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CONTAMINATED SUSPENDED SEDIMENTS TOXIC TO AN ANTARCTIC FILTER FEEDER: AQUEOUS- AND PARTICULATE-PHASE EFFECTS

NICOLE A. HILL,*†‡ CATHERINE K. KING,‡ LISA A. PERRETT,† and EMMA L. JOHNSTON†
†Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences,
University of New South Wales, Kensington, New South Wales 2052, Australia
‡Environmental Protection and Change, Australian Antarctic Division, Channel Hwy, Kingston, Tasmania 7050, Australia

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Abstract—Disturbances such as dredging, storms, and bioturbation result in the resuspension of sediments. This may affect sessile organisms that live on hard substrates directly above the sediment. Localized sediment contamination exists around many Antarctic research stations, often resulting in elevated contamination loads in marine sediments. To our knowledge, the potential impact of resuspended contaminated sediments on sessile fauna has not been considered, so in the present study, we assessed the sensitivity of Antarctic spirorbid polychaetes to aqueous metals and to metal-contaminated sediments that had been experimentally resuspended. Worms were first exposed to aqueous metals, both singly and in combination, over 10 d. Spirorbid mortality was tolerant to copper (median lethal concentration [LC50], 570 μg/L), zinc (LC50, >4,910 μg/L), and lead (LC50, >2,905 μg/L); however, spirorbid behavior responded to copper concentrations as low as 20μg/L. When in combination, zinc significantly reduced mortality caused by copper. A novel technique was used to resuspend sediments spiked with four concentrations of three metals (up to 450 μg/g dry wt of copper, 525 μg/g dry wt of lead, and 2,035 μg/g dry wt of zinc). The response of spirorbids to unfiltered suspended sediment solutions and filtered solutions (aqueous metal exposure) was measured. Suspended sediments were toxic to filter-feeding spirorbids at concentrations approximating those found in contaminated Antarctica areas. Toxicity resulted both from aqueous metals and from metals associated with the suspended sediments, although suspended clean sediments had no impact. To our knowledge, the present study is the first to show that resuspension of contaminated sediments can be an important pathway for toxicity to Antarctic hard substrate organisms. Based on the present results, current sediment-quality guidelines used in the evaluation of Australian sediments may be applicable to Antarctic ecosystems.

Keywords—Spirorbid Resuspended Heavy metals Total suspended solids Aqueous

INTRODUCTION

Marine sediments have long been recognized as a sink for contaminants. Historical and current sources of pollution have produced sediment metal concentrations in some urbanized regions of the world that are several orders of magnitude greater than background levels [1]. Localized contamination also exists around many Antarctic stations, largely as a consequence of previous waste management practices [2]. Elevated sediment metal concentrations have direct consequences for sediment infauna and generally result in communities that are less diverse and dominated by opportunistic species [3–5]. Sediment contaminants also have the potential to be remobilized by disturbances such as dredging, trawling, storm turbulence, tidal movements, and smaller scale processes (e.g., bioturbation) [6]. In addition, wind-generated currents and iceberg scouring in near-shore polar environments can lead to significant disturbances of sediment [7].

The resuspension of contaminated sediments is a mechanism by which invertebrates that live on hard substrates may be negatively impacted. These animals may be smothered by sediments [8], be exposed to dissolved metals leaching from sediment resuspensions, or actively filter resuspended particulates from the water column and, thereby, expose themselves to contaminants via ingestion [6]. Contaminant concentrations generally are highest in the fine fraction (particle size, <63 μm) of aquatic sediments because of the large surface area to volume ratio and the availability of binding phases, such as organic carbon and sulfides [9]. This sediment fraction also is the most easily resuspended and stays in suspension the longest. In addition, disturbances to contaminated sediments cause changes to sediment chemistry, such as increased redox potential and decreased pH, both of which act to increase the bioavailability of metals [9]. As a result, the resuspension of contaminated sediments poses a hazard that reaches beyond the immediate benthic environment.

In Antarctica, concentrations of hydrocarbons and metals, including copper, lead, zinc, and cadmium, commonly are elevated in sediments adjacent to station facilities, and these contaminants negatively affect sediment infauna [10,11]. Many shallow Antarctic bays also consist of muddy sediments interspersed with boulders that are covered by diverse assemblages of filter-feeding invertebrates, including tube worms, sponges, and bryozoans [12]. Over the summer, the ice that covers such bays in Antarctica typically melts and breaks out [13], leaving bays exposed to severe winds and associated currents that have the potential to create resuspension events. In addition, as coastal ice melts, a large influx of melt water into adjacent bays takes place. Where melt water passes through areas impacted by human activities (e.g., old waste dumps), the melt water picks up high loads of particulates that may be contaminated with metals and hydrocarbons [14].

The overall aim of the present study was to determine if the resuspension of metal-contaminated sediments has the potential to affect hard substrate organisms, particularly those in Antarctic near-shore ecosystems. Spirorbid polychaetes (tube

* To whom correspondence may be addressed (n.hill@unsw.edu.au).
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worms) were chosen as a test species, because they are a dominant organism on boulders and algae and have a ubiquitous distribution in East Antarctica [13,15]. Only a handful of studies have examined the toxicity of metal contaminants, either aqueous or sediment bound, to polar organisms, and none of these has focused on the response of a hard substrate organism. The present study had three specific aims: First, to assess the sensitivity of spirorbids to aqueous exposures of copper, lead, and zinc, both singly and in combination; second, to determine if the resuspension of metal-contaminated sediments affects spirorbid behavior and health; and third, to determine if any observed toxicity results from the presence of sediment in the water column, increased concentrations of aqueous metals, contaminated suspended particulates, or a combination of these factors.

