Meiofaunal and benthic foraminiferal response to lead

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The response of cultured meiofaunal and benthic foraminiferal communities to lead contamination: results from mesocosm experiments

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Abstract

Lead (Pb) mimicking other biologically essential metal has been regarded as a very toxic element and poses serious threat to biota. A mesocosm experiment has been implemented to assess the influence of Pb on meiofaunal and benthic foraminiferal communities. To this end, sediments bearing such communities were incubated in mesocosm, exposed to different levels of Pb in seawater and monitored up to eight weeks. Concentrations of Pb below 1 mg/L in water do not promote a significant increase of this metal in sediments. Relatively high concentrations of Pb seem to affect meiofaunal and benthic foraminiferal communities by reducing their richness or diversity and the sensitive behavior of most taxa can be defined. The mesocosm approach is here considered as an effective method to document the responses of meiofaunal and benthic foraminiferal communities to various kinds and concentrations of pollutants over time and validating the field study outcomes.

Keywords: meiofauna, foraminifera, lead, mesocosm

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1. INTRODUCTION

Increasing human activities have deeply impacted marine and estuarine ecosystems, affected living organisms therein and degraded the environment quality. Because of the toxicity, bioaccumulative, and non-biodegradable nature, trace elements, also known as heavy metals, might pose a serious threat to organisms (Stankovic et al. 2014). Among them, lead (Pb) has been regarded as very toxic and easily exposed and is of very great concern as it mimics other biologically essential metals (Lidsky and Schneider 2003; Flora et al. 2012). In light of it, Pb represents a toxic element for biota even at low concentrations (Sousa Bispo et al. 2002). Although Pb seawater concentrations range from 0.002 to 0.2 μg/L in open ocean water, concentrations greater than 1 μg/L might be found in coastal area due to natural sources and anthropogenic activities (Neff 2002; Lavilla et al. 2011). Lead in water is then prone to be sorbed to suspended solids and sediments, where the latter represents one of the most important sink. Accordingly, sediments contain considerably higher levels of Pb than surface waters that vary, in coastal sediments, up to 912 mg/kg with a mean value of 87 mg/kg (EPA 1982; Nriagu 1978). The effects range low (ERL) and effects range median (ERM) of Pb concentrations in sediment are 46.7 and 218 ppm, respectively (Long et al. 1995). Thus, the understanding of the effects of heavy metals, and specifically of Pb, on biota is particularly important and can be pursued through different approaches: field studies (i.e. monitoring programs), laboratory cultures (i.e. exposure of a single species to pollutants) and micro- and mesocosm experiments (i.e. exposure of sediments and the entire community living therein to pollutants). The latter approach represents a very effective and direct method to assess the effect of a single parameter (i.e. pollutant) on the biota through different concentrations and time (Frontalini et al. 2018). In fact, micro- and mesocosms experiments are intended to reduce and possibly eliminate the temporal and spatial environmental variability allowing the investigators to focus on one or a combination of variables (i.e. pollutant) and to establish cause-and-effect relationships.

Meiofaunal organisms are known to play a key role in the benthic ‘small food web’ as well as to be a trophic source for pelagic organisms through juvenile fishes or epibenthic crustaceans (Zeppilli et al. 2016). Fichet et al. (1999) observed that meiofauna, and in particular nematodes may be an important route for metals transfer from sediment to living resources through the food web. The release of bioavailable metals represent a risk to the biota (Amiard et al. 1995). However, only a relatively limited number of meiofaunal and foraminiferal micro- and mesocosm experiments have been conducted so far on heavy metals (e.g. Austen and McEvoy 1997; Gustafsson et al. 2000; Ernst et al. 2006; Gyedu-Ababio and Baird 2006; Hedfi et al. 2007; Mahmoudi et al. 2007; Hermi et al. 2009; Beyrem et al. 2011; Boufahja et al. 2011; Frontalini and Coccioni 2012; Frontalini et al. 2018) and a low number of them was focused on the effects of Pb (i.e. Austen and McEvoy 1997; Millward et al. 2001; Gyedu-Ababio and Baird 2006; Mahmoudi et al. 2007).

As an example, Austen and McEvoy (1997) treated offshore meiobenthic communities with different levels of Pb (up to 1580 μg/g) and documented significant variations on meiofauna at medium concentrations of Pb (1343 μg/g). A reduction in meiofaunal diversity and nematodes density and diversity was related to higher concentration of Pb (Gyedu-Ababio and Baird 2006). Similar results with a reduction of diversity, density, evenness and alteration of the composition of the nematode assemblages was observed to high concentration of Pb in a microcosm experiment (Mahmoudi et al. 2007).

The main aim of the present paper is to document the response of a meiofaunal community incubated in mesocosm when exposed to selected concentrations of Pb.

2. MATERIALS AND METHODS

2.1 Sampling and experiment setup

Sediment collection was at 14 m water depth site (43°33'54" N, 13°39'52" E) in the coastal area off the Mt. Conero (central Adriatic Sea) characterized by oligo-mesotrophic conditions and low influence of human activity (Frontalini and Coccioni 2008). Physico-chemical parameters, namely temperature, pH, salinity, Eh and dissolved oxygen of water were measured by using a multiparametric CTD (Conductivity, Temperature and Depth) probe in vertical profile. Sediments were collected by multiple deployments of Van Veen grab and the sampling of the uppermost part of sediment (ca. 2 cm). Once on board, sediment was highly homogenized and sieved over a 500-µm sieve tissue to remove bioturbators. The remaining fraction was placed in an insulated box covered by ambient seawater, and kept near ambient temperature until arrival at our shore-based laboratory.

