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Acute toxicity of zinc and arsenic to the warmwater aquatic oligochaete *Branchiura sowerbyi* as compared to its coldwater counterpart *Tubifex tubifex* (Annelida, Clitellata)

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Abstract
Purpose This study aimed at evaluating the acute effects of arsenic and zinc to the warmwater aquatic oligochaete *Branchiura sowerbyi*. Relative sensitivity with the coldwater species *Tubifex tubifex* was compared. Implications for the use of *B. sowerbyi* in the risk assessment of sediments in the tropics are discussed.
Materials and methods Water-only (96 h) and sediment (14 days) toxicity tests were conducted with both species evaluating a concentration series of arsenic and zinc. The tests were conducted considering the environmental conditions in the natural habitat of *T. tubifex* (predominantly temperate) and *B. sowerbyi* (predominantly tropical). Both lethal and sublethal endpoints (autotomy of the posterior body parts, abnormal behavior and appearance) were determined in the tests. The lethal (LC_{10} and LC_{50}) and effect (EC_{10} and EC_{50}) concentrations were also determined to assess metal sensitivity for both species.
Results and discussion Both test species were more sensitive to Zn than As in water-only tests, which is in agreement with previous studies evaluating the toxicity of these metals to aquatic oligochaetes. Sublethal effects were generally noted at concentrations lower than those leading to mortality. The warmwater oligochaete *B. sowerbyi* was more sensitive to both metals tested than the coldwater species *T. tubifex*.
Conclusions Study findings support the need for using indigenous tropical species in risk assessments in the tropics. In addition, sublethal effect parameters should be included in toxicity testing with aquatic oligochaetes.

Keywords Autotomy · Bioassay · Ecotoxicology · Survival · Tropics · Tubificines

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1 Introduction

The tropical zone contains the highest species diversity on the planet. On the other hand, many countries in the tropics are developing nations, heavily populated, and rapidly becoming industrialized but lack the money, infrastructure, and other resources for advanced pollution controls (Kwok et al. 2007). Subsequently, aquatic risk assessments in tropical countries often rely on studies from temperate countries, even though the fate and effects of contaminants may be different between climatically distinct regions (Daam and Van den Brink 2010). The use of temperate toxicity data in tropical risk assessments has been disputed (e.g. Lahr et al. 2001; Do Hong et al. 2004; Lopes et al. 2007; Freitas and Rocha 2011).

Oligochaetes feed on bulk sediment and penetrate the sediment through the construction of burrows that extent into anoxic sediments. These traits make them especially vulnerable to sediment contamination (Warren et al. 1998). By bioturbation of the sediment and by serving as prey these animals can have a strong influence on the bioavailability of contaminants to other organisms, e.g., benthivorous fish (OECD 2007).

The oligochaete *Tubifex tubifex* (Müller 1774) (Clitellata, Tubificinae) is widely available and abundant in temperate world regions, easy to keep in the laboratory, and can be easily cultured under laboratory conditions. Subsequently, this species has often been used in ecotoxicology testing and test protocols have been developed (e.g., ASTM 2005; OECD 2008). *T. tubifex* is most commonly encountered in temperate regions and is associated with cold water in tropical regions (Bonacina et al. 1987).

Branchiura sowerbyi (Beddard 1892) (Clitellata, Rhyacodrilinae) has only more recently been indicated to be an adequate alternative aquatic oligochate test species for ecotoxicology testing (e.g. Marchese and Brinkhurst 1996; Ducrot et al. 2007; Saha and Kaviraj 2008; Ducrot et al. 2010; Del Piero et al. 2014; Lobo and Espíndola 2014). *B. sowerbyi* appears to prefer warm waters and in temperate latitudes is often found in artificially heated habitats, although it is not restricted to them (Bonacina et al. 1994).

The most common endpoint in acute toxicity testing with oligochaetes is mortality (e.g., ASTM 2005; OECD 2007). Another reaction of oligochaetes to metal stress is autotomy ("self-amputation") of the posterior body parts, which aids the worms in escaping from predators as well as in body detoxification (Fleming et al. 2007). Oligochaetes accumulate metals in their posterior body segments, which can be discarded when the concentration reaches critical values (Lucan-Bouché et al. 1999; Rathore and Khangarot 2003).

Arsenic may be present in the aquatic environment from natural and anthropogenic sources. In Brazil, arsenic contamination has been encountered in mining regions, with sediment concentrations of up to 3300 mg kg^{-1} d.w. (Deschamps et al. 2002). Zinc has also been detected in high concentrations in the aquatic environment. For example, concentrations up to 574 mg Zn kg^{-1} d.w. were reported in sediment from the Tietê river basin (São Paulo State, Southeast region of Brazil), a concentration that is 80 times greater than the global geological reference level of 6 mg kg^{-1} d.w. (Nascimento and Mozeto 2008). Despite the frequently detected contamination of sediments with arsenic and zinc, little is presently known about the (sublethal) effects of these substances to aquatic oligochaetes, especially in tropical regions.