MATERIALS AND METHODS

Animal collection and handling

Polychaetes of the species *Spirorbis nordenskjoldi* Ehlers, 1,900 were collected from Beall Island, Windmill Islands, East Antarctica, a clean site located approximately 2 km from Casey Station (66°17′S, 110°32′E) and from any known sources of contamination [2]. Animals attached to brown algae (*Desmarestia* sp.) were collected in shallow, subtidal areas (depth, <10 m) by deploying grappling hooks from inflatable boats. Algae and animals were transported in plastic containers back to Casey Station, where they were kept in an aquarium facility in seawater maintained at −1°C and a 12-h light:dark photoperiod. Seawater was constantly aerated and changed every 5 to 6 d. Spirorbids were used within two weeks of their collection and fed once per week with plankton collected in the field by a 5-min plankton tow. Seawater used for holding animals and as the diluent in tests was collected locally from uncontaminated sites in the Windmill Islands and had a salinity of 34.4 ± 0.5 practical salinity units.

Spirorbids were removed from the surface of algal blades 24 h before the start of a test, taking care not to damage their fragile tubes, and were placed in a constant-temperature chamber (CTC) at 0.5°C to recover and acclimate. Immediately before the start of tests, spirorbids were examined, and only healthy individuals were selected and added to test vials. Spirorbids were determined to be healthy if their tentacular crown (fan) was extended and retracted quickly in response to stimuli. During handling outside of the aquarium or CTC, containers with spirorbids were kept on ice to prevent heat stress.

Aqueous exposures

General procedures. All test equipment was soaked overnight in 10% nitric acid, rinsed twice with MilliQ® (Millipore, Bedford, MA, USA) water, and dried before use. Seventy-milliliter polycarbonate vials were used as test containers and were presoaked overnight with test solutions to minimize metal adsorption to the container walls during the tests. Metal stock solutions were prepared for copper using $CuSO_4 \cdot 5H_2O$ (250 and 500 mg/L), zinc using ZnCl (500 and 1,000 mg/L), and lead using $Pb(NO_3)_2$ (500 mg/L). Lead nitrate was used, because lead is relatively insoluble in seawater and this is one of the most soluble forms. The lead stock solution was warmed on a hot plate during mixing to encourage dissolution, and no salt crystals were visible at the end of this process. Test solutions were created by diluting stock solutions to the required concentration with clean seawater. Five replicates of each treatment, each with 10 randomly selected, healthy spirorbids, were set up with 40 ml of test solution. Tests ran for 10 d in a CTC at 0.5°C, with three grolux tubes per shelf set for a 12-h light: dark photoperiod. Because of the small volume of test containers, the CTC was set at 0.5°C to avoid the possibility of the surface water freezing. This temperature is within natural limits that can be experienced by spirorbids at shallow water sites [16]. Spirorbids were not fed during the test period. Thirty milliliters of test solution were renewed on days 4 and 8. Metal analysis was conducted on a composite sample from two replicates of the old test solutions and from a subsample of the new test solutions of each treatment. The measured concentrations in the present paper represent the average concentrations to which spirorbids were exposed over the 10-d test period. Temperature, pH, and salinity of test solutions also were measured at each renewal using a multiprobe YSI 556 MPS meter (YSI, Yellow Springs, OH, USA).

The response of spirorbids to metal treatments was observed after 1, 2, 4, 7, and 10 d and scored according to the following criteria: Worms were considered to be healthy if their tentacular crown was extended and retracted quickly in response to a touch stimulus, to be avoiding the solution if their operculum was closed, to be unhealthy if their tentacular crown was wholly or partially extended but they exhibited a slow response to stimuli, and to be dead if their collar was hanging out of the tube and/or signs of necrosis were present.

Healthy spirorbids retract quickly in response to stimuli and take a few minutes to emerge after being disturbed. Observations of healthy spirorbids indicated that from 70 to 100% had their fans extended at any one time. Tests were considered to be acceptable if survival in controls was 90% or greater and if 70% or more of control spirorbids were scored as healthy.

Single-metal exposures. Spirorbids were exposed to five or six dissolved metal concentrations up to nominal values of 1,000 μg/L of copper, 5,000 μg/L of zinc, and 4,000 μg/L of lead. Two to three replicate tests were run over two consecutive field seasons. A median lethal concentration (LC50) for copper and median effect concentrations (EC50s) for copper, zinc, and lead were generated from measured metal concentrations using either maximum-likelihood probit regression or the trimmed Spearman–Karber method if the data did not fit the probit model. Abbott's correction was used when necessary. No-observed-effect concentrations (NOECs) and lowest-observed-effect concentrations (LOECs) were calculated for the number of healthy spirorbids. No-observed-lethal and lowest-observed-lethal concentrations also were calculated using either Dunnett's multiple-comparison test or Steel's many one-rank test on arc sine–transformed data. All analyses were performed using Toxcalc™ (Ver 5.0; Tidepool Scientific, McKinnleyville, CA, USA).

