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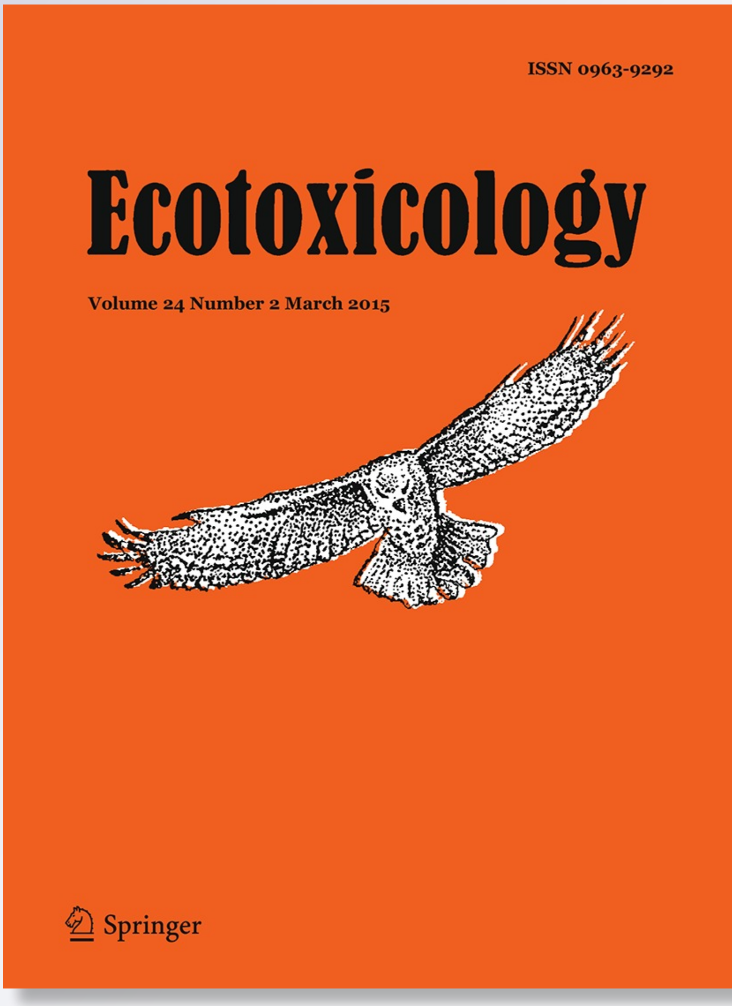
Ecotoxicology

ISSN 0963-9292
Volume 24
Number 2

Ecotoxicology (2015) 24:414-423
DOI 10.1007/s10646-014-1390-8



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Accepted: 18 November 2014 / Published online: 27 November 2014

Abstract The ecotoxicity of a dredged sediment from the Guanabara Bay (Rio de Janeiro, RJ, Brazil) was evaluated using reproduction tests with *Eisenia andrei*, *Folsomia candida* and *Enchytraeus crypticus*, and avoidance and feeding inhibition tests with *Folsomia candida*. The sediment was mixed with artificial soil to obtain the following doses: 1.25, 2.5, 5.0, 10.0, 20.0 and 40.0 %. Lead, nickel, chromium, copper and zinc concentrations were determined in the test mixtures. In reproduction tests, *E. andrei* was the most sensitive species (EC50 = 2.94 %), followed by *F. candida* (EC50 = 7.72 %) and *E. crypticus* (EC50 = 10.10 %). The percentage of initial weight of earthworms was significantly higher in all test concentrations compared to the control except at the highest one where earthworms biomass significantly decreased. No feeding inhibition of *F. candida* was observed for any test mixture and the number of organisms with a dark gut (the fed collembolans) generally increased with the increasing dose of sediment. Significant avoidance responses of *F. candida* were observed towards all test mixtures, however, the avoidance behaviour was the less sensitive endpoint after feeding inhibition. The results showed that chemical analysis is not sufficient to foresee toxic effects in terrestrial systems resulting from sediment disposal in soil if not complemented with an ecotoxicological evaluation.

Keywords Dredged sediment · Metals · Earthworms · Collembolan · Enchytraeids

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Introduction

Soil contamination resulting from disposal of dredged sediments in soils has been an environmental concern in developing countries (Maranho et al. 2009; Machado et al. 2011; Cesar et al. 2014a). It is well-known that contaminated sediments cause adverse effects on aquatic organisms, wildlife and human health (Ho et al. 2002; Machado et al. 2011; Vašíčková et al. 2013). Moreover, sediments dredging of rivers are often required to keep these waterways navigable and to avoid the eutrophication of aquatic systems (Munns et al. 2002; Bidone and Lacerda 2004). The dredged sediments may be used in the optimization of materials for beach nourishments and buildings, or may be applied to the soil for expansion of wetlands or as soil amendments for agriculture (Ho et al. 2002; Munns et al. 2002; Vacha et al. 2011). When dredged sediments are applied to the soil, the metals, organic contaminants and pathogens, often present in these materials, may provoke serious damages to edaphic biota (Vašíčková et al. 2013; Vacha et al. 2011; Cesar et al. 2014a).