The aim of the present study was to study the lethal and sublethal (autotomy of the posterior body parts, abnormal behavior and appearance) effects of arsenic and zinc to the warmwater oligochaete *B. sowerbyi*. Differences in sensitivity with the coldwater oligochaete *T. tubifex* were determined by also conducting acute tests with the latter species. Tests for both species were conducted in the presence and absence of sediment with the objective to determine the sensitivity differences of the test organisms to water and sediment exposures.

2 Materials and methods

The tests with *T. tubifex* and *B. sowerbyi* were conducted at the Animal Ecotoxicology and Biodiversity Laboratory of the University of the Basque Country (UPV/EHU, Spain) and Nucleus for Ecotoxicology and Applied Ecology (NEEA) of the University of São Paulo (Brazil), respectively. The tests were conducted considering the environmental conditions in the natural habitat of *T. tubifex* (predominantly temperate) and *B. sowerbyi* (predominantly tropical).

2.1 Culture and maintenance of test organisms

The test organisms were obtained from existing in-house cultures of the laboratories where the tests were conducted. The *T. tubifex* culture has been maintained in the Animal Ecotoxicology and Biodiversity Laboratory for over 20 years. Animals are kept at 22 ± 1 °C in complete darkness in vessels (length 15 cm; width 15 cm; height 10 cm) containing a 3-cm sediment layer (grain size <0.25 mm) obtained from an uncontaminated pond in the mountains of Álava (Iturbatz, Spain) supplied with groundwater. The 3-cm water column is made up of dechlorinated tap water (pH = 6.8 ± 0.2; electrical conductivity (EC) = 279 ± 3 μS cm^{-1}; total hardness = 127 mg $CaCO_3$ L^{-1}) and is moderately aerated. To ensure that tests are performed with similarly aged animals, fresh cultures are initiated at 6 to 7 weeks (i.e., the time for animals to reach maturity) before testing by transferring 100 to 150 small juvenile animals from the existing stock culture to a new vessel. A sediment with poorer organic content from Barrundia (Álava, Spain) was sampled for the performance of the toxicity tests with *T. tubifex.*

B. sowerbyi has been maintained at NEEA for approximately 4 years under 25 ± 1 °C in the dark with moderate aeration. Each 3.5-L vessel contains a sediment layer (grain size <0.50 mm) of approximately 5 cm derived from the waterbody (Perdizes stream) alongside a spring in Brotas (São Paulo State, Brazil). Reconstituted test water was used with the following physical-chemical characteristics: pH = 7.0; EC = 100 μS cm^{-1}; total hardness = 40 mg L^{-1} (as $CaCO_3$). Test organisms were acclimated by transferring 80 small juveniles to a newly started culture 6 to 9 weeks prior to the start of the tests, which is the maturity time of this species.

In the field, 0.5 kg (wet weight) sediment was collected with a stainless steel spade and sieved through 0.5 mm to remove associated macrofauna, which may influence the subsequent analytical results (Reynoldson 1994). The sediment was assessed for particle size distribution (by sieving), total organic matter content (calculated through the percent loss of organic matter by ignition at 450 °C for 6 h), water content (105 °C overnight), and metal concentrations (using ICP-AES in

Spain and AAS in Brazil). Results from sediment analyses as used in the test cultures and toxicity tests are presented in Table 1.

2.2 Water-only exposure (96 h)

The 96-h water-only tests with both *B. sowerbyi* and *T. tubifex* followed the method as described by Maestre et al. (2009). Each test included the exposure of worms to each metals separately and consisted of a control and five metal concentrations ranging for arsenic (spiked as $HAsNa_2O_4 \cdot 7H_2O$; purity 98 %) from 0 to 81 mg As L^{-1} in *B. sowerbyi* and from 0.03 to 118 mg As L^{-1} in *T. tubifex* exposure; and for zinc (spiked as $ZnSO_4 \cdot 7H_2O$; purity 99 %) from 0 to 8.9 mg Zn L^{-1} in *B. sowerbyi* and 0.03 to 41 mg Zn L^{-1} in *T. tubifex* exposure (Table 2). The tested concentrations were chosen based on a literature review and on preliminary experiments conducted in the laboratory (unpublished data). Tests were conducted in 250-mL beakers (pre-washed with 10 % nitric acid) with 100 mL test solution, five replicates per concentration, and five worms per replicate. The test lasted for 96 h, in the dark and with neither food nor aeration. Nominal and measured concentrations tested are provided in Table 2. The test organisms from *B. sowerbyi* cultures were 7–8 weeks old (sexually mature with visible eggs in the ovisac), and 6–8 weeks old in the case of *T. tubifex* (sexually mature with a well-developed clitellum). At the beginning of the test, a 10-mL water sample was taken from each metal concentration of the treatments, acidified with 70 % nitric acid (150 μL for each 100 mL sample) and stored at 4 °C until chemical analyses (see Section 2.4).

