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Impact of historical sulfide mine tailings discharge on meiofaunal assemblages (Portmán Bay, Mediterranean Sea)

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Abstract

Portmán Bay is one of the most contaminated and chronically impacted coastal marine areas of the world. Here, from the 1957 to 1990, about 60 million tons of mine tailings from the processing of sulfide ores were dumped directly at the shoreline. The resulting deposit provides an unique opportunity to assess the impact of mine tailings on coastal marine ecosystems after ca 30 years since the discharge has ceased. We investigated meiofaunal abundance, biomass and biodiversity along a gradient of metal concentration that overlaps with a bathymetric gradient from 30 to 60m depth. Despite the localized presence of extremely high concentration of metals, the bay was not a biological desert, but, nevertheless, was characterized by evident signs of impact on benthic diversity. Meiofaunal variables increased significantly with decreasing metal contamination, eventually reaching values comparable to other uncontaminated coastal sediments. Our results show that mine tailings influenced the spatial distribution of meiofaunal taxa and nematode species composition. In particular, we report here that the bay was characterized by the dominance of nematode opportunistic species tolerant to high metal concentration. The effects of mine tailing discharge on meiofaunal biodiversity and composition were still evident ca 30 years after the end of the mining activities. Overall, this study provides new insights on the potential impact of mine tailings disposal and metal contamination in coastal sediments and can also contribute to predict the potential long-term consequences of ever-expanding deep-sea mining industry on benthic environments.

Key words: historical metal contamination, metal-rich mine tailings, meiofauna, nematode biodiversity, Portmán Bay, Mediterranean Sea

Highlights

- The mine tailings off Portmán Bay are not a biological desert.
- Meiofaunal diversity and composition are negatively related to metal content.
- Higher food availability along with low metal concentration support a higher diversity.
- The highly contaminated deposit is mostly characterized by opportunistic nematode species.
- Investigating mining impacts is crucial to implement a sustainable deep-sea mining industry.

1. Introduction

The mining industry at the global scale produces several thousand million tonnes per year of waste (overburden rock and tailings), comparable to the weight of Earth materials moved by global geological processes (Hudson-Edwards et al., 2011). Although most of this waste is disposed on land, there is also a long history of mining practices that has resulted in intentional and accidental dumping of tailings into water systems, either in rivers that flow out to the sea or directly in shallow-coastal areas (Brunskill, 2012; Koski, 2012; Ramirez-Llodra et al., 2015; de Oliveira Gomes et al., 2017; Queiroz et al., 2018). The mining and processing of ores to recover different metals have been documented to affect numerous coastal environments at global scale (38 sites have been listed by Dold, 2014) with severe consequences on ecosystems (including bays, estuaries, lagoons and fjords), organisms and human health (Conesa et al., 2008; Ramirez-Llodra et al., 2015).

This is certainly the case of the Cartagena-La Union Mining District and of Portmán Bay, one of the most important historical mining and metal smelting areas of Spain. From 1957 to 1990, about 60 million tons of mining wastes were discharged directly into the Portmán Bay, facing the mining district (Martinez-Frias, 1997; Oyarzun et al., 2013). Part of this material completely infilled the ancient bay, which originally was more than 10 m deep in its center, and subsequently displacing the shoreline 700 m seawards. The remaining half was swept onto the continental shelf (Conesa and Schulin, 2010). Portmán Bay has been classified as the hottest spot for metals contamination in the entire Mediterranean basin (Cesar et al., 2009), and likely is one of the worldwide marine areas most contaminated by mine tailings (Martinez-Frias, 1997; Koski, 2012; Dold, 2014). Previous investigations carried out in Portmán Bay have been mainly dedicated to assessing the concentration of metals, their distribution, mobility and toxicity to target-species (Cesar et al., 2004; -2009, Martínez-Sánchez et al., 2008; Fernandez et al., 2010; -2012, Oyarzun et al., 2013; Gómez-García et al., 2015; Benhamed et al., 2016; Mestre et al., 2017). So far, few studies were carried out to assess the ecological status of the bay (Marín-Guirao et al., 2005; Benedicto et al., 2008; Cesar et

al., 2009) and the effects of metal contamination on anatomical and physiological traits of benthic marine fauna, including the alteration of the bioluminescence of *Amphipholis squamata* (Ophiuroidea: Echinodermata; Deheyn et al., 2000), the assessment of metal content in calcareous skeletons of sea urchins (Auernheimer and Chinchon, 1997), and the environmental hazard assessment using biomarkers in mussel tissues (Mestre et al., 2017).

Portmán Bay represents a unique laboratory in the Mediterranean Basin to study the long-term impact of mine tailings and metal contaminations on benthic ecosystem. Here, we investigated meiofaunal assemblages along a coast-offshore putative decreasing gradient of metal contamination. Given its high ecological relevance in benthic ecosystems and the lack of larval dispersion, metazoan meiofauna represents a reliable and sensitive tool for investigating the anthropogenic impacts on structural and functional features of natural assemblages at local scale (Wilson and Kakouli-Duarte, 2009 for a review). Among meiofaunal taxa, nematodes are the most abundant multicellular organisms on Earth, accounting for about 2/3 of all metazoans (Giere, 2009). Nematodes diversity and their life strategies are widely used as proxies for health assessment of environmental ecosystem and to detect the effect of different impacts from coastal to deep-sea ecosystems (Bongers, 1990; Danovaro et al., 1995; Bongers and Bongers, 1998; Schratzberger et al., 2000; Ahnert and Schriever, 2001; Ferris and Bongers, 2006; Frascetti et al., 2006; -2016; Moreno et al., 2008; -2011; Gambi et al., 2009; -2020; Goodsell et al., 2009; Losi et al., 2013; Pusceddu et al., 2014). Moreover, the analysis of their functional traits, such as buccal apparatus, allows gathering important insights on their functional diversity (Danovaro et al., 2008).

The aim of our investigation is to assess the potential persistent effects of historical (dating back ca 30 years ago) metal-rich mine tailings on the diversity (at the taxon level for meiofauna and at the species level for nematodes) of meiofaunal assemblages along a decreasing gradient of metal contamination that is overlapped with the increasing water depth and distance from the coast over the inner continental shelf. We tested the null hypothesis by which meiofauna is not affected by the mine tailings and chronic contamination in the Portmán Bay and, therefore, that it does not display

any significant change along the contamination gradient ca 30 years after the cessation of mining waste dumping. Lessons learnt from the Portmán Bay case study provide new insights on the impact of mine tailings disposal and metal contamination in coastal sediments and can contribute to predict the potential long-term consequences of ever-expanding deep-sea mining industry on benthic ecosystems of Areas Beyond National Jurisdiction that have been tentatively identified (Danovaro et al., 2017; van Dover et al., 2017; Simon-Lledó et al., 2019; Washburn et al., 2019).

2. Material and Methods

2.1 Sampling strategy

Portmán Bay is located in La Unión municipality, province of Murcia in the southeastern coast of Spain (Western Mediterranean Sea; Fig. 1). Sediment samples were collected with a multicorer in August 2014 during the MIDAS-Portmán research cruise on board R/V Ángeles Alvariño. Sampling stations were selected along the expected decreasing gradient of mine tailings and metals contamination (Table 1 and Fig. 1). At all stations, independent replicates of sediment samples were collected for the analysis of heavy metal concentrations (Ni, Zn, Fe, Cr, Cu, Pb, Cd, As and Hg), grain size, total phytopigments and the biochemical composition of organic matter, and meiofaunal (nematode) abundance, biomass and diversity (0-15 cm). Sediments samples were preserved at -20°C until chemical and biochemical analyses were performed in the laboratory.

