TOXICITY OF HOLMIUM-LABELLED CLAY TO FOUR BENTHIC INVERTEBRATES

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ABSTRACT

Montmorillonite clay adsorbed with the rare earth element holmium (Ho-clay) is a new material produced for tracing movements of fine particles in aquatic systems. However, little is known about the toxicity of Ho-clay, and its potential environmental effects. To investigate effects of Ho-clay on aquatic biota, four benthic invertebrate species (*Chironomus riparius*, *Hexagenia* spp., *Hyalella azteca*, *Tubifex tubifex*) were exposed to Ho-clay alone and mixtures of 10, 25 and 50% Ho-clay with field-collected reference sediment for 10-28 days in standard laboratory toxicity tests. Overall, only the 100% Ho-clay treatment resulted in significantly higher toxicity than the reference sediment. Mean survival at the end of the exposures to 100% Ho-clay was 7, 53, 2 and 100% for *Chironomus*, *Hexagenia*, *Hyalella* and *Tubifex*, respectively. Exposure to a negative control treatment of 100% clay resulted in minor reductions in growth, but no lethal responses. Highest concentrations of Ho at which no toxicity was observed were 1400 µg/g for sediment and 11 µg/L for water. Potential impacts of Ho-clay released into natural waters would be expected only where Ho-clay persists in sediment at proportions >50% for at least several days.

RÉSUMÉ

La montmorillonite adsorbée avec la terre rare holmium (Ho-argile) est une nouvelle matière qui permet de suivre les déplacements des particules fines dans les réseaux aquatiques. Toutefois, on ne connaît que peu de choses sur la toxicité de Ho-argile et sur ses effets possibles dans l'environnement. Pour étudier les effets du mélange Ho-argile sur le biote aquatique, on a exposé pendant 10 à 28 jours, dans un laboratoire ordinaire de toxicologie, quatre taxons d'invertébrés benthiques (*Chironomus riparius*, *Hexagenia* spp., *Hyalella azteca* et *Tubifex tubifex*) au mélange Ho-argile seul, ainsi qu'à des mélanges de 10, 25 et 50 % de Ho-argile avec des sédiments témoins recueillis sur place. Dans l'ensemble, seulement le traitement à 100% de Ho-argile a causé une toxicité notablement supérieure à celle des sédiments témoins. Le taux moyen de survie à la fin de l'exposition à 100 % de Ho-argile était de 7, 53, 2 et 100 % pour *Chironomus*, *Hexagenia, Hyalella* et *Tubifex*, respectivement. L'exposition à un témoin négatif composé de 100% d'argile a causé de faibles réductions de la croissance, sans aucune réaction létale. La plus forte concentration de Ho pour laquelle on n'observait aucune toxicité était de 1 400 µg/g pour les sédiments et de 11 µg/L pour l'eau. Les mélanges Ho-argile libérés dans des eaux naturelles ne devraient avoir des effets nocifs que s'ils persistent dans les sédiments pendant plusieurs jours, dans des proportions de plus de 50 %.

INTRODUCTION

Many contaminants discharged to aquatic systems are associated with fine sediment particles. Determining the transportation pathways of fine sediments in surface waters is an important step in understanding the environmental fate of contaminants. Clay labelled with the rare earth element holmium (Ho-clay), is a recently developed tracer material with potential use in field experiments for examining fine sediment transportation and fate (Suzuki and Spencer 2005). However, the toxicity of Ho-clay to aquatic organisms is not well known.

Ho-clay is composed of holmium adsorbed to aggregates of montmorillonite clay minerals. In freshwater and marine environments, a fraction of the bound Ho can be desorbed through cation exchange (Suzuki and Spencer 2005). Thus both particle-bound and dissolved Ho could be bioavailable. In an assessment of the toxicity of dissolved forms of 63 metal and metalloid elements to the freshwater amphipod *Hyalella azteca* (Borgmann et al. 2005), Ho ranked in the middle for relative toxicity. Environmentally important elements Cu, Ni and Zn were several times more toxic that of Ho based on median lethal concentrations (LC50s); metals showing the lowest LC50s (Cd, Ag, Pb, Hg) were 75–600 times more toxic than Ho. Whether a solid phase form of Ho, such as Ho-clay, also lies at the middle for relative toxicity is uncertain, because toxicity of a contaminant can be strongly related to its solubility and lability.

In order to assess the ecological risk of releasing Ho-labelled clay to the environment, a series of laboratory toxicity tests with four benthic macroinvertebrate species was conducted. The tests involved exposures to mixtures of Ho-clay and pond sediment following a standard methodology developed by Environment Canada for assessing toxicity of contaminated sediments in the Great Lakes (Reynoldson and Day 1998). These tests were conducted in advance of a planned field experiment in which a slurry of Ho-clay will be discharged to a stormwater pond in the Region of Halton, Ontario, in October 2007.

The purpose of the toxicity assessment was to characterize relationships between exposure to Ho-clay (in terms of the proportion of Ho-clay mixed with natural pond sediment) and the ecotoxicological responses of four macroinvertebrate taxa (midge, mayfly, amphipod, oligochaete worm) representing a range of feeding habits and lifestyles.

METHODS

Experimental design

The overall experimental design is shown in Fig. 1. Four sets of toxicity tests were conducted involving seven sediment treatments. Each set of tests corresponded to one of the following test organisms and responses:

- *Chironomus riparius* 10-day survival and growth,
- *Hexagenia* spp. 21-day survival and growth,
- *Hyalella azteca* 28-day survival and growth, and
- *Tubifex tubifex* 28-day survival and reproduction.

Five sediment treatments involved a range Ho-clay and pond sediment mixtures:

- 100% Ho-clay / 0% pond sediment
- 50% Ho-clay / 50% pond sediment
- 25% Ho-clay / 75% pond sediment
- 10% Ho-clay / 90% pond sediment
- 0% Ho-clay / 100% pond sediment

The pond sediment used in the above experiments was collected from the stormwater pond of the Regional Municipality of Halton's Waste Management Site located in Milton, Ontario,

Canada. A Ponar grab sampler was used to collect the bed sediment from the middle of the pond. The collected sediment was composed primarily of deposited clays (67% <5µm), silts (32% >5µm <63µm) and an insignificant amount of sand (1% >63µm). The collected sediment was wet sieved through a 250 µm screen. Organic content was not determined for the sediment; however, given its dark hue, it is believed to possess a moderate amount consistent with pond/river sediments.

In addition to the 100% pond sediment reference treatment, two other reference treatments were tested: 100% montmorillonite clay as a negative control for effects of the Ho "carrier", and sediment from an uncontaminated site in Lake Erie (Long Point Marsh), which is used as a standard laboratory control sediment.

The test containers (=experimental units) were glass beakers containing sediment and overlying water. Toxicity tests were conducted in triplicate for each sediment treatment and test organism combination. A fourth treatment replicate was run with each test for the sampling of water and sediment for analyses of Ho concentrations. Sediment and water were allowed to equilibrate for one week before introducing test organisms. All sediment treatments were tested concurrently for each organism, although not all test sets were run concurrently.

Sediment treatment preparation

The Ho-labelled clay tracer was prepared by first making up stocks of holmium chloride ($HoCl_3$) and sodium nitrate ($NaNO_3$). From these stock solutions, 400 mL of $HoCl_3$ and 1600 mL of $NaNO_3$ were added to 10 2000-mL bottles and the pH adjusted to between 4.9 and 5.1 using a few drops of dilute HNO_3. Next, 200 g of montmorillonite clay was added to each of these working solutions. These working solutions were placed on a flat shaker at 100 rpm for 72 hours, and left to stand overnight. The supernatant was then decanted from each bottle and the

sediment transferred into 4 200-mL centrifuge bottles. The 200-mL centrifuge bottles were topped up with distilled water and centrifuged for 15 minutes at 3000 rpm. Supernatant was poured off and the bottles were refilled with distilled water and centrifuged again for 15 minutes at 3000 rpm. The supernatant was poured off again and the sediment was washed into foil trays using distilled water and dried in a conventional oven at 95°C overnight. The sediment was then placed into a furnace at 500°C for 7 hours. The resulting Ho-clay sediment was grinded to <63 µm and stored until use.