Most of the legislation for regulation of terrestrial disposal of dredged sediments in South America countries only requires chemical analysis to evaluate the potential risk of these materials (e.g. 2004, 2009). However, the chemical analysis only refers to total or partial (selective extractions) contamination, do not necessarily reflecting its availability for biota (Selivanovskaya and Latypova 2003) and the multi-contaminant interactions (Natal-da-Luz et al. 2011a). In this context, ecotoxicological tests may constitute important tools for monitoring the risks of disposing dredged sediments when applied to the soil. The ecotoxicological evaluation should include different key soil dwelling species in order to take into consideration different physiologies and routes of exposure to contaminants. Earthworms (*Eisenia andrei* and *Eisenia fetida*), collembolans (*Folsomia candida*) and potworms (*Enchytraeus crypticus* and *Enchytraeus albidus*) have been widely used as test organisms for this purpose (Lukkari et al. 2005; Natal-da-Luz et al. 2009; Kobeticova et al. 2011; Van Gestel et al. 2011). While in earthworms and potworms the uptake of contaminants occurs predominantly via skin, in collembolans the uptake of contaminants occurs mainly by food ingestion (Vijver et al. 2003). These organisms are relatively easy to be kept in laboratory conditions and they have high sensitivity against chemicals presence in soil.

The sediments of Guanabara Bay basin (Rio de Janeiro, RJ, Brazil) are highly impacted by domestic and industrial wastes (Machado et al. 2002; Silva et al. 2007; Silveira et al. 2010; Rodrigues et al. 2011). The ecotoxicological evaluation of these sediments may support the establishment of environmentally sustainable strategies for their disposal in local soils. A recent study provided information on physical and chemical properties of local soils and soil metal availability aiming to support Brazilian authorities in maintaining the environmental quality of the terrestrial and aquatic systems from the bay in Rio de Janeiro State (Cesar et al. 2012; Cesar et al. 2014b). Another study developed by (Cesar et al. 2014a) presented a preliminary assessment based on screening tests with soil organisms, suggesting that the application of dredged sediments from Guanabara Bay basin in local soils may cause hazardous effects on soil biota even at low doses. The present manuscript reports a refined ecotoxicological evaluation of the same dredged sediment from the Guanabara Bay Basin based on two screening tests with the collembolans *F. candida* and chronic tests with *E. andrei*, *F. candida* and *E. crypticus* and using standard artificial soil as substrate. Working hypothesis assumed that: (i) the ecotoxicity of test mixtures increases with the increase of the dredged sediment dose; and (ii) the application of the dredged sediment at low doses benefit the biomass of *E. andrei* (Hormetic response) due to the organic matter provided by the sediment.

Materials and methods

Sample processing

Dredged sediment was sampled in three different points (station 1: 22° 51′ 46.77″S, 43° 14′ 0.92″O; station 2: 22° 51′ 41.63″S, 43° 14′ 10.90″O; station 3: 22° 50′ 48.28″S, 43° 14′ 29.48″O), located at the Cunha Estuary (Guanabara Bay Basin, Rio de Janeiro, RJ, Brazil). The test sediment is mainly contaminated with metals, pathogenic microorganisms and petroleum hydrocarbons (Machado et al. 2002; Silva et al. 2007, 2010; Rodrigues et al. 2011).

After drying at 20 °C for 1 month, the sediment samples were sieved (1.7 mm) to remove large stones and other larger particles, and then mixed and ground to generate a unique composite sample. (Cesar et al. 2014a) determined total metal concentrations in the same composite sample used in this study. Metal concentrations measured were generally below those established as fourth level of risk (high probability of adverse effects on biota) by Brazilian legislation for dredged sediments disposal (2004; Table 1). The exception was the mercury concentration that was higher than the maximum values allowed by Brazilian law. (Cesar et al. 2014a) measured an organic matter content of 19.1 % in the same sediment sample. The range of sediment doses used in the present study was defined according to the results obtained in avoidance and acute toxicity tests previously performed with *E. andrei* (Cesar et al. 2014a) using mixtures of two tropical local soils (ferralsol and chernosol) and the same sediment. Thus, the doses used in the tests were 1.25, 2.5, 5, 10 and 20 % (considering the dry weight mass). These doses were prepared mixing the dredged sediment with artificial soil in different

Table 1 Total metal concentrations of the dredged sediment and the upper limit values of metals allowed for dredged sediments to be disposed in soil according to (2004). Table was taken from (Cesar et al. 2014a)

Metal	Dredged sediment (mg/kg)	Limit values (mg/kg)	
		Level three[a]	Level four[b]
Hg	1.08	0.15	0.71
Cu	92.0	34	270
Zn	329	150	410
Pb	124	46.7	218
Ni	20.3	20.9	51.6
Cr	94.5	81	370

[a] Means limit of low probability of adverse effects on biota; and [b] means limit of high probability of adverse effects on biota (2004)

proportions. The artificial soil was composed by 70 % of quartz sand, 20 % of kaolin, 9–9.5 % of ground *Sphagnum* peat and 0.5–1 % of calcium carbonate to adjust the pH to 6.0 ± 0.5, according to the ISO guideline 1268-2 ((International Organization for Standardization) 1998). The moisture content of the test mixtures was adjusted to 50 % of the water-holding capacity immediately before being used in the tests. All tests were incubated at 20 ± 2 °C and under a photoperiod of 16:8 h light:dark. Test species (*Eisenia andrei*, *Folsomia candida* and *Enchytraeus crypticus*) were obtained from laboratory cultures of the University of Coimbra (Portugal).