Artificial Sea Water (ASW) was prepared following the methods of Ciacci et al. (2012), stored in the dark, aerated and mixed under in situ temperature. A total of seven Pb-ASW concentrations plus control were prepared. Lead (II) chloride ($PbCl_2$, CAS Number 7758-95-4, Sigma-Aldrich) 98% pure was used for stock solutions. The final pollutant concentrations for experimental media were obtained by adding appropriate volumes of stock solutions to ASW. The selected concentrations were control (ctrl), 10, 100, 200, 500 µg/L (ppb) and 1, 5, 10 mg/L (ppm). Eight tanks (aquarium, 60 cm X 40 cm X 20 cm) were filled with approximately 20 L of the Pb-ASW solutions. A total of twelve mesocosms (15 cm x 8 cm x 3 cm) containing 1 cm thick sediment were placed inside each tank (Fig. 1). Multichannel pumps were used to circulate and to oxygenate water through silicone rubber tubing anchored between the tanks' bottom and plastic grids. Tanks were placed in a controlled environment with air temperature of 14-16 °C, uniformly maintained throughout the experiment. Dissolved Oxygen (DO), Salinity (S), Temperature (T), Oxidation Reduction potential (ORP) and pH of the seawater were routinely monitored by a set of HQ40d portable multi-parameter probes.

2.2 Subsampling

From each mesocosm and at every sampling time (one week, T1; two weeks, T2; three weeks, T3; four weeks T4, six weeks T5 and 8 weeks, T6), ca. 50 cm^3 of sediment and 3+3 replicates of 10 cm^3 of sediment were collected from each mesocosm for chemical, meiofaunal and foraminiferal analyses, respectively. Additionally, 50 mL of water from each tank was also sampled.

Water sample was placed into 50-mL centrifuge tube, immediately acidified with 50 µL of nitric acid 65% and refrigerated at 4 °C until chemical analysis. Sediment sample was placed into 50-mL centrifuge tube and frozen upon collection. Three replicates of 10 cm^3 of sediment were treated with a 2g/L Rose Bengal solution and used for foraminiferal analyses whereas the other 3 replicates were treated with a 7% $MgCl_2$ aqueous solution for narcotizing fauna, fixed in a 4% formaldehyde solution in buffered sea-water, and stained with Rose Bengal (2 g/L).

2.3 Lead analyses in water and sediment

The methodological description of analyses for lead concentrations in water and sediment was reported in Maccotta et al. (2016). Briefly, sediment was dried in an oven at 40 °C for 48 h, powdered, and digested (HNO_3–H_2O_2–HF). A Perkin Elmer AAnalyst 800 atomic absorption spectrometer with graphite furnace was used to measure Pb concentrations in water and sediments.

2.4 Meiofaunal and foraminiferal analyses

Only samples collected at T1, T2, T4 and T6 for control and 100 µg/L, 1 mg/L and 10 mg/L were considered for meiofaunal and foraminiferal analyses.

In the laboratory, samples for meiofauna were carefully washed through a 42 µm sieves for retaining the meiofaunal component (Frontalini et al. 2014). The resulting fraction was used to extract meiobenthos using the Ludox HS30-flotation method (Semprucci et al. 2014). All the meiofaunal organisms were sorted and counted into major taxa (mainly Phylum and Order level of rank) under a stereomicroscope (Leica G26) from the three replicates of each mesocosm. Temporary slides were prepared for soft-body meiofaunal groups (e.g. Platyhelminthes, Nemertea) to obtain an exact identification under a 100x oil immersion objective using Nomarski Differential Interference Contrast illumination (Optiphot-2 Nikon). The richness (number of taxa) was calculated at major taxon level.

Foraminiferal samples treated with rose Bengal were gently washed through 63 µm sieve to remove any excess stain, and were then oven dried at 50°C. All samples and replicates were used for benthic foraminiferal counts on >63 µm fraction. Benthic foraminiferal specimens were taxonomically identified following Cimerman and Langer (1991). The following indices were calculated: Shannon-Wiener diversity index (H', log2) and Pielou-evenness (J').

2.5 Statistical analyses

Statistical analyses were performed on the relative abundances of the meiofaunal major taxa and foraminiferal species. A Principal Component Analysis (PCA) was performed to determine the meiofaunal and foraminiferal community's responses to the increasing Pb concentrations and progressive exposure time. Prior to PCA, all of the biotic and abiotic data were normalized by applying an additive logarithmic transformation log(1+X). For foraminifera, only taxa with a relative abundance exceeding 3% were taken into consideration. Even if rare species, commonly considered the most sensitive ones, were down-weighted in this way, their contribution was accounted by means of the calculation of Shannon and Pielou indices. In detail, the relative abundances of the benthic components were projected on the factor plane as primary variables, while Pb concentration of both water and sediment matrices were used as secondary variables without contributing to the results of the analysis. These statistical tests are performed using STATISTICA v.8 computer program. Analysis of Similarity (ANOSIM) was used to test the significance of the differences between treatments. A transformation log(1+X) and Bray-Curtis similarity measure were applied to the data of both meiofaunal and foraminiferal communities. The multivariate data analysis followed the methods described by Clarke and Gorley (2006) using the PRIMER Version 5 software package.