2.2 Grain size and heavy metal content

Sediment grain size was determined using a Coulter LS230 Laser Diffraction Particle Size Analyser. The concentrations of trace elements in the sediment were determined after acid digestion and the extracts were analysed by inductively coupled plasma-atomic emission spectrometry (ICP-AES). In both cases we followed the procedures reported in Mestre et al. (2017).

2.3 Sedimentary organic matter content and biochemical composition

Phytopigments were extracted using 3-5 mL 90% acetone (at 4°C in the dark for 12 h) from 0.5 g sediment samples. Chlorophyll-a and, after acidification of extracts with 200 µl 0.1 N HCl, phaeopigment contents were determined fluorometrically (Danovaro, 2010). Proteins, carbohydrates and lipids were determined spectrophotometrically, following the protocols detailed in Danovaro (2010), and their sedimentary contents (mg g dry sediment⁻¹) expressed as bovine serum albumin, glucose and tripalmitine equivalents, respectively. Carbohydrate, protein and lipid sedimentary contents were converted into carbon equivalents using the conversion factors of 0.40, 0.49 and 0.75 µgC µg⁻¹, respectively, and their sum defined as Biopolymeric Carbon (BPC; Fabiano et al., 1995).

2.4 Meiofaunal abundance, biomass and diversity

Each sediment sample was treated with ultrasounds (for 1 min 3 times, with 30 s intervals) to detach organisms from the grain particle surface and, then, sieved through a 1000-µm and a 30-µm mesh net to retain the smallest organisms (Danovaro, 2010). The fraction remaining on the latter sieve was re-suspended and centrifuged three times with Ludox HS40 diluted with water to a final density of 1.18 gcm⁻³ (Heip et al., 1985). All specimens from three independent replicates per station were counted and sorted by taxa, under a stereomicroscope and after staining with Rose Bengal (0.5 gL⁻¹). Meiofaunal taxa representing <1% of the total abundance were defined as rare taxa (Bianchelli et al., 2010). Meiofaunal biomass was assessed by bio-volumetric measurements of all retrieved specimens. Nematode biomass was calculated from their biovolume, using the Andrassy (1956) formula ($V=L \times W^2 \times 0.063 \times 10^{-5}$, in which body length, L, and width, W, are expressed in µm). Body volumes of all other taxa were derived from measurements of body length (L, in mm) and width (W, in mm), using the formula $V=L \times W^2 \times C$, where C is a dimensionless factor (specific for each meiofaunal taxon) used to convert $L \times W^2$ to body volume, according to models relating body dimensions and volume (Feller and Warwick, 1988). Each body volume was multiplied by an average density of 1.13 g cm⁻³ to obtain the biomass (µg dry weight: µg wet

weight = 0.25; Wieser, 1953) and the carbon content was considered to be 40% of the dry weight (Feller and Warwick, 1988).

2.5 Nematode biodiversity

One-hundred and twenty specimens were randomly picked up from the three replicates per station and were mounted on slides using the formalin-ethanol-glycerol technique (Seinhorst, 1959). Nematodes were identified to the species level or morphotypes (*sensu* De Mesel et al., 2006) according to Platt and Warwick (1983; -1988), Warwick et al. (1998) and the NeMys database (Bezerra et al., 2020). Nematode diversity was estimated as the expected species number (Sanders, 1968, as modified by Hurlbert, 1971) for a theoretical sample of 51 specimens, ES(51) (Gambi et al., 2014). Species evenness was measured using the Pielou's index (Pielou, 1975).

The trophic group of nematodes was determined according to the classification of Wieser (1953). Each nematode species was assigned to one of the following 4 trophic groups, based on the buccal morphology: (1A) selective (bacterial) feeders: no buccal cavity or a fine tubular one; (1B) non-selective deposit feeders: large but unarmed buccal cavity; (2A) epistrate or epi-growth (diatom) feeders: buccal cavity with scraping tooth or teeth and (2B) predators/omnivores: buccal cavity with large jaws. The index of trophic diversity (ITD) was calculated as $ITD = g_1^2 + g_2^2 + g_3^2 \dots + g_n^2$, where g is the relative contribution of each trophic group to the total number of individuals and n is the number of trophic groups ($n=4$ in the present study) (Heip et al., 1985; Gambi et al., 2003).

The nematode life strategy was estimated as the Maturity Index (MI), calculated according to the weighted mean of the individual genus scores: $MI = \sum v(i) f(i)$, where v is the c-p value (colonisers-persisters) of genus i as given in the Appendix of Borgers et al. (1991) and $f(i)$ is the frequency of that genus. The colonizer-persister scale is composed of five classes: 1-5; the colonizers receive a low value (c-p=1/2) while the persisters are allocated to c-p=4/5.

2.6 Statistical analyses

To assess the differences in total meiofaunal abundance, biomass, richness of taxa, community composition (both higher and rare taxa) and nematode diversity among stations along the coast-offshore gradient of metals contamination, we applied one-way distance-based permutational analyses of variance (PERMANOVA) using unrestricted permutations of the raw data. The analyses were carried out using stations (7 fixed levels) as main sources of variance. The analyses were carried out on Bray-Curtis similarity matrices of no transformed data (for meiofaunal higher and rare taxa and nematode species composition), using 999 permutations of the residuals under a reduced model. The same approach described above was applied to metal concentrations, grain size, biochemical components of the organic matter, meiofaunal abundance, biomass and number of taxa, nematode diversity indices using Euclidean distance matrices commonly utilized for both environmental data (Clarke and Gorley, 2006) and faunal variables (Alves et al., 2015). For those PERMANOVA tests providing significant differences among stations, pair-wise tests were also carried out. Because of the restricted number of unique permutations in the pair-wise tests, p values were obtained from Monte Carlo samplings (Anderson and Robinson, 2003).

To visualize differences in the meiofaunal taxonomic composition among stations, bi-plots after a Canonical Analysis of Principal Coordinates (CAP) were also prepared (Anderson and Willis, 2003).

SIMPER analyses were performed to assess the turnover diversity as percentage of dissimilarity in meiofaunal and nematode community composition among stations along the coast-offshore contamination gradient. A ranked matrix of Bray-Curtis similarities, constructed on previously no transformed data, was used as input for the SIMPER (Similarity percentage analysis) tests (Gray, 2000). Moreover, since the variation of the species composition of assemblages may reflect two different phenomena, spatial species turnover and nestedness of assemblages, we used also the model proposed by Balsega (2012) and we estimated: i) Jaccard dissimilarity; ii) turnover component and iii) nestedness-resultant component of the Jaccard dissimilarity.

