1.05 ±0.36	0.88 ±0.13	4.75 ±2.0 a	0.83 ±0.13 a	0.82 ±0.13	0.98 ±0.10 a	2.97 ±0.59 a	1.56 ±0.42
Permanent [PE]	1.36 ±0.50 b	2.72 ±1.02 a	1.16 ± 0.37 b	5.34 ± 1.06	1.69 ±0.31	1.19 ±0.34 a	1.90 ±0.27 b	1.43 ±0.14	1.07 ±0.18	2.09 ±0.96 b	1.01 ±0.04 ab	2.47 ±0.67	1.56 ±0.19 b	1.08 ±0.12 b	1.50 ±0.17
Reseeded [RS]	3.61 ±0.72 c	5.27 ±0.35 b	1.14 ±0.09 b	5.91 ± 0.97	2.85 ±0.30	2.25 ±0.07 b	1.58 ±0.15 ab	1.24 ±0.06	0.82 ±0.07	1.84 ±0.29 c	1.09 ±0.06 b	2.44 ±0.67	1.85 ±0.15 c	1.32 ±0.09 b	1.45 ±0.09

pasturelands, along with a moderate enrichment (EF 2–5) with V, Co and Cu, all associated with anthropogenic activities. The **PE** pasturelands presented a moderate enrichment for Cd, Ni and Rb. The **RS** pasturelands revealed a major enrichment for Cd and moderate enrichment values for Cu, As, Rb and Hg.

3.3. Trace metal bioaccumulation in earthworm tissues

Results of generalized linear models (Gaussian) applied to the concentration of trace metals in earthworms are represented in Table 3, and the evolution of concentrations for each metal are given in Fig. 2 (see also Table 3 SM). Global complementary discriminant analyses, separating samples according to soil type or exposure time, are provided as supplementary material (Fig. 2 SM, 3 SM). Here, the main results, all in agreement with results from the two discriminant analyses (Fig. 2 SM, 3 SM), are summarized for each TM:

V and Co - There was a significant effect of soil type and of exposure time, with no significant interaction (Table 3). Values were significantly larger for **SN** than for **PE** and **RS**; there was an increase in time but without significant differences between T7 and T56 for V, while for Co there was a decrease in time with a significant difference between T7 and T56 (Sidak test in Table 4 SM).

Mn, Mo, Pb and Li - There was a significant effect of soil type and of exposure time, with no significant interaction (Table 3). Values were significantly smaller for **SN** than for **PE** and **RS**; there was an increase in time, with a significant difference between T7 and T56 for Mn, Pb and Li and, with significant differences between T56 and the remaining times of exposure for Mo (Sidak test in Table 4 SM) (Fig. 2).

Ni and Zn - There was no significant effect of soil type and of exposure time, with no significant interaction (Table 3, Fig. 2).

Cu - There was only a significant effect of soil type (Table 3). Values were larger for **SN** than for **PE** and **RS**, but the Sidak test was not significant (Table 4 SM) (Fig. 2).

As and Cr - There was only a significant effect of exposure time (Table 3). There was a tendency for a decrease with time; As values were significantly larger for T7 than for T56, while Cr values were significantly larger for T14 and T28 than for T56, and T7 was not significantly larger than T56 (Sidak test in Table 4 SM) (Fig. 2).

Rb - There was a significant effect of soil type and of exposure time, with no significant interaction (Table 3). Values were significantly different among the three soil types, decreasing in the direction **RS** - **PE** - **SN**; there was a significant peak at T14, but the values later decreased, without significant differences between T7 and T56 (Sidak test in Table 4 SM) (Fig. 2).

Cd - There was only a significant effect of exposure time (Table 3). There was a tendency for a decrease for times T14 and T28, and an increase for T56, but without significant differences between T7 and T56 (Sidak test in Table 4 SM) (Fig. 2).

U - There was a significant effect of soil type and of exposure time, with a significant interaction (Table 3). Values were significantly smaller for **SN** than for **PE** and **RS**; there was an increase in time, with a peak at T14, with significant differences between T7 and T56 (Sidak test in Table 4 SM) (Fig. 2).

Hg - There was a significant effect of soil type and of time of exposure, with a significant interaction (Table 3). Values were significantly larger for **RS** than for **PE** and **SN**; there was a considerable fluctuation in time, with a peak at T14, and significant differences between T7 and T56 (Sidak test in Table 4 SM) (Fig. 2). The increase in concentration by T56 was much more accentuated for **PE**.