Bioassays with Folsomia candida

Laboratory cultures of *F. candida* were maintained as described by Natal-da-Luz et al. (2009). Springtails 10–12 days old were taken from synchronized cultures and used in the experiments.

Two-chamber avoidance tests were performed following the ISO guideline 17512-2 (ISO (International Organization for Standardization) 2007). Cylindrical plastic boxes (11 cm diameter and 4 cm height) were used as test containers divided into two equal sections by a plastic card (ISO (International Organization for Standardization) 2007) transversally introduced in the middle of the vessels. One section was filled with 30 g of artificial soil (fresh weight equivalent; FW) and the other one with the same amount of the test mixtures. Each test mixture was combined with pure artificial soil and five replicates were prepared per combination. A combination with pure artificial soil in both sections of the replicates was also tested (dual-control test) to control the homogeneous distribution of the springtails in the soil (Natal-da-Luz et al. 2009). After soils addition in the test containers, the plastic card was removed and 20 springtails were placed in the middle line between soils. After 48 h, the plastic card was reintroduced with the same position in the test vessels and water with some drops of blue ink was added in the test vessels. The number of animals was determined in each section by counting the animals floating on the water surface. Missing individuals were considered dead. An additional replicate without organisms was prepared per combination for moisture and pH measurements after the test period. These two parameters were measured at the beginning and at the end of the test, for each combination. Validity criteria for avoidance tests assumed an average mortality lower than 20 % and an average number of springtails in one section within 40–60 % of the total number of organisms in the dual-control tests.

A feeding inhibition test based on the procedures described by Domene et al. (2007) was performed. The same dredged sediment doses were tested, however, particularly for this test, an additional concentration of 40 % was prepared and the dredged sediment was diluted in a mixture of 78 % of quartz sand and 22 % of kaolin instead of standard artificial soil. Therefore, no *Sphagnum* peat was used in any of the mixtures tested in the feeding inhibition test only. By this way dredged sediment was the only food source available for collembolans in the test mixtures. In each replicate, 15 organisms were exposed to 5 g of fresh test material using 50-ml polyethylene containers covered with a plastic lid as test vessels. After 48 h, the test containers were flooded with water, and the number of organisms floating on the water surface with dark gut was determined in each replicate. Moisture and pH of the test mixtures were measured only at the beginning of the test and missing organisms were considered dead. Validity criteria assumed mortality lower than 20 % and none individuals with dark gut in the 0 % dose (control) at the end of the test.

A collembola reproduction test was conducted following the ISO guideline 11267 (ISO (International Organization for Standardization) 1999). Five replicates with 10 springtails and 30 g (FW) of pure artificial soil or test mixtures were prepared, using cylindrical glass flasks (4 cm diameter and 7 cm height) as test containers. An additional replicate without organisms was prepared for each treatment for soil moisture and pH determination at the end of the test. These two parameters were measured at the beginning and at the end of the experiment. Test containers were aerated and moisture content was adjusted weekly by compensating weight losses with distilled water. About 2 mg of granulated dry yeast was given as food in each test vessel at the beginning and at the 14th day of the test. After 28 days of exposure, the content of each test vessel was transferred to a larger vessel that was filled with water and a few drops of blue ink were added. After gentle stirring, the animals floating on the water surface were photographed (using a digital camera) and the number of juveniles and adults was determined using the Image Tool software (http://ddsdx.uthscsa.edu/dig/itdesc.html). The missing adult springtails were considered dead. Validity criteria assumed average mortality lower than 20 %, coefficient of variance of reproduction no higher than 30 % and more than 100 juveniles per test container in the 0 % treatment (control) at the end of the test period.