To identify the potential (metals, environmental and trophic conditions) drivers of meiofaunal variables (including nematodes), when significant differences were present among stations, non-parametric multivariate multiple regression analyses based on Euclidean distances were carried out, using the routine DISTLM (distance-based multivariate analysis for a linear model using forward selection), the forward selection procedure and R^2 as the selection criterion (McArdle and Anderson, 2001). The forward selection of the predictor variables was carried out with tests by permutation. P values were obtained using 999 permutations of the raw data for the marginal tests (test of individual variables), whereas for all of the conditional tests, the routine used 999 permutations of residuals under a reduced model. To run this test, meiofaunal and nematode variables were used, separately, as dependent variables whereas metal contents, depth, grain size (as % silt-clay), total phytopigments and Biopolymeric C were used as potential explanatory variables. Since meiofaunal abundance and (nematode) diversity were negatively related with increasing metal contaminations, we test if the effect was still significant after the removal of covariates such as depth, grain size and food sources using a linear model (DISTLM) forward (Anderson, 2004). P values were obtained with 999 permutations of residuals under the reduced model (Anderson, 2001).

dbRDA (Distance-based redundancy analysis) ordination after DISTLM forward analysis, was carried out to fitted model of meiofaunal taxa and nematode species composition (based on Bray-Curtis similarities of no transformed data) versus metals, and environmental and trophic variables.

The PERMANOVA, SIMPER, CAP, DISTLM and dbRDA analyses were performed using the routines included in the PRIMER 6+ software including the PERMANOVA add-on (Clarke and Gorley, 2006). Jaccard dissimilarity, turnover component and nestedness-resultant component were calculated according to the formulas reported in Baselga (2012).

3. Results

3.1 Environmental characteristics and contamination by heavy metals

Grain size analyses revealed that silt-clay dominated in Sts 1 and 4 at 43m, and Sts 5 and 6 at ca 60m depth (Table 1). The silt-clay fraction generally increased significantly with increasing water depth, except for St7 (Table 2).

The concentration of the investigated heavy metals is reported in Table 3. The results of PERMANOVA analysis revealed that the concentration of heavy metals in Sts 2 and 3 (at ca 30m depth) was significantly higher than in all other investigated stations, whereas Sts 1 and 4 (at ca 43m) showed concentrations significantly higher than in the deepest stations (Sts 5, 6 and 7; ca 60m-depth) (Tables 2 and 3). The results of PERMANOVA carried out separately for Ni, Zn, Fe, Cu, Pb, Cd, As and Hg concentrations revealed that the values were significantly higher in the shallowest stations (Sts 2 and 3) than in the deepest ones (Sts 5, 6 and 7; Supplemental online material Table S1). Among the investigated metals, Zn, Fe, Pb, Cd and As generally showed decreasing concentrations with increasing water depth (Supplemental online material Table S1).

3.2 Total phytopigments and sedimentary organic matter

Total phytopigments and Biopolymeric C sedimentary contents (Table 1) varied significantly among stations, with the highest concentrations occurring at St5 (50m-depth), and the lowest in the shallowest ones (Sts 3 and 2; Table 2).

3.3. Meiofaunal abundance, biomass and diversity

Meiofaunal abundance varied significantly among stations, with values in Sts 2 and 3 (30m-depth) lower than those in all other stations (except St5 at 50m-depth; Fig. 2a and Table 4) while total meiofaunal biomass did not vary significantly among sampling stations (Fig. 2b and Table 4).

The richness of meiofaunal taxa ranged from 6 to 12, with values in St5 significantly higher than in Sts 1-4 (Fig. 2c and Table 4). Overall, 13 taxa were identified: nematodes, copepods, polychaetes, ostracods, kinorhynchs, oligochaetes, cumaceans, amphipods, isopods, tanaidaceans, acarians, nemertean and decapods larvae. Nematodes were the dominant taxon (77-91% of total meiofaunal abundance) at all sampling stations, followed by copepods (including their nauplii 4-18%) and polychaetes (1-5%). All other taxa accounted each for <1% of the total meiofaunal abundance and were here reported as “rare taxa”. The composition of either higher and rare taxa assemblages varied significantly among stations (Table 4). The CAP analyses on higher meiofaunal taxa highlighted the presence of assemblages in Sts 2 and 3 clearly different from those in all other stations (Fig 3a). The same analysis carried out on meiofaunal rare taxa identified again three distinct groups of stations (Fig. 3b).

The turnover diversity as dissimilarity in (whole) taxa assemblage composition ranged from 16% (St4 vs St6) to 47% (St2 vs St7) while the dissimilarity in rare taxa assemblage composition was higher and ranged from 37% (St6 vs St7) to 75% (St1 vs St7).

3.4 Nematode biodiversity

Overall, 188 species of nematodes belonging to 105 genera were identified in the Portmán Bay sediments. Rare species (each <1% of the total number of species) represented 75% of the nematode species identified. The results of PERMANOVA analyses revealed that ES(51) varied among stations (Fig. 4a) and showed values in Sts 5 and 7 significantly higher than those in all other stations (Table 4). Evenness ranged from 0.865 ± 0.016 to 0.929 ± 0.023 and did not vary among stations while nematodes assemblage composition varied significantly between Sts 3, 2 and all other stations and between Sts 1-4 and Sts 5-7 (Table 4).

The output of the CAP analyses revealed the presence of different nematode assemblages segregating according to the contamination gradient present at different depths (Fig. 3c). The

SIMPER analysis showed that nematode turnover diversity was high as dissimilarity in species composition between couples of stations varied from 63% (between Sts 2 and 3 and between Sts 6 and 7) to 88% (between Sts 3 and 5).

The Jaccard dissimilarity ranged from 0.66 (Sts 2-3 vs Sts 1-4) to 0.80 (Sts 2-3 vs Sts 5-7), the turnover component of dissimilarity ranged from 0.59 (Sts 1-4 vs Sts 5-7) to 0.65 (Sts 2-3 vs Sts 5-7) while the nestedness-resultant component varied between 0.04 (Sts 2-3 vs Sts 1-4) and 0.14 (Sts 2-3 and Sts 1-4 vs Sts 5-7).

The trophic structure of nematode assemblages is reported in Fig. 4b. Deposit feeders (selective plus non-selective trophic groups) represented from 64 to 82% of the entire nematode assemblages dominated in all stations at 43m and 60m, whereas epistrate feeders provided a prominent contribution (48-65%) at the shallowest stations located at 30m. Predators accounted for from 1 to 11% of nematode assemblages. The contribution of the different groups to the nematodes trophic structure varied significantly among the shallowest stations (dominated by epistrate feeders) and all other sampling sites (dominated by deposit feeders; Fig. 4b and Table 4).

The Index of Trophic Diversity did not vary among stations (Fig. 5a and Table 4), whereas the Maturity Index, in Sts 3, 5, 6 and 7, showed values higher than in St2 (Table 4; Fig. 5b).

3.5 Drivers of meiofaunal assemblages and nematode diversity

The results of the DISTLM analyses revealed that depth (49%), Cr (19%), Ni (10%) Cd (6%) and Biopolymeric C (2%) accounted for 86% of total variance of meiofaunal abundance (Table 5). Depth (41%), Cr (25%), Pb (12%), Zn (4%) and total phytopigments (3%) accounted for 85% of total variance of meiofaunal higher taxa composition while Biopolymeric C and Cd (both 18%), grain size (13%), total phytopigments (11%) and Fe, Pb and Zn (6-4%) accounted for 75% of the total variance of rare taxa composition. Cd (54%), grain size (11%), depth (9%), Cu (8%), total phytopigments and Hg and Ni (2-3%) accounted for 91% of the total variance of nematode diversity while Zn (22%), Cd (10%), total phytopigments (9%), Biopolymeric C (7%), Fe and Pb(5-6%)

accounted for 59% of the total variance of species composition. The effect of metal contamination on meiofaunal abundance and (nematode) diversity and composition accounted for 91% of variance after the removal of the covariables (depth, grain size and food source) (Supplemental online material Table S2).

dbRDA1 explained 76% variance of environmental parameters (food sources and depth) and was highly positively related to Ni and Cr and negatively related to As for meiofaunal higher taxa composition (Fig. 6a). The results of the dbRDA1 explained 31% variance of environmental parameters (food sources and depth) and was positively related to Cu and Cd while resulted negatively related to Pb for rare taxa composition (Fig. 6b). dbRDA1 explained only 23% variance of environmental parameters (food sources, depth and grain size) and was positively related to Cr, Pb and Hg and negatively related to Ni, Cu, Cd and As for nematode species composition (Fig. 6 c).