For **SN** the BSAF values (Table 5 SM) presented high variability between TM (ranging from 0.1 up to 82.1), with the highest BSAF values observed for As (57–71) and Cd (25–26), followed by Cu

Table 3
Results of generalized linear models (Gaussian) applied to the concentration of trace metals in earthworms, considering the type of pasture from which the soil was taken, and exposure time. Source of variation, trace metal, Wald Chi-Square statistic, degrees of freedom and significance value. Values in bold indicate significant effects.

Source	Metal	Wald	d.f.	p	Metal	Wald	d.f.	p	Metal	Wald	d.f.	p
(Intercept)	V	820.7	1	0.000	Zn	23837.1	1	0.000	Pb	467.3	1	0.000
Pasture		17.5	2	**0.000**		4.7	2	0.096		28.0	2	**0.000**
Exposure time		13.5	3	**0.004**		4.0	3	0.265		48.6	3	**0.000**
Pasture * Exposure time		2.6	6	0.856		8.8	6	0.184		10.5	6	0.106
(Intercept)	Co	3838.7	1	0.000	As	1606.2	1	0.000	U	686.1	1	0.000
Pasture		15.6	2	**0.000**		0.6	2	0.757		75.0	2	**0.000**
Exposure time		12.4	3	**0.006**		13.0	3	**0.005**		57.0	3	**0.000**
Pasture * Exposure time		2.8	6	0.832		9.3	6	0.157		14.8	6	**0.022**
(Intercept)	Mn	746.0	1	0.000	Rb	1907.6	1	0.000	Hg	109.7	1	0.000
Pasture		12.2	2	**0.002**		56.5	2	**0.000**		47.6	2	**0.000**
Exposure time		42.1	3	**0.000**		20.8	3	**0.000**		26.1	3	**0.000**
Pasture * Exposure time		7.0	6	0.321		10.3	6	0.113		41.6	6	**0.000**
(Intercept)	Ni	173.8	1	0.000	Mo	2658.7	1	0.000	Li	417.1	1	0.000
Pasture		5.2	2	0.073		17.6	2	**0.000**		44.5	2	**0.000**
Exposure time		6.7	3	0.083		70.2	3	**0.000**		39.4	3	**0.000**
Pasture * Exposure time		2.7	6	0.843		11.0	6	0.088		9.0	6	0.173
(Intercept)	Cu	227.7	1	0.000	Cd	2500.9	1	0.000	Cr	382.4	1	0.000
Pasture		6.4	2	**0.041**		5.2	2	0.074		3.3	2	0.195
Exposure time		0.6	3	0.892		9.5	3	**0.023**		11.3	3	**0.010**
Pasture * Exposure time		4.9	6	0.561		9.5	6	0.145		5.2	6	0.523

(3–4), Rb (2–3) and Zn (~2). BSAF values for **PE** ranged from 0.1 up to 25.0, where the highest BSAF values were also observed for As (13–19) and Cd (15–20), followed by Cu (~5), Rb (1–2) and Zn (2–3), as well as for Co (2–3). Regarding **RS**, BSAF values showed smaller ranges (0.1–11.7), with the higher mean BSAF values observed for As (5–6) and Cd (8–10), followed by Rb (1–2) and Zn (2–3), as well as for Co (2–3). The major observed pattern is the reduction in the BSAF values for As and Cd, from **SN** to **PE** and **RS**.

3.4. Ecotoxicological assays

At day 14, a general decrease of SOD activity (ranging from 11% to 23%) was observed in earthworms exposed to all experimental soils compared to the enzymatic activity observed in earthworms exposed to LUFA soil (Table 4); yet, after 28 days, a recovery of SOD activity was observed in earthworms exposed to **SN** and **PE** soils, a pattern maintained until the end of the experiment. SOD activity in **RS** was below the control until the end of the assay, although only significantly different from **PE** soils at 56 days.

Regardless of the experimental soil, the AChE activity showed a tendency to increase with exposure time, ending above the control for all soil types, and with a significantly higher value for **RS** than for **PE** and **SN**.