The three pond sediment and Ho-clay mixtures were prepared based on dry weights of proportions. After drying a sample of the pond sediment to determine the water content (73.8%), dry weight-equivalent wet weights of pond sediment and dry Ho-clay were combined with some distilled water in 2-L containers and homogenized with a drill mixer. Appropriate amounts of the resultant slurries were then distributed to the test containers.

Toxicity tests

Static toxicity tests were conducted in aerated glass beakers with dechlorinated tap water from Lake Ontario. Ratios of water to sediment were about 4:1 by volume for all tests except *Tubifex*, which used a 1.5:1 ratio. Details of sediment handling procedures and toxicity test methods are described in Borgmann and Munawar (1989), Borgmann et al. (1989), Krantzberg (1990), Reynoldson et al. (1991) and Reynoldson et al. (1998). Brief descriptions of each test are provided below.

The *Hyalella* test was conducted for 28 days using 15 2 -10 day old organisms. On day 28, the contents of each beaker were rinsed through a 250-µm screen and the surviving amphipods counted. Amphipods were then dried at 60 °C for 24 hours and dry weights recorded. (Initial weights were considered negligible.)

The *Chironomus* test was conducted for 10 days using 15 first instar organisms. On day 10, the contents of each beaker were wet sieved through a 250-μm screen and the surviving chironomids counted. Chironomids were then dried at 60 °C for 24 hours and dry weights recorded. (Initial weights were considered negligible.)

The *Hexagenia* spp. test was conducted for 21 days using 10 preweighed nymphs (5 - 8 mg wet weight/nymph). On day 21, the contents of each jar were wet sieved through a 500-μm screen and surviving mayfly nymphs counted. Nymphs were then dried at 60 °C for 24 hours and dry weights recorded. The relationship between mayfly wet and dry weights was determined previously by regression analysis. Initial dry weights were calculated using the equation:

$$\log(\text{dry weight}) = -0.905 + 0.968 \log(\text{wet weight}); \; r^2=0.86$$

Final growth was determined as final dry weight minus initial dry weight.

The *Tubifex* test was conducted for 28 days using 4 sexually mature worms. On day 28, the contents of each beaker were sequentially rinsed through 500-μm and 250-μm sieves. The number of surviving adults, full cocoons, empty cocoons, and large immature worms were counted from the 500-μm sieve and the numbers of small immature worms were counted from the 250-μm sieve. Reproduction was measured with three endpoints: total number of cocoons per adult, percent cocoons hatched, and total number of young per adult.

For a set of tests to be acceptable, survival in the reference or laboratory control sediment had to exceed specific minimum levels: 80% for *H. azteca* and 70% for *C. riparius* (USEPA 1994; ASTM 1995); 80% for *Hexagenia* spp., and 75% for *T. tubifex* (Reynoldson et al. 1998).

In each replicate test beaker, pH, dissolved oxygen, conductivity, temperature, and total ammonia + ammonium were measured at the start (day 0 – prior to the introduction of organisms) and the completion of the test (day 10, day 21, or day 28). Evaporated water was replaced with dechlorinated tap water. Tests were run under static conditions in environmental chambers at 23 ±1 °C, under a photoperiod of 16L: 8D and an illumination of 500–1000 lux, with the exception of *T. tubifex* test, which was run in the dark.

Water and sediment sampling

Samples of water and sediment were collected from the fourth replicate beaker of each treatment during each test. Water (30 mL) was sampled in duplicate on day 0 (before placement of test organisms in beakers), day 1, midway through the test, and on the last day of the test. The water was collected using one-use10-mL sterile glass pipettes, filtered on 0.45-µm cellulose acetate filters and preserved with concentrated HNO_3 (pH to 2) within polyethylene bottles. During the tests, water removed for Ho analysis was replaced with dechlorinated tap water.

Sediment (10g wet weight) was sampled in duplicate at the beginning and end of the test. The initial sediment sample was collected by simply pouring into pill jar containers directly from the bulk sample. The final set of sediment samples were taken at the end of each test, after the last set of water samples had been taken and the remaining overlying water had been siphoned off. The sediment remaining in each treatment beaker was transferred into pill jar containers using one-use sterile plastic scoops.

Ho analyses

Sediment samples were first dried at 105° C until a consistent weight was achieved. Samples were then ground followed by microwave digestion with 0.5g of concentrated nitric acid. The

nitric acid was then diluted to 50 mL by deionised water and injected into a Perkin Elmer Optima 5300v ICP-OES system. Dissolved concentrations of Ho were determined by direct injection into the ICP-OES.

Data analyses

Effects of Ho-clay on water and sediment were examined graphically by plotting Ho concentrations by sediment treatment and against proportion of Ho-clay.

Differences in responses of test organisms among sediment treatments were analyzed by one-way ANOVA of each toxicity endpoint and Tukey multiple comparison of treatment means. Differences among test organisms in sensitivity to Ho-clay were assessed by two-way ANOVA of survival data for the five Ho-clay and pond sediment mixtures. Dose-response relationships were examined for the five Ho-clay and pond sediment mixtures by plotting survival, growth and reproductive endpoints against Ho concentrations in sediment and water. Sediment and water LC50s for Ho were estimated by the trimmed Spearman-Karber method (Hamilton et al. 1977).

Joint toxicological responses were assessed by Principal Components Analysis (PCA) of the endpoints. Eigenanalysis was conducted on a correlation matrix of untransformed endpoint data. Scores for the first two principal components were plotted and tested for differences among treatments by ANOVA.

RESULTS

Ho concentrations in sediment and water

Concentrations of Ho in sediment sampled from the test beakers were low or not detected for the three treatments containing no Ho-clay, and increased approximately linearly with the proportion of Ho-clay (Fig.2, Fig. 4A). Within-beaker differences in concentrations between the

start and end of tests were not significant. Among the four sets of tests, mean Ho levels in sediment agreed well for all given treatments (Fig. 4A), suggesting that the measured Ho in sediment from the fourth treatment replicate beakers were similar to those in the other three treatment replicates.

All sediment treatments containing Ho-clay resulted in dissolved Ho in the overlying water (Fig. 3). (Dissolved Ho was also detected in some samples from non-Ho-clay beakers, likely due to cross-contamination during sample analyses.) In contrast to the sediment Ho, relationships between Ho water concentration and proportion of Ho-clay in sediment were strongly curvilinear, with the 100% Ho-clay treatment resulting in dissolved Ho levels 2-3 orders of magnitude higher than those for the other treatments (Fig. 4B). Differences in mean dissolved Ho concentrations among tests were also greater than those for sediment Ho, particularly for the 100% Ho-clay treatment, where Ho in the *Hyalella* and *Tubifex* beakers were almost 2 times those for *Chironomus* and *Hexagenia*.

Among treatments overall, differences in Ho concentrations in sediment and, to a lesser extent, water indicate that the test organisms were exposed to a range of Ho levels.

Toxicological responses

Effects of treatments

Exposure to the sediment treatments had significant ($P \leq 0.0035$) effects on all toxicity endpoints except *Tubifex* survival and "percent of cocoons hatched". The general pattern was of the 100% Ho-clay treatment resulting in substantially higher toxicity than all other treatments (Table 1; Fig. 5A,B,C).

For the lethal endpoints (Fig. 5A), mean survival was ≥73% for all treatments and taxa except for the 100% Ho-clay treatment, in which mean survival was only 7, 53 and 2% for *Chironomus*, *Hexagenia* and *Hyalella*, respectively. Survival of *Tubifex* was 100% in all beakers. For all taxa, survival in the negative control sediment (100% clay) was as high as in the laboratory control and the pond reference sediments.