4. Discussion

4.1 Impact of historical mining tailings on meiofaunal assemblages

Although ore deposits occur in a variety of continental settings around the globe, many of them are located in coastal regions and the disposal of tailings, that results from mining activities, was poorly regulated and commonly placed directly into the sea until recently (Medina et al., 2005; Koski, 2012). The dramatic consequences of tailings disposal have been observed in different coastal areas (including bays, estuaries, lagoons and fjords) and on different benthic components (e.g. algae, meiofauna, macrofauna and fishes; Koski, 2012; Dold, 2014; Ramirez-Llodra et al., 2015). As observed in the Portmán bay, widespread and permanent topographic and bathymetric alteration of other coastal areas due to the large amount of the tailings discharged for decades has been documented: Calancan Bay (Philippines), about 200 million tons from 1975-1991; Chañaral Bay (Chile), about 150 million tons from 1975-1990, Jøssingfjorden and Dyngadjupe (Norway): about 50 million tons from 1960-1994 (Koski, 2012; Oyarzun et al., 2013; Vogt, 2013; Dold, 2014; Schaanning et al., 2019). In such coastal areas severely impacted by long-term mine tailing deposition changes in benthic fauna taxon/species composition, presence of opportunistic species and overall low biodiversity independently by the benthic group investigated have been reported (Lee et al., 2001; Josefson et al., 2009; Schaanning et al., 2019). Moreover, all case studies show that after cessation of the mining activity, the re-establishment of a faunal community similar to the original state may take several decades (Burd, 2002; Josefson et al., 2009) or probably does not occur especially in cases where the bathymetry has been severely modified (Tranum et al., 2018). The pumping of millions of tons of tailings enriched in galena (PbS), sphalerite (ZnS) and pyrite (FeS) directly into the Portmán Bay triggered the enrichment of marine sediments in Mn, Fe, Zn, Pb and As, among others (Cerdà-Domenech et al., 2020) continuously for ca 30 years (Martinez-Frias, 1997). As a result, Zn, As and Pb sedimentary contents in modern Portmán Bay bay reached values ca 66, 13 and 37 higher than their average concentration in overall Mediterranean sediments at

similar depths (Martínez-Frías, 1997; Martínez-Gómez et al., 2012). This provides evidence of a relevant contamination, which is still massively persistent nowadays ca 30 years after the cessation of the dumping. In addition, our results show a clear decreasing gradient of metal contamination with the increasing distance from the shore (and, therefore, with increasing water depth). This gradient could be explained because the mine tailings were discharged from the coastline. However, while previous studies carried out in Portmán Bay reported evident signs of contamination at a distance of >7 km from the shore (Cesar et al., 2009), our results indicate that metal contamination currently persists within hundreds of meters from the coast, down to a depth of ca 60 m. At higher distances from the coast and larger depths Ni, Cr, As, Cd and Hg sedimentary contents were indistinguishable from those measured in other coastal areas not-influenced by the dumping of mine tailings and even lower than the safety values considered by European and Spanish laws (CIEM, 2015). We can not exclude that natural-like sedimentation conditions at the end of the mine tailings dumping may have buried and hid the trace metal signal at least from the very surface sediments (Cerdà-Domeènech et al., 2020).

Despite the presence of very high metal concentrations found in the sediments at short distance from the shoreline (e.g., Sts 2 and 3), Portmán Bay was not a biological desert, as meiofaunal abundance and biomass were overall similar to values reported in other not contaminated coastal areas (Danovaro et al., 2000; Gambi et al., 2009). This would suggest that meiofaunal assemblages, if ever disturbed, showed a high resilience to the impact of mine tailings in Portmán Bay. However, since the dumping of the mine tailing ceased ca 30 years before our study, we cannot, in principle, exclude that the weak or apparent lack of response of meiofauna (in terms of abundance and biomass) is the result of their progressive adaptation to persistently contaminated sediments. Moreover, the limited impact of Cu contamination on meiofaunal abundance and biomass could be also related to a limited bioavailability (and hence toxicity) of metals released in Portmán Bay. This hypothesis is supported by previous investigations, which revealed that in the sediments of Portmán Bay, Pb, Cu and As are tightly correlated to the high concentrations of magnetite, which is known

to act as a captor of metals, definitely limiting their bioavailability for benthic fauna (Gómez-García et al., 2015). More recently, a spectroscopy synchrotron study on the speciation and bioavailability of As in the mine tailings deposit of Portmán Bay has demonstrated that a succession of oxidation, dissolution and re-precipitation processes effectively block the release of this highly toxic metalloid to the surrounding environment (Roqué et al., 2019).

We could also infer that the observed spatial pattern in meiofaunal abundance increasing at increasing distance from the shoreline in Portmán Bay could be affected by an overimposed pattern due to water depth and organic C contents. However, the effects of heavy metals contamination on meiofauna persist also after the removal of covariates (i.e., depth, grain size composition and food availability), suggesting that the metal concentration (in particular Cd and Ni) influences the meiofaunal spatial distribution along the coast-off shore gradient in Portmán Bay.

The diversity and richness of meiofaunal taxa are generally lower in polluted and stressed environments, due to the loss of some sensitive taxa (e.g., copepods, polychaetes, ostracods, gastrotrichs and tardigrades; Lee et al., 2001; Lee and Correa, 2005; Gopalakrishnan et al., 2007; Giere, 2009; Wilson and Kakouli-Duarte, 2009). This typically results in meiofaunal assemblages increasingly dominated by highly tolerant organisms, such as nematodes (Danovaro et al., 2009). However, this is not the case of the Portmán Bay sediments, where meiofauna showed an assemblage structure including, apart nematodes (which dominated), also copepods, polychaetes and a large group of “other”, relatively rare, taxa in all sediments, independently by the degree of heavy metals contamination. The presence of exclusive taxa (cumaceans and nemerteans) in the most contaminated sediments lets us to hypothesize a possible taxon-specific response to metal contamination and that their limited bio-availability along with the food availability can explain the presence, at least of the most sensitive taxa in Portmán Bay.

Heavy metal contamination has a prominent role in driving the spatial distribution of meiofaunal composition since three different assemblages can be identified according to the metal concentration at increasing distance from the shoreline along the bathymetric gradient. We can

explain this pattern as the result of the spatial distribution of taxa, especially rare groups, with different levels of tolerance to the metals contamination along the increasing distance from the shoreline and the increasing of food sources availability.