4. Discussion

4.1. Trace element profile in volcanic grasslands soils and their source

At the **SN** soils, that represent the less disturbed system, the highest soil TM concentrations were observed for V, Co, Ni and Zn, compared to the other pastureland systems. The total TM concentrations in these soils ranked as follows: Zn > V > Cr > Ni > Co. The results also revealed that those TM concentrations were associated with the agricultural management activities experienced in these systems (enrichment factors > 2), except: for Zn, whose concentrations were mainly associated with natural processes, i.e. the volcanic nature of the bedrock material; and, with the inclusion of Cu, also with an enrichment factor above 2. Despite this apparent soil contamination, the observed values were similar to or lower than those observed in other regions with volcanic soils (Cabral Pinto et al., 2015; Doelsch et al., 2006; Mendoza-Grimón et al., 2014; Palumbo et al., 2000), except for Cr, with higher values (16.50 mg kg^{-1}) than the undisturbed **RF** volcanic soil (3.00 mg kg^{-1}) and in other soils of the island (i.e., Picos Fissural Volcanic System - 12.60 mg kg^{-1}) (Parelho et al., 2014). A study performed by Zhao et al. (2014) associated long-term cattle manure input with the increase of Zn, Cu and Cr soil concentrations. Both Cu and Zn are commonly found in cattle manure because of their use as feed additives to promote animal growth and to control diseases (Nicholson et al., 2003). Despite the lower grazing intensity in the **SN** pasturelands, the increase of Zn, Cu, and Cr in the soils could be traced to the mass loading of cattle manure, as the higher animal density in cell grazing systems could explain the observed increase of TM soil concentrations. Moreover, even in **SN** pastureland systems, the dairy cattle are frequently supplemented with feed additives (mineral lick blocks and supplementary feed), fortified with several essential elements (e.g. Cu, Zn, Co, Fe, Mn; Franco-Uría et al., 2009) to overcome the low nutritional value of the natural vegetation. Consequently, the TM surplus can be released to the soil along with animal faeces and urine, contributing to the observed increase of the concentrations for V, Co, Ni and Cu in **SN** pasturelands.

Soils from **RS** pasturelands showed significant levels of contamination with As, Cd, and Hg. The results revealed that intensive agricultural management of volcanic pasturelands constitutes a major source of soil contamination for As, Cd and Hg (EF > 2). Despite the natural enrichment of volcanic soils with TM, the long-term and intensive management of volcanic soils could lead to the depletion of soil TM, including essential elements to plants and animals (macro and micronutrients). Thus, large quantities of fertilizers are usually added to intensive commercial farm soils to provide sufficient essential nutrients to plant growth and animal well-being (Alves et al., 2016). It is well known that inorganic fertilizers contain trace amounts of potentially toxic elements such as As, Cd, Pb, U, Rb and Hg (Sakizadeh et al., 2016; Zahra et al., 2010). Our data also suggests that the agricultural activities experienced in both **RS** and **PE** pastureland systems are a common anthropogenic source of soil contamination with Rb and U, since the use of inorganic fertilizers is a common practice in the Azores pastureland. Phosphate fertilizers, processed from phosphate rocks of sedimentary origin, have been identified as the predominant source for soil U, Th, and Rb (Sahu et al., 2014). Thus, the use for many years of inorganic fertilizers in volcanic soils, neglecting their