3. RESULTS

3.1. Physico-chemical parameters and Pb concentrations

Physico-chemical parameters remained quite constant throughout the experiment. The mean value of salinity was 36.9‰ with a slight increase (ca. 1‰) during the experiment. The mean DO value in the tanks was 9.37 mg/L with some fluctuations. A significant decrease of Pb concentrations in water was mirrored by an increase in the sediment. Very high values of Pb in sediments were associated with 10 ppm at T4 (38 ppm) and T6 (127.5 ppm) with the latter exceeding the ERL (Fig. 2).

3.2 Meiofauna

Total meiofaunal abundance varied from 189±45 ind./10 cm^3 at T6 1 ppm to 371±41 at T1 ctrl ind. 10 cm^3. A total of 12 meiofaunal taxa was identified in the study area: Foraminifera, Platyhelminthes, Nematoda, Kinorhyncha, Copepoda, Crustacea *nauplii*, Ostracoda, Polychaeta, Oligochaeta, Bivalvia, Gasteropoda and Halacaroidea. Among them, the dominant taxa were Nematoda (75.1±10.3%) and Foraminifera (23.6±10.7%), all the other taxa showed very low percentages (<1%). Meiofaunal richness (namely number of taxa) was lower at T4 100 ppb and T6 100 ppb and (3 taxa) and higher at T1 1ppm (8 taxa) and T1 ctrl (7 taxa).

3.3 Benthic foraminifera

A total of 26 benthic foraminiferal taxa was recognized in the studied samples. The most abundant taxa were *Ammonia parkinsoniana* (50.0%, on average), *Ammonia tepida* (26.6%, on average), *Eggerelloides scaber* (5.9%, on average), *Aubygnina perlucida* (3.6%, on average), *Haynesina depressula* (3.1%, on average), *Bolivina spathulata* (2.8%, on average), *Elphidium advenum* (2.0%, on average), *Bulimina elongata* (1.5%, on average) and *Bolivina striatula* (1.3%, on average). The H' varied from 0.88 T6 1 ppm to 1.88 T1 ctrl. The J values ranged from 0.59 T6 1 ppm to 0.75 T2 ctrl.

3.4 Statistical analyses

ANOSIM revealed a significant difference of the meiofaunal community between samples (Global R=0.40; p=0.001). The PCA based on meiofaunal taxa revealed that ~53% of total variance (inertia) can be explained by the first two principal components (factors) (Fig. 3a). These exhibit eigenvalues greater than one and have therefore been considered. Most of the meiofaunal taxa and specifically Nematoda, Kinorhyncha, Polychaeta, and Platyhelminthes as well as meiofaunal richness showed an opposite trend of the Pb concentration in sediment. The only taxon exhibiting a positively relation to Pb concentration in sediment was Ostracoda. Bivalvia appeared to be negatively related to both Pb water and sediment contents, whereas a positive relation was found between Pb content in water and Gasteropoda, Copepoda, Polychaeta and *nauplii*. When projecting samples on the factor plans, most of the Pb-enriched samples were placed in the negative part of the first component (Fig. 3b). ANOSIM revealed a significant difference of the Foraminifera community between samples (Global R=0.33; p=0.001). The PCA based on foraminiferal taxa showed that ~52% of total variance (inertia) can be explained by the first two principal components (factors) (Fig. 3c). Similar to meiofaunal taxa, most of the benthic foraminiferal species as well as H’ and J exhibited negative relations with Pb concentration in sediment. Only *A. tepida* and *B.*

spathulata appear to be positively related to Pb concentration in sediment. The most Pb-enriched samples were located in the positive values of the first component (Fig. 3d).

4. DISCUSSION

Lead (Pb) has been considered as a very toxic and is of particular concern by mimicking other biologically essential metals (Lidsky and Schneider 2003; Flora et al. 2012). In our experiment, very high nominal concentrations (i.e. 1 and 10 ppm that is mg/L) were used for testing the response of meiofaunal and benthic foraminiferal assemblages. These concentrations were much higher than those found in open ocean water (0.002 to 0.2 μg/L) or coastal area (>1 μg/L) (Neff 2002; Lavilla et al. 2011). The choice of the targeted concentration was driven to ensure a real enrichment in the sediments where our considered biota live. In fact, Pb is absorbed to suspended solids and sediments, where the latter represents one of the most important sink. The initial concentration of Pb in sediment, in our experiment, was 13.2 mg/kg that was lower than the Italian Sediment Quality Guidelines (LCB, chemical base level 37 mg/kg for mud over 25% and LCL chemical limit level 70 mg/kg) or the Effect Range Low (46.7 mg/kg) (Long et al. 1995). Following the available data and interpretation of Maccotta et al. (2016), a clear temporal evolution of Pb release in seawater and absorption in sediment was observed (Fig. 2). Concentrations lower than 1 mg/L in seawater did not lead to any appreciable increase in sediments as these concentrations were comparable lower by order of magnitude than the initial Pb levels naturally present in the sediments. A significant decrease in Pb concentrations of seawater coupled with a concurrent increase of Pb in sediment was evidenced at T3 that was four weeks after the beginning of the experiment. In particular, higher concentrations than 20 mg/kg in sediments were found for 1 ppm experiment at T4 and T6 and for 10 ppm one at T1 and T2. The highest concentrations were confined for 10 ppm experiment at T4 (38 mg/kg) and T6 (127.6 mg/kg) that were both higher of the Italian thresholds. In lights of it, only samples retrieved from 10 ppm tank at T4 and T6 could be considered polluted (P) whereas all the other samples were regarded as unpolluted (UP) (Fig. 2).