The number of dead animals and any visible sublethal effects (i.e., abnormal behavior and appearance) were noted daily. A worm was considered to be dead when there was no response in the 10 s after receiving a slight disturbance with a bar (following Maestre et al. 2009 and references therein). Dead animals were removed to avoid any adverse influence on physical-chemical characteristics resulting from decomposition.

Autotomy, i.e., self-amputation of segments through a local macroscopic constriction of the circular muscles of the caudal region of a worm has frequently been described as a response to toxic stress induced by metals (Maestre et al. 2009 and references therein). Therefore, the number of organisms that suffered autotomy was also quantified at the end of the 96-h experimental period.

2.3 Sediment toxicity test (14 days)

The sediment toxicity tests were conducted with arsenic and zinc for *T. tubifex* and with Zn for *B. sowerbyi*. For logistic reasons, the test with *B. sowerbyi* evaluating arsenic could unfortunately not be conducted.

Test sediments were spiked with metal solutions to achieve five concentrations, following the recommendations of OECD (2007). The sediment volume required to conduct the tests and the quantity of the test compound to obtain the desired nominal concentrations were mixed in a 5-L vessel with dilution water in a ratio of 1:4 (sediment weight to water volume). Tetramin® (80 g/100 mL sediment) was added, after which the vessels were shaken for 4 h at moderate speed (160–170 rpm) on an orbital shaker (KS501D, IKA Labortechnik

Table 1 Metal concentrations (mean ± SE; in mg kg^{-1} dry weight) and physical characteristics of the natural sediment used for the cultures and tests in Brazil (Perdizes), and culture (Iturbaz) and tests (Barrundia) in Spain with *B. sowerbyi* and *T. tubifex*, respectively

	Perdizes	Iturbaz	Barrundia
Metal concentrations			
As	5.0 ± 0.7	4.3 ± 1.9	2.7 ± 1.4
Cd	0.004 ± 0.009	0.5 ± 0.5	0.2 ± 0.1
Co	0	3.7 ± 4.7	8.9 ± 0.3
Cr	0.030 ± 0.035	13 ± 5	22 ± 25
Cu	0.006 ± 0.009	2.4 ± 1.0	3.3 ± 1.6
Hg	ND	0.09 ± 0.01	0.2 ± 0.2
Ni	0.01 ± 0.02	10 ± 5	16 ± 0.2
Pb	0.1 ± 0.1	7.9 ± 1.6	8.6 ± 6.4
Zn	56 ± 25	9.6 ± 2.9	27 ± 28
Physical characteristics of the sediment			
% OM	0.8 ± 0.4	3.1 ± 0.5	1.7 ± 0.1
% SDW	78 ± 0.3	ND	70 ± 2
% grain size fractions	46 % 0.5–0.25 mm	3 % gravel	49 % 0.5–0,25 mm
	48 % 0.25–0.105 mm	68 % sand	39 % 0.25–0.125 mm
	5 % 0.105–0.53 mm	29 % silt-clay	8 % 0.125–0.063 mm
	1 % <0.053 mm		4 % <0.063 mm

OM organic matter content, *SDW* soil dry weight fraction, *ND* not determined

Table 2 Nominal and measured test concentrations of arsenic and zinc in the water-only (96 h; in mg L^{-1}), and sediment (in mg kg^{-1} d.w.) and pore water (in mg L^{-1}) of the sediment (14 days) toxicity tests performed with *Tubifex tubifex* and *Branchiura sowerbyi*