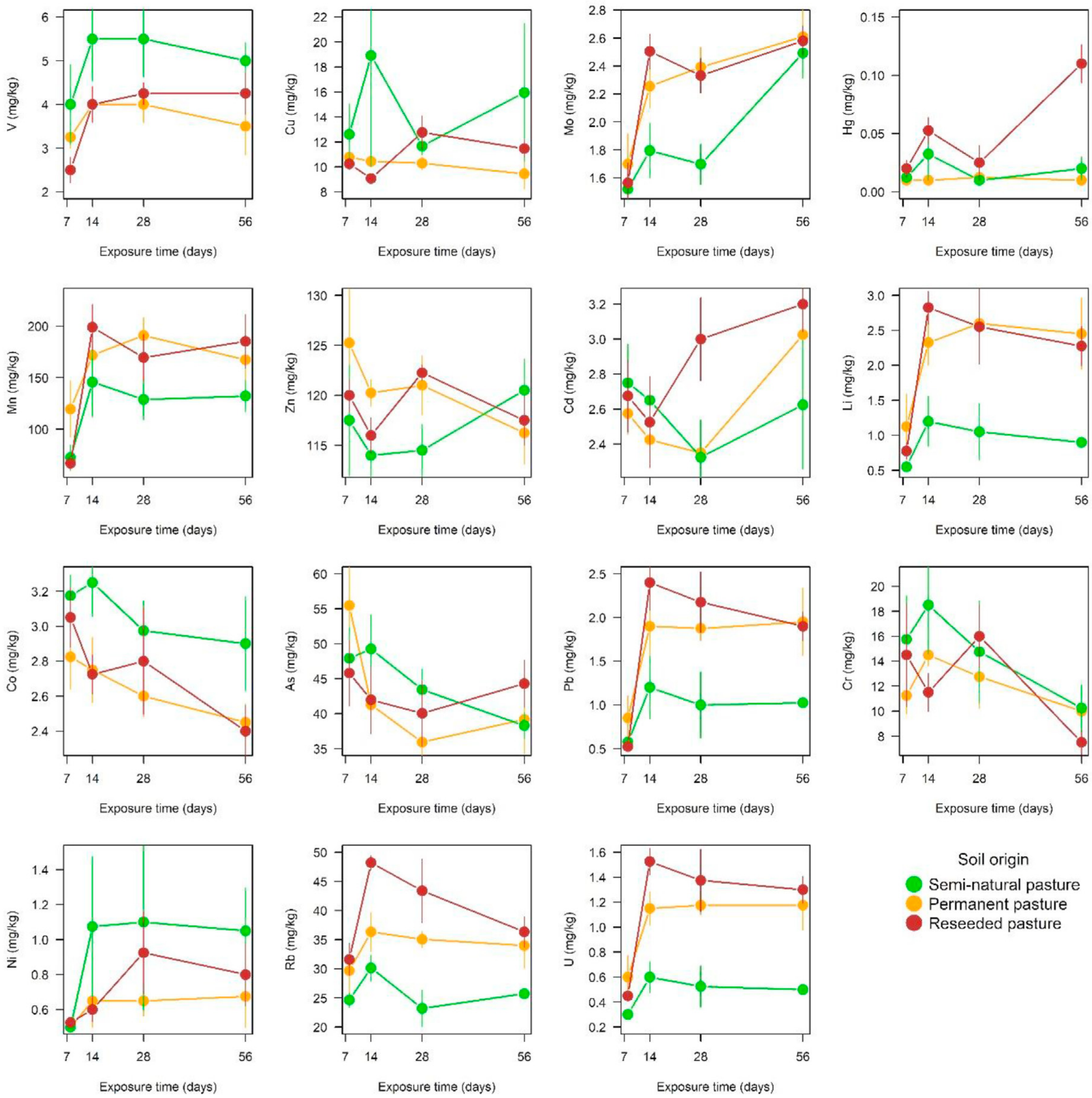

Fig. 2. Earthworms body trace metal concentrations (mean ± SD) after exposure during 7, 14, 28 and 56 days to the experimental soils: semi-natural (green); permanent (orange) and reseeded (red) pasturelands. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Biochemical responses from acetylcholinesterase (AChE) and superoxide dismutase (SOD) activities, of *Eisenia fetida* earthworms exposed (7, 14, 28 and 56 days) to the experimental soils (semi-natural [SN], permanent [PE] and reseeded [RS] pasturelands). Values are percentages of the enzymatic activities found in LUFA reference soil. Means (±SD) within each line followed by different letters indicate significant differences regarding time (at $p < 0.05$, Tukey test applied after ANOVA). Means (±SD) within each column followed by different numbers indicate significant differences between test soils (at $p < 0.05$, Tukey test applied after ANOVA).

	Exposure Time			
	7 days	14 days	28 days	*56 days*
SOD (%)				
Semi-natural [**SN**]	98.8 ± 7.2 ab	83.3 ± 8.5 a	129.4 ± 10.2 b (I)	103.8 ± 11.2 ab (I/II)
Permanent [**PE**]	115.6 ± 5.9 a	76.9 ± 6.8 b	102.3 ± 7.7 ab (I/II)	109.1 ± 9.4 a (I)
Reseeded [**RS**]	106.3 ± 8.7	88.9 ± 6.7	96.3 ± 9.8 (II)	88.8 ± 8.9 (II)
AChE (%)				
Semi-natural [**SN**]	85.0 ± 19.1 a	82.7 ± 12.2 a	104.4 ± 15.3 ab	151.5 ± 18.7 b (I)
Permanent [**PE**]	86.6 ± 25.3 a	101.2 ± 23.7 ab	124.5 ± 18.4 ab	153.5 ± 19.7 b (I)
Reseeded [**RS**]	68.4 ± 11.8 a	104.7 ± 22.9 ab	122.7 ± 20.6 ab	206.4 ± 36.7 b (II)

