Environ Monit Assess
DOI 10.1007/s10661-013-3468-9

Disposal of dredged sediments in tropical soils: ecotoxicological effects on earthworms

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Received: 10 May 2013 / Accepted: 1 October 2013

Abstract The upper limit concentrations of metals established by international legislations for dredged sediment disposal and soil quality do not take into consideration the properties of tropical soils (generally submitted to more intense weathering processes) on metal availability and ecotoxicity. Aiming to perform an evaluation on the suitability of these threshold values in tropical regions, the ecotoxicity of metal-contaminated dredged sediment from the Guanabara Bay (Rio de Janeiro, Brazil) was investigated. Acute and avoidance tests with *Eisenia andrei* were performed with mixtures of dredged sediment with a ferralsol (0.00, 6.66, 13.12, 19.98, and 33.30 %) and a chernosol (0.00, 6.58, 13.16, 19.74, and 32.90 %). Mercury, lead, nickel, chromium, copper, and zinc concentrations were measured in test mixtures and in tissues of surviving earthworms from the acute tests. While ferralsol test mixtures provoked significant earthworm avoidance response at concentrations ≥13.31 %, the chernosol mixtures showed significant avoidance behavior only at the 19.74 % concentration. The acute tests showed higher toxicity in ferralsol mixtures (LC50=9.9 %) compared to chernosol mixtures (LC50=16.5 %), and biomass increased at the lowest sediment doses in treatments of both test soils. Most probably, the expansive clay minerals present in chernosol contributed to reduce metal availability in chernosol mixtures, and consequently, the ecotoxicity of these treatments. The bioconcentration factors (BCF) for zinc and copper were lower with increasing concentrations of the dredged sediment, indicating the existence of internal regulating processes. Although the BCF for mercury also decreased with the increasing test concentrations, the known no biological function of this metal in the earthworms metabolism lead to suppose that Hg measured was not present in bioaccumulable forms. BCFs estimated for the other metals were generally higher in the highest dredged sediment doses.

Keywords Bioconcentration factor · *Eisenia andrei* · Avoidance tests · Acute tests · Metals

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Published online: 11 October 2013

Introduction

The expansion of urban areas in tropical countries has originated the generation of high amounts of industrial and domestic wastes. Due to that reason, the definition of appropriate destinations to industrial and domestic wastes is an issue of increasing environmental concern. In South America, most of these wastes have been discharged into aquatic systems without an adequate treatment, contaminating superficial water and sediments, and affecting aquatic biota. In addition, the frequent dredging of these waterways followed by sediment incorporation on adjacent areas is contaminating agricultural fields. Due to this reality, the effect of the incorporation of dredged sediments for terrestrial systems is actually an issue of concern to the environmental authorities in Brazil (Machado et al. 2002; Junior et al. 2002; Luiz-Silva et al. 2006; Loureiro et al. 2009).

Actual Brazilian legislation on dredged sediments disposal (*Conselho Nacional de Meio Ambiente* (CONAMA) 2004, 2009) is essentially based on Canadian and Dutch guidelines. For this reason, the limits for metal concentrations established in those guidelines were defined for temperate ecosystems and, consequently, do not take into consideration the particular features of tropical regions in Brazil. These regions are characterized by the occurrence of intense weathering processes; specific mineralogy, with an abundance of 1:1 clay minerals (kaolinite) and high contents of aluminum and iron oxi-hydroxides. Therefore, the generation of ecotoxicological reference values for metal concentration in soils relying on the natural properties of tropical systems is urgent. To fill this gap, adapted limits must be defined.

Most studies on metal contamination in tropical soils have been based on total chemical analysis, chemical sequential extractions, or geochemical speciation (Roulet and Lucotte 1995; Windmöller et al. 1995; Rodrigues-Filho and Maddock 1997; Amir et al. 2005; Cesar et al. 2011). Although these analytical approaches provide relevant information on the chemical characterization of the test area, these analyses do not take into consideration additive, antagonistic and synergistic effects resulting from interactions between contaminants (Selivanovskaya and Latypova 2003; Burton et al. 2006; Straalen et al. 2005; Fjällborg et al. 2006). Moreover, physical, chemical, and mineralogical interactions between soil and dredged sediments play a crucial role in the mobility, speciation, and bioavailability of metals in soil and, consequently, interfere in their ecotoxicity to soil fauna. Several studies have demonstrated that variations on pH, redox potential, electric conductivity, salinity, cation exchange capacity (CEC), clay minerals, fertility, texture, permeability, porosity, moisture content, and iron and aluminum oxi-hydroxides influence the behavior of metals in soil (Roulet and Lucotte 1995; Yin et al. 1996; Rodrigues-Filho and Maddock 1997; Peijnenburg and Jager 2003; Wasserman et al. 2003; Cesar et al. 2011). In this context, laboratory bioassays using key species representative of soil fauna are very useful tools to evaluate the ecological risk associated to the disposal of contaminated dredged sediments in soil since it takes into consideration all factors involved in the ecotoxicity of the test substance towards soil fauna, constituting a complement of physical and geochemical analysis.