Reproduction tests with Eisenia andrei

The maintenance of the earthworm laboratory cultures used in this test followed the procedures described by Natal-da-Luz et al. (2009). The organisms used in the tests were sexually mature (with fully developed clitellum) and had an average fresh weight (± standard deviation, $n = 240$) of 372.8 ± 0.026 mg. Earthworm reproduction test performed in the present study followed the procedures described in the ISO guideline 11268-2 (ISO (International Organization for Standardization) 1998). Cylindrical plastic containers (11 cm diameter and 12 cm height) filled with 500 g of pure artificial soil or test mixture (dry weight equivalent; DW) and 10 earthworms (previously washed and weighted) were used in each replicate. Earthworms were acclimatized in pure artificial soil for 24 h before being used in the test. 5 g (DW) of finely ground and wetted horse dung were given as food in each replicate at the beginning and at the 14th day of the experiment. Four replicates were prepared per treatment. The test containers were opened once a week to allow air circulation and moisture content was adjusted by reestablishing the initial weight adding distilled water. After 28 days of exposure, the surviving adults were collected by hand, counted, washed, weighted to determine the percentage of initial biomass and finally discarded. The test soils were incubated for additional 28 days and another portion of 5 g of wetted ground dung was given in each replicate. After this period the number of juveniles was determined in each replicate by placing the test containers in a water bath at 60 °C as described in annex B of the ISO guideline 11268-2 (ISO (International Organization for Standardization) 1998). Soil moisture and pH were measured at the beginning and at the end of the experiment. Validity criteria assumed mortality lower than 10 % and a coefficient of variance no higher than 30 % in replicates of control (0 % dose).

Reproduction tests with Enchytraeus crypticus

The enchytraeids were cultured in Petri dishes containing agar prepared by mixing, in a proportion of 1:1 (v:v), a four salt solution composed of calcium chloride, magnesium sulfate, potassium chloride and sodium bicarbonate and an agar solution composed of bacti-agar (Oxoid, Agar N0. 1), sodium bicarbonate, potassium chloride and demineralized water. Both solutions were previously autoclaved at 121 °C for 30 min and then stored at 4 °C. Petri dishes with agar were kept at room temperature during at least 2 h before being used as substrate for enchytraeid cultures. About 50 mg of ground rolled oats were given as food once a week. Every 2 months the organisms were transferred to a new substrate. The cultures were maintained at 20 ± 2 °C, under a photoperiod of 16:8 h light:dark.

The enchytraeids reproduction test was based on the ISO guideline 16387 (ISO (International Organization for Standardization) 2004). Replicates consisted of cylindrical glass flasks (5 cm diameter and 9 cm height) with 20 g (DW) of pure artificial soil or a test mixture and 10 potworms with a visible well-developed clitellum. Five replicates per treatment were prepared. Additionally, a replicate without organisms was prepared for each treatment for soil moisture and pH measurements at the end of the experiment. Once a week, the test containers were opened to allow air circulation and the moisture content was adjusted when needed (by reestablishing the initial weight adding distilled water). 25 mg of ground rolled oats were given as food in each replicate at the beginning and at the 14th day of experiment. After 28 days, each replicate was filled with ethanol (at 70 %) up to a depth of 4 cm and 200–300 µL of Bengal red (1 % solution in ethanol) were added. After gently stirred, the replicates were left to rest overnight. After that period the replicates content was washed individually through a sieve (0.25 mm) with tap water, and transferred to a Petri dish to count juveniles under a binocular microscope. The soil moisture and pH of each treatment were measured at the beginning and at the end of the test. Validity criteria assumed mortality lower than 20 %, a number of juveniles higher than 25 per test vessel and a coefficient of variation no higher than 50 % in the replicates of control (0 % dose).

Chemical and mineralogical characterization

Total concentration of aluminum (Al) and iron (Fe) was determined in the dredged sediment by atomic absorption technique, according to the procedures described in (EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) 1997).

In the test mixtures, the total concentration of zinc (Zn), copper (Cu), chromium (Cr), nickel (Ni) and lead (Pb) was determined in triplicate using 80-100 mg of sample previously homogenized (using an acid-washed porcelain pestle) per replicate. The samples were extracted with 2 mL of HNO_3 (69 %), using a PDS-6 pressure digestion systems at 150 °C for 10 h, according to the method described by Natal-da-Luz et al. (2011b). Potentially bioavailable concentrations of Zn, Cu, Cr, Ni and Pb were measured in the test mixtures used for reproduction tests and collembola avoidance tests. These extractions were performed in duplicate by using one gram of sample and 25 mL of a 0.1 M HCl solution (DePaula and Mozeto 2001) per replicate. This mixture was shaken for 2 h (200 rpm) and then filtered using Whatman No. 1 filter paper discs (Cat. No. 1001150, Maidstone, England). Both total and potentially bioavailable metal concentrations were determined in the respective analytical solutions by flame AAS (2380 Absorption Atomic Spectrometer, Perkin-Elmer). The detection limits were 0.04, 0.1, 0.0012, 0.06 and 0.05 mg/kg, for Cu, Pb, Zn, Ni and Cr, respectively. The standard reference material SRM 2709 (San Joaquin Soil), certified by National Institute of Standards and Technology (Department of Commerce, USA), was used to check the accuracy of total metal analyses.

Statistical analysis

The significant avoidance responses observed in collembolan avoidance tests were determined using the Fisher exact test as described by Natal-da-Luz et al. (2009). Significant losses on habitat function were considered when less than 30 % of the test organisms were found in the section with the test mixture (percentage of avoidance ≥70 %; ISO, ISO (International Organization for Standardization) 2007). The dose of dredged sediment that provokes an avoidance response of 50 % (avoidance EC50) and the respective 95 % confidence limits (CL) was estimated using the PriProbit 1.63 software (Sakuma 1998; http://bru.gmprc.ksu.edu/proj/priprobit/download.asp). Prior to the statistical analysis, the number of dead or missing organisms in each combination was equally distributed by both sections of each replicate.