4.2 Impact of mine tailings on nematode diversity

The effect of heavy metal contamination due to mine tailing discharge on benthic fauna is evident also at the nematode species level and, in particular, nematode diversity is highest in deepest sediments (Sts 5 and 7), where metals contamination is the lowest. The tolerance to metal pollutants has been shown to vary widely among nematode species (Hagerbaumer et al., 2015 and references therein), and it has been observed that a long exposure to metals can lead to the selection of tolerant nematode species (Millward and Grant 1995; Bastami et al., 2017; Gambi et al., 2020). Accordingly, our results reveal that highly contaminated sediments host specific nematode assemblages, characterized by a few epistrate feeders species. As for the whole meiofaunal community, heavy metal sedimentary content is the main factor influencing the spatial distribution of nematode species in Portmán Bay. Changes in nematode species composition, indeed, allow discriminating again three groups whose dissimilarity in species composition is high and increases when assemblages from stations with high and low metals concentrations are compared. This is also confirmed by the average pairwise dissimilarity estimated according to Balsega (2010, -2013). Our results indeed suggest that the variation of the nematode species composition is mainly due to species replacement among assemblages at increasing distance from the shoreline and thus at decreasing metals contamination while the variation in species composition derived from nested patterns is negligible.

The dominant nematode species in the most contaminated sediments belong to the genera *Sabatieria*, *Spilophorella*, *Desmodora* and *Pseudochromadora*, which are known to tolerate different impacts, including sediments affected by long-term heavy metals' contamination (e.g., Tietjen, 1980; Hendelberg and Jensen, 1993; Steyaert et al., 1999; Heip et al., 1990; Vanreusel,

1990; Montagna and Harper, 1996; Schratzberger et al., 2006; Moreno et al., 2009; Frascchetti et al., 2016). On the other hand nematode assemblages in the less contaminated sediments are dominated by species belonging to the genera *Richtersia* and *Desmoscolex*, sensitive to different sources of impact (Moreno et al., 2011). Our investigation reveals that changes in species composition are also related to changes in the nematode trophic guilds composition (deposit vs epistrate feeders) suggesting that the high levels of contamination and the low food availability can act as a physical barrier in the shallower stations at 30m-depth. These patterns reflect changes also in the Maturity Index, thus suggesting the presence of nematode assemblages with different trophic and life strategies. Indeed highly contaminated sediments with lower food availability show a large contribution of “colonisers” (as opportunistic species), while sediments with low metals concentration and higher food availability host “persister” species (Bongers et al., 1991; Bongers and Ferris, 1999). Food availability and heavy metals content play an important role in influencing nematode assemblages suggesting a similar response observed for macrofauna inhabiting contaminated sediments where the competition for food sources is an important driver to define species composition (Ryu et al., 2011).

Huge progress has been made in the last years toward technologically feasible and economically viable seafloor mining (Jones et al., 2017). While mining techniques differ depending on the resources to be exploited and the ecosystems hosting them, many of the potential impacts on the ecosystem are similar (Levin et al., 2016; Van Dover et al., 2017). Upcoming industrial seabed mining is expected to determine habitat modifications and species mortality due to mechanical disturbances (Ellis, 2001; Van Dover, 2014; Jones et al., 2017) and impacts due to the release and spread of toxin-laden water from the generation of plumes (Ellis, 2001; Boschen et al., 2013). Recent field works have shown that deep-sea sites impacted by simulated mining activities may take decades to recover, and that while faunal abundance recovers more quickly than diversity, the latter can remain low even after multiple decades (Gollner et al., 2017; Jones et al., 2017; Van Dover et al., 2017). However, the consequences of mine tailings disposal and associated metals, as

documented in several marine coastal systems worldwide (Lee et al., 2001; Josefson et al., 2009; Dold, 2014; Schaanning et al., 2019; the present study) are still largely unknown.

The results reported here open new perspectives on the potential impact of mining activities over marine benthic ecosystems and suggest that: 1) high concentration of heavy metals can have an impact even if the environmental conditions limit the bioavailability of the xenobiotic compounds, and 2) the effect of long-term tailing discharge on benthic diversity is still clearly evident after several decades from the end of the mining activities also in coastal areas.

The more dynamic and rapid ecological responses that characterize coastal ecosystems cannot be expected in the deep sea, where the comparatively slower growth rates and longer life span of deep-sea organisms inevitably slow down the ecological recovery of grounds exposed to mining. With the increasing global interest in deep-sea mining and tailing disposal, it is therefore of the utmost relevance to take into consideration the findings in Portmán Bay, which may herald even more harmful impacts on the relevant habitats in deep-sea environments. Moreover, a severe and rigorous assessment and monitoring of the impacts of mining on deep-sea ecosystems is a priority.

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Caption of figures

Figure 1. Location map of the study area. Reported are the location of Portmán Bay and the spatial distribution of sampling stations at increasing distance from the shoreline and hence water depth.

Figure 2. Meiofaunal assemblages in Portmán Bay. Reported are meiofaunal: a) abundance (ind 10 cm⁻²), b) biomass (µgC 10 cm⁻²) and c) richness of taxa (as minimum and maximum number of taxa among three replicates) in all investigated stations. Reported are average value ± standard deviation for abundance and biomass.

Figure 3. Outputs of the CAP analysis based on meiofaunal and nematode composition in Portmán Bay. Reported are taxonomic composition of a) all meiofaunal taxa and b) rare taxa and c) nematode species in all investigated stations. Stations and depth are reported in Table 1.

Figure 4. Nematode diversity in Portmán Bay. Reported are nematode a) Expected Species number [ES(51)] and b) trophic composition (1A: selective deposit feeders; 1B: non selective deposit feeders; 2A: epistrate feeders and 2B: predators) in all investigated stations. Reported are average value ± standard deviation for ES(51).

Figure 5. Nematode functional diversity in Portmán bay. Reported are a) Index of Trophic Diversity and b) Maturity Index in all investigated stations. Reported are average value ± standard deviation.

Figure 6. dbRDA ordination after DISTLM forward analysis, describing the relationship between a) meiofaunal higher and b) rare taxonomic composition and c) nematode species composition with heavy metal concentration, depth, grain size and food availability (total phytopigments -Tot phyt- and Biopolymeric C -BPC-). Vectors are proportional to the % of observed variation.