Water-only 96 h						
	As			Zn		
	Nominal	Measured	Recovery rate[a]	Nominal	Measured	Recovery rate[a]
B. sowerbyi	Control	0	–	Control	0	–
	8	6.9	86	0.62	0.50	80
	16	13	82	1.3	0.85	68
	32	27	85	2.5	2.0	79
	64	52	81	5	4.3	87
	128	81	63	10	8.9	89
T. tubifex	Control	0.03	–	Control	0.03	–
	4	4.0	100	1.25	1.34	107
	8	7.98	99	2.5	2.75	110
	16	15	96	5	5.5	110
	32	32	100	10	13	134
	64	66	103	20	21	106
	128	118	92	40	41	103
Sediment-water 14 days						
	As[b]			Zn		
	Nominal	Sediment	Pore water	Nominal	Sediment	Pore water
B. sowerbyi	–	–	–	Control	80	0.03
	–	–	–	50	168	0.63
	–	–	–	87.5	230	1.9
	–	–	–	153	280	8.6
	–	–	–	268	358	15
	–	–	–	469	373	28
T. tubifex	Control	12	0.05	Control	181	0.009
	3.2	29	0.03	10	152	0.008
	10	44	0.12	31.6	259	0.009
	31.6	95	0.45	100	383	0.016
	100	208	3.84	316	565	0.43
	316	421	18.79	1000	679	9.57

[a] Recovery rate: ratio between measured and nominal concentration

[b] The sediment 14-day toxicity test for As with *B. sowerbyi* was not performed due to logistic constraints

GmbH, Staufen, Germany). Subsequently, vessels were maintained for 1 week at test temperature (22 ± 1 °C for *T. tubifex* and 25 ± 1 °C for *B. sowerbyi*) to allow sediment and porewater partitioning equilibrium. After this period, the overlying water was carefully removed through siphoning, mechanically homogenized, and distributed over the test containers. Each test container received 100 mL sediment and 100 mL fresh dilution water and was kept at their respective test temperature for 48 h under moderate aeration. Then, test animals were added at which time the experimental observation period commenced.

Toxicity tests were conducted, with modifications, according to ASTM (2005). To this end, three (*T. tubifex*) or five (*B. sowerbyi*) replicates were prepared for each five test concentration as well as for the untreated controls, where one replicate for chemical analysis at the start of the toxicity tests, and the others replicates were used for effect assessments. Test concentrations ranged from 12 to 41 mg As kg^{-1} d.w. and 181 to 679 mg Zn kg^{-1} d.w. for *T. tubifex* exposure, and from 181 to 679 mg Zn kg^{-1} d.w. for *B. sowerbyi* exposure (Table 2). Each replicate contained four test organisms in their start of the first reproductive cycle (6–7 weeks for *T. tubifex* and 7–8 weeks for *B. sowerbyi*), all obtained from the same cohort. At the end of the experimental period (i.e., 14 days after introducing the test organisms), the sediments were washed over a 0.5 mesh sieve and the collected organisms assessed for mortality and autotomy.

2.4 Physical-chemical water quality parameters

In all the water-only and sediment toxicity tests, the following parameters were measured daily: DO (Yellow Springs YSI-55, OH, USA), pH (Micronal B374, São Paulo, Brazil), EC (Thermo Orion M145, Beverly, USA), and ammonia (spectrophotometry method, APHA 1995) in the *B. sowerbyi* test. DO, pH, and EC were measured with a Thermo Orion 5-Star Plus (Beverly, USA) meter in *T. tubifex* toxicity tests.

2.5 Metal analyses

Metal concentrations in the water-only and sediment tests were measured as described below. In the latter, the whole-sediment and the porewater concentrations were analyzed from the replicate used for chemical analyses. The porewater was removed from the sediment through centrifugation of 50 mL sediment (3000 rpm for 30 min at 4 °C), and subsequently filtered through a 0.45-μm filter before chemical analyses (Méndez-Fernández et al. 2013). The whole-sediment sample was dried at ambient temperature, sieved through a 0.063-mm mesh before digestion, and analyzed as outlined below.

Arsenic and zinc concentrations in water from *T. tubifex* tests were performed by SGIker at the University of Basque Country using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) or ICP-mass spectroscopy (ICP-MS), depending on metal concentrations. Detection limits (dl) were 0.05 and 0.10 mg L^{-1} for ICP-AES and 5 and 0.3 μg L^{-1} for ICP-MS, for arsenic and zinc, respectively, and analytical recoveries were 113 % for arsenic (NIST 1643e) and 96–104 % for zinc (TMDA 52.3—NIST 1643e). Sediment metal concentration was measured by Analytical Service of Sosprocan Unit (University of Cantabria, Santander, Spain), following the EPA 3052 protocol for microwave-assisted acid digestion of siliceous- and organically based matrices and the UNE-EN 13656:2003-03-28 method for microwave-assisted digestion with hydrofluoric (HF), nitric (HNO_3), and hydrochloric (HCl) acid mixture for subsequent determination of elements. After digestion, metal concentrations were determined using ICP-MS (dl = 5 μg L^{-1} for Zn and 0.3 μg L^{-1} for As). Buffalo River sediment reference material (RM8704, NIST USA) was also analyzed for quality control, and recoveries were within certified values (mean of 87 % for zinc).