natural background (natural enrichment with TM) and unique soil properties (presence of high adsorbent clay mineral, Harsh et al., 2002) can contribute to soil accumulation of potentially toxic elements (Rodríguez Martín et al., 2006). Therefore, volcanic soils from **PE** and **RS** pastures, due to their soil physicochemical properties (pH, EC, organic matter, clay mineral content), are more sensitive to subtle changes in TM contents that may lead to an increased bioavailability of potentially toxic elements (As, Cd, Pb, U, Rb, Hg) from soil to resident biota (Sakizadeh et al., 2016; Zahra et al., 2010), compromising their health and the soil-based ecosystem services.

4.2. Trace metal bioaccumulation in earthworm tissues

It should be noted that the only cases where an increase in TM concentration along the exposure to the studied soils was detected, included Mn (with lower values for **SN**), Mo (with lower values for **SN**), PB (with lower values for **SN**), U (with lower values for **SN**), Hg (with lower values for **SN** and **PE**), and Li (with lower values for **SN**). However the BSAF results indicated an inverse trend (BSAF=SN > PE > RE) from that described above for the earthworms' tissue TM concentrations (TM bioaccumulation = SN < PE = RE), revealing that the particular physicochemical soil properties modulate the metal bioavailability from soil to biota (Sun et al., 2018; Xiao et al., 2020; Xu et al., 2018). In **SN** pasturelands an increased transfer of TM from soil to biota was observed (>BSAF), most probably due to the soil lower OM content; however, lower TM tissue concentrations were verified for the earthworms exposed to this soil. This could be attributed to the lower soil concentration of some of the assayed TM; thus, no adverse biological effects are expected in soil organisms inhabiting **SN** pastureland systems. However, in more intensively managed volcanic soils, due to the natural background and agricultural TM inputs, soil properties are more labile, and slight modifications in soil physicochemical properties could lead to a higher accumulation of TM in the biota, further associated with damaging effects on soil organisms (Gupta et al., 2019).

4.3. Ecotoxicological effects of soil management intensity

Our results revealed that the long-term exposure of earthworms to soils from **RS** pastureland was associated with a higher Hg accumulation and oxidative damaging effects in their tissues. Within the cell, SOD activity constitutes the first line of defence against ROS (Fulda et al., 2010; Ighodaro and Akinloye, 2018). Enhanced production of ROS, a general pathway of toxicity induced by organic and inorganic pollutants (Drzeżdżon et al., 2018; Livingstone et al., 1990), leads to a condition of oxidative stress and subsequent alteration in antioxidant defence mechanism. An increase in SOD activity is expected as a short-term response against oxidative stress. Yet, the excess of ROS molecules produced during long-term exposure to oxidant pollutants is a potential threat to normal cellular function, disrupting the antioxidant defence mechanism that results in a decrease of SOD activity and, consequently, in oxidative stress (Markad et al., 2012). This increase-decrease-recovery pattern of the SOD was observed in this study for earthworms exposed to **SN** and **PE** soils, but not to **RS** soil, where a tendency for a decrease of SOD activity was kept after day 7. These results indicate that earthworms have internal mechanisms (homeostasis), such as the antioxidant defence mechanism, that allow them to regulate the effects of some TM (e.g. V, Rb, Pb, U and Li), but not of others (e.g. Hg) (Xiao et al., 2020). The Hg earthworm tissue concentrations after exposure to the **RS** soil doubled after 7 days (from 0.01 ± 0.00 to 0.02 ± 0.01 mg kg^{-1}) and was eleven-fold higher after 56 days of exposure (0.11 ± 0.02 mg kg-1). Toxicologically, Hg displays a high bioaccumulation factor (Burton et al., 2006; Gray, 2002; Li et al., 2018) and acts as a pro-oxidant through the generation of hydrogen peroxide (Bjørklund et al., 2019; Lund et al., 1993). In general, organisms exposed to Hg show a disturbance of the SOD homeostasis (Huang et al., 2010; Lund et al., 1993; Malqui et al., 2018). Thus, the relatively smaller SOD activity in earthworms exposed to soils from **RS** can be attributed to the disruptive effect of long-term exposure to toxic TM and constitute an indicator of oxidative stress to resident soil organisms (Dzul-Caamal et al., 2020).