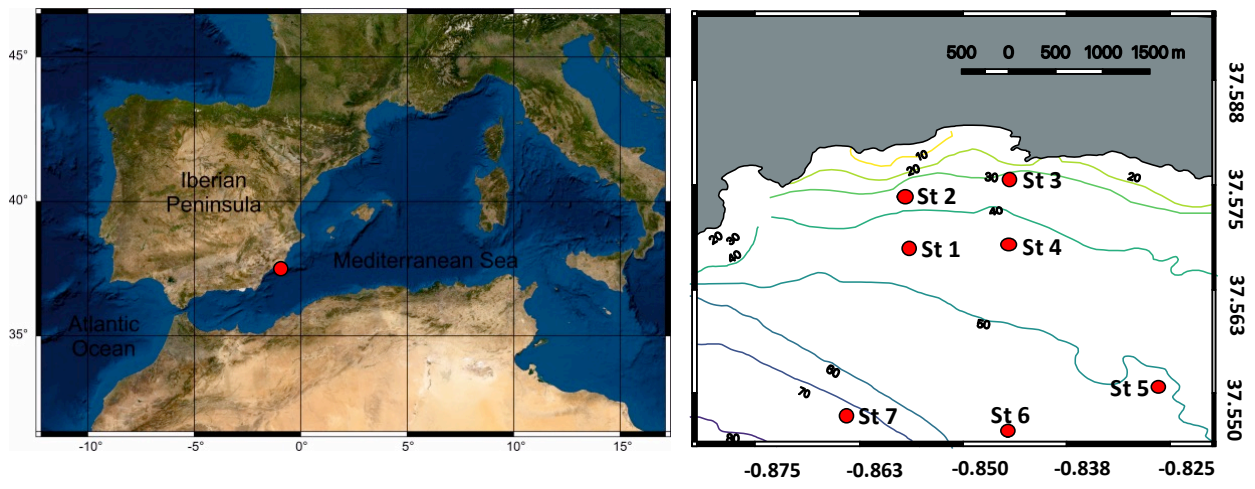


Figure 1

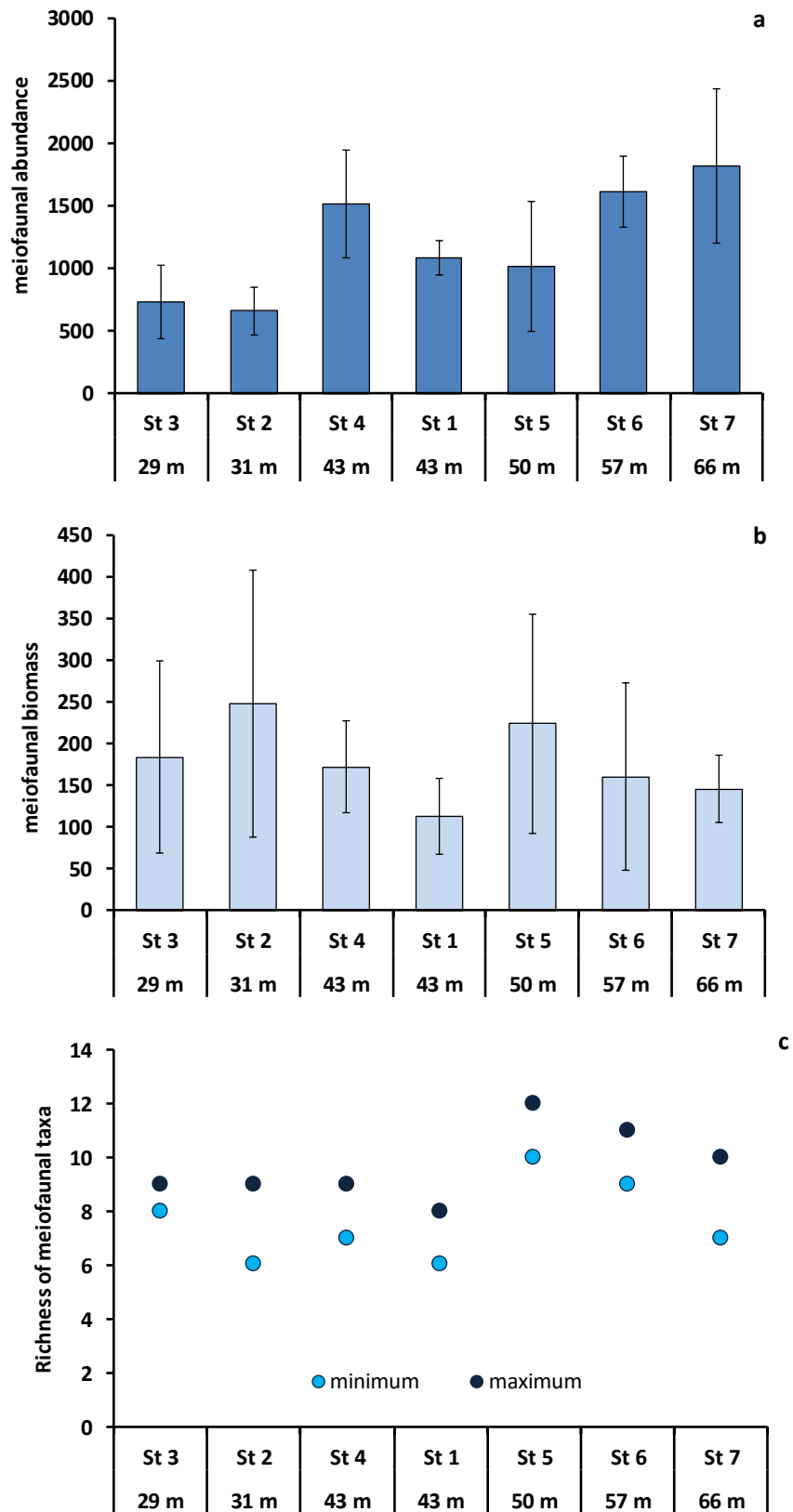


Figure 2

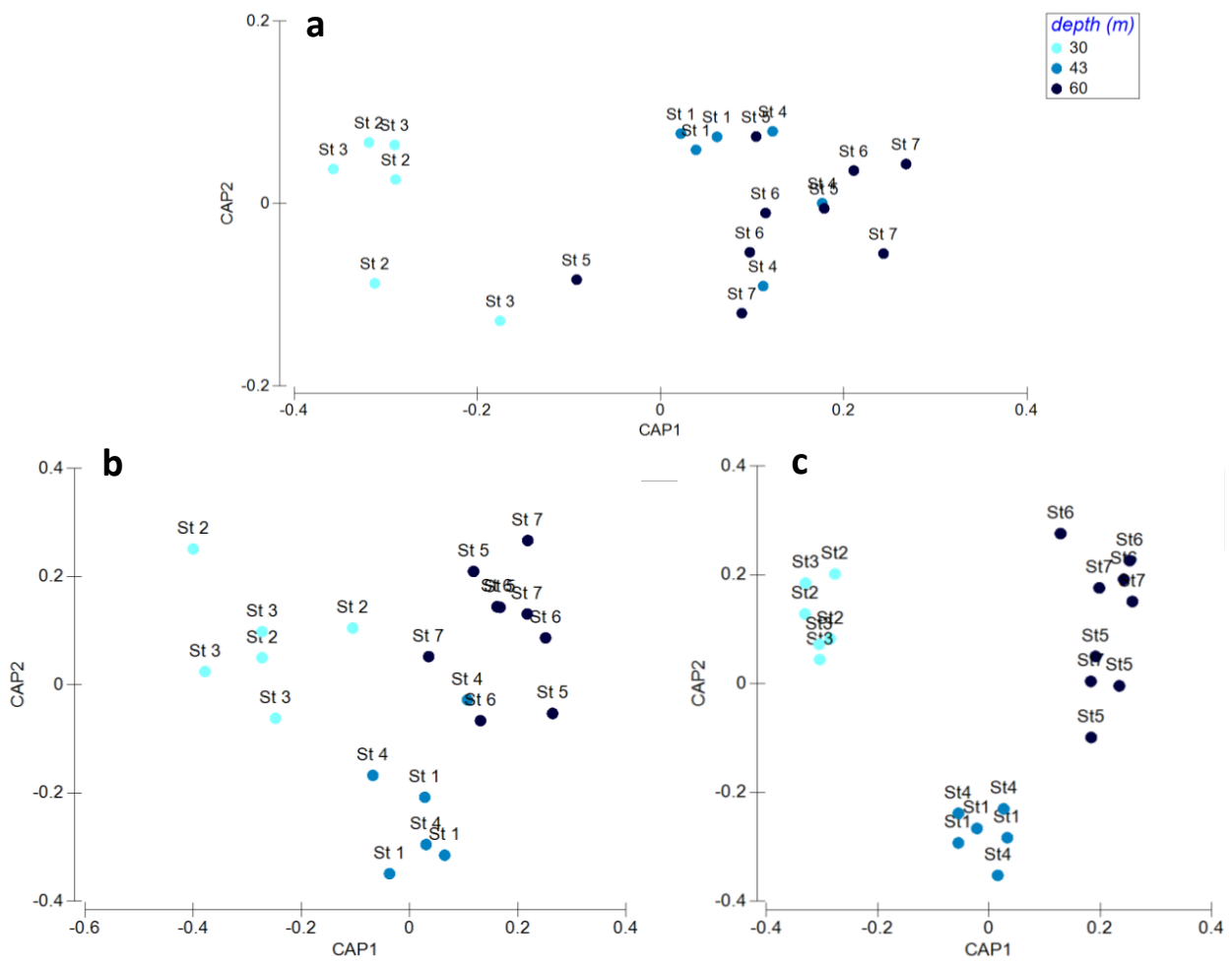


Figure 3

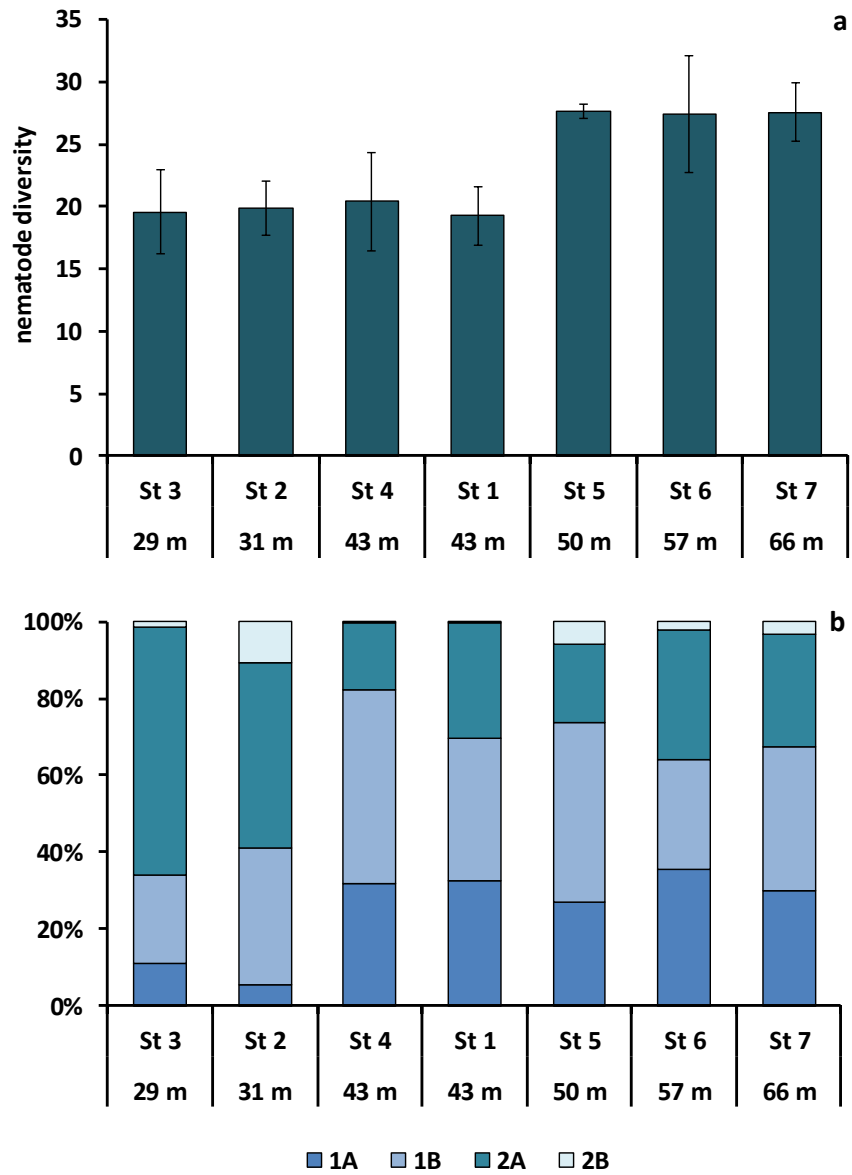


Figure 4

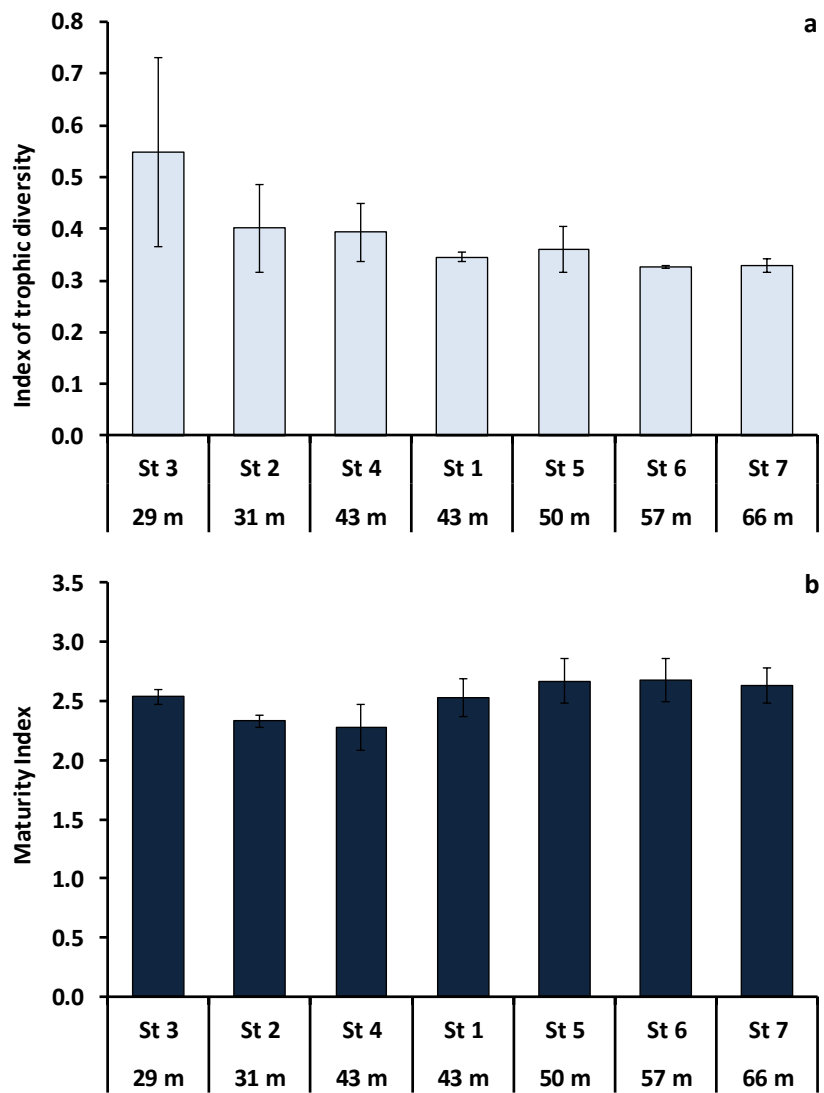


Figure 5

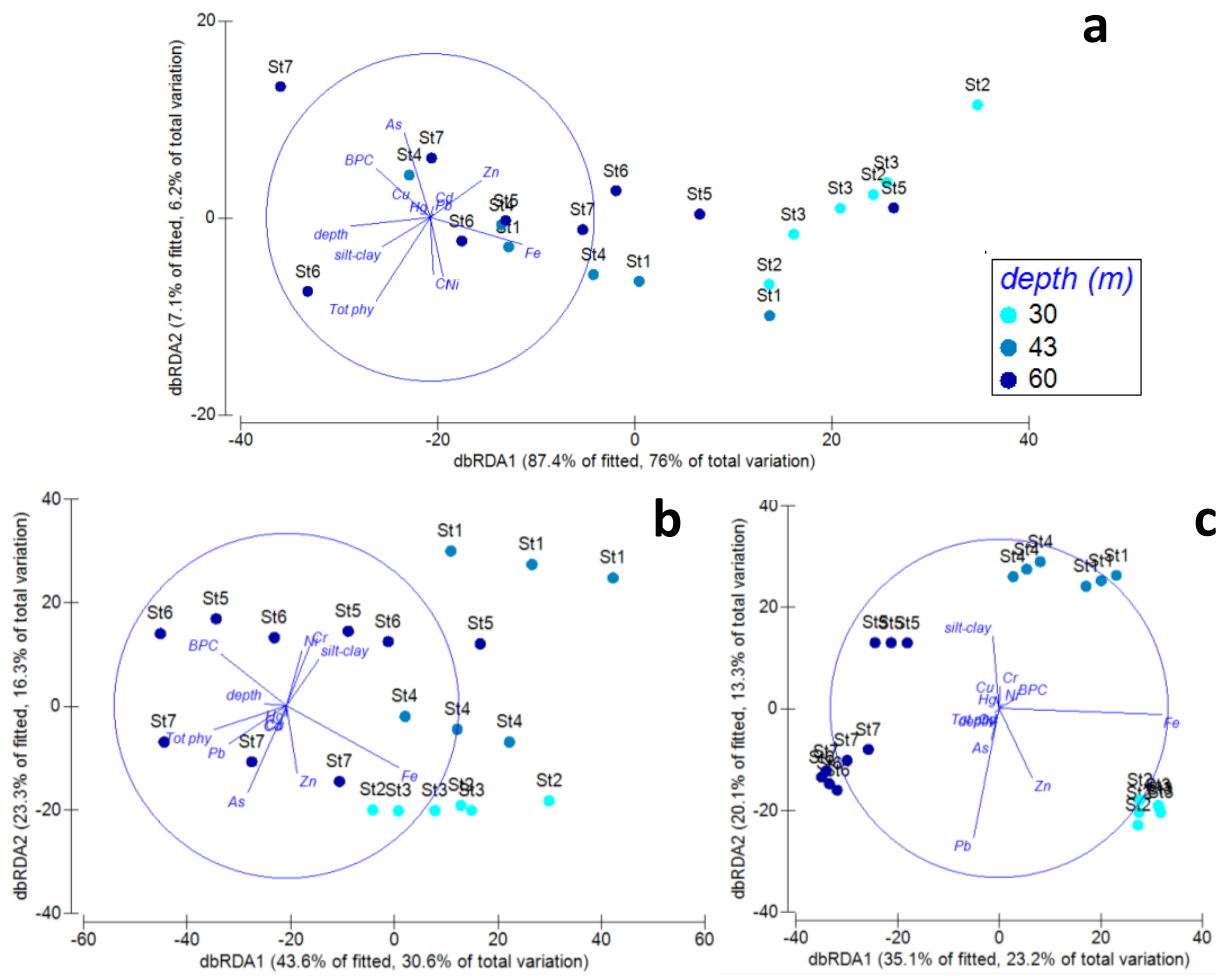


Figure 6

Supplemental online material

Table S1. Output of the PERMANOVA analysis carried out to test for differences in the selected heavy metals concentrations in Portmán Bay (DF=degrees of freedom; MS=mean square; F=F statistic; P=probability level; ***=p<0.001).

	Source	DF	MS	F	P	Pair-wise tests
Ni	Station	6	505	97	***	St2 and St3>St4, St5, St6 and St7; St1 and St4>St5, St6 and St7; St5 and St6>St7
	Residual	14	5			
	Total	20				
Zn	Station	6	14979000	173	***	St 2 and St3>all; St 1 and St4>St5, St6 and St7;
	Residual	14	86559			
	Total	20				
Fe	Station	6	14541000000	142	***	St2>all; St1, St 3and St4>St5, St6 and St7; St5 and St6>St7
	Residual	14	102180000			