Multiple-metal exposures. Two replicate tests were run to examine the response of spirorbids exposed to multiple metals. Spirorbids were exposed to a nominal copper concentration of 600 μg/L, because this was close to the LC50 determined from single-contaminant tests and to nominal zinc concentrations ranging from 0 to 5,000 μg/L. Six treatments were tested: A test control with clean seawater, a copper-only treatment with 600 μg/L of copper, and four mixture treatments with 600 μg/L of copper combined with either 500, 1,000, 2,500, or 5,000 μg/L of zinc. Data from tests were arc sine square-root transformed and analyzed using Dunnett's multiple-comparison test (Dunnett Program, Ver 1.5; U.S. Environmental Pro-

Table 1. Concentrations of particulate metals in spiked sediments at the beginning of the sediment resuspension experiment[a]

Treatment	Cu	Pb	Zn
Clean	11 (0.1)	21 (0.0)	49 (0.1)
Low	55 (0.6)	27 (0.3)	244 (0.0)
Moderate	245 (12)	347 (52)	1,110 (45)
High	445 (4.5)	525 (40)	2,035 (35)

[a] Values presented are the mean concentration of metals in the bulk sediment (μg/g dry wt), with the standard error in parentheses.

tection Agency, Cincinnati, OH), which tested the mixture treatments against the copper-only treatment.

Sediment exposures

Sediment spiking. The test sediment was constructed from clean sediment sourced from Sydney (NSW, Australia) to minimize disturbance to Antarctic ecosystems. The composition of constructed sediment was designed to resemble that of sediment found in the bays near Casey Station, which generally consists of poorly sorted sediment with from 20 to 40% fines and 2% total organic carbon [11,17]. Test sediment consisted of 20% fine material (silt and clay; particle size, <63 μm) sourced from Bonnet Bay (Woronora River, NSW, Australia), a site that has been used previously for spiked sediment studies and has approximately 12% total organic carbon [18], and 80% sand (particle size, 63 μm to 1 mm) sourced from Chowder Bay (Sydney Harbor, NSW, Australia; average metal concentrations: Copper, 6 μg/g dry wt; lead, 20 μg/g dry wt; zinc, 25 μg/g dry wt). Sediment was sieved through a 1-mm mesh before spiking to remove animals and large organic matter. Four treatments were created by spiking sediments with metal concentrations that simulated a range from clean to highly contaminated sediments (Table 1). The fine fraction of the sediment was spiked with $CuSO_4 \cdot SH_2O$, $PbSO_4$, and ZnCl dissolved in deoxygenated seawater according to the methods described by Simpson et al. [18]. The pH was initially adjusted to 8.3, and sediments were thoroughly mixed and pH readjusted every 5 d for three weeks. The sand fraction of the sediment was then combined with the fine fraction. The composite sediment was regularly mixed, and the pH was adjusted and equilibrated for a further four weeks. Sediments were kept in an anoxic environment (a nitrogen glove box) throughout the spiking process (a total of seven weeks). Before shipping to Antarctica, sediments were autoclaved at 121°C for 30 min to satisfy quarantine requirements. Sediments were then kept refrigerated and capped with nitrogen or argon. At the beginning of the experiment, sediment subsamples were taken for total particulate metals and pore-water metals analyses.

Sediment resuspension. Treatments for the suspended sediment test were created using a novel paddle stirring device adapted from a larval mixer described by Strathmann [19]. This consisted of a frame with a series of acrylic paddles attached to an electric motor with an acentric disk. The disk rotated at a slow speed (28 rpm), thus moving the paddles within a water–sediment solution. This method of suspending sediments was chosen to mimic resuspension that would occur in the field because of water movement generated by storms and currents. The amount of spiked sediment required to give a concentration of 1 g dry wt/L of each treatment was weighed into 5-ml centrifuge tubes and centrifuged at 3,500 rpm for 5 min. To prevent unbound metals in the pore water from contributing to aqueous metal concentrations, the pore-water supernatant was removed and the sediments rehydrated with clean seawater and mixed with a vortex mixer. The sediment was added to 1-L plastic containers and the seawater volume made up to 830 ml. The device was set to stir for 6 h before the beginning of each water change.

Initially, this test was run using spirorbids that had recruited to settlement plates deployed in the field for 11 months. Plates were placed directly into containers within the stirring device, and the device was set to stir for 24 h. This pilot study, however, determined that the stirring motion itself affected spirorbid behavior and confounded results. To remove this effect, further tests were run in 70-ml containers and spirorbids exposed to solutions collected from the resuspension device described above. Spirorbids for this experiment also were sourced from algal blades.

Resuspension solutions were collected after a 6-h stirring period. Approximately 600 ml of overlying water were carefully decanted from each treatment container into a Nalgene bottle and mixed thoroughly. Half of this mixture was then vacuum filtered through acid-washed, dried, and preweighed cellulose acetate filters (pore size, 0.45 μm; Whatman, Clifton, NJ, USA). The filtrate was used as the aqueous-only metal treatment. The unfiltered half of the mixture containing the suspended solids was added directly to test containers. Filters were then dried for 24 h at 60°C, allowed to cool in a desiccator, and reweighed to give the amount of total suspended solids (TSS) in each treatment at the beginning of each water change. Six replicates of each exposure type and combination of metal concentrations were used, with 10 spirorbids per replicate. A complete water change was performed every 2 d, and a composite sample of two replicates was taken from the old test solution and a subsample from the new test solution of each treatment for metal analyses. Spirorbids were scored after 1, 2, 4, 7, and 10 d as previously described. A two-factor analysis of variance was used to determine the effect of exposure type (aqueous vs suspended sediment) and metal treatment at day 10.