Earthworms have been widely used in laboratory ecotoxicological tests as test organisms because they are relatively easy to be kept in the laboratory and are sensitive towards contaminants. In addition, earthworms are abundant in tropical and temperate soils, representing about 90 % of soil fauna biomass (Hinton and Veiga 2002; Nahmani et al. 2007). Recently, earthworm acute tests and avoidance tests with *Eisenia andrei* or *Eisenia fetida* made part of a ring test to characterize three representative waste types (Moser and Römbke 2009). The ring test comprised a basic test battery of five ecotoxicological tests (three aquatic and two terrestrial tests including earthworm acute tests) and ten additional tests (five aquatic and five terrestrial tests including earthworm avoidance tests). Experiments were performed in several laboratories from different European countries showing that the earthworm acute test is a robust method (results with low variability and coefficients of variation between 20 and 31 %), and the earthworm avoidance tests are short-period tests with high sensitivity for waste characterization (Moser and Römbke 2009). Other studies have been conducted to investigate the earthworm body burden as a function of soil metal contamination (Nahmani et al. 2007). Although metal bioaccumulation does not forcibly imply adverse effects (Vijver et al. 2004), concentrations inside the organisms are directly related with the bioavailable fraction in soil and, consequently, to the potential risk of adverse effects.

The present study aims to (1) to make a preliminary assessment of the effects associated with the disposal of dredged sediments (predominantly contaminated with metals) in tropical soils to edaphic fauna using survival, metal bioaccumulation, and avoidance behavior of earthworms as endpoints and (2) to evaluate the adequacy of the concentration limits of metals established by Brazilian law (CONAMA) (2004, 2009) for dredged sediment disposal and soil quality. To attain these purposes, laboratory lethal and sublethal tests were performed exposing the earthworms *E. andrei* to a dredged sediment mixed with two natural soils (ferralsol and chernosol), typical from tropical and sub-tropical regions, with different properties, including clay mineralogy, cation exchange capacity, and nutrient contents. Working hypothesis were as follows: (1) the ecotoxicological parameters measured are adequate to a preliminary evaluation of the effects of dredged sediments to soil fauna; (2) the threshold limits established by Brazilian legislation for metals are not adequate to prevent toxicity to terrestrial systems; and (3) the toxicity of dredged sediment to earthworms is dependent on soil properties (e.g., mineral composition and nutrients content).

Materials and methods

Dredged sediment, natural soils, and test mixtures

Dredged sediment was obtained from three collecting stations located at the Cunha estuary (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), part of the Guanabara Bay basin (Rio de Janeiro municipality, Rio de Janeiro, Brazil). The Cunha estuary has been impacted with silting processes and discharges of residues from industrial and domestic wastes over the last decades. Guanabara Bay basin is characterized by high concentrations of metals, petroleum hydrocarbons, and domestic wastes in sediments, water, and biota (Machado et al. 2002; Bidone and Lacerda 2004; Silva et al. 2007; Silveira et al. 2010; Rodrigues et al. 2011). The same amount of sediment was collected from three dredging points along the estuary and fractioned using nylon sieves (1.7 mm), to remove stones, roots, and other larger particles. Sediment samples from each point were then mixed and homogenized to generate a composite sample. The organic carbon content of the dredged sediment was measured by elementary analysis, using LECO SNS-2000 equipment. The organic matter concentration was estimated multiplying the organic carbon content with a factor of 1.724 (EMBRAPA 1997). The texture of the sediment was still determined using the pipette method (EMBRAPA 1997).

To investigate the influence of soil properties in the ecotoxicity of the dredged sediment, two natural soils representative of the Guanabara Bay basin were used. A ferralsol and a chernosol were sampled at the Rio de Janeiro State, (chernosol: 220 51′22.5″S, 430 30′0.7″W; ferralsol: 220 41′ 34.2″S, 430 17′ 14.5″W). Cesar et al. (2012) and Alamino et al. (2007) characterized the physical and chemical and mineralogical properties of these soils (Table 1). While the mineralogy of clay fraction of ferralsol is mainly composed by kaolinite, gibbsite, and goethite, the chernosol has a mineralogy essentially composed by 2:1 clay minerals (illite, interstratified illite–smectite and vermiculite) and kaolinite. The different properties of the test soils are mainly due to intense chemical weathering processes that ferralsols have been submitted. While the ferralsols are one of the most abundant tropical soil classes (especially in Brazil), have low CEC and high concentrations of Fe and Al oxi-hydroxides, the chernosols are widely distributed in temperate and sub-tropical areas (especially in southern Brazil) and are often characterized by high levels of fertility and CEC. Both soils cover a large area used for agriculture.