	Total	20				
Cr	Station	6	410	35	***	St 2, St3, St4 and St1>St5, St6, St7; St5 and St6>St7
	Residual	14	12			
	Total	20				
Cu	Station	6	175	76	***	St 2, St3, St4 and St1> t5, St6, St7; St5 and St6>St7
	Residual	14	2			
	Total	20				
Pb	Station	6	531890	137	***	St2>all; St1, St3, St4>St5, St6 and St7; St5>St6 and St7
	Residual	14	3877			
	Total	20				
Cd	Station	6	51.339	167.03	***	St2 and St3>all; St1 and St4>St5, St6 and St7; St5>St6 and St7
	Residual	14	0.30737			
	Total	20				
As	Station	6	94585	178.78	***	St2>all; St1>St3, St4, St5, St6 and St7; St 3 and St4>St5, St6 and St7; St5>St6 and St7
	Residual	14	529.06			
	Total	20				
Hg	Station	6	4.69E-03	25.352	***	St2>St4 and St6; St3>StSt4, St6 and St7; St1>St4,

St6 and St7; St4>ST5, St6 and St7; St5>St6 and St7

Residual	14	1.85E-04
Total	20	

Table S2. Effects of metals contamination on abundance, biodiversity and nematode expected species number ES(51) after the removal of the covariables' effect: depth, grain