Chemical analyses

Samples taken for chemical analysis from aqueous tests at each water change were filtered through acid-washed, 0.45-μm syringe filters. Samples were acidified to 2% (v/v) with Tracepur™ nitric acid (Merck, Darmstadt, Germany) and refrigerated until analyzed. Water samples of tests solutions from the suspended sediment test also were acidified as described above and filtered through acid-washed, 0.45-μm syringe filters immediately before analysis. Copper, lead, and zinc were analyzed using an inductively coupled plasma–atomic emission spectrometer (Spectroflame EOP; Spectro Analytical Instruments, Kleve, Germany). Two seawater blanks were included in each run, and samples were analyzed against matrix-matched standards (QCD Analysts, Englewood, FL, USA).

In addition to water samples taken during the suspended sediment test, pore water and sediment were sampled, and the pH and redox of spiked sediment were measured at the start of the test. Duplicate pore-water samples were obtained from each treatment by centrifuging 50 ml of sediment at 3,500 rpm for 10 min, then filtering (pore size, 0.45 μm) and acidifying the supernatant. For total particulate metals analysis, two subsamples of spiked sediment from each treatment were oven-dried at 110°C overnight and ground with a mortar and pestle, after which 0.25 g dry weight was digested overnight with 0.5 ml of Tracepur nitric acid and 1 ml of hydrochloric acid. Samples were then placed in sealed glass jars and microwaved at 10% power in a 1,100-W microwave oven inside a fume hood for 40 min. Samples were allowed to cool and diluted to 25 ml with MilliQ. Two replicates of sediment reference material (PACS-2; National Research Council Canada, Ottawa, ON) and two blanks were analyzed with each batch of samples for quality-control purposes. All samples were analyzed for copper, lead, and zinc against matrix-matched standards using inductively coupled plasma–atomic emission spectrometry. Reference samples were within 10% of the certified value, and blanks were below detection limits.

RESULTS

Aqueous exposures

Single-metal exposures. Survival in control treatments was 90% or greater, and the proportion classified as healthy was greater than 70% for all tests. Water-quality parameters remained reasonably constant within a test: Temperature within the CTC chamber remained at 0.5°C, pH varied by less than 0.5, and salinity varied by less than one practical salinity unit. For copper and zinc tests, 90% of the average measured metal concentrations over the test duration were within 15% of nominal values. In the two lead tests, the measured concentrations were within 27% of the nominal concentrations, with the highest nominal concentration (4,000 μg/L) showing the greatest deficit (2,905 μg/L). Measured concentrations were used to calculate LC50s, EC50s, NOECs, LOECs, and so on and are reported here unless otherwise indicated.

For the sake of brevity and because of the slow response of spirorbids to metal treatments, only 10-d results are presented. In the first set of tests, no healthy spirorbids were observed at the lowest copper treatment of 200 μg/L (nominal), and mortality increased steadily with increasing copper concentration (Fig. 1A). The average LC50 was 570 μg/L (95% confidence interval [CI], 420–1,100 μg/L). The average no-observed-lethal concentration was 215 μg/L, and the average lowest-observed-lethal concentration was 365 μg/L (Table 2).

Based on the results from these experiments, a second set of copper tests was run at lower concentrations to further examine sublethal responses and to determine the EC50 for healthy spirorbids. Nominal copper concentrations in these tests ranged from 0 to 200 μg/L. The proportion of healthy spirorbids steadily decreased with increasing copper concentration, and again, no healthy spirorbids were observed at a nominal concentration of 200 μg/L. Most animals in this treatment were avoiding the test solution (Fig. 1B). The average NOEC was 11 μg/L, and the LOEC was 24 μg/L. The average EC50 was 20 μg/L (95% CI, 17–24 μg/L) (Table 2).

No significant mortality of spirorbids exposed to zinc concentrations of up to 4,910 μg/L (highest average measured concentration among the tests) was observed. Instead, spirorbids avoided the test solution as zinc concentration increased (Fig. 1C). In the two highest zinc concentrations, between 5 and 10% of spirorbids were unhealthy. The average EC50 for healthy spirorbids was 1,210 μg/L (95% CI, 900–1,470 μg/L) (Table 2).