Soils were collected at the B horizon and also sieved (1.7 mm). A concentration gradient of the test sediment with each natural soil was prepared by mixing soil with

Table 1 Physical and chemical properties of ferralsol and chernosol used in bioassays

Soil parameters		Ferralsol	Chernosol
Texture (%, n=3)	Clay	58	24
	Silt	6	35
	Sand	36	41
pH (n=3)	H_2O	4.2	6.2
	KCl	3.6	4.1
Sorption complex ($cmol_c/dm^3$, n=3)	$Mg^{+2} + Ca^{+2}$	0.2	40.5
	K^+	0.02	0.03
	Na^+	0.03	1.44
	CEC	2.3	48.3
Total concentrations (%, n=3)	Al	26.4	18.9
	Fe	11.3	7.9
Organic matter (%, n=3)		0.22	0.34

Data are taken from Alamino et al. (2007) and Cesar et al. (2012)
CEC cation exchange capacity

sediment in different proportions. The range of concentrations used in the experiments was based on data obtained in a previous study conducted by Cesar et al. (2012). The authors performed acute tests with *E. andrei* using concentration gradients of a domestic sewage sludge (with a level of metal contamination comparable to that of the sediment used in the present study) in the same natural soils: mixtures in ferralsol—0, 6.66, 13.32, 19.98, and 33.3 %; mixtures in chernosol—0, 6.58, 13.16, 19.74, and 32.9 % (Table 2).

Acute toxicity test with *Eisenia andrei*

The earthworm acute test was performed according to the ASTM (2004) protocol with some differences. Three replicates per test concentration were prepared, each one consisting of a cylindrical plastic box (3.8-cm diameter and 6.4-cm height) with 200 g (dry weight equivalent) of uncontaminated soils or mixtures of soil and dredged sediment. Ten adult organisms (previously acclimated in the natural soils for more than 1 day, washed with distilled water, and weighed) were inoculated in each replicate. Earthworms with an average weight of 0.548 ±0.08 g (average ± standard deviation, n=300) were used in the test. Moisture content of test mixtures was previously adjusted to 50 % of its water-holding capacity. The test was performed under constant illumination and controlled temperature (20±2 °C). Additional replicates with pure tropical artificial soil were prepared to attest the good condition of the test organisms. The artificial soil was prepared according to Garcia (2004) consisting of 70 % of quartz sand, 20 % of kaolin, and 10 % of coconut shells dust. After 14 days of exposure, mortality and biomass change of the surviving earthworms were determined. After being counted, washed, and weighted, surviving organisms were maintained in moistened absorbent paper for 24 h to purge the gut before being stored for metal content determination. The validity criterion of acute tests assumed earthworms' mortality to be lower than 10 % in replicates with artificial soil and pure ferralsol and chernosol.

Avoidance tests with *Eisenia andrei*

Earthworm avoidance tests were performed following the ISO 17512–1 (2008) with some modifications. Plastic boxes (20-cm length, 12-cm width, and 5-cm height) were vertically divided into two equal sections using a plastic card. One section was filled with uncontaminated soil (0 %) and the other one with the mixtures of soil and dredged sediment. The soil moisture was previously adjusted to 50 % of its water-holding capacity in both sections. Three replicates were prepared per combination. The plastic card was then removed and ten adult earthworms were introduced in the midline of the replicates in the soil surface. The test ran at a temperature of 20±2 °C and a photoperiod of 16:8-h light/dark. After 48 h, the number of surviving organisms was determined in the soil of each section. The avoidance tests were considered valid when the number of dead or missing worms was no higher than 10 % per combination.