The sublethal endpoints showed more variability that the lethal ones to the sediment treatments. While the 100% Ho-clay treatment was again substantially more toxic than the other Ho-clay mixtures and the 100% pond sediment for all endpoints except "percent of cocoons hatched", the laboratory control sediment and 100% clay treatments also resulted in some apparent toxicity: reduced growth for *Hexagenia* and *Hyalella* (Fig. 5B). However, because the growth responses in the laboratory control sediment were within QA/QC ranges for the *Hexagenia* test, these differences may result in part from enhanced growth in the treatments with pond sediment, which appeared to be high in organic content. Conversely to the lower growth, production of *Tubifex* young was higher in the laboratory control, 100% clay and 50% Ho-clay treatments compared to the 100% pond sediment (Fig. 5C).

The adverse effects of the laboratory control sediment on growth, together with the *Hyalella* survival of <80%, are typically indicative of potentially unhealthy test organisms. However, given that survival, growth, and 2 of the 3 *Tubifex* reproduction endpoints were as high or higher in the 100% pond sediment than in any of the other treatments, it is likely that the laboratory control sediment treatment itself was affecting the endpoints. Therefore, the 100% pond sediment treatment alone was considered the appropriate reference for comparisons to the Ho-clay exposures.

The multivariate toxicological response to sediment exposures is shown in a plot of the treatment replicate scores for the first two principal components from the PCA of nine measured endpoints (Fig. 6). (*Tubifex* survival was invariant and therefore excluded from the PCA.) Of the total variance in the endpoints, 83% was represented by PC1 and PC2. PC1 is strongly, inversely related to toxicity for all endpoints. PC2 is related to increasing *Tubifex* cocoon hatch and production of young, and decreasing *Hexagenia* and *Hyalella* growth.

In terms of joint responses, the 100% Ho-clay treatment was substantially more toxic than the other treatments ($P<0.0001$ from ANOVA of PC1). Responses to all other Ho-clay treatments, though, were similar to those for the 100% pond sediment, as indicated by the overlapping distributions of the treatment replicate scores and the ANOVA results. The laboratory control sediment and 100% clay treatments produced effects that were similar to each other, but distinct from the other treatments (mainly by PC2). Although the 100% clay treatment resulted in slightly elevated toxicity compared to the 100% pond sediment, the effect is much lower than the toxicity produced by the 100% Ho-clay.

Effects of Ho concentration

Dose-responses relationships between the toxicity endpoints and measured Ho concentrations in sediment and water for the five Ho-clay and pond sediment mixtures are shown in Fig. 7 and Fig. 8. Sediment with concentration of Ho up to about 1400 µg/g was not more toxic than the pond reference sediment by any of the endpoints, whereas sediment with 4400-4800 µg/g significantly ($P<0.0015$) reduced survival and growth for *Chironomus*, *Hexagenia* and *Hyalella*, and production of cocoons and young for *Tubifex* (Fig. 7). Shapes of the curves were similar for Ho in water with concentration on the log scale. Concentrations of dissolved Ho up to 11 µg/L were not toxic to test organisms, whereas significantly reduced survival and growth for

Chironomus, *Hexagenia* and *Hyalella*, and production of cocoons and young for *Tubifex* were observed in beakers with dissolved Ho of 330 to 546 µg/L (Fig. 8).

LC50s could be determined only for the *Chironomus* and *Hyalella* tests, for which at least one Ho concentration produced <50% survival. For Ho in sediment, LC50s (and 95% confidence intervals) were 2611 (2409-2829) and 2378 (2258-2504) µg/g for *Chironomus* and *Hyalella*, respectively. For Ho in overlying water, the LC50 for *Chironomus* was 35.0 µg/L (CI could not be estimated). The water Ho LC50 (and 95% CI) for *Hyalella* was 52.2 (42.8-63.6) µg/L. Results from the tests with *Hexagenia* and *Tubifex* suggest that LC50s for Ho in sediment and water exceed the highest concentrations measured in the treatments. For sediment, these were 4720 and 4723 µg/g for *Hexagenia* and *Tubifex*, respectively; for water, 230 and 542 µg/L, for *Hexagenia* and *Tubifex*, respectively. However, the *Hexagenia* LC50s are not likely much greater than the maximum measured Ho concentrations because survival in the 100% Ho-clay treatment was 53%.

Differences among taxa in tolerance of treatments

Variability among taxa in survival of treatments was significant ($P<0.0001$, two-way ANOVA with treatment and taxon as factors). Although the treatment-taxon interaction was significant, examination of the interaction plot, which is similar to the survival vs. sediment Ho concentration curves in Fig. 7, indicated that *Chironomus* was most sensitive to Ho-clay, followed by *Hyalella*, *Hexagenia* and *Tubifex*. Consideration of the treatment exposure times for the tests further supports these ranks. Despite the *Chironomus* test being the shortest (10 days), mortality at test end was highest. The *Tubifex* test lasted 28 days, but resulted in lower mortality than the 21-day *Hexagenia*, 28-day *Hyalella*, and 10-day *Chironomus* tests. The difference in sensitivity between *Hexagenia* and *Hyalella* is less significant due to the unequal Ho-clay exposure periods.

DISCUSSION

Toxicity of Ho-clay

Sediment containing up to 50% Ho-clay did not prove toxic for any of the four benthic invertebrates in chronic laboratory toxicity tests, but exposure to 100% Ho-clay resulted in substantially reduced survival for three of the four test organisms. Although fine sediment, such as clay, can cause reductions in survival and growth of some benthic organisms (e.g., DeWitt et al. 1988), adverse effects with the 100% clay were observed only for the *Hexagenia* and *Hyalella* growth endpoints (Fig. 5A-C). Therefore, effects of clay do not account for low survival (nor reductions for 3 of 5 sublethal responses) in the 100% Ho-clay treatment.

Ho appeared to desorb from Ho-clay in the toxicity tests, but not very readily. Dissolved Ho was measured in water from beakers with Ho-clay, but not in proportion to the amount of Ho-clay in the sediment. Whereas concentrations of Ho reached only as high as 17 µg/L in water overlying sediment with up to 50% Ho-clay, in the 100% Ho-clay treatment beakers concentrations ranged from 116 to 954 µg/L (Fig. 3; note log-scale). In static test conditions with low water:sediment volume ratios (as in these tests), concentrations of metals in overlying waters probably reflect concentrations in porewater of sediment (Borgmann and Norwood 1999). Because concentrations of metals in porewater are often better related to toxicological responses of benthic invertebrates than bulk metal concentrations in sediment, elevated toxicity observed in the 100% Ho-clay treatment may be explained by highly elevated dissolved Ho levels. Sediment mixtures with ≤50% Ho-clay may not be toxic because Ho is not bioavailable to the test organisms.

LC50s for Ho in water were estimated to be 35.0, 52.2, >230 and >542 µg/L for *Chironomus*, *Hyalella*, *Hexagenia* and *Tubifex*, respectively. Although the exposure times were not equal for all the tests, they do provide a tentative ranking of relative sensitivities to Ho. The oligochaete

worm, *Tubifex tubifex*, the least sensitive of the taxa tested, is known to be tolerant to a variety of contaminants and environmental stressors, and was not adversely affected by the 100% Ho-clay exposure.

The only known published Ho toxicity test with a benthic invertebrate was conducted by Borgmann et al. (2005). The test was conducted on *Hyalella azteca* with a 7-day, water only exposure to Ho from a 2% HCl solution. Test conditions were otherwise similar to those of the present study, including the use of dechlorinated Lake Ontario tap water. Tests were also conducted with tap water diluted to 10% with deionized water. The trimmed Spearman-Karber LC50 estimated by Borgmann et al. for *Hyalella* was 755 µg/L based on the nominal Ho concentration. If measured Ho concentrations (which were not available) were used for the estimate, the LC50 would undoubtedly be lower, perhaps about 220 µg/L based on the ratio of measured:nominal Ho LC50s obtained for the softwater tests. Our estimated LC50 for *Hyalella* exposed to Ho for 4 times the duration of Borgmann et al.'s test is about 25% of this LC50. However, the presence of sediment in our test complicates the comparison.