Analytical quantification of the test compounds in water and sediment from the *B. sowerbyi* tests was performed in the Poços de Caldas laboratory (LAPOC) of the National Nuclear Energy Commission (CNEN) in Brazil. Water samples were analyzed by flame atomic absorption spectroscopy (F-AAS; dl = 20 μg L^{-1}) for zinc and by hydride generation atomic absorption spectrometry (HG-ASS; dl = 6 μg L^{-1}) for arsenic. Reference water (NIST 1643e) yielded an analytical recovery of 103 and 107 % for zinc and arsenic, respectively. Sediment was digested following EPA 3052 and analyzed using F-AAS (for zinc) and HG-AAS (for arsenic), as described above. The reference sediment of Buffalo River yielded a recovery rate of 90 % for zinc.

2.6 Statistical analyses

Statistical analyses were conducted using the measured test concentrations. Two-parameter log-logistic (LL.2) regression models were used to calculate the effective and lethal concentrations (EC_{10}, EC_{50}, LC_{10}, and LC_{50}). Significant differences with controls were assessed by Fisher's exact test. Significance level was $\alpha = 0.05$. Statistical analyses were performed using the free R software (R core team 2013) and extension package *drc* (Ritz and Streiberg 2005).

3 Results and discussion

3.1 Test performance

The physical-chemical conditions were comparable between the different experiments and varied little between treatments as indicated by the low standard deviation values (Table 3). Conditions were within the ranges as indicated in existing standard protocols for oligochaete testing, e.g., pH between 6 and 9 (OECD 2007, 2008) and dissolved oxygen concentration above 2.5 mg L^{-1} in the overlying water (ASTM 2005). Ammonia concentrations remained below the levels considered toxic to oligochaetes (5 mg L^{-1}: Schubauer-Berigan et al. 1995; see Table 3).

Table 3 Physical-chemical water quality parameters (mean ± SD) in the water-only (96 h) and sediment (14 days) tests test evaluating the toxicity of arsenic and zinc to *Tubifex tubifex* and *Branchiura sowerbyi*

	As				Zn			
	pH (−)	DO (mg L^{-1})	EC (μS cm^{-1})	Ammonia (mg L^{-1})	pH (−)	DO (mg L^{-1})	EC (μS cm^{-1})	Ammonia (mg L^{-1})
Water-only 96 h								
B. sowerbyi	7.4 ± 0.3	6.6 ± 0.8	141 ± 27	0.4 ± 0.1	7.1 ± 0.3	6.5 ± 1.0	118 ± 12	0.4 ± 0.1
T. tubifex	7.7 ± 0.2	7.7 ± 0.6	290 ± 35	0.5 ± 0.1	7.1 ± 0.1	7.7 ± 0.5	293 ± 33	0.4 ± 0.2
Sediment-water 14 days								
B. sowerbyi	[a]	[a]	[a]	[a]	7.4 ± 0.5	6.6 ± 0.3	204 ± 47	2.1 ± 0.2
T. tubifex	7.9 ± 0.3	7.6 ± 0.4	310 ± 41	1.5 ± 0.2	8.0 ± 0.5	7.7 ± 0.3	325 ± 49	1.9 ± 0.2

[a] The sediment 14-day toxicity test for As with *B. sowerbyi* was not performed due to logistic constraints

Appropriate test conditions were also confirmed by the control treatment performances. In the water-only test with *T. tubifex*, one test organism had died (which equals 5 % of the total test population) and another one showed autotomy of the posterior body region (5 %). No other lethal or sublethal effects on control organisms were recorded.

3.2 Effects of arsenic and zinc on oligochaete survival

The effects of zinc and arsenic on survival and autotomy of posterior body section observed in the water-only tests with *T. tubifex* and *B. sowerbyi* are illustrated in Fig. 1. The effective concentrations calculated for all water-only as well as the sediment tests are summarized in Table 4.

To the authors' knowledge, this study is the first to evaluate the toxicity of arsenic to *B. sowerbyi*, whereas two water-only studies are available for *T. tubifex*. These studies reported a 96 h-LC_{50} of 8.9 mg As L^{-1} (Khangarot 1991) and 127 mg As L^{-1} (Fargašová 1994). The results of the present study are in line with the latter study (28 % mortality at the highest test concentration: 118 mg As L^{-1}). The greater toxicity as noted in the study by Khangarot (1991) may be related to one or more of the following factors in the experimental design of that study: (i) test water was renewed every 24 h; (ii) possible greater sensitivity of the test organism culture; (iii) the metal salt tested in the present study ($HAsNa_2O_4.7H_2O$) was different from the one used by Fargašová (1994) (Na_3AsO_3); and (iv) test temperature was 30 °C, instead of 25 and 22 °C in Fargašová (1994) and the present study, respectively. The last factor has indeed been demonstrated to influence the toxicity of metals, and several studies have reported greater toxicity with increasing temperatures (Wang 1987; Rathore and Khangarot 2002).