The results show that exposure during 56 days to the **RS** soils triggers an intensification of AChE activity in earthworms' tissues, which indicates the presence of anti-AChE molecules in these soils. This enzyme activity is used as an exposure (to anti-AChE molecules) and effect (neurotoxicity) biomarker. Even though the quantification of pesticide residues was not assessed in this study, organophosphate compounds and carbamate pesticides (both fungicide, insecticide and herbicide formulations), are used in the local agricultural context, more intensively in **RS** but also in **PE** pastureland systems, for animal and crop pest control. These compounds bind and phosphorylate the enzyme AChE, disrupting its normal activities (Colovic et al., 2013). Our results showed a time-related increase (of about 200% in **RS** soil) of AChE activity along the assay. This distinctive pattern of biological response is consistent with previous findings (Parelho et al., 2018), where earthworms of the species *Amynthas gracilis* exposed during 14 days to intensive livestock production soils also displayed an increase in AChE activity. Other studies also observed this AChE pattern in several earthworm species (e.g. *Drawida willsi*, Panda and Sahu, 2004; *E. andrei*, Velki and Hackenberger, 2012; *E. fetida*, Gambi et al., 2007; *Lumbricus terrestris*, González Vejares et al., 2010), and have attributed this to the capacity of the animal to detoxify and eliminate the pesticide through an enhanced metabolism, a parallel detoxification process, such as an increased synthesis of carboxylesterase, to protect the nervous system. Our results suggest the presence of higher concentrations of anti-AChE molecules in **RS** soil, while no neurotoxic effect should be expected, as AChE activity recovers after long-term exposure to these pollutants.

4.4. Soil management

Despite the selected study sites are located in the same geological unit, thus sharing the same volcanic bedrock and pedological conditions, results revealed that different soil management activities entail a distinct soil metal footprint for each agricultural system. In volcanic areas, the land use management type based on general directives (CAP and European Habitats Directive for 2014–2020) should not be dissociated from the soil chemical heritage and agricultural land use history. A sustainable agricultural land use management in volcanic areas must consider the natural TM background, present day and historical input of agrochemicals, soil management intensity and potential biological effects to resident biota. Earthworms actively take part in processes that influence the soil properties and health, therefore they are recognized as suited indicators of the effects of pollutants to soil organisms (Asencio et al., 2013). The integrated analysis of our results indicates that in more intensively managed volcanic pasturelands (**RS**), agricultural activities are important sources of soil contamination with As, Cd, and Hg (TM soil concentrations and EF), neurotoxic compounds (observed through the pattern of response of AChE), as well as other pollutants (revealed by SOD activity). The particular physicochemical soil properties in **RS** pasturelands modulates the TM bioavailability from soil to biota, leading to a greater Hg bioaccumulation in earthworm tissues associated with adverse effects in key biological processes (such as neurotransmission and antioxidant defence mechanisms).

5. Conclusion

In volcanic outermost islands, the remoteness and economic dependence on agriculture, and therefore on soil resources, claims to land use management a key role in the provision of soil-based ecosystem services. Framed by the particularities of volcanic soils and by the fast growth of the agricultural sector, this study aimed to understand the link between management intensity, TM contamination and their joint effects on soil ecosystem, to assist a well-balanced ecosystem supporting of local, regional, and global environmental health.

Overall the results show that agricultural management activities experienced in volcanic pasturelands lead to significant metal soil enrichment: V, Co and Ni in semi-natural pasturelands; As and Hg in reseeded pasturelands; and, Rb and Cd in both permanent and reseeded pasturelands. The soil particular physicochemical properties in reseeded pastureland systems (higher EC values associated with the moderately acid pH value) modulates the metal bioavailability from soil to biota, leading to a greater Hg bioaccumulation in earthworm tissues associated with adverse biological effects, encompassing key processes such as neurotransmission and antioxidant defence mechanisms.

This study point towards the increased importance of semi-natural and permanent pastureland management, over the intensive management (reseeded pasturelands), in favour of more sustainable ecosystems. Moreover, it highlights that volcanic pasturelands management must take into account the natural TM background, present day and historical agrochemicals inputs, soil management intensity and potential biological effects to the resident biota.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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