Risk of Ho-clay to natural surface waters

Overall results of the toxicity tests suggest that potential impacts of Ho-clay released into natural waters would be expected only where Ho-clay persists in sediment at proportions >50% for at least several days. However, the extrapolation of laboratory toxicity test results to predict *in situ* effects of contaminants is limited by differences between the two environments in various conditions, including the spatial and temporal scales of exposure to contaminants, physicochemical factors that affect contaminant transportation and fate, and the composition and organization of the exposed biological community.

Exposures of Ho-clay in the toxicity tests likely represent worst-case conditions in natural water bodies. A field Ho-clay exposure would likely be a short term pulse rather than a continuous press as in toxicity tests. Spatially, discharges of Ho-clay should be diluted exponentially across the receiving environment. Thus, the duration and area of high Ho-clay concentration and, consequently the degree biological impact, should be restricted. Our observed toxicity test responses, therefore, are likely to overestimate field impacts to benthic invertebrates.

The toxicity tests in this assessment involved several species, multiple endpoints (including lethal and sublethal responses), and exposures to sediment over a large fraction of the organisms' generation times, all of which improve the ecological relevance of the results. Responses of several taxa occupying different microhabitats and feeding niches involve more contaminant exposure pathways than responses in single species tests. Measurements of lethal and sublethal endpoints provide a broad characterization of ecotoxicological responses. Exposure of a range of life stages to Ho-clay improves the toxicity assessment by integrating potential age- and size-related variability in organism sensitivity.

Although conditions of benthic invertebrates are commonly examined in sediment assessments, their responses to contaminants and other stressors are not necessarily indicative of those of other biological groups, such as microbial communities, algae and fishes. Some contaminants, such as polycyclic aromatic hydrocarbons and contaminants that biomagnify, are rarely toxic to benthic invertebrates at concentrations that affect can fishes and other vertebrates (e.g., Fuchsman et al. 2006). Also, testing of organisms alone in artificial conditions isolated from the receiving environment, does not allow observations of interactions and other complex effects that can occur in the field (Clements 1997). Further assessment of Ho-clay effects should, therefore, involve tests with other biological groups and exposures in intact natural ecosystems.

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Table 1. Survival, growth and reproduction of macroinvertebrate test organisms after exposure to sediment treatments in Ho-clay toxicity test.

Treatment	Replicate	*Chironomus riparius*		*Hexagenia* spp.		*Hyalella azteca*		*Tubifex tubifex*			
		Survival (%)	Growth (mg/indiv.)	Survival (%)	Growth (mg/indiv.)	Survival (%)	Growth (mg/indiv.)	Survival (%)	#cocoons/ adult	% hatched	young/ adult
Laboratory control sediment	1	80.0	0.462	100	5.22	46.7	0.184	100	10.8	55.8	32.0
	2	80.0	0.555	100	4.56	100.0	0.218	100	11.0	52.3	32.0
	3	100.0	0.563	100	4.64	73.3	0.242	100	12.3	61.2	30.8
100% Pond sediment	1	66.7	0.890	100	9.17	100.0	0.933	100	10.0	57.5	14.8
	2	86.7	0.846	100	9.29	100.0	0.754	100	10.3	56.1	14.3
	3	93.3	0.816	100	10.15	100.0	0.743	100	10.0	47.5	12.5
100% clay	1	86.7	0.702	90	1.03	73.3	0.339	100	10.5	64.3	25.8
	2	100.0	0.655	100	1.51	100.0	0.283	100	8.5	64.7	20.0
	3	93.3	0.661	100	1.11	100.0	0.309	100	9.8	64.1	26.3
10% Ho-clay / 90% Pond sediment	1	86.7	0.878	100	7.86	86.7	0.649	100	10.5	59.5	13.0
	2	100.0	0.665	100	8.80	86.7	0.967	100	10.8	58.1	15.0
	3	73.3	0.745	100	8.58	93.3	0.737	100	10.5	64.3	14.0
25% Ho-clay / 75% Pond sediment	1	100.0	0.591	100	7.91	100.0	0.891	100	9.0	63.9	14.3
	2	86.7	0.685	100	6.76	93.3	0.810	100	9.8	61.5	14.3
	3	86.7	0.801	90	7.48	100.0	0.853	100	10.3	58.5	15.8
50% Ho-clay / 50% Pond sediment	1	66.7	0.940	90	7.67	93.3	0.734	100	11.0	61.4	22.5
	2	80.0	0.680	100	7.11	100.0	0.751	100	12.0	60.4	20.3
	3	93.3	0.788	100	6.85	100.0	0.773	100	10.5	61.9	17.5
100% Ho-clay	1	0	0	60	0.52	0	0	100	0.3	100.0	0.3
	2	20.0	0.047	30	0.02	0	0	100	0	0	0.3
	3	0	0	70	-0.03	6.7	0.55	100	0	0	0

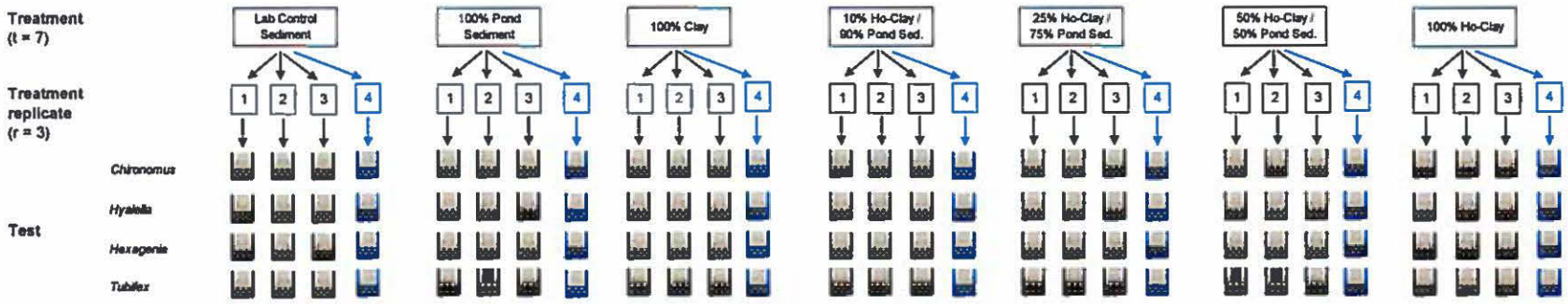

Figure 1. Experimental design for Ho-clay toxicity tests. Responses of test organisms were observed from three replicate experiment units per test. The fourth replicate was sampled for water and sediment for analyses of Ho concentrations.