Metal analysis

Total concentrations of copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), and chromium (Cr) were determined in the test mixtures (including pure soils) and dredged sediment through the solubilization of 1 g of homogenized soil sample in 40 ml of an acid mixture of $HF/HCl/HClO_4$ (2:1:1). The concentration of metals in soil extracts was determined by inductively coupled plasma–atomic emission spectrometry (HORIBA Jobin Yvon, Ultima 2). Hg concentrations were extracted from 0.5 g of sample using LUMEX equipment (RA-915+, Zeeman mercury spectrometer). Detection limits were 0.2, 0.2, 0.005, 1.4, 0.2,

Table 2 Estimation of organic matter contents in mixtures of dredged sediment with ferralsol or chernosol, and their respective values of pH (determined using KCl)

	Doses in ferralsol (%)					Doses in chernosol (%)				
	0.00	6.66	13.31	19.98	33.30	0.00	6.58	13.16	19.74	32.90
pH	3.6	6.20	6.30	7.10	7.30	4.1	6.50	6.55	6.90	7.35
MO (%)	0.22	1.57	2.80	4.02	6.48	0.34	1.47	2.72	3.97	6.47

MO organic matter

and 0.01 mg/kg, for Cu, Zn, Hg, Pb, Ni, and Cr, respectively.

Surviving earthworms from the acute tests were lyophilized before being submitted to metal extraction using the same methodology used for soil samples. Lyophilized organisms from each treatment were previously mixed and grounded before being analyzed in triplicate. The earthworm metal concentrations measured were used to calculate the bioconcentration factor (BCF) of each metal in the test treatments. Previous studies have shown that for several metals, bioaccumulation in earthworms may reach a steady state within 14 days of exposure (e.g., Pearson et al. 2000; Conder and Lanno 2000; Laskowski et al. 2010). BCFs were determined by the ratio between metal concentration in earthworm tissue and soil metal concentration. When earthworm metal concentration was lower than the detection limit, the BCF was determined considering the detection limit value for that metal. Certified reference materials (NIST 2709 San Joaquin Soil and IAEA 407—fish homogenate, for soil and earthworm samples, respectively) were used to check the accuracy of metal analysis for both soil and earthworm samples. Metal recovery levels were always ≥90 %.

Heavy metal concentrations determined in the dredged sediment were compared to the threshold limits established by the Brazilian legislation for the disposal of this type of materials in soil (CONAMA 2004). The total metal concentrations of the test mixtures were compared with the metal limits established in Brazilian protocols for soil quality (Companhia de Tecnologia de Saneamento Ambiental (CETESB) 2005; CONAMA 2009). Metal concentrations measured in the dredged sediment were used to calculate the geoaccumulation indices (IGEOs) for each metal, using background values determined in the standard shale (Müller 1979) through the following equation:

$$IGEO = Log_2 Me/NBN_{Me} \tag{1}$$

where, Me is the metal concentration in the sediment, and NBN_{Me} is the metal geochemical background in the standard shale (Hg=40 mg/kg; Zn=95 mg/kg; Cu=39 mg/kg; Cr=90 mg/kg; Pb=23 mg/kg; Ni=68 mg/kg; Turekian and Wedepohl 1961). This index consists of a logarithmic scale varying between 0 and 6 that is used to classify the samples into different levels of pollution (Müller 1979).

Statistical analysis

Earthworm weight change, and the earthworm mortality found in the test mixtures of soil and sediment were compared to that in the respective controls (uncontaminated soils—0 %) by one-way ANOVA analysis followed by Dunnett's post hoc test, to detect significant differences (using the software STATISTICA, version 6). The number of surviving earthworms (considering escape earthworms dead) in the acute toxicity tests was used to calculate the earthworm median lethal concentration (LC50) through Probit analysis (using the software PriProbit 1.63; Sakuma 1998).

In the avoidance tests, when the number of earthworms found in the test section (section with test mixture) was lower than 20 %, it was assumed that test mixture had limited habitat function (ISO 2008). Significant avoidance behavior was assumed when the number of organisms found in the test section was significantly lower ($p \leq 0.05$) than that found in the control section (section with uncontaminated soil). The significance of these differences was evaluated by the one-tailed Fisher's exact test (Natal-da-Luz et al. 2009). The concentration of dredged sediment that causes 50 % of avoidance behavior by the test organisms (avoidance EC50) was estimated by Probit analysis (Sakuma 1998).

Results

Dredged sediment and test mixtures

The organic carbon measured in the dredged sediment was 11.08 % and the organic matter content estimated was 19.1 %. The sediment was mainly composed by clay particles (82.3 %), followed by 14.1 and 3.6 of sand and silt factions, respectively. The pH and organic matter contents of the test mixtures increased with increasing dredged concentrations in both test soils (Table 2).

According to the actual Brazilian legislation (CONAMA 2004), Cu, Zn, Pb, and Cr concentrations measured in the dredged sediment were between the third (low probability of adverse effects on biota) and the fourth level of contamination (high probability of adverse effects on biota; Table 3). Ni and Hg concentrations were lower than the third level and higher than the fourth level of contamination, respectively (Table 3).