The EC50 values for collembola, earthworm and potworm reproduction tests and for earthworms biomass were estimated through non-linear regression models using the software Statistica version 7.0. Significant differences between the control (0 % dose) and the test treatments in reproduction and biomass (earthworms only) were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett post hoc test. Prior to these statistical analyses, the assumptions of normality and homoscedasticity were checked through Kolmogorov–Smirnov and Levene's tests (for $p > 0.05$), respectively. When these assumptions were not fulfilled, values were log transformed. Treatments where the reproduction was lower than 1 % of the control were not considered for one-way ANOVAs. When the assumptions to the ANOVA analysis were not fulfilled, even after log transformation, a Kruskal–Wallis test, followed by Fisher LSD test on ranks, was performed.

Results

Metal concentrations in the artificial soil and test mixtures

The addition of sediment increased pH of the test mixtures (from 5.8 in the control to the maximum value of 7.19 in the 20 % dose). Total metal concentration of mixtures used in the tests generally increased with the increasing sediment dose. Metal concentrations followed the increasing order Cr > Pb > Zn > Ni > Cu in mixtures used in reproduction and avoidance tests (Table 2) and Zn > Cr > Pb > Ni > Cu in mixtures used in feeding inhibition test (Table 3). In mixtures with standard artificial soil, Cu and Zn were the metals with the highest percentage of total concentration as potentially bioavailable fraction, presenting generally 50 % of the total metal concentration of the test mixtures followed by Pb with about 20 % in all test doses, except at the highest one (20 %) with 78 %.

Avoidance and feeding inhibition tests with Folsomia candida

The validity criteria were fulfilled in both screening tests. In avoidance tests the mortality was not dose related and was always lower than 20 % (data not shown). *F. candida* significantly avoided all soil-sediment mixtures (Fisher exact test; $p < 0.001$), but only the highest test dose showed limited habitat function (avoidance behaviour higher than 70 %; Fig. 1). The avoidance EC20 and EC50 values estimated were 18.47 and 20.28 %, respectively (Table 4).

In the feeding inhibition tests, the number of organisms without dark gut increased with the increase of the sediment dose indicating that no feeding inhibition was observed along the sediment dose gradient (Fig. 2). At the end of the test, collembolan moults were observed only in

Table 2 Total and potentially bioavailable (Bio) concentrations of chromium, copper, nickel, lead and zinc in the test mixtures used in the reproduction and avoidance tests. The test mixtures were composed by dredged sediment and standard artificial soil (70 % of quartz sand, 20 % of kaolin, 9 to 9.5 % of ground *Sphagnum* peat and 0.5 to 1 % of calcium carbonate)

Test Mixtures (%)	Cr (mg/kg)			Cu (mg/kg)			Ni (mg/kg)			Pb (mg/kg)			Zn (mg/kg)		
	Total	Bio	Bio (%)	Total	Bio	Bio (%)	Total	Bio	Bio (%)	Total	Bio	Bio (%)	Total	Bio	Bio (%)
0.00	38.39	0.96	2.51	0.11	0.02	22.74	10.68	3.51	32.89	44.34	5.16	11.63	2.84	0.92	32.34
1.25	42.98	1.21	2.81	0.11	0.05	43.58	11.11	4.33	38.98	30.09	3.10	10.30	12.25	2.07	16.90
2.5	50.19	1.52	3.02	0.17	0.09	52.52	3.59	0.17	4.74	32.56	5.48	16.82	16.72	8.48	50.74
5.0	51.67	0.40	0.77	0.26	0.12	47.95	16.97	0.12	0.74	48.22	8.07	16.74	26.73	13.25	49.57
10.0	48.39	2.53	5.23	0.48	0.19	39.79	14.73	0.19	1.30	48.68	9.96	20.47	55.51	25.53	46.00
20.0	49.14	3.61	7.35	0.89	0.37	42.26	18.96	0.37	1.98	23.23	18.04	78.09	125.56	51.10	40.69

the two highest sediment doses (11 and 13 moults were found in the 20 and 40 % sediment doses, respectively).

Reproduction tests

The validity criteria were fulfilled in the reproduction tests with the three test organisms. Considering all mixtures tested, collembolan mortality was lower or equal to 4 %, while in earthworms no mortality was found. The reproduction EC20 and EC50 values estimated showed that *E. andrei* was the most sensitive species (EC20 = 1.26 % and EC50 = 2.94 %) followed by *F. candida* (EC20 = 3.70 % and EC50 = 7.72 %) and *E. crypticus* (EC20 = 6.91 % and EC50 = 10.10 %; Table 4). The three highest sediment doses (5, 10 and 20 %) significantly inhibited (one-way ANOVA, F = 42.37, $p < 0.001$) the reproduction of *F. candida*, while only the two highest doses (10 and 20 %) significantly affected the reproduction of *E. crypticus* (Kruskall Wallis, $p < 0.05$). The reproduction of *E. andrei* was significantly affected in all test mixtures (one-way ANOVA, F = 36.42; $p < 0.001$; Fig. 3).