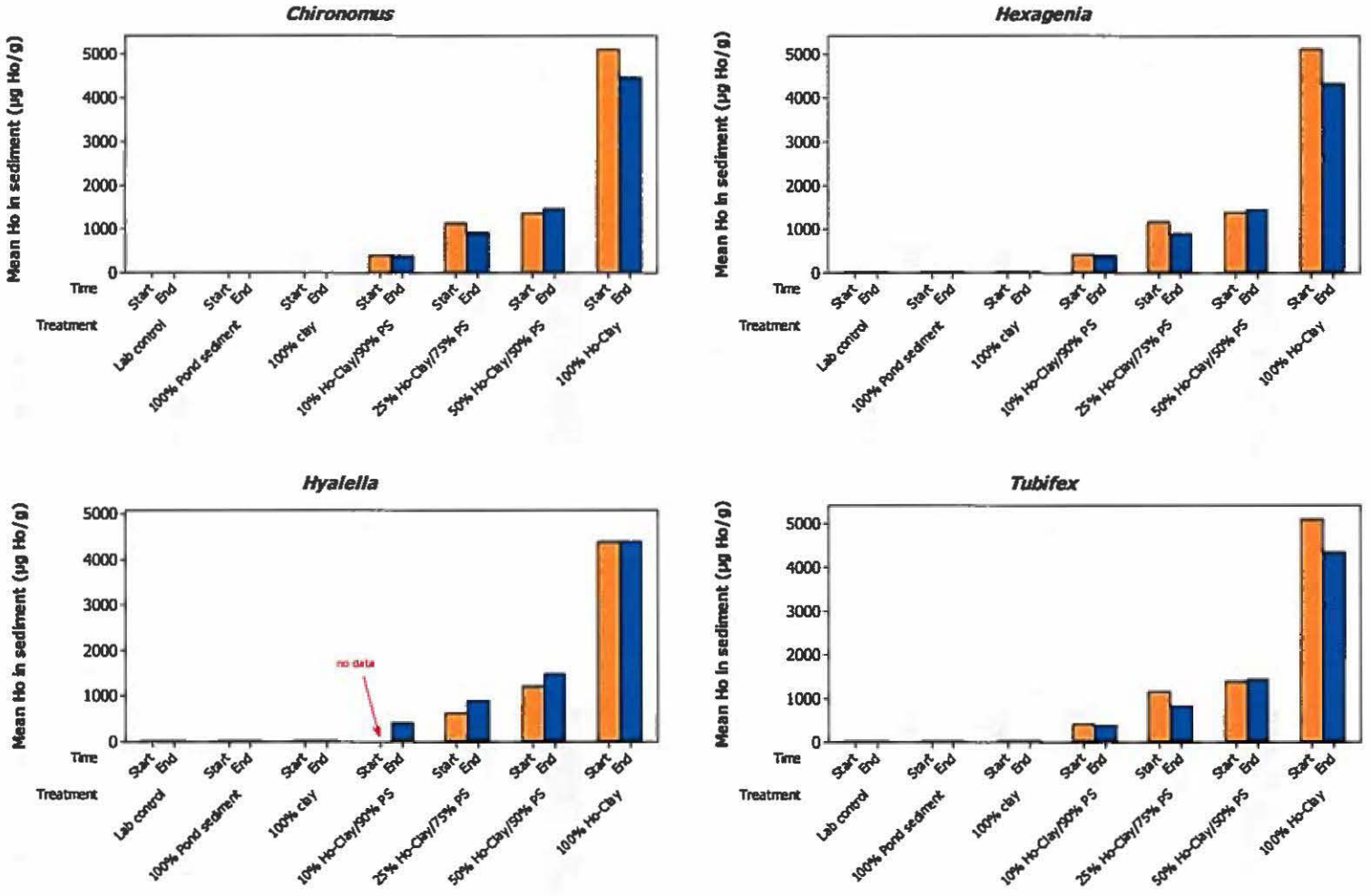

Figure 2. Measured concentrations of Ho in sediment of beakers (mean of duplicate samples) at start and end of toxicity tests for exposures of seven sediment treatments to four test organisms.

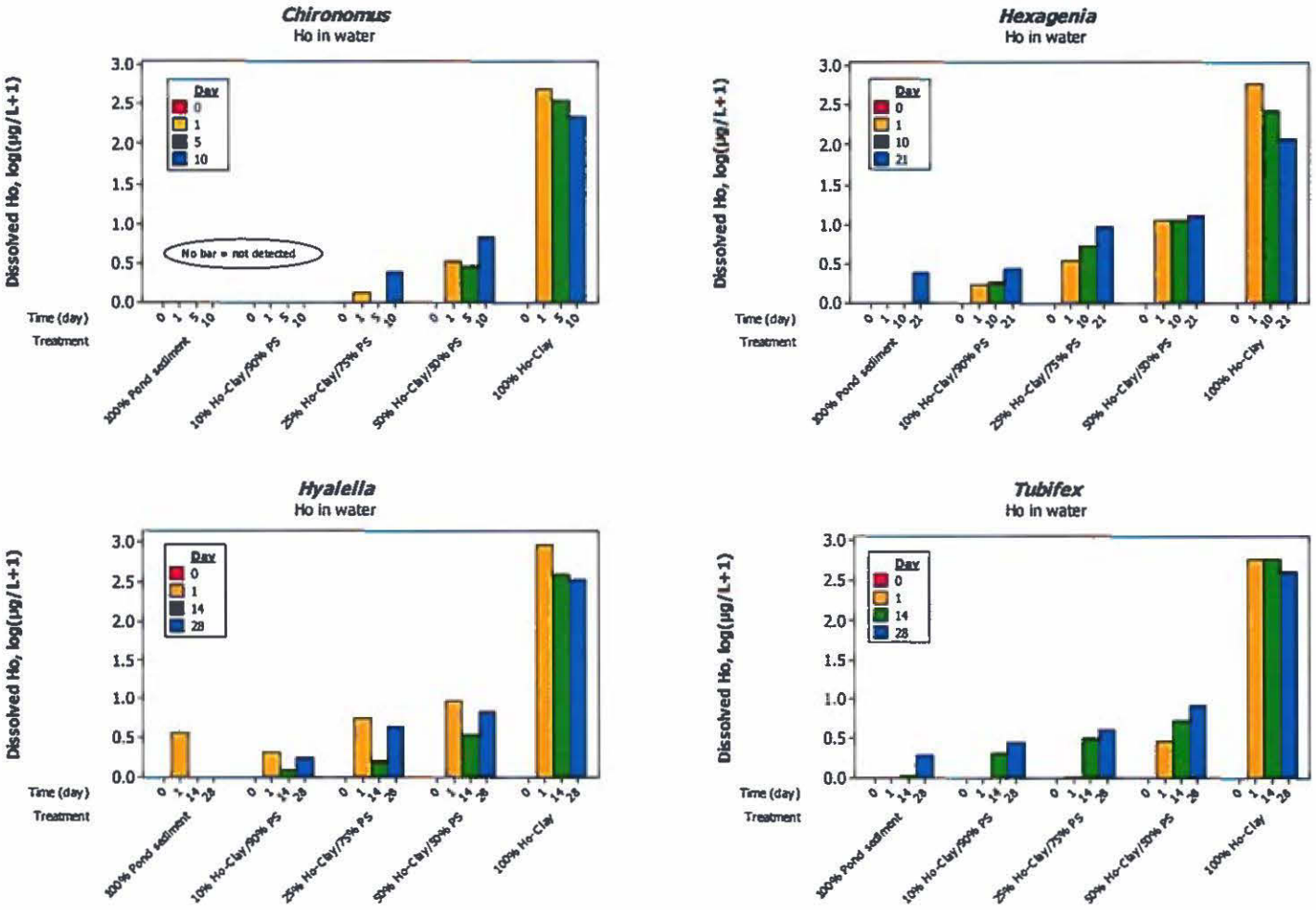

Figure 3. Measured concentrations of Ho in filtered water from beakers (mean of duplicate samples) at start, middle and end of toxicity tests. Note log-scale for Ho concentration.