Table 3 Total metal concentrations of the dredged sediment studied and the upper limit values of metals allowed for dredged sediments to be disposed in soil according to CONAMA (2004)

Metal	Dredged sediment (mg/kg)	Limit values (mg/kg)	
		Level III[a]	Level IV[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] Limit of low probability of adverse effects on biota

[b] Limit of high probability of adverse effects on biota (CONAMA 2004)

The estimated IGEOs indicated absence of pollution (0 class) for Cr and Ni concentrations and “low to moderately,” “strongly,” and “strongly to very strongly” pollution (1, 4, and 5 classes, respectively) for Cu, Pb, and Hg, respectively (IGEO classes according to Müller 1979).

Metal concentrations measured in the test mixtures were lower than the "prevention" (for ecological receptors) and "intervention" limits (for human health) defined by law (CETESB 2005; CONAMA 2004). The concentrations of Hg, Zn, Cu, and Pb were higher than the reference limits recommended by CETESB 2005 in some test mixtures (Table 4).

Earthworm acute toxicity test

In both ferralsol and chernosol mixtures, the pH values increased with the increasing dose of sediment (Table 2). The validity criterion of the earthworm acute test was fulfilled since no mortality was found in replicates with artificial soil and pure ferralsol and chernosol (0 % doses). The mortality observed in the mixtures of soil and sediment showed dose–response relationships for treatments of both natural soils. Mortality found in ferralsol mixtures was significantly higher than that in control for concentrations ≥13.31 % and the LC50 estimated was 9.9 % (7.9–11.9). In the chernosol mixtures, mortality was significantly higher than that in control for concentrations ≥19.74 %, providing a LC50 (and 95 % confidence limits) of 16.5 % (15.9–17.1) (Fig. 1). The biomass loss was higher in ferralsol mixtures compared to chernosol treatments. The organisms increased their biomass levels at low doses of sediment application, in mixtures of both soils (6.66 % in ferralsol, and 6.58 and 13.16 % in chernosol).

Earthworm bioconcentration factors

Metal concentrations in earthworms exposed to the 13.31 and 19.74 % mixtures of ferralsol and chernosol, respectively, were determined in duplicate using only 0.5 to 0.8 mg of lyophilized earthworm tissue (instead of 1 g per replicate as used in the other treatments where the BCF was estimated) due to the low number of surviving organisms found in these treatments (3 and 2, respectively) at the end of the acute test. In 19.98 and 33.30 % treatments of ferralsol and 32.90 % treatment of chernosol, no BCFs could be estimated since no surviving earthworms were found. Hg concentrations measured in the earthworms exposed to the test mixtures were the lowest among the other metals and its BCF values (ratio between internal metal concentration and metal concentration in soil) were <1 for mixtures of both test soils (except for 0 % treatments), decreasing with the increased dredged sediment dose. Earthworm Zn and Cu concentrations were the highest among measured metals with BCFs >1 for mixtures of both soils, decreasing with the increased dredged sediment dose. The earthworm Pb concentrations were lower than the Pb detection limits in all test mixtures. The concentrations of Cr and Ni and their respective BCF values were generally low (≤0.1), except for the 13.31 % ferralsol treatment that were 2.76 and 6.6 for Cr and Ni, respectively (Table 5).

Avoidance tests

No mortality was observed at the end of the test in any combination. While ferralsol test mixtures provoked significant earthworm avoidance response at concentrations ≥13.31 %, the chernosol mixtures showed significant avoidance behavior only at the 19.74 % concentration. In combination with the 6.66 % concentration of ferralsol mixtures and 13.16 % concentration of chernosol mixtures, a higher number of organisms was found in the test section (section with sediment mixture) compared to the number of organisms found in the section with uncontaminated soil (Fig. 2). A limited habitat function (<20 % of the total organisms in the test section) was observed only for the highest concentrations of both chernosol and ferralsol mixtures.

Table 4 Total metal concentrations in the test mixtures of dredged sediment and ferralsol or chernosol and the reference and upper limit values of metals established by Brazilian legislation for soil quality (CETESB 2005; CONAMA 2009)

Metal	Doses in ferralsol (%)					Doses in chernosol (%)					Limit values		
	0.00 (mg/kg)	6.66	13.31	19.98	33.30	0.00 (mg/kg)	6.58	13.16	19.74	32.90	Reference[a] (mg/kg)	Prevention[b]	Intervention[c]
Hg	0.097	0.162	0.227	0.292	0.358	0.032	0.101	0.169	0.238	0.307	0.05	0.5	12
Cu	6.80	12.5	18.2	23.8	29.5	15.8	20.8	25.8	30.8	35.9	35	60	200
Zn	48.6	67.3	86.0	105	123	40.0	59.0	78.0	97.1	116	60	300	450
Pb	13.0	20.4	27.8	35.2	42.6	5.20	13.0	20.8	28.7	36.5	17	72	180
Ni	2.90	4.06	5.22	6.38	7.54	1.60	2.83	4.06	5.29	6.52	13	30	70
Cr	7.20	13.1	18.8	24.6	30.5	3.10	9.11	15.1	21.1	27.2	40	75	150