Table 4 Values of the lethal concentrations (LC_{10} and LC_{50}) and effect concentrations (EC_{10} and EC_{50} for autotomy) for *Branchiura sowerbyi* and *Tubifex tubifex* exposed to As and Zn in the 96-h water-only (in mg L^{-1}) and 14-day sediment-water (in mg kg^{-1} d.w.) tests (value ± SE)

Test	Metal	Species	Endpoint	LC_{10}/EC_{10}	LC_{50}/EC_{50}
96 h	As	*B. sowerbyi*	Mortality	15.35 ± 2.13	22 ± 1.8
			Autotomy	14.84 ± 1.93	21 ± 1.8
		T. tubifex	Mortality	74.60 ± 11.79	>118.18
	Zn	*B. sowerbyi*	Mortality	0.85 ± 0.03	0.97 ± 0.07
			Autotomy	0.77 ± 0.34	0.88 ± 0.11
		T. tubifex	Mortality	4.56 ± 0.67	8.7 ± 0.84
			Autotomy	4.57 ± 0.48	6.9 ± 0.59
14 days	As	*T. tubifex*	Mortality	116.01 ± 34.70	251 ± 47
			Autotomy	80.98 ± 26.81	210 ± 44
	Zn	*B. sowerbyi*	Mortality	269.11 ± 46.14	280 ± 2.3
		T. tubifex	Mortality	675 ± 28.80	>679
			Autotomy	556 ± 38.59	635 ± 25

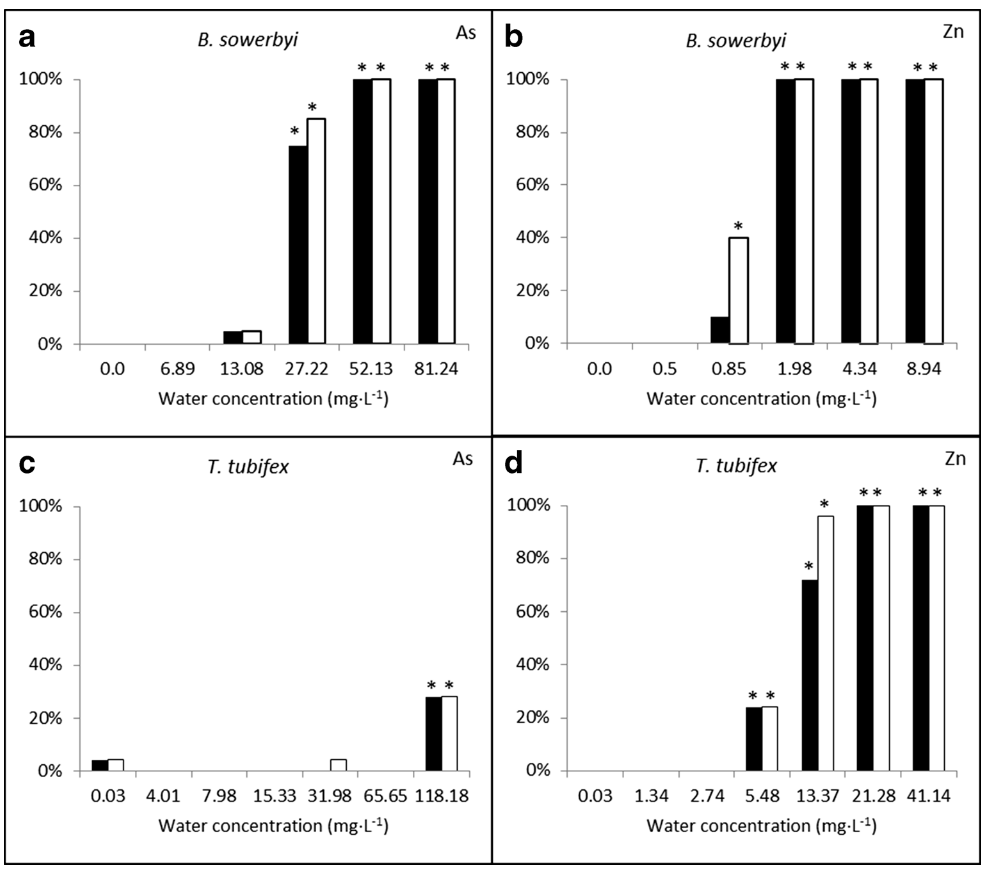

Fig. 1 **a**–**d** Percentage mortality (*black columns*) and autotomy (*white columns*) noted in the water-only tests with *B. sowerbyi* and *T. tubifex* exposed to a concentration series of As and Zn for 96 h. *Asterisks* indicate significant differences as compared to the control treatment (Fisher's exact test; $p = 0.05$)