At the end of the test period, the surviving adult earthworms (*E. andrei*) found at the lowest sediment doses (1.25, 2.5, 5 and 10 %) had a percentage of initial biomass significantly higher than that of the earthworms from control, and significantly lower than that of the earthworms from the highest dose (20 %: Fig. 4) (one-way ANOVA, F = 35.24, $p < 0.001$). The biomass EC20 and EC50 values estimated were higher than the highest sediment dose tested (22.04 and 44.47 %, respectively; Table 4). The surviving adult earthworms exposed to the highest sediment dose (20 %) presented visible morphological damages on their epidermis.

Discussion

Total metal concentrations of test mixtures are generally below the threshold limit for ecological risk on soil biota

Table 3 - Total concentrations of chromium, copper, nickel, lead and zinc in the test mixtures used in the feeding inhibition tests with *Folsomia candida*. For these tests, test mixtures were composed by dredged sediment and artificial soil without *Sphagnum* peat (78 % of quartz sand and 22 % of kaolin)

Test Mixtures (%)	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
0.00	21.67	0.11	14.45	nd	4.92
1.25	34.02	0.18	21.70	nd	15.34
2.5	33.63	0.00	26.88	0.77	25.85
5.0	34.59	0.37	21.41	nd	41.18
10.0	40.89	0.55	20.24	11.84	80.32
20.0	60.29	0.58	23.4	17.44	107.04
40.0	93.19	1.83	23.38	67.24	208.83

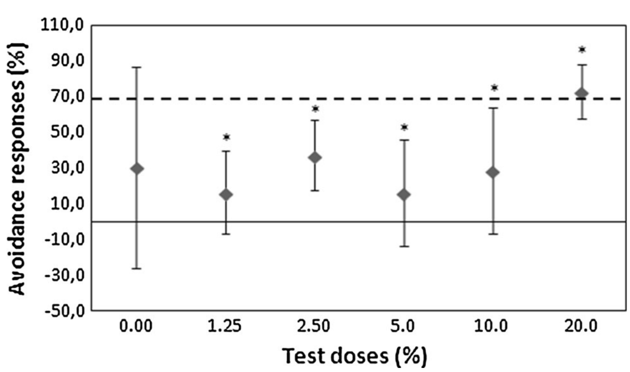

Fig. 1 Avoidance response of *Folsomia candida* to different mixtures of a dredged sediment at different doses (0, 1. 25, 2.5, 5, 10 and 20 %) with standard artificial soil. Values are the average percentages of avoidance (± standard deviation; $n = 5$). *Asterisk* means significantly higher percentage of organisms in the control section than in the test section

Table 4 - Median effective dose (EC50) with 95 % confidence limits inside curve brackets, for all tests performed with a gradient of mixtures of standard artificial soil and a dredged sediment. Estimated values are expressed in percentage of dredged sediment (considering dry weight)

Test species	Endpoint	EC20	EC50
Folsomia candida	48 h-avoidance	18.47 (-)[a]	20.28 (-)[a]
	28 days-reproduction	3.70 (2.33–5.08)	7.72 (6.33–9.11)
Eisenia andrei	28 days-growth	22.04 (18.67–25.40)	44.47 (26.23–62.71)
	56 days-reproduction	1.26 (0.95–1.57)	2.94 (2.59–3.30)
Enchytraeus crypticus	28 days-reproduction	6.91 (5.29–8.53)	10.10 (9.20–11.00)

[a] Data do not allow estimation of a 95 % confidence interval

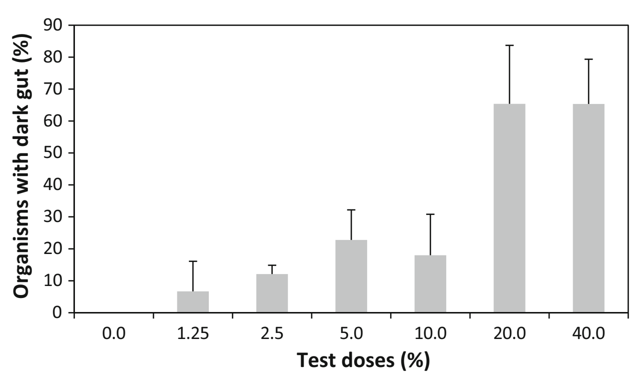

Fig. 2 Percentage of *Folsomia candida* (average + standard deviation; $n = 5$) with a dark gut found in mixtures of a dredged sediment in the doses 0, 1. 25, 2.5, 5, 10, 20 and 40 % with quartz sand and kaolin after 48 h of exposure