The investigated communities, namely meiofauna and benthic foraminifera, resulted negatively affected over time and exposure to the lead treatment. In particular, meiofaunal structure appears negatively affected by the increase of Pb in the sediment, with the exception of Ostracoda that seem to be positively correlated with Pb increasing. Ostracoda are generally reported as sensitive to environmental stress, but several species have been documented to have adaptive behaviors to numerous environmental changes (Mirto et al. 2012; Vandekerkhove et al. 2013). Contrary to the general tolerance of Nematoda observed in field studies (Mirto et al. 2004; Semprucci et al. 2015), their abundances appeared directly and negatively affected by Pb concentrations in sediment. This trend is in agreement with the results of other microcosm experiments that documented a significant decrease of nematodes in relation to the increased trace element concentrations (Gyedu-Ababio and Baird 2006; Hedfi et al. 2007; Boufahja et al. 2011; Chaaban Santos et al. 2018). Recent studies on *Caenorhabditis elegans* have highlighted that this species avoids food spots even containing low concentrations of Pb that likely interferes with its food finding (Monteiro et al. 2014). Kinorhyncha, Polychaeta and Platyhelminthes are also negatively affected by Pb. In particular, Kinorhyncha are recognized as a very sensitive taxon to anthropogenic stress (Gyedu-Ababio and Baird 2006; Mirto et al. 2012; Dal Zotto et al. 2016). In our samples, Kinorhyncha are mainly represented by *Pycnophyes communis* that, given the negative correlation found with Pb, may be regarded as a *k*-strategist species for Pb impact. Dal Zotto et al. (2016) proposed the Nematoda/Kinorhyncha (Ne/Ki) ratio as a tool to assess the human impact because these taxa have an opposite auto-ecological behavior. However, the trends observed in our experiment suggest that Ne/Ki ratio cannot be applied to detect the trace element effects on the meiobenthic compartment given that both taxa decrease with its enhancement. Nematoda and Foraminifera are removed from the meiobenthos community to track differences between unpolluted (UP) and polluted (P) samples (Fig. 4). Among the minor groups, Copepoda (36%), Platyhelminthes (30%), Kinorhyncha (22%), followed by Bivalvia (5%), Ostracoda, crustacean nauplii and Polychaeta (2%) characterized the UP samples, while Copepoda (47%), Platyhelminthes (28%), Kinorhyncha (19%), followed by

Ostracoda (5%) characterized the P samples (Fig. 4). Decreases in relative abundance of Platyhelminthes and Kinorhyncha were documented in P samples. Meiofaunal richness showed a clear decline in the samples that showed the highest Pb concentrations (Fig. 4). Similarly, a reduction of diversity and abundance and changes in meiofaunal community structure (Nematoda) were observed in a microcosm experiment treated with several heavy metals (i.e. Cu, Zn, Cd and Pb) (Austen and McEvoy, 1997). Four targeted concentrations were selected for Pb that are from 56 µg/g that is mg/kg (control), 247 mg/kg (low), 1343 mg/kg (medium) and 1680 mg/kg (high) (Austen and McEvoy, 1997). Their concentrations were much higher than those considered in the present experiment and only the Pb-low experiment showed comparable concentration with 10 ppm T6 (127.6 mg/kg). Taking into account that the nematodes viability was only checked on the preservation, the same authors reported that Nematoda were significantly affected by medium concentration of Pb and not the low dose. Similarly, Millward et al. (2001) addressed the impact on a meiofauna-dominated salt marsh community of a mixture of Cu, Cr, Cd, Pb, and Hg at three concentrations up to a month in microcosm experiments. Lead contents in sediment were analyzed both through 1 N HCl and total (HNO_3) extractions. The second extraction is similar to that used in the present study and the resulting Pb concentrations spanned from 48 to 242 mg/kg that match well with those of our experiment. They noted that deposit feeders (i.e. bivalves, and gastropods) were more sensitive to metal contamination than particle feeders (Nematoda, Ostracoda, and Copepoda) and hypothesized the feeding strategy and therefore the metal uptake as responsible for the specific sensitivity. In our experiment, when Nematoda are not considered in the meiofaunal communities (Fig. 4), Copepoda and Ostracoda seem to be more abundant at higher Pb concentrations, with the latter interestingly supported by the PCA (Fig. 2). The response of meiofaunal and nematode assemblages in terms of density, diversity, and composition to different environmental contaminants including Pb was evaluated with a microcosm experiment with estuarine sediment (Gyedu-Ababio and Baird, 2006). The author documented a lowering in meiofaunal diversity and nematode abundance associated with Pb treatment. Interestingly, they observed the most marked reduction of nematode density to Pb and Zn treatments than with organic carbon, Cu, and Fe treatments. Similarly, we observed a significant reduction of nematode abundance (Fig. 2). Again, a reduction of nematodes' diversity was found in Pb treated microcosms (Mahmoudi et al. 2007). It is particularly interesting the opposite trend between meiofaunal richness and Pb concentrations in the sediment. This faunal parameter is commonly used to assess the ecological quality status of the marine sediments by means of the meiofaunal community (e.g. Bianchelli et al. 2016; Semprucci et al. 2017) and the results here obtained could further support its use in studies focused on the trace element impact.