Regarding the effects of zinc on survival, the 96 h-LC_{50} calculated in the water-only test with *T. tubifex* of the present study (8.7 mg L^{-1}) was similar to that reported in a study by Rathore and Khangarot (2002; 8.9 mg L^{-1}). *B. sowerbyi*, however, was more sensitive in the sediment test (EC_{10} 269.11 ± 46.14 mg kg^{-1} d.w.) as compared with a study conducted by Ducrot et al. (2010). The latter study only demonstrated a significant decrease in *B. sowerbyi* juvenile survival rates in worms exposed to zinc concentrations exceeding 1819 mg kg^{-1} d.w. for 14, 21, and 28 days. Differences in the adopted experimental design (e.g., temperature and pH of water, characteristics of natural sediment used, and sediment equilibration period) may be partly responsible for this difference in observed effects. For example, Lee et al. (2004) demonstrated that LC_{50} values for zinc obtained for the amphipod *Leptocheirus plumulosus* were twice as high in sediment-water tests with an equilibration period of 20 days as compared to tests allowing only a 5-day equilibration period after sediment spiking. In the present study, an equilibration period of 10 days was used, whereas Ducrot et al. (2010) only allowed the sediment to equilibrate for 3 days.

3.3 Sublethal effects on autotomy and behavior

Autotomy was noted in the water-only and sediment experiments of both species and metals (Fig. 2; Table 4). Other studies have also reported autotomy in oligochaetes as a response and elimination mechanism to metal stress (e.g. Lucan-Bouché et al. 1999; Rathore and Khangarot 2003; Méndez-Fernández et al. 2013). For example, EC_{50} values reported for autotomy by Méndez-Fernández et al. (2013) were five to ten times lower than the LC_{50} values for the three metals (Cd, Cu, and Cr) tested on *T. tubifex*. In the present study, however, little difference in toxicity levels for autotomy and mortality was found after short-term water-only exposure: Autotomy occurred at the same concentrations as mortality in most cases. Only for intermediate Zn concentrations also surviving organisms showed autotomy (Fig.1b, d). This is also reflected in the EC_{50} and EC_{10} values, where no differences were found between mortality and autotomy for both species (Table 4). Regarding autotomy in sediment tests after short-term exposures, autotomy was noted at lower concentrations than mortality for both metals in *T. tubifex* exposure, although the standard error (SE) of the effective concentration indicates that the 95 % confidence intervals overlapped (Table 4).

Abnormal appearance and behavior of the test organisms were observed at concentrations below those leading to mortality or autotomy (Fig. 2). For example, swelling of the body of *T. tubifex* was noted for 13 and 43 % of the worms exposed for 24 h to concentrations above 2.75 mg Zn L^{-1} and 7.98 mg As L^{-1}, respectively (Fig. 2a). Swelling was accompanied by an increase in the number of granules in the coelomic cavity (Fig. 2b). After 96-h exposure to 118 mg As L^{-1}, one *T. tubifex* individual showed a deformed posterior body (Fig. 2c). In the tests with *B. sowerbyi*, swelling of the body, posterior part lacking gills and with ring constrictions were observed (Fig. 2d, e). Similar deformations have previously been demonstrated in oligochaetes exposed to insecticides (Komala 1992), cadmium (Bailey and Liu 1980), and okadaic acid (Franchini and Marchetti 2006).