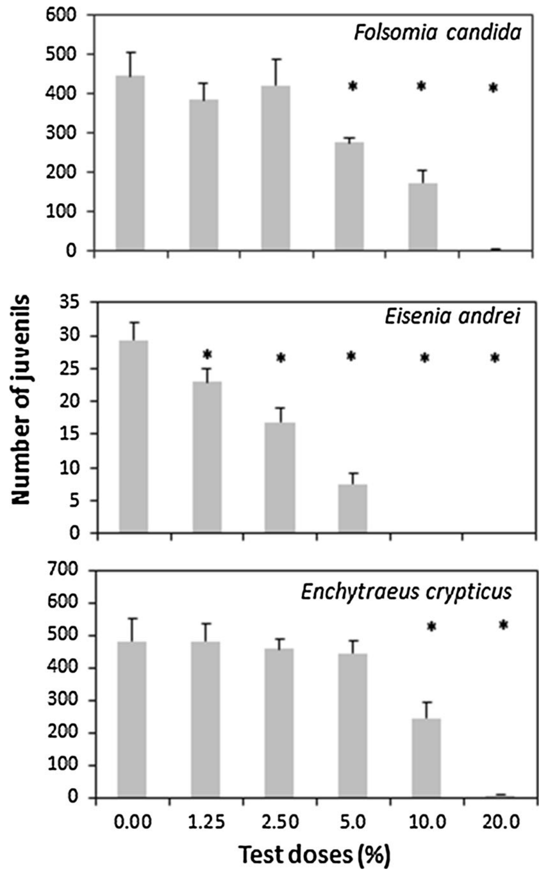

Fig. 3 Reproduction tests with *Folsomia candida* (first graph; $n = 5$), *Eisenia andrei* (second graph; $n = 4$) and *Enchytraeus crypticus* (third graph; $n = 5$). Reproduction is expressed in number of juveniles (average + standard deviation) when exposed to mixtures of standard artificial soil and a dredged sediment in the doses 0, 1. 25, 2.5, 5, 10 and 20 % of dredged sediment. *Asterisk* number of juveniles significantly different from the 0 % dose (control)

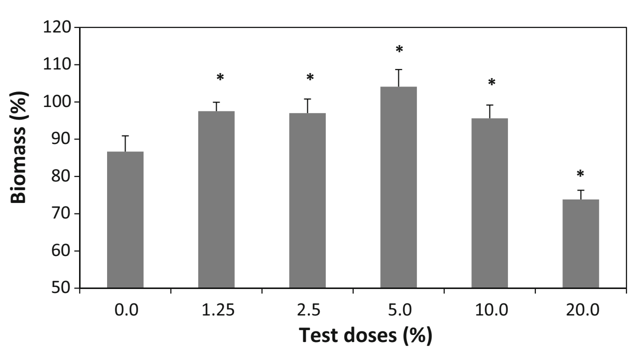

Fig. 4 Percentage of initial biomass (average + standard deviation: $n = 4$) of surviving adults of *Eisenia andrei* exposed to mixtures of standard artificial soil and a dredged sediment in the doses 0, 1. 25, 2.5, 5, 10 and 20 % of dredged sediment for 28 days

established by Brazilian law (2009). In spite of that, significant toxic effects were observed in the reproduction and avoidance tests in several test mixtures. Such results may be due to synergistic effects resulting from multi-contaminant interactions and/or the presence of contaminants other than metals (for which chemical analyses are not required by Brazilian law) that might have an important role in the toxicity observed.

The ecotoxicological tests performed in the present study were generally less sensitive to the dredged sediment than those previously performed in a preliminary evaluation using mixtures of sediment diluted in two natural soils in earthworm acute and avoidance tests (Cesar et al. 2014a). Most probably, the high organic matter content of the standard artificial soil promoted the adsorption of contaminants from sediment mitigating the toxicity of the dredged sediment to the test organisms.

The avoidance and feeding inhibition tests were the least sensitive among the ecotoxicological tests achieved. Although significant avoidance behaviour was observed since the lowest test concentration (1.25 %), the avoidance EC20 and EC50 values estimated were higher than those estimated for the reproduction of the three test species. The 19.1 % of organic matter content of the dredged sediment increased the organic matter content of the test mixtures as higher the sediment dose. This could mitigate the repellent effect of the test mixtures to *F. candida* (Natal-da-Luz et al. 2008). This type of interference was reported by Natal-da-Luz et al. (2009) who observed an avoidance response of *F. candida* towards a natural soil when combining it with a mixture of soil and an organic sludge.