Similar to other meiofaunal groups, most of benthic foraminiferal species are negatively affected by increasing concentrations of Pb in sediment. An opposite behavior was only noted for *Ammonia tepida* and *Bolivina spathulata*. The former is a species typical of shallow marine environments, lagoons and deltaic zones and has been widely considered as a tolerant taxon to chemical and thermal pollution, fertilizing products, and hydrocarbons (i.e. Ferraro et al. 2006; Frontalini et al. 2009). When the UN and P samples are compared, *A. parkinsoniana* (53%), *A. tepida* (24%), *E. scaber* (6%), *A. perlucida* (4%), *B. spathulata*, *H. depressula* (3%), *B. elongata, B. striatula, E. advenum* (2%), *B. punctata* and *P. granosum* (1%) mainly characterized the UP samples, while *A. parkinsoniana* (52%), *A. tepida* (30%), *E. scaber* (6%), *A perlucida* (4%), *B. spathulata, H. depressula* (2%), *B. elongata, B. striatula* and *E. advenum* (1%) characterized the P ones (Fig. 4). Most of the studies carried out in polluted environments have evidenced that a lowering in foraminiferal diversity can be viewed as a measure of environmental stress on benthic foraminiferal communities caused by pollution (Frontalini and Coccioni, 2011). In our experiment, the lower values of diversity were associated with higher concentrations of Pb (Fig. 4). A reduction of diversity (Shannon) was documented in a similar laboratory experiment with Hg (Frontalini et al. 2018). Remarkably, this reduction was documented for both morphological (CellTracker Green, CTG, CMFDA labelling and Rose Bengal, RB, dying) and molecular (environmental DNA) analyses. It was reported that comparatively more negative correlations between diversity indexes and Hg were documented in the CTG dataset than the RB ones. Although widely used as standard dying in ecological and environmental studies, the RB might lead to an overestimation of the abundance (Bernhard et al. 2006). In light of this, the application of RB staining might have included in our dataset some false positive (stained but not living) and slightly blurred and underestimate the real effects of Pb contamination.

Laboratory experiments based on meso- and microcosm represent a valuable approach by which the response of meiofaunal and benthic foraminiferal communities to various types and concentrations of pollutants can be monitored through time. The approach allows the direct evaluation of the effect of a single pollutant on organisms living in their original setting (sediment). Such experiments have the advantages of avoiding inadequate reference sites, mixtures of different pollutants, the establishment of cause-effect relationships, and the great natural variability both in time and space of field studies (Gyedu-Ababio and Baird 2006). Similar experiments targeting the response of benthic foraminifera have been performed with tributyltin (Gustafsson et al. 2000), oil (Ernst et al. 2006), Cu (Frontalini and Coccioni 2012) and Hg (Frontalini et al. 2018). Indeed, meiofaunal communities' experiments, in micro- and mesocosm, have also been carried out to test the effects of pollutants (i.e. Austen and McEvoy 1997; Gyedu-Ababio and Baird 2006; Hedfi et al. 2007; Mahmoudi et al. 2007; Hermi et al. 2009; Beyrem et al. 2011; Boufahja et al. 2011).

Differently from other experiments that mixed sediments containing living meiofaunal communities with defaunated sediments with different targeted concentrations of pollutants (Austen and McEvoy 1997; Gyedu-Ababio and Baird 2006), our experiment incubated meiofaunal and foraminiferal communities retrieved from unpolluted sites and treated with Pb in seawater that allow a gradual release of the pollutant from water to sediment preventing a sudden exposure to biota.

5. CONCLUSION

The results of the present study further strengthen the application of meiofaunal and benthic foraminiferal communities as environmental proxies. It also reinforces the consideration of laboratory experiments (i.e. micro- and mesocosms) as a methodological approach by which the effect of a single or a set of mixed, either organic or inorganic pollutants can be studied on biota through time. The results from our study reveal a reduction of diversity both in meiofaunal and benthic foraminiferal communities and the specific behavior of some taxa within these two communities. These findings highlight the importance of using meiofaunal and foraminiferal communities in laboratory experiment to assess the dose-response relationships that allow the validation of field study outcomes.