Although two replicate lead tests were run, only one test produced results that could be analyzed using concentration–response curves. In the test that was not analyzed, fewer spirorbids were healthy in the 100 μg/L treatment than in the 500 μg/L treatment, resulting in an inconsistent response with increasing concentration. In the test that conformed to statistical assumptions, no mortality of spirorbids exposed to lead con-

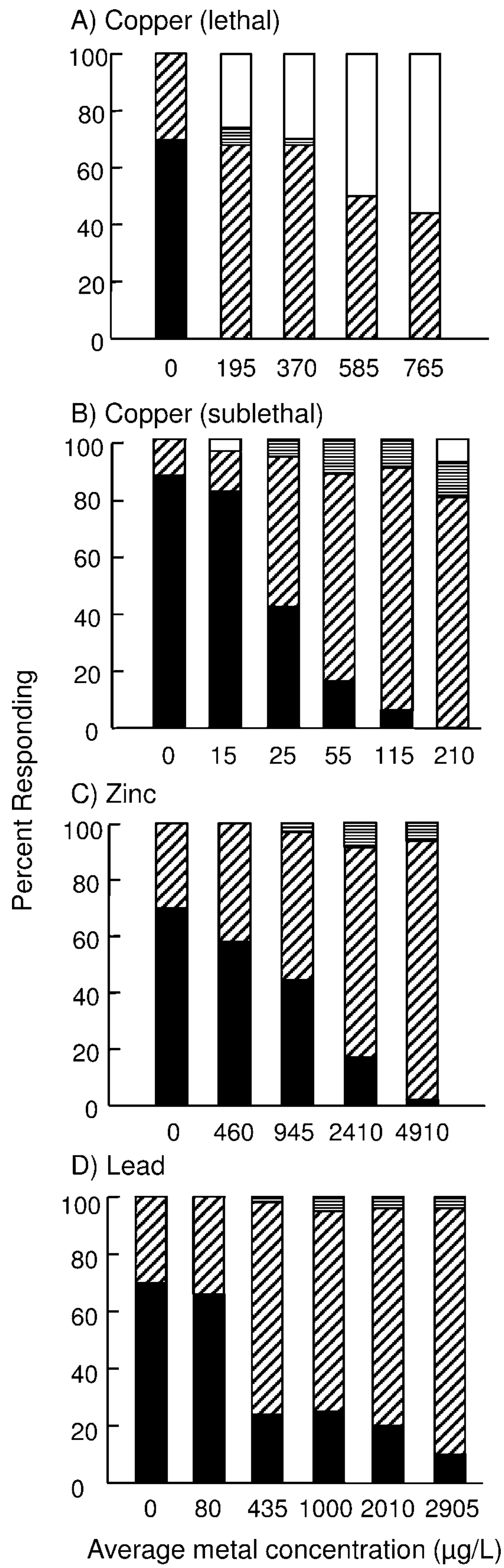

Fig. 1. Response of spirorbids after 10 d of exposure to (**A**) lethal copper, (**B**) sublethal copper, (**C**) sublethal zinc, and (**D**) sublethal lead concentrations. Categories scored were healthy spirorbids (■), spirorbids avoiding test solution (▨), unhealthy (▤), and dead (□). Concentrations are the average measured value in each treatment over the 10 d, and each graph represents one test run. Standard error was less than 10% for all response categories across all tests.

Table 2. Average hypothesis test and point estimate values for copper, zinc, and lead tests[a]

	Lethal endpoint				Sublethal endpoint[b]			
	LC50 (μg/L)	NOLC (μg/L)	LOLC (μg/L)	Tests (n)	EC50 (μg/L)	NOEC (μg/L)	LOEC (μg/L)	Tests (n)
Cu	570 (420–1,100)	215	365	3	20 (17–24)	11	24	2
Zn	—	4,910	—	3	1,210 (900–1,470)	770	1,660	3
Pb	—	2,905	—	2	365 (140–620)	80	435	1

[a] Values are based on average measured concentrations over the duration of the test. Median lethal concentration (LC50) and median effect concentration (EC50) values are presented with the associated 95% confidence interval in parentheses. LOEC = lowest-observed-effect concentration; LOLC = lowest-observed-lethal concentration; NOEC = no-observed-effect concentration; NOLC = no-observed-lethal concentration; — = no significant mortality occurred and values could not be calculated.
[b] The sublethal endpoint used was the proportion of healthy individuals.

centrations of up to 2,905 μg/L was observed (Fig. 1D). Spirorbids showed significant avoidance of the test solutions, however, at concentrations of 435 μg/L and greater. The EC50 based on the proportion of healthy spirorbids in this test was 365 μg/L (95% CI, 140–620 μg/L), with a NOEC of 80 μg/L.

Multiple-metal exposures. Survival in the test control was 100%. Temperature within the CTC remained at 0.5°C, pH between 7.6 to 8.0, and salinity between 34.4 to 35.3 practical salinity units throughout the test. Measured copper and zinc concentrations were within 10% of nominal values over the duration of the tests.

The effect of exposure to aqueous zinc and copper in combination was not additive (Fig. 2). Spirorbids were exposed to an average copper concentration of 550 and 600 μg/L across all treatments in the two replicate tests. The average mortality in the copper-only treatment was 56 and 43%, respectively. Exposure to 470 μg/L of zinc had no effect on the toxicity of the copper test solution. The addition of zinc at concentrations of 930 μg/L and greater, however, significantly reduced spirorbid mortality, making the mixtures less toxic than the copper-only treatment. Increasing zinc concentrations did not further decrease mortality.