size, total phytopigments and biopolymeric C. F= F statistic; P= probability level; Var(%)= percentage of the variance explained by that explanatory variable; Cumul.(%)= cumulative percentage of variance explained by the explanatory variables. Reported are those variables that display a P level: *p<0.05; **p<0.01; ***p<0.001.

	Variable	F	P	Var (%)	Cumul.(%)	heavy metals
Abundance	Ar	15.9	***	9	9	57
	Cd	16.0	***	20	29	
	Ni	7.4	**	28	57	
Taxa Richness	Ni	10.9	***	36	36	48
	Pb	4.3	*	12	48	
Meiofaunal composition	Ar	5.6	**	6	6	64
	Cd	10.7	***	16	22	
	Cr	6.3	***	19	41	
	Ni	5.6	*	23	64	
Meiofaunal rare taxa	Cd	4.4	**	9	9	60
	Cu	6.6	***	19	28	
	Ni	3.3	**	15	43	
	Pb	3.0	*	7	50	
	Zn	2.5	*	10	60	
Nematode ES(51)	Ar	5.3	*	3	3	91
	Cd	10.0	***	6	9	
	Cu	26.1	***	28	37	
	Ni	22.5	***	54	91	
Maturity Index	Cd	57.3	***	15	15	81
	Cu	34.3	***	43	58	
	Ni	5.8	*	23	81	
Nematode species	Cr	2.5	***	9	9	62
	Cu	2.1	***	7	16	
	Fe	1.8	***	7	23	
	Ni	5.4	***	22	45	
	Pb	2.6	***	7	52	
	Zn	2.5	***	10	62	