[a] Values representing pedogeochemical background of the São Paulo State, Brazil (CETESB 2005)

[b] Represent the limit of occurrence of adverse effects on soil ecological receptors

[c] Represent the limit of occurrence of adverse effects on human health (CETESB 2005; CONAMA 2009)

Discussion

The toxicity observed in the earthworm acute tests agrees with the avoidance behavior observed in the avoidance tests. The mixtures with dredged sediment that were significantly avoided by the earthworms (concentrations ≥13.31 % in ferralsol mixtures and 19.74 % in chernosol mixtures) were the same mixtures where the earthworm mortality, in acute tests, was higher than 80 %. In avoidance tests, the higher number of organisms found in the 13.16 % chernosol mixture when combined with chernosol (0 %) most probably due to the low toxicity of this test mixture (as supported in the acute test) allied to its higher organic matter content (from the dredged sediment fraction) compared to the chernosol. Though the organic matter content of the test mixtures were higher as the sediment dose got higher (due to the organic component of the test sediment), supposedly, the test organisms avoided a test mixture only when the repellence effect of its contamination prevailed against the organic matter attraction. In fact, the preference of earthworms by soils amended with weakly contaminated organic matrices has been reported in previous studies (Natal-da-Luz et al. 2009). Both in acute and avoidance tests, the low variability of results obtained in replicates from the same treatments (acute tests) or combinations (avoidance tests) lead to suppose that the use of four replicates per treatment (as advised in ASTM 2004 for acute tests) or five replicates per combination (as advised in ISO 2008 for avoidance tests) instead of three would not provide results significantly different from those obtained in the present

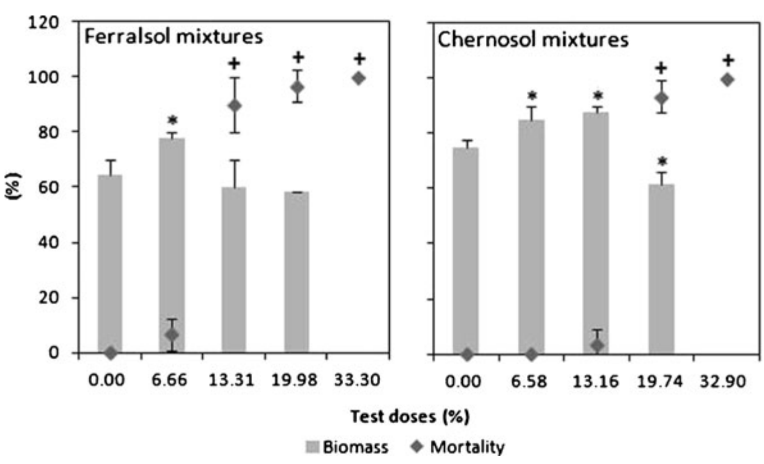

Fig. 1 Acute toxicity tests with *Eisenia andrei*. Mortality (*diamonds*) and biomass change (*bars*) of organisms (average ± standard deviation, n=3) exposed to mixtures of a ferralsols or a chernosol with a dredged sediment. *Biomass change significantly different from control (p≤0.05). [+]Mortality significantly different from control (p≤0.05)

Table 5 Bioconcentration factors measured in three composite samples (average, $n=3$) obtained from the surviving earthworms (30 individuals in treatments without mortality) after 14 days of exposure in mixtures of dredged sediment with ferralsol or chernosol

	Doses in ferralsol (%)					Doses in chernosol (%)				
Metal	0.00	6.66[a]	13.31[b]	19.98	33.30	0.00	6.58	13.16[a]	19.74[c]	32.90
Hg	1.30	0.31	0.23	–	–	1.90	0.31	0.30	0.21	–
Cu	1.83	1.21	0.12	–	–	0.72	0.50	0.41	0.10	–
Zn	1.81	1.32	0.53	–	–	1.93	1.10	0.82	0.01	–
Pb	0.10	0.10	0.10	–	–	0.30	0.10	0.10	0.05	–
Ni	0.10	0.05	6.60	–	–	0.10	0.10	0.05	0.04	–
Cr	0.02	0.02	2.76	–	–	0.10	0.02	0.01	0.01	–

[a] Data obtained from 28 surviving earthworms

[b] Data obtained from 3 surviving earthworms

[c] Data obtained from 2 surviving earthworms

study. In general, the ecotoxicological parameters measured in the present study were sufficiently sensitive to detect toxicity in the test mixtures, which supports the first working hypotheses. It is important to note that the toxicity levels observed reflect not only the presence of metals but also the presence of contaminants other than metals (e.g., petroleum hydrocarbons—not measured in the present work).