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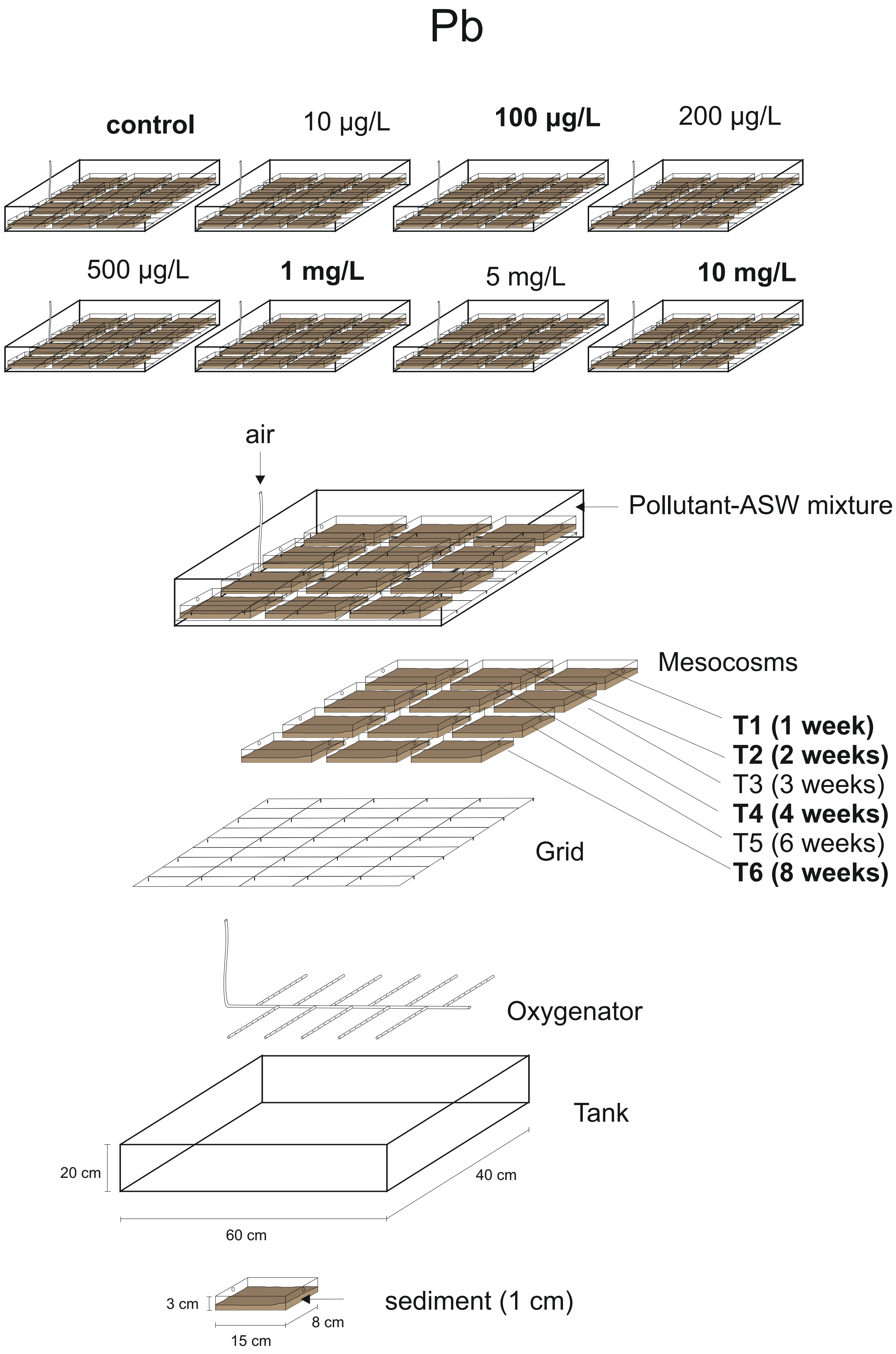
Figure captions:

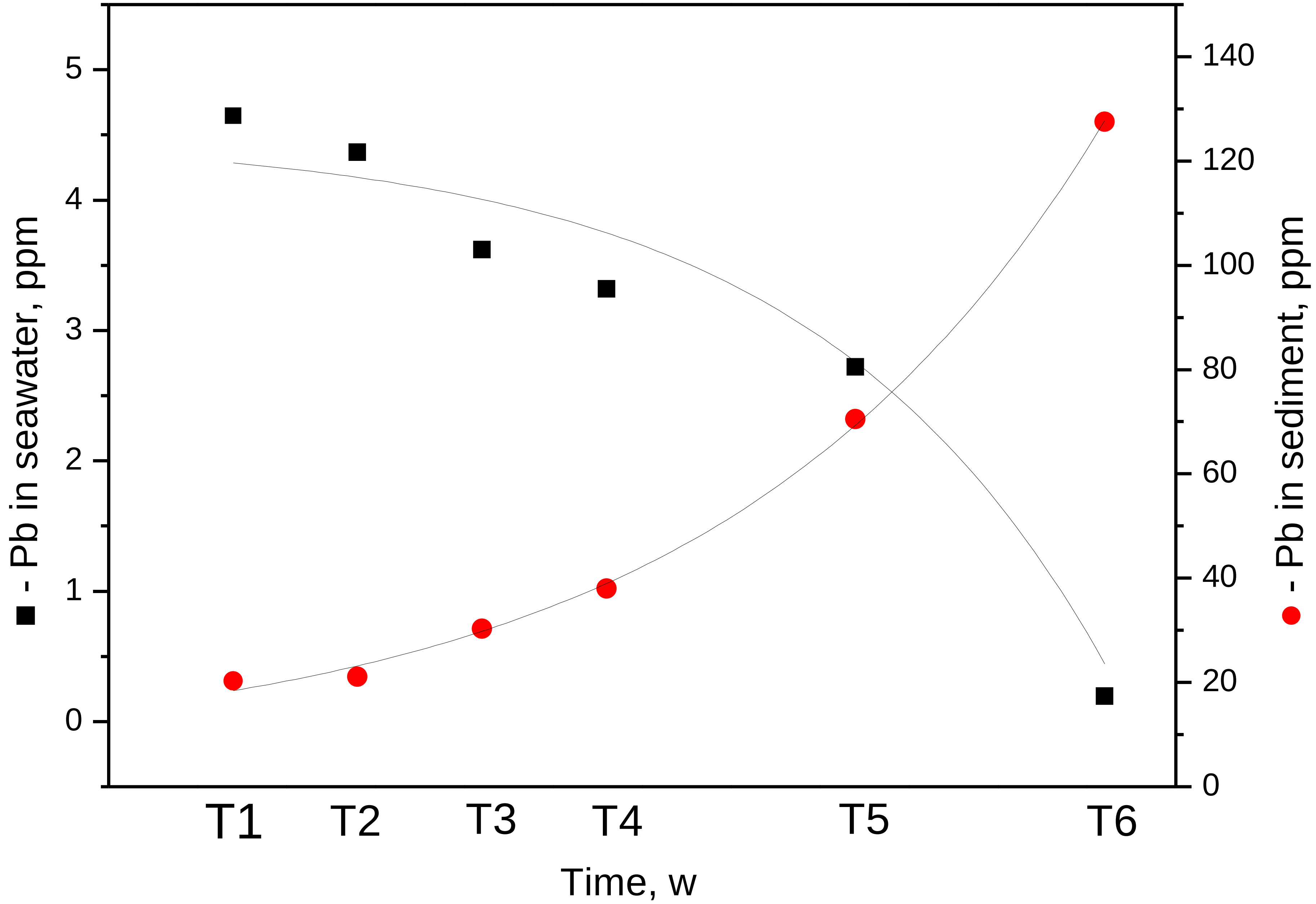
Figure 1. Schematic design of the experiment. Tanks (60 cm × 40 cm × 20 cm) represent different concentrations of Pb in in artificial sea water. Lids are used over the tank to prevent evaporation (not shown). Mesocosms (15 cm× 8 cm × 3 cm) are filled with 1 cm-thick of <500-μm sediments and represent the sampling interval. Multichannel pumps are used to circulate and oxygenate water. In bold concentration and time considered for meiofaunal and benthic foraminiferal communities.