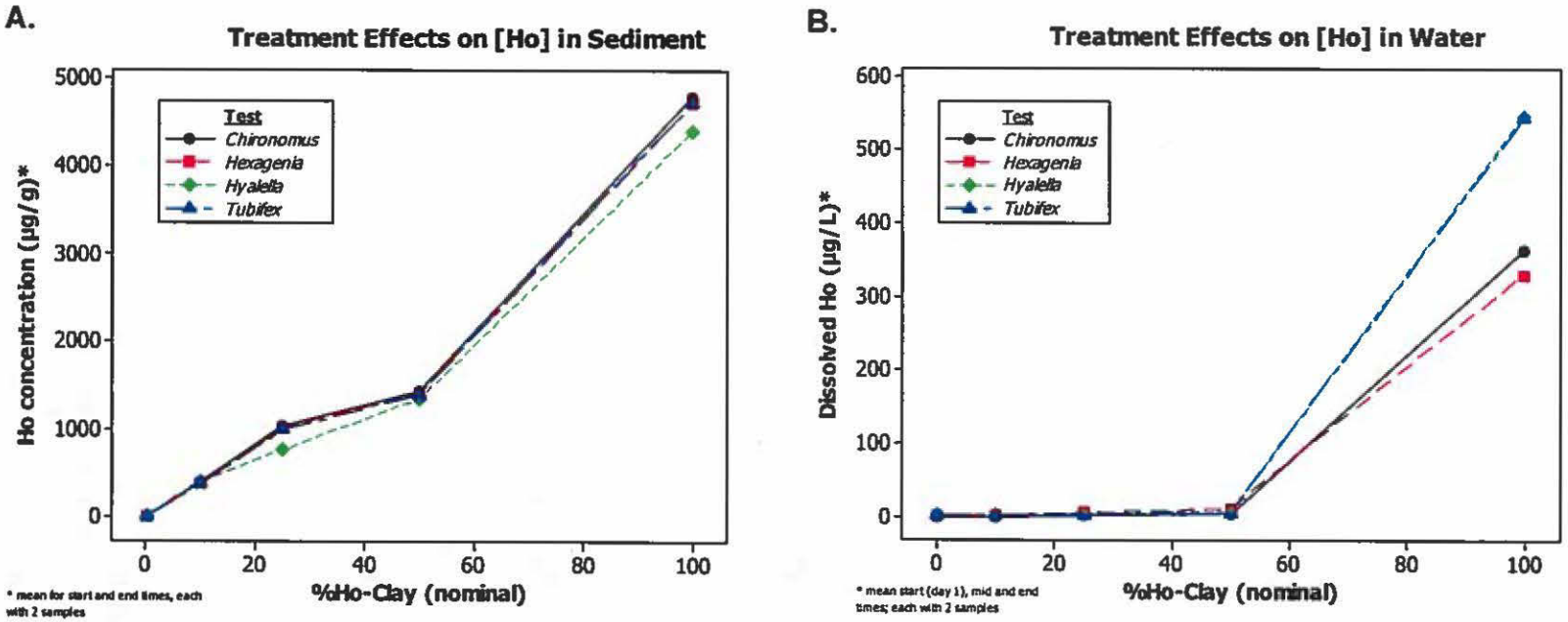

Figure 4. Concentrations of Ho in sediment (A) and overlying water (B) of toxicity test in relation to percent of Ho-clay in treatment sediment. Concentration values are means of all samples, except the day 0 water sample.

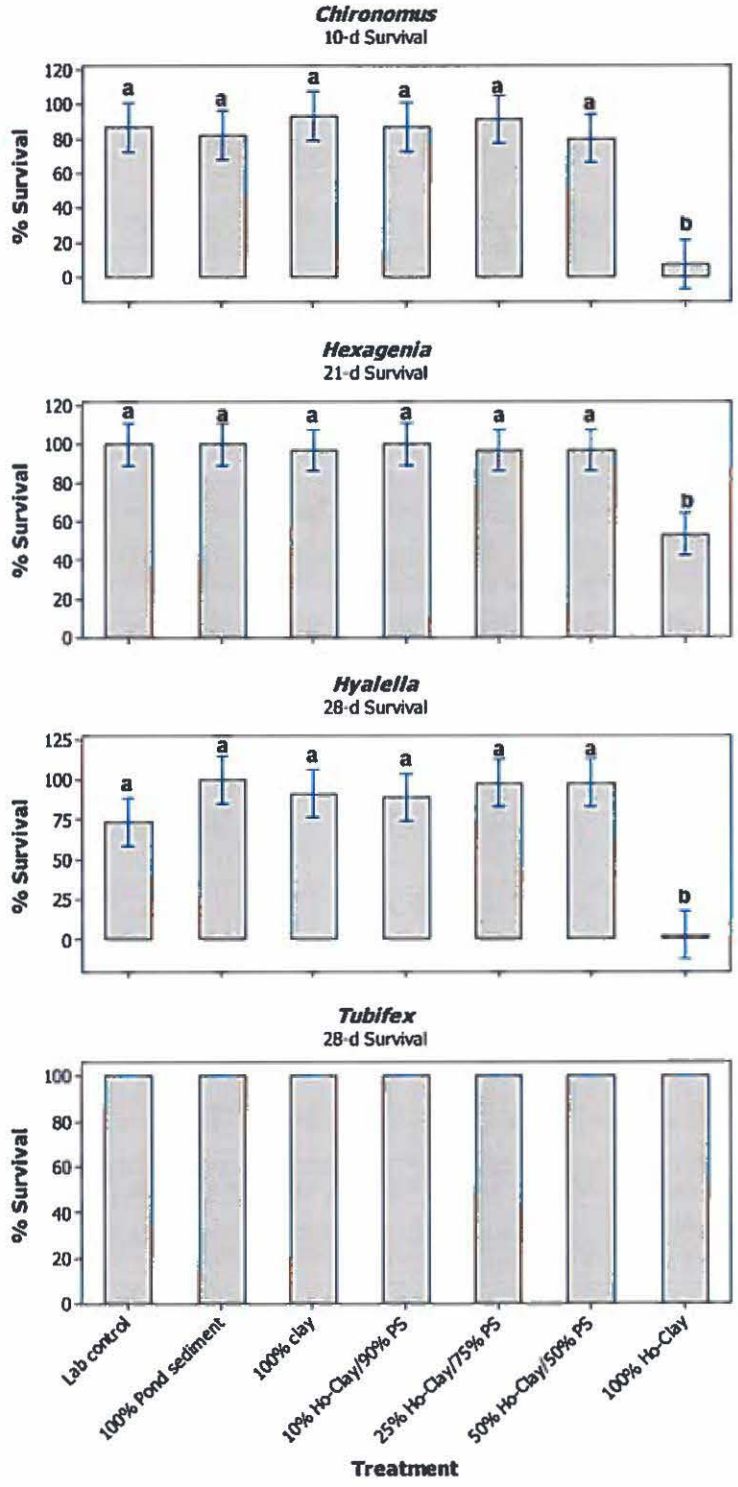

Figure 5A. Effects of seven sediment treatments on survival of four benthic invertebrates in toxicity tests. Bars are means for treatment replicates with pooled-error 95% CIs. Bars labelled with different letters are significantly different by Tukey comparisons with family error rate α=0.05.