Metal concentrations in the dredged sediment were generally higher than the metal limits for the third level of contamination (low probability of adverse effects on biota), and Hg concentration was even higher than the limits established for a high probability of adverse effects on biota (CONAMA 2004; Table 3). This level of contamination was confirmed by the toxicity found in the bioassays performed. Toxicity measured in the ecotoxicological tests also supports the IGEOs estimated for Cu, Pb, and Hg (1, 4, and 5 classes, respectively). However, toxicity data are not in agreement with the fact that metal concentrations in the test mixtures were lower than metal limits established by CETESB (2005) and CONAMA (2009) for soil quality (Table 4). Although the reference values established by CETESB (2005) are based on soil geochemical backgrounds for the São Paulo State, such values do not necessarily represent risks to biota. This suggests that these limits may not be adequate for soils of Brazil and highlights the need for an ecotoxicological evaluation with local soils (to take into consideration local soil properties). Such assumptions support the second working hypothesis.

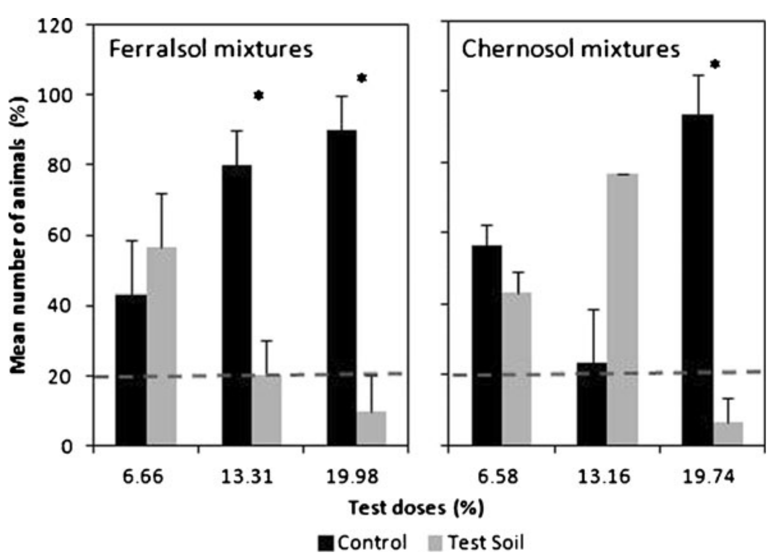

Fig. 2 Percentage of *Eisenia andrei* (average ± standard deviation, $n=3$) on a ferralsol or a chernosol (control section; *black bars*) against mixtures of these soils with a dredged sediment (test section; *gray bars*) in different doses. Note: Percentages of the test section below the *dashed line* means limited habitat function of the test mixture (ISO, 2008). *Significant avoidance behavior ($p \leq 0.05$)

Data on mortality, biomass change, and avoidance behavior obtained in the present study seem to indicate lower toxicity of dredged sediment when mixed with chernosol compared to that when mixed with ferralsol, which confirm the third-working hypothesis. The different mineral composition of the test soils seems to have influence on the toxicity of the dredged sediment. The abundance of expansive clay minerals (illite, interstratified illite–smectite and vermiculite) with high CEC in the chernosol might have reduced metal concentrations in soil solution (due to metal adsorption by such clay minerals) and, consequently, the ecotoxicity of these test mixtures. Cesar et al. (2012) and Matzke et al. (2008) also reported the lowest mortality and biomass losses when testing the ecotoxicity of metals and organic substances to *E. andrei*, *Triticum aestivum* and *Lepidium sativum* in the natural soils with the highest contents of expansive clay minerals, including vermiculite. The decrease of metal concentrations in pore water (soil solution) reduces metal uptake through earthworms derma, which is one of the most important pathway of metal exposure in earthworms (Vijver et al. 2003), decreasing the toxicity of the test mixture.