Concerning the feeding inhibition test, the number of dark gut individuals was higher especially at the two highest sediment doses (20 and 40 %). This suggests that the sediment consumption by the test organisms was mainly related with the availability of organic matter (from the sediment). Apparently, metal contamination of the test sediment was not sufficiently high to inhibit the feeding activity of collembolans. This behaviour was distinct from the feeding inhibition found by Domene et al. (2007) when exposing *F. candida* to dose gradients of a dried pig slurry and six sewage sludges with different origins and pre- and post-treatments. On the other hand, according to Fountain and Hopkins (2001), who maintained *F. candida* in Plaster of Paris/graphite substrate supplying metal contaminated yeast as food for 7 weeks, these collembolans could have great tolerance of moderate metal contamination in the diet due to their ability to excrete assimilated metals at moulting. This agrees with the observed behaviour of collembolans at the highest test doses (20 and 40 %) where several moults were observed after the test period. On the other hand, the presence of several moults in the highest treatments could be a result of a faster growth rate due to high organic matter content (and therefore to high amount of food) in the test mixtures. Analysis of the moults would be needed to clarify the reasons behind this behaviour.

The first working hypothesis, assuming an increased ecotoxicity of the test mixtures as higher the sediment dose, was confirmed to the avoidance test and reproduction test with *E. andrei*, but it was rejected to the feeding inhibition test. The present study evidenced the existence of some factors that may condition the outcome of the two screening tests performed. More studies (e.g. testing the influence of other organic residues and different types of natural soils in the outcome of the screening tests) are needed to better identify and describe these conditioning factors.

In reproduction tests, *E. andrei* was the most sensitive organism tested. This is in agreement with data reported by Vašíčková et al. (2013), who studied the reproduction response of *E. fetida*, *E. crypticus* and *F. candida* when exposed to dredged sediments. Vašíčková et al. (2013) found a sensitivity of *E. fetida* higher than that of *F. candida* and *E. crypticus*.

In the earthworm reproduction tests the percentage of initial biomass of the surviving adults at the lowest sediment doses agrees to the biomass change observed in the earthworm acute test performed by (Cesar et al. 2014a) using mixtures of increasing doses of the same sediment in two natural soils. The biomass change confirm the second working hypothesis and suggests that the earthworms used the test sediment as food. This also agrees with Carbonell et al. (2009) who reported an increase of earthworms biomass (using *E. fetida* as test organism) when exposed to a multi-species-soil-system composed by natural soils amended with a domestic sewage sludge, contaminated with Zn, Pb, Cu, Cd and Ni, in different application rates. Moreover, the high amount of food available in test mixtures could compensate for the toxic effect. In this way, Postma et al. (1994) observed in *Chironomus riparius* (Insecta, Diptera) larvae that an increase of the amount of food allows to compensate for the direct negative effects of cadmium exposure until a critical threshold concentration. Indeed, in the present study, the percentage of initial biomass was lower than that of control only at the highest sediment dose (20 %). In such treatment (20 %), the energy reserves are probably mobilized for detoxification processes leading to a reduction of the energy allocated to the growth.

Up to the 5 % sediment dose, the increase of the percentage of initial earthworms biomass was followed by the reduction of their reproduction. This inverse relationship was also reported by Van Gestel and Hoogerwerf (2001) and (Santurofu et al. 2012) in artificial and natural metal-contaminated soils, respectively. Such fact may be explained by the increasing investment of energy to the body growth which, consequently results in a reduction of cocoons production. However, the ecological implications and the mechanisms behind this trade-off are still unknown (Van Gestel and Hoogerwerf 2001). Another hypothesis consists on the fact that the dredged sediment studied contains a mixture of organic and mineral compounds. These toxic compounds have different mode of actions and, therefore, different effects to the test organisms. For example, some endocrine disruptors can affect the reproduction and not necessarily the growth (Kavlock et al. 1996). By the light of this hypothesis, a reduction of cocoons production and no effect on growth would be expectable.

Conclusion

The results observed in the present study showed that chemical analysis of dredged sediments is not sufficient to prevent toxic effects in terrestrial systems after sediment application in soil. Therefore, the results obtained reinforce the need for an ecotoxicological evaluation of dredged sediments to complement chemical analyses required by Brazilian law. The chronic tests performed with the three test species showed robust and sensitive performances, while the screening tests with *F. candida* (both avoidance and feeding inhibition tests) might be influenced by factors inherent to the sediment nature (e.g. high OM content). More research is needed to increase the knowledge about the influence of these factors in the outcome of collembola avoidance and feeding inhibition tests. In the chronic tests, the earthworm *E. andrei* was the most sensitive species tested followed by *F. candida* and *E. crypticus*.

The present study showed that the dredged sediment collected is toxic for key-invertebrate species. However, it is known that the toxicity of contaminants is highly dependent on soil properties, like clay mineralogy, texture and organic matter type and content. Because of that, ecotoxicological tests using gradients of sediment dilutions in local soils would be desirable. In addition, dredged sediments from other regions should be analyzed in order to generate ecotoxicological reference values that reflect the main characteristics and diversity of the local dredged sediments, supporting decision-makers in future programs of environmental management.

Acknowledgments We would like to thank CNPq (National Brazilian Council for Scientific and Technological Development) and CAPES (Coordination for the Improvement of Higher Level -or Education- Personnel) for providing grants for Ricardo Cesar (Doctorage/CNPq and Sandwich Doctorage/CAPES). Without such financial support, this research could not be executed.

Conflict of interest The authors declare that they have no conflict of interest.

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