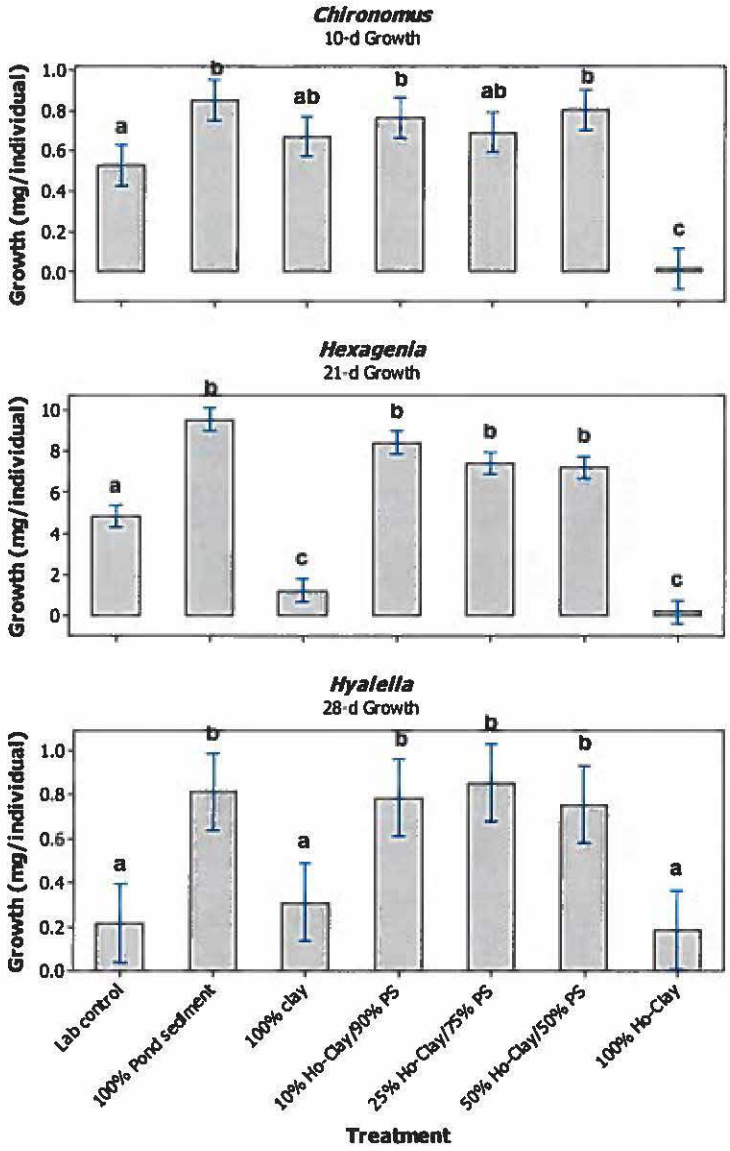

Figure 5B. Effects of seven sediment treatments on growth of three benthic invertebrates in toxicity tests. Bars are means for treatment replicates with pooled-error 95% CIs. Bars labelled with different letters are significantly different by Tukey comparisons.

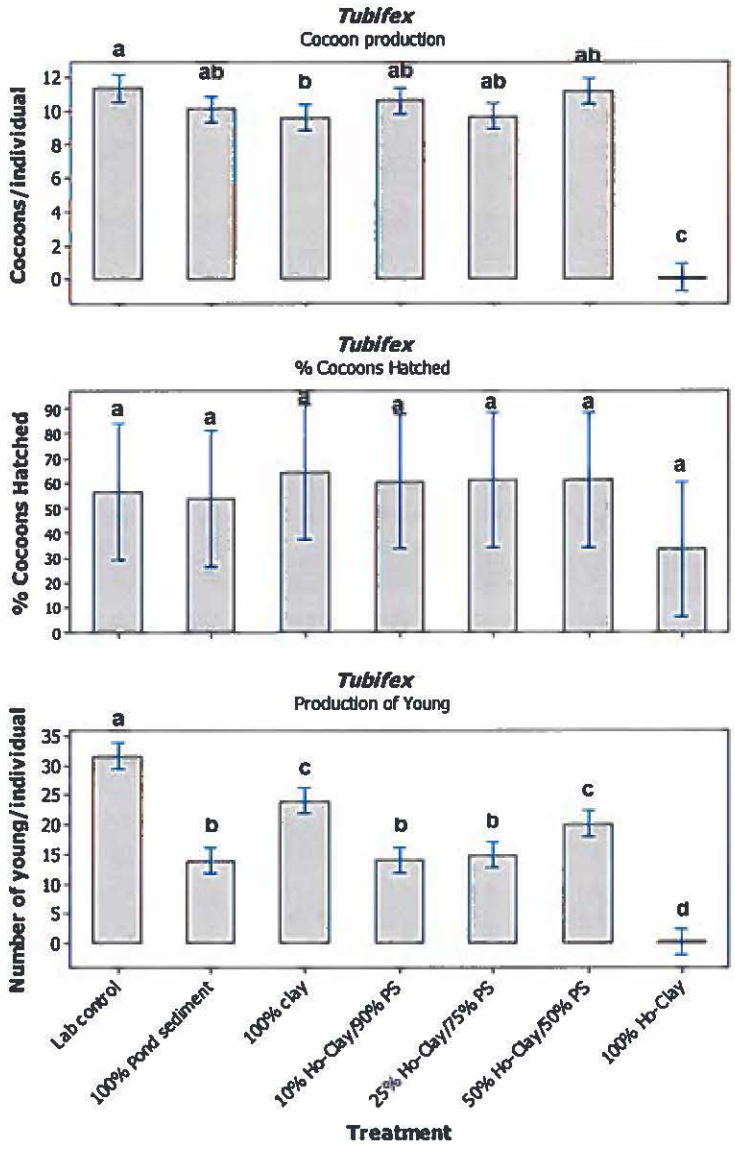

Figure 5C. Effects of seven sediment treatments on reproduction of *Tubifex* in toxicity tests. Bars are means for treatment replicates with pooled-error 95% CIs. Bars labelled with different letters are significantly different by Tukey comparisons.

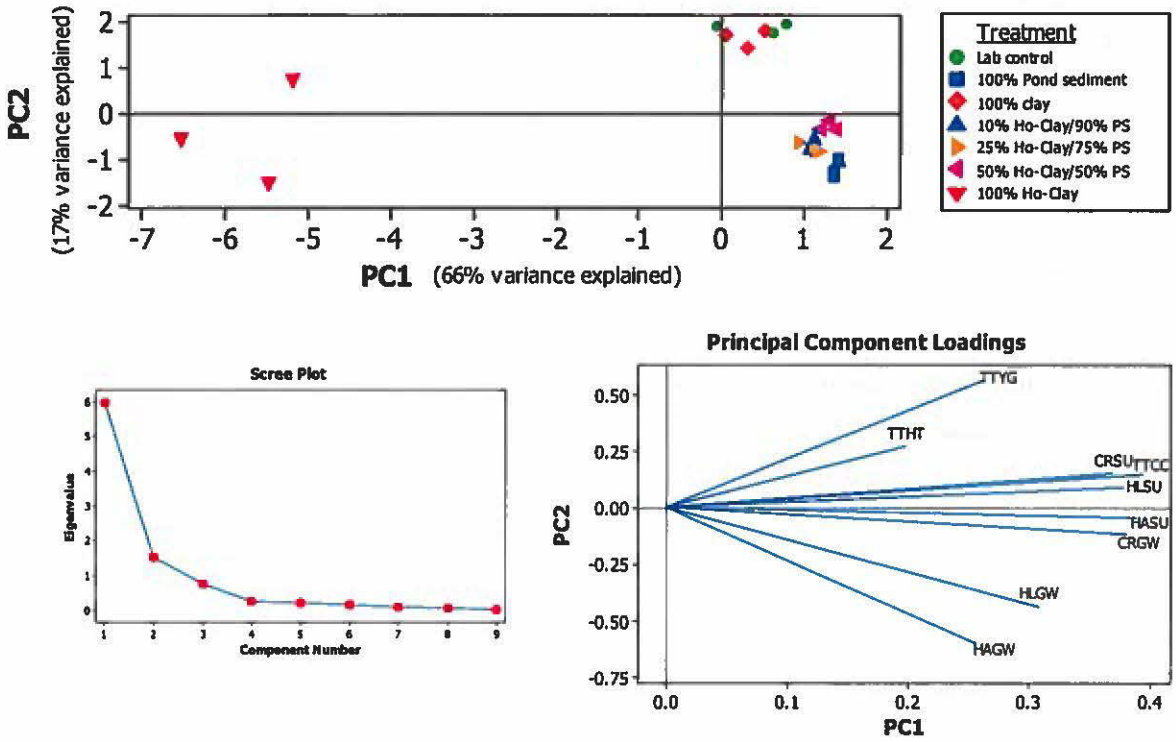

Figure 6. Results of PCA of nine toxicity endpoints from Ho-clay toxicity tests. Upper figure shows variation among treatment replicates in scores for the first two principal components. Scree plot shows amount of variance explained by each component. Loadings plot (lower right) relates the measured variables to the component variables. Endpoint abbreviations: CRSU = *Chironomus* survival; CRGW = *Chironomus* growth; HASU = *Hyalella* survival; HAGW = *Hyalella* growth; HLSU = *Hexagenia* survival; HLGW = *Hexagenia* growth; TTSU = *Tubifex* survival; TTCC = *Tubifex* cocoons/adult; TTHT = *Tubifex* % hatch; TTYG = *Tubifex* young/adult.