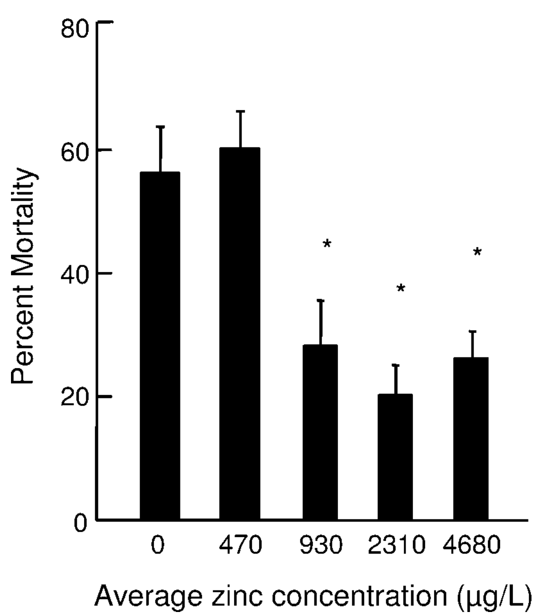

Fig. 2. Percentage mortality of spirorbids in one test of the multiple-metal exposures. Spirorbids were exposed to an average measured copper concentration of 550 μg/L across all treatments and varying concentrations of zinc. Asterisks indicate treatments that have significantly less ($p < 0.05$) mortality than the copper-only treatment using Dunnett's multiple-comparison test.

Sediment exposures

The spiking procedure was effective, with less than 1% of the initial concentration of metals added remaining in the pore water at the start of the test. This pore water was removed by centrifuging the whole sediment and removing the supernatant before adding the sediment to the stirring device. The pH of the spiked sediment before addition to seawater ranged from 7.7 to 7.9. Redox values ranged from −238 mV in the low-metal treatment to −17 mV in the high-metal treatment. The stirring method produced an average of 68 ± 6 mg/L of suspended sediment in each treatment.

The average concentration of copper, lead, and zinc in the filtered clean and low-metal treatments was less than 10 μg/L. Copper, lead, and zinc concentrations reached an average of 38, 50, and 195 μg/L, respectively, in the filtered high-metal treatments (Fig. 3). The difference in the metal concentrations between old and new test solutions in these filtered treatments was minimal. Metal concentrations in the unfiltered clean and low-metals treatment were similar at each solution change (e.g., an average of 7–8 μg/L of copper over the 10 d). These concentrations also were similar to their corresponding filtered treatments. In the moderately and highly contaminated sediments, however, the unfiltered treatments had much higher metal concentrations in the new compared with the old solutions at each water change. For example, the average copper concentration immediately after a water change in the unfiltered high-metal treatment was 135 μg/L of copper (Fig. 3), but after 2 d, this concentration had dropped and was comparable to that in the filtered high-metal treatment (e.g., 45 μg/L).

Evidence (in the form of fecal pellets) was found that spirorbids were ingesting (or at least processing) the suspended particulates in all except the highest-metal suspension treatment. The addition of clean suspended sediment did not affect the health of spirorbids (Fig. 4). A significant interaction was observed between metal concentration and the filtering treatment (two-factor analysis of variance; mean square = 0.050; degrees of freedom = 3, 40; p = 0.001). Increasing metal concentrations generally decreased the percentage of healthy spirorbids, but this was accentuated in the low and mid-range spiked treatments by the presence of particulate metals. Spirorbids displayed a similar response to the high-metal treatment regardless of exposure type.

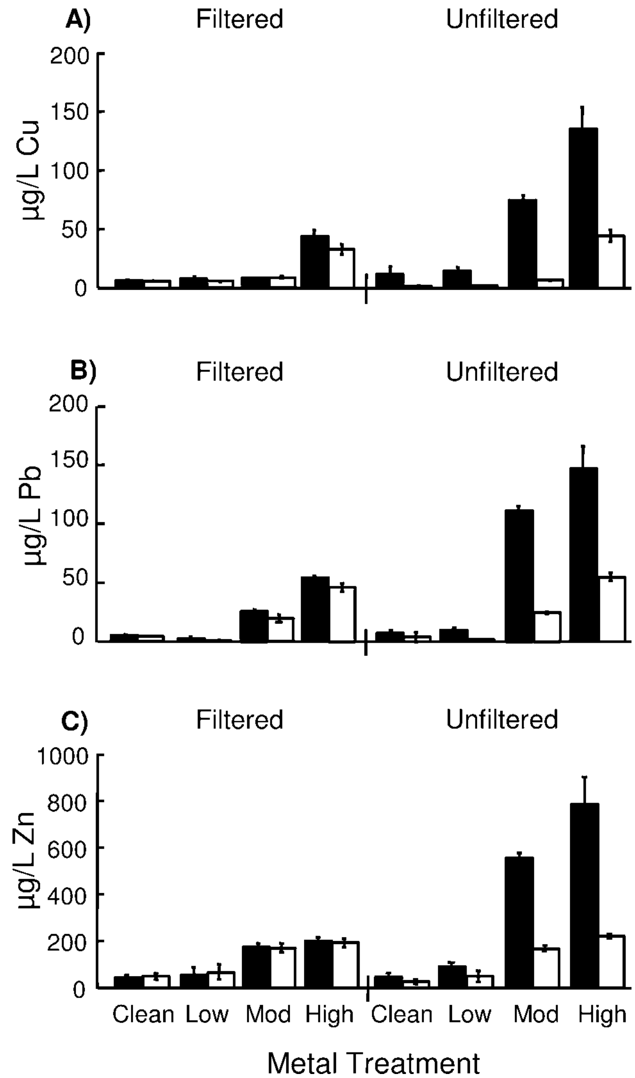

Fig. 3. Average concentration of (**A**) copper, (**B**) lead, and (**C**) zinc in filtered (aqueous metals only) and unfiltered (suspended sediment) treatments at the renewal of test solutions. ■ = new test solutions; □ = 48-h-old test solutions.