The increase of pH values in the test mixtures with the increasing dose of dredged sediment (Table 2) is most probably due to the presence of carbonated minerals in the sediment that are typical from estuarine sediments. The variations of pH (beyond other factors as described below) may influence the oxidation and geochemical availability of metals in the environment (Peijnenburg and Jager 2003). The higher contents of nutrients in the chernosol compared to ferralsol (Table 1) may explain the lower earthworm biomass losses observed in the chernosol treatments compared to the similar doses of ferralsol mixtures. The lower earthworm biomass loss found at the lowest test concentrations was probably due to the high organic matter content of the test sediment (19.1 %) that could be used as food source to the earthworms. This occurred only in the test mixtures where the metal contamination levels were not lethal to the earthworms (mortality <20 %). This agrees with Carbonell et al. (2009) and Natal-da-Luz et al. (2009) who reported the reduction of *E. andrei* biomass loss when performing bioassays with soils treated with increasing doses of wastes (sewage sludges) characterized by high organic matter concentrations.

As mentioned above, organic matter content of soil may reduce metal concentrations in soil solution (due to metal complexation) and, consequently, their potential availability and toxicity to earthworms (Lukkari et al. 2006; Vijver et al. 2004). In this respect, the geochemistry of the organic matter in the test mixtures may have an important influence in the complexation of metals (Ritschie and Perdue 2003), especially due to the influence of different humic and fulvic acid types on metal complexation. Although the test soils had low organic matter content (since they were collected at B horizon), in mixtures of both soil types, the organic matter concentrations were similar within the same sediment dose, a fact which therefore, could not explain the difference of toxicity observed between such soils. Perhaps, the presence of expansive clay minerals and high levels of fertility in chernosol could mitigate the toxicity of chernosol mixtures.

Hg is a highly toxic metal well-known by its ability to cause serious damages on biota, even at low concentrations (Burton et al. 2006; Castilhos et al. 2006; Rodrigues et al. 2011). Given the fact that the earthworm BCFs found for Hg in the present study were low and decreased with the increasing sediment concentration in both soils, apparently, the Hg was not highly available to the test organisms and did not occur in bioaccumulable forms (e.g., divalent or methylated form).

For Cr and Ni, while the BCFs estimated for the 13.31 % concentration in ferralsol were 2.76 and 6.6, respectively, the BCFs estimated for the 13.16 and 19.74 % concentrations in chernosol were <1. These BCFs evidenced that Cr and Ni uptake happened only in ferralsol mixtures which indicate a higher bioavailability of Cr and Ni in the ferralsol treatments compared to mixtures of chernosol. This agrees with the LC50 estimated for dredged sediment in ferralsol (10.7 %) that is lower than that estimated for chernosol mixtures (15.7 %). On the other hand, the lower the BCFs found for Zn and Cu as the higher the doses of sediment application (13.31 % ferralsol and 19.74 % chernosol treatments) reflects the constant Zn and Cu concentration in earthworms with increasing Zn and Cu concentration in soil derived from the increasing sediment dose. This is probably due to the ability of the earthworms to internally regulate these metals. In fact, according to the literature, earthworms are able to regulate their internal Zn and Cu concentrations since those elements are essential for its physiology (Hopkin 1989; Lukkari et al. 2005). Zinc plays a crucial role in the cell metabolism, and in the development, growth, and regeneration of some tissues, while copper participates in the transport of substances among cells and tissues

(Lukkari et al. 2005). However, the interpretation of data obtained from the measurement of earthworm metal concentrations should be made with care. The low number of surviving earthworms in some treatments (13.31 % in ferralsol and 19.74 % in chernosol) implicated the measurement of metals in earthworms using a biomass lower than 1 g per replicate (0.5—0.8 mg), reducing the representativeness of the samples which, consequently, could interfere in metal measurements and respective BCFs.

Conclusions

The bioassays performed in the present study were adequate for a preliminary evaluation of the effects of the dredged sediment to soil biota. Data obtained suggest that the threshold limits established by Brazilian law are not adequate to prevent noxious effects on local soil systems. Results also suggest that soil properties play a crucial role in the ecotoxicity of the dredged sediment. The presence of expansive clay minerals and the nutrient content of soils are properties that apparently had influence on the toxicity of the test mixtures. More robust ecotoxicological tests (e.g., reproduction tests) using test species representative of different routes of exposure (e.g., collembolans) are needed to confirm and further investigate the attainment of these findings.

Acknowledgments The authors would like to thank the colleagues from the Mineral Analysis Group (COAM) of the Centre for Mineral Technology (CETEM/MCT) for their help in the chemical determinations. Ricardo Cesar and Juan Colonese were supported by grants from the National Brazilian Council for Scientific and Technological Development (CNPq) and CAPES (Coordination for the Improvement of Higher Level -or Education- Personnel—Sandwich Doctorate). Without such financial help, this research could not be executed.

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