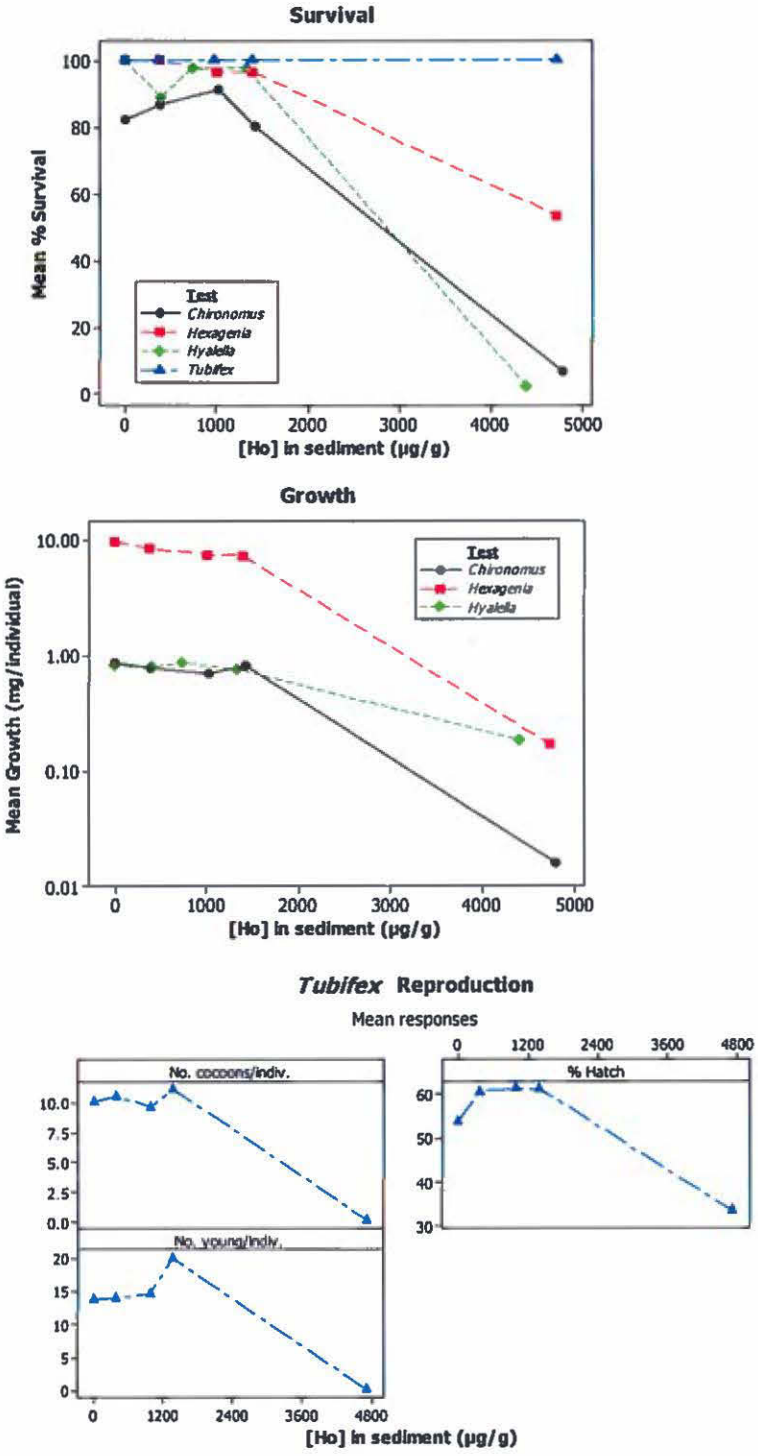

Figure 7. Relationships between toxicity endpoints and Ho concentration in sediment from test beakers for Ho-clay and pond sediment treatments. Note log scale for growth.

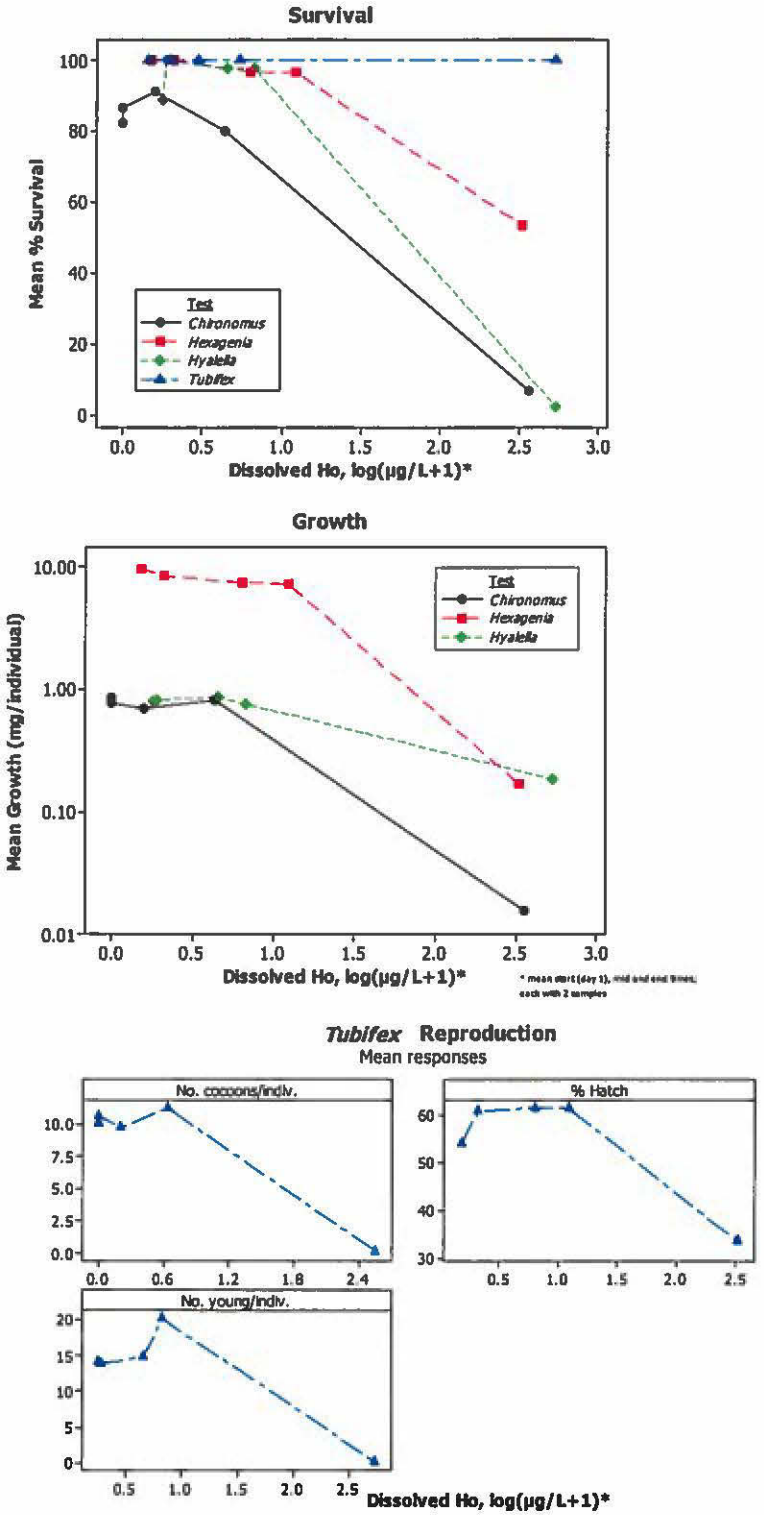

Figure 8. Relationships between toxicity endpoints and Ho concentration in overlying water from test beakers for Ho-clay and pond sediment treatments. Note log scales for growth and dissolved Ho concentration.