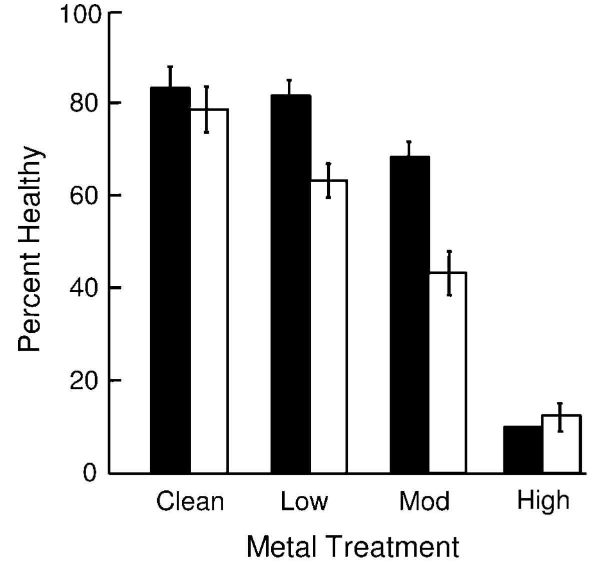

Fig. 4. Percentage of spirorbids healthy after 10 d of exposure to either filtered (■; aqueous metals only) or unfiltered (□; suspended sediment) treatments at four spiked metal concentrations.

DISCUSSION

Aqueous metal exposures

To our knowledge, the present study is the first to examine the toxicity of metals to a hard substrate invertebrate from a polar ecosystem. Mortality was a relatively insensitive endpoint for this Antarctic spirorbid, and this finding agrees with previous results for other polar organisms [20–23]. The sublethal response of spirorbids to metals was an order of magnitude more sensitive, however, and spirorbids were able to detect and avoid solutions containing much lower concentrations of copper than either lead or zinc. This may be because zinc ions simply are not very toxic to this species, or the spirorbid may efficiently regulate accumulation or detoxify high concentrations of zinc. Regulation of zinc accumulation may occur because of an increased rate of zinc excretion, as is the case for many crustaceans [24]. Alternatively, spirorbids may have the ability to detoxify zinc by binding it in unavailable forms, such as metallothionein or zinc phosphate granules, as barnacles do [25]. Lead is a nonessential metal, so any accumulated metal needs to be detoxified to avoid harmful effects [24]. Spirorbids, however, also were relatively insensitive to lead exposure. This may be caused by the low activity of chemicals in near-freezing water, the low solubility of lead in seawater, or a combination of both.

In their natural environment, organisms rarely are exposed to single contaminants, and single-metal tests likely are inadequate for determining the effect of metal mixtures [26–28]. In the present study, high zinc concentrations significantly mitigated the mortality of spirorbids exposed to copper. This antagonistic interaction between copper and zinc has been observed previously for marine invertebrates, such as shrimp [27], sea urchin larvae [28], and amphipods [29]. Less-than-additive combined toxicity may be explained by competition between metals of similar activity and may occur during uptake at cell membranes or at specific binding sites within the cell [30]. The present study further reinforces the idea that the effect of contaminant mixtures on organisms may not be predicted by their response to individual toxicants.

In a review of the available toxicity data for polar, temperate, and tropical regions, Chapman et al. [31] concluded that temperate and polar organisms were equally sensitive to copper but that organisms in polar regions were the least sensitive to lead and zinc. The present results tentatively support this conclusion for copper, although toxicity tests on hard substrate organisms are rare. *Hydroides elegans*, a serpulid tube worm, is the most comparable subtropical invertebrate for which aqueous toxicity data are available. This species has a 48-h LC50 for copper of 715 µg/L [32]. Despite the different lengths of the two tests, this suggests that the Antarctic species *S. nordenskjoldi* is similarly tolerant of copper, although to our knowledge, no comparable studies for zinc and lead have been reported. The apparent tolerance of polar organisms to contaminants most likely relates to the reduced activity of chemicals at low temperatures and the slower metabolism of organisms in cold environments [33]. In addition, many polar organisms are slow-growing, long-lived, and have long developmental phases [34]. This has two consequences. First, using fixed-length toxicity tests to compare sensitivities across latitudes may underestimate toxicity to polar organism; a better approach would be to use comparative life history or developmental stages [21]. Second, any observed sublethal effects of contaminants, such as a reduction in feeding, will reduce the fitness of individuals and may have effects that scale up to the population and community levels. For these reasons, we should be cautious in suggesting that polar organisms are relatively insensitive to metal contaminants.