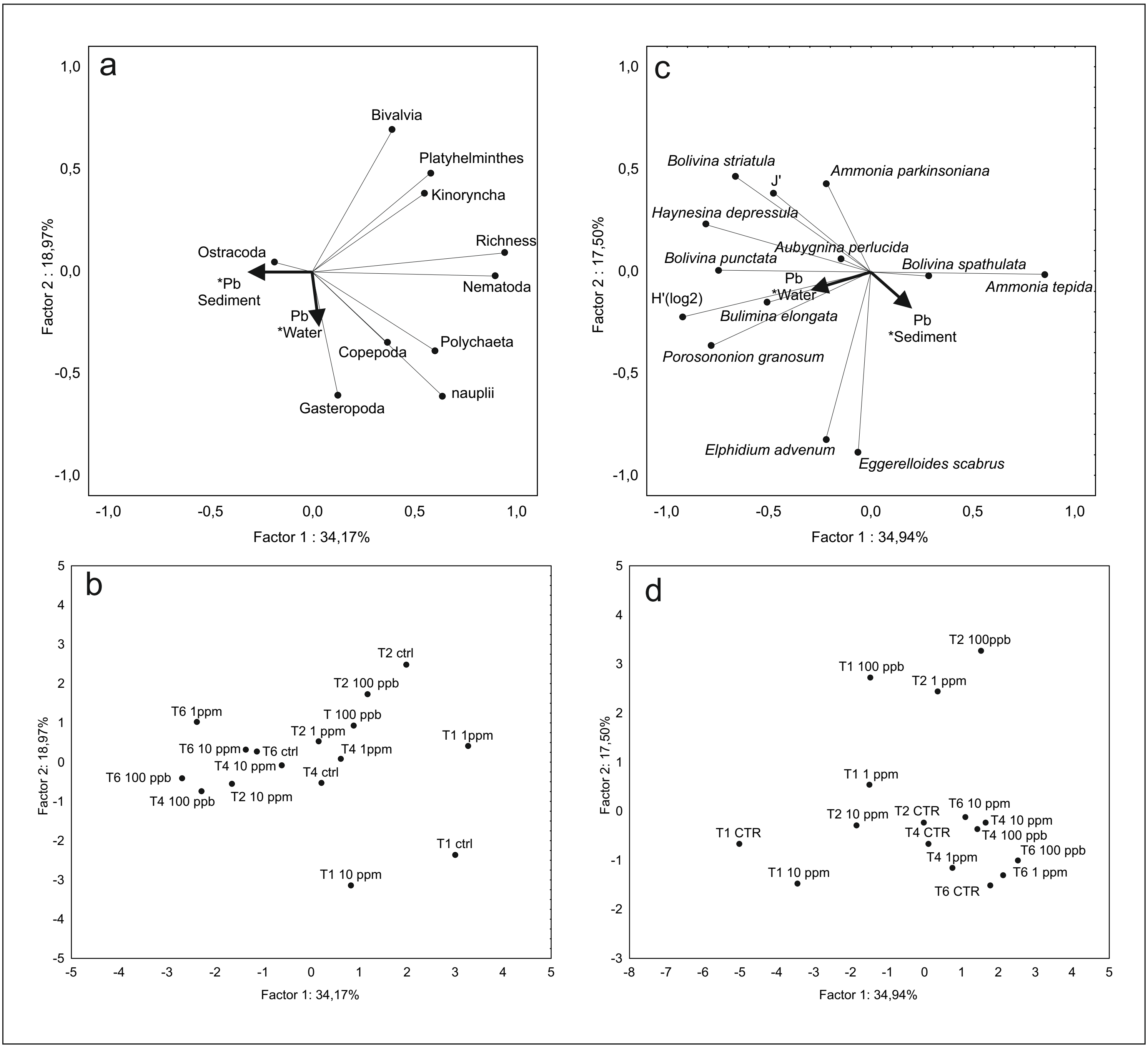
Figure 2. Trend of lead concentration over the time in mesocosm containing an initial concentration of 10 mg L−1. Points represent experimental data, solid curve is the fit to the data by the forms [A]τ = [A]0 e±kτ and [A]τ = [A]0 +C e±kτ.