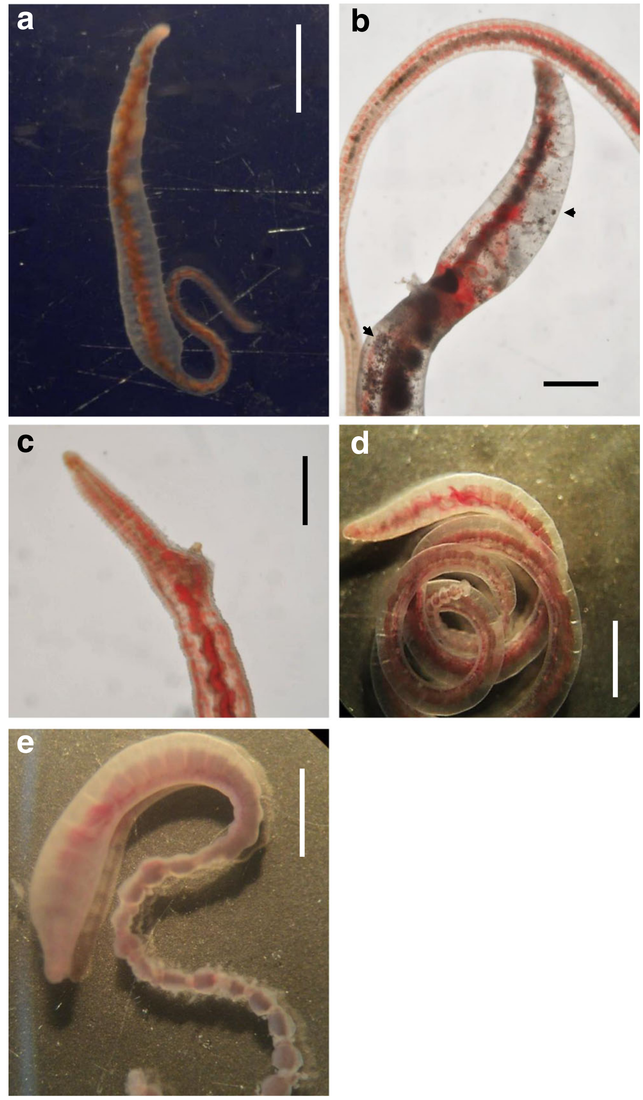

Fig. 2 Body appearance transformations observed in the tests with *T. tubifex* and *B. sowerbyi*: **a** *T. tubifex* exposed for 14 days to Zn (679 mg kg^{-1} d.w.): swollen anterior body parts. **b** *T. tubifex* exposed to Zn (1577 mg L^{-1}) for 96 h: with swollen body and increased number of granules in the coelomatic cavity (as indicated by the *arrows*). **c** *T. tubifex* after 96-h exposure to 118 mg As L^{-1}: deformed body posterior appearing the formation of a bifid tail. **d, e** *B. sowerbyi* exposed to Zn (0.85 mg L^{-1}; **d**) and As (52 mg L^{-1}; **e**) with parts of the posterior body lacking gills and with ring constrictions. *Scale bars*: **a**, **d**, **e** 2 mm; **b** 1 mm; **c** 0.5 mm

At the end of the 14-day exposure period in the sediment tests, five *T. tubifex* had a swollen body cavity after exposure to arsenic concentrations greater than 208 mg As kg^{-1} d.w. No other effects on general appearance were recorded. The presence of sediment has frequently been reported to potentially decrease toxicity through binding of the toxicant to the sediment (e.g. Chapman et al. 1982). On the other hand, endobenthic aquatic oligochaetes ingest sediment particles which may hence be a relevant uptake route and subsequently increase toxicity. In the higher Zn (>358 mg Zn kg^{-1} d.w. for *B. sowerbyi* and 679 mg Zn kg^{-1} d.w. for *T. tubifex*) and As (421 mg As kg^{-1} d.w. for *T. tubifex*) treatments, however, organisms were noted to remain on the sediment surface and hence avoiding penetrating the sediment, which may have reduced the toxicity due to a lesser sediment intake and contact. However, this behavioral effect as well as those on general appearance described above is likely to increase predation in natural environments and, thus, the relevance and importance of such behavior needs further attention in future studies.

4 Conclusions and implications of study findings for the use of *B. sowerbyi* in tropical aquatic risk assessments

In the water-only toxicity tests with both species, toxicity values as calculated for zinc were lower than those established for arsenic (Table 4). This is in agreement with previous studies into the sensitivity of oligochaetes to metals that report a relatively high toxicity of zinc and a relatively low toxicity of arsenic among the metal tested (e.g., Fargašová 1994; Rathore and Khangarot 2002). Effects on behavior and appearance of the test organisms were noted at concentrations lower than those leading to significant mortality levels. Subsequently, such parameters should be evaluated with care when conducting aquatic risk assessment studies.

The warmwater oligochaete *B. sowerbyi* was more sensitive to both metals tested than the coldwater species *T. tubifex*. This may be partly related to differences in experimental setup of the tests with these species (e.g., temperature, water chemistry, and physical-chemical characteristics of the sediment). In the light of these results, and in accordance with previous authors (e.g. Daam and Van den Brink 2010 and reference therein), we recommend the use of tropical species in the risk assessment of tropical regions. There is thus also a need for the development of adequate protocols for sediment toxicity assessment in these areas.

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Compliance with ethical standards

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

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