Importance of suspended sediment in toxicity

To our knowledge, the present study is the first to examine the toxicity of resuspended sediments on a polar marine organism. The method used to resuspend sediment was effective and produced average suspended sediment concentrations of 68 mg/L. This value is well within the range of TSS generated by a variety of natural processes, such as storms (20–200 mg/L [35,36]) and high-density bioturbation (177 mg/L [37]). Suspended sediments were toxic to the tested species of spirorbid within the range of metal concentrations examined, and spirorbids were not affected by the presence of clean suspended sediment. Aqueous toxicity tests revealed spirorbids to be far more sensitive to copper than to zinc or lead; therefore, the toxicity observed in the resuspended sediment test may be attributed primarily to copper. In the resuspension test, aqueous copper concentrations in the filtered treatments only exceeded the EC50 (20 μg/L) generated from aqueous tests in the highest metal treatment. Total copper concentrations in the moderate and high unfiltered treatments, however, also exceeded this EC50, and spirorbid health in these treatments was less than 50%. This suggests that total copper concentrations correlate well with toxic effects but that the toxicity of contaminated sediments may be underestimated in tests that only consider exposure through the aqueous phase.

Several studies have examined the effect of clean resuspended sediments generated from a variety of process, such as floods, storms, and bioturbation in a variety of environments. In many cases, an increase in TSS alone has negative impacts on organisms. Shellfish [38], fish [39,40], and coral larvae [41] are all negatively affected by environmentally relevant loads of suspended sediment, and organisms such as flagellates and daphnids, which actively ingest clay-sized particles as food, suffer reductions in their growth and fitness [42,43]. In general, high levels of TSS would be expected to pose a smothering hazard for sessile hard substrate organisms [8]. In the present study, however, spirorbid health was not affected by clean suspended sediment. Animals actively filtered the water column, ingested material, and produced feces. In filter-feeding mussels, processing increased TSS with a high inorganic content affects their ability to assimilate food and comes at a significant metabolic cost [44]. It is unknown if active water filtering in spirorbids is simply a response to avoid smothering or an attempt to enhance feeding and if processing inorganic suspended sediment has an overall negative metabolic cost.

The resuspension of metal-contaminated suspended sediments also can be acutely toxic to organisms such as algae [45], oyster larvae [46,47], amphipods [48], and cladocera [48–51]. Similarly, the Antarctic filter feeder used in the present study was negatively affected by the resuspension of metal-contaminated sediments at environmentally relevant concentrations. Very few studies have attempted to identify the underlying cause of toxicity, however, and these studies have reported mixed results. For example, some studies have found that metals in the dissolved phase were more important in determining the toxicity of metal-contaminated suspensions [49,50,52], whereas others have found that suspended particulates were important [47,51]. The present study indicates that uptake from the aqueous phase is an important pathway in toxicity, because spirorbid health decreased as aqueous metal concentrations increased. At low and moderate metals concentrations, spirorbid health also was affected by the presence of contaminated particulates. This indicates that the ingestion of metals attached to particulates is an important route of exposure and that any observed toxicity is the result of both aqueous and particulate metals. To further examine the underlying physiological mechanisms of toxicity, biodynamic modeling studies that use radiolabeled tracers or isotopes to investigate the uptake and bioaccumulation of metals from aqueous and dietary sources may be useful [53].

The lowest sediment metal concentrations used in the present study are representative of concentrations at contaminated sites in Antarctica [54,55]. Resuspended sediments at this concentration also had a negative impact on spirorbid health. With the exception of zinc, these values in Antarctica can exceed the Australian and New Zealand Interim Sediment Quality Guidelines trigger values [56]. Currently, these guidelines are used in Australia for determining the concentration of individual metals that may cause biological impacts (the trigger value) and should be further investigated. The highest-metal sediment used in the present study was representative of contaminated sediments in highly urbanized regions of the world [1]. The resupension of sediments at this concentration also had a significant negative effect on spirorbids because of aqueous copper concentrations that exceeded the previously determined EC50s. Concentrations in this treatment are well above the high value of the interim sediment-quality guidelines and almost certainly have negative effects on benthic organisms [56]. The present study represents the response and sensitivity of only one species, but based on these results, current sediment-quality guidelines used in the evaluation of Australian sediments may be applicable to Antarctic ecosystems.

CONCLUSION

Mortality was not a particularly sensitive endpoint in this species of spirorbid, and the present results agree with the relatively few toxicity tests conducted on other polar organisms. No additive effect of zinc on copper toxicity was found. In fact, high concentrations of zinc mitigated mortality caused by copper, suggesting competition for binding sites between these metals. The apparent tolerance of polar organisms to contaminants may be attributed to the reduced activity of chemicals at low temperatures as well as the slower metabolism and, therefore, uptake and processing of contaminants by organisms. Spirorbids did, however, exhibit a behavioral response to much lower, more environmentally relevant copper concentrations. Any behavioral changes that reduce the feeding capacity of an organism obviously will have a negative impact over the longer term. To address this issue and make toxicity tests more relevant and comparable across latitudes, longer-term toxicity tests using equivalent developmental periods should be used in the future.

Suspended sediments were toxic to filter-feeding spirorbids over the range of metal concentrations tested. These concentrations approximate those found in contaminated areas in Antarctica as well as highly contaminated sediments in urbanized regions of the world. Toxicity resulted both from the aqueous metal fraction and from metals associated with the suspended sediments, although suspended sediment per se did not have an impact on the spirorbids. The present study has shown that metal-contaminated particulates can be toxic to hard substrate, Antarctic filter feeders and, therefore, that contaminated sediments have the potential to affect hard substrate communities. Sediment-quality guidelines currently used in the evaluation of Australian sediments would be protective of this hard substrate species, and these guidelines may be applicable to Antarctic ecosystems.

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