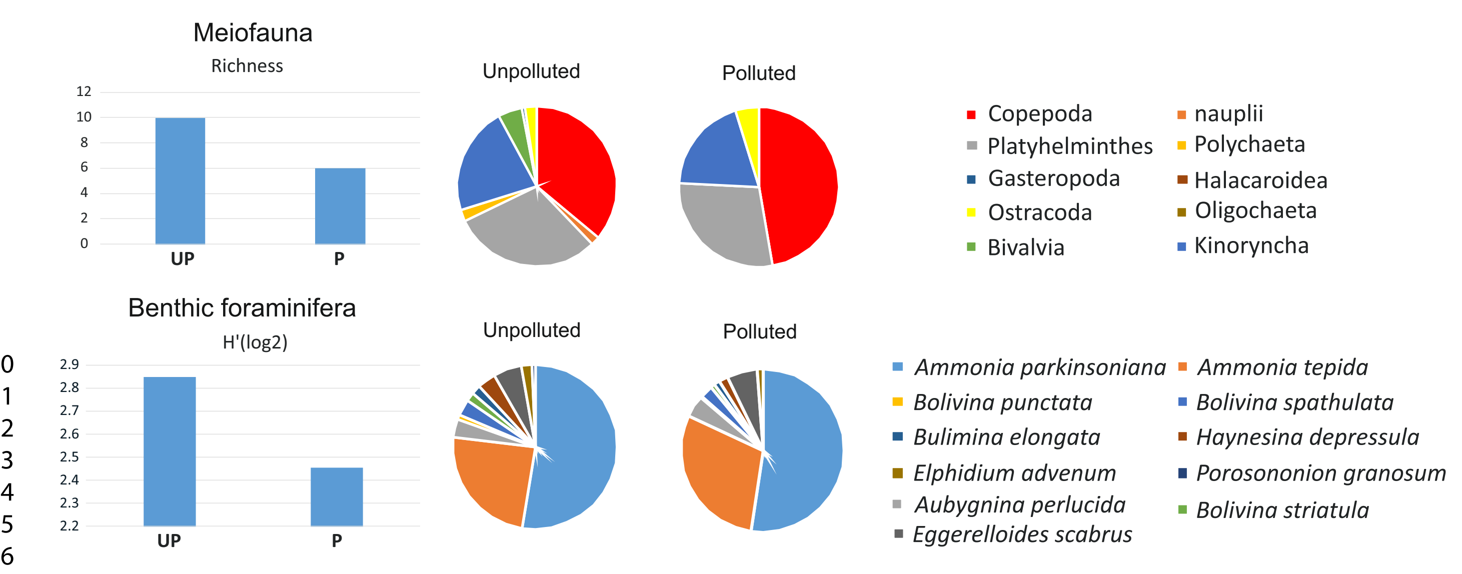
Figure 3. PCA ordination diagram based on meiofaunal (a, b) and benthic foraminiferal (c, d) communities. Concentrations of Pb in sediment and water are here used as secondary variables.

Figure 4. Summary of the meiofaunal structure and richness and benthic foraminiferal composition and diversity reported as Pb-unpolluted (UP) *vs*. polluted (P) conditions.


Pb
control
10 µg/L
100 µg/L
200 µg/L
500 µg/L
1 mg/L
5 mg/L
10 mg/L
air
Pollutant-ASW mixture
Mesocosms
T1 (1 week)
T2 (2 weeks)
T3 (3 weeks)
T4 (4 weeks)
T5 (6 weeks)
T6 (8 weeks)
Grid
Oxygenator
Tank
20 cm
40 cm
60 cm
3 cm
sediment (1 cm)
8 cm
15 cm


■ - Pb in seawater, ppm
● - Pb in sediment, ppm
Time, w
T1
T2
T3
T4
T5
T6
0
1
2
3
4
5
0
20
40
60
80
100
120
140


a
Factor 2 : 18,97%
Factor 1 : 34,17%
Bivalvia
Platyhelminthes
Kinoryncha
Richness
Nematoda
Ostracoda
*Pb
Sediment
Pb
*Water
Copepoda
Polychaeta
nauplii
Gasteropoda
c
Factor 2 : 17,50%
Factor 1 : 34,94%
Bolivina striatula
J'
Ammonia parkinsoniana
Haynesina depressula
Aubygnina perlucida
Bolivina punctata
Bolivina spathulata
Ammonia tepida
Pb
*Water
H'(log2)
Bulimina elongata
Pb
*Sediment
Porosononion granosum
Elphidium advenum
Eggerelloides scabrus
b
Factor 2: 18,97%
Factor 1: 34,17%
T2 ctrl
T2 100 ppb
T 100 ppb
T6 1ppm
T2 1 ppm
T1 1ppm
T6 10 ppm
T6 ctrl
T4 1ppm
T4 10 ppm
T4 ctrl
T6 100 ppb
T4 100 ppb
T2 10 ppm
T1 ctrl
T1 10 ppm
d
Factor 2: 17,50%
Factor 1: 34,94%
T2 100ppb
T1 100 ppb
T2 1 ppm
T1 1 ppm
T6 10 ppm
T2 CTR
T2 10 ppm
T4 10 ppm
T4 100 ppb
T1 CTR
T4 CTR
T6 100 ppb
T4 1ppm
T1 10 ppm
T6 1 ppm
T6 CTR


Meiofauna
Richness
12
10
8
6
4
2
0
UP
P
Unpolluted
Polluted
Copepoda
nauplii
Platyhelminthes
Polychaeta
Gasteropoda
Halacaroidea
Ostracoda
Oligochaeta
Bivalvia
Kinorhyncha
Benthic foraminifera
H'(log2)
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
UP
P
Unpolluted
Polluted
Ammonia parkinsoniana
Ammonia tepida
Bolivina punctata
Bolivina spathulata
Bulimina elongata
Haynesina depressula
Elphidium advenum
Porosononion granosum
Aubygnina perlucida
Bolivina striatula
Eggerelloides scabrus