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

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32

33 **Abstract**

34 Portmán Bay is one of the most contaminated and chronically impacted coastal marine areas of
35 the world. Here, from the 1957 to 1990, about 60 million tons of mine tailings from the
36 processing of sulfide ores were dumped directly at the shoreline. The resulting deposit provides
37 an unique opportunity to assess the impact of mine tailings on coastal marine ecosystems after ca
38 30 years since the discharge has ceased. We investigated meiofaunal abundance, biomass and
39 biodiversity along a gradient of metal concentration that overlaps with a bathymetric gradient
40 from 30 to 60m depth. Despite the localized presence of extremely high concentration of metals,
41 the bay was not a biological desert, but, nevertheless, was characterized by evident signs of
42 impact on benthic diversity. Meiofaunal variables increased significantly with decreasing metal
43 contamination, eventually reaching values comparable to other uncontaminated coastal
44 sediments. Our results show that mine tailings influenced the spatial distribution of meiofaunal
45 taxa and nematode species composition. In particular, we report here that the bay was
46 characterized by the dominance of nematode opportunistic species tolerant to high metal
47 concentration. The effects of mine tailing discharge on meiofaunal biodiversity and composition
48 were still evident ca 30 years after the end of the mining activities. Overall, this study provides
49 new insights on the potential impact of mine tailings disposal and metal contamination in coastal
50 sediments, and, can also contribute to predict the potential long-term consequences of ever-
51 expanding deep-sea mining industry on benthic environments.

52

53

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

56

57

58 **Highlights**

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

66

67 **1. Introduction**

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

79 This is certainly the case of the Cartagena-La Union Mining District and of Portmán Bay, one of
80 the most important historical mining and metal smelting areas of Spain. From 1957 to 1990,
81 about 60 million tons of mining wastes were discharged directly into the Portmán Bay, facing the
82 mining district (Martinez-Frias, 1997; Oyarzun et al., 2013). Part of this material completely
83 infilled the ancient bay, which originally was more than 10 m deep in its center, and
84 subsequently displacing the shoreline 700 m seawards. The remaining half was swept onto the
85 continental shelf (Conesa and Schulin, 2010). Portmán Bay has been classified as the hottest spot
86 for metals contamination in the entire Mediterranean basin (Cesar et al., 2009), and likely is one
87 of the worldwide marine areas most contaminated by mine tailings (Martinez-Frias, 1997; Koski,
88 2012; Dold, 2014). Previous investigations carried out in Portmán Bay have been mainly
89 dedicated to assess the concentration of metals, their distribution, mobility and toxicity to target-
90 species (Cesar et al., 2004; -2009, Martínez-Sánchez et al., 2008; Fernandez et al., 2010; -2012,
91 Oyarzun et al., 2013; Gómez-García et al., 2015; Benhamed et al., 2016; Mestre et al., 2017). So
92 far, few studies were carried out to assess the ecological status of the bay (Marín-Guirao et al.,

93 2005; Benedicto et al., 2008; Cesar et al., 2009) and the effects of metal contamination on
94 anatomical and physiological traits of benthic marine fauna, including the alteration of the
95 bioluminescence of *Amphipholis squamata* (Ophiuroidea: Echinodermata; Deheyn et al., 2000),
96 the assessment of metal content in calcareous skeletons of sea urchins (Auernheimer and
97 Chinchon, 1997), and the environmental hazard assessment using biomarkers in mussel tissues
98 (Mestre et al., 2017).

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

115 The aim of our investigation is to assess the potential persistent effects of historical (dating back
116 ca 30 years ago) metal-rich mine tailings on the diversity (at the taxon level for meiofauna and at
117 the species level for nematodes) of meiofaunal assemblages along a decreasing gradient of metal
118 contamination that is overlapped with the increasing water depth and distance from the coast

119 over the inner continental shelf. We tested the null hypothesis by which meiofauna is not
120 affected by the mine tailings and chronic contamination in the Portmán Bay and, therefore, that it
121 does not display any significant change along the contamination gradient ca 30 years after the
122 cessation of mining waste dumping. Lessons learnt from the Portmán Bay case study provide
123 new insights on the impact of mine tailings disposal and metal contamination in coastal
124 sediments and can contribute to predict the potential long-term consequences of ever-expanding
125 deep-sea mining industry on benthic ecosystems of Areas Beyond National Jurisdiction that have
126 been tentatively identified (Danovaro et al., 2017; van Dover et al., 2017; Simon-Lledó et al.,
127 2019; Washburn et al., 2019).

128

129 **2. Material and Methods**

130 *2.1 Sampling strategy*

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

141

142 *2.2 Grain size and heavy metal content*

143 Sediment grain size was determined using a Coulter LS230 Laser Diffraction Particle Size
144 Analyser. The concentrations of trace elements in the sediment were determined after acid

145 digestion and the extracts were analysed by inductively coupled plasma-atomic emission
146 spectrometry (ICP-AES). In both cases we followed the procedures reported in Mestre et al.
147 (2017).

148

149 *2.3 Sedimentary organic matter content and biochemical composition*

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

159

160 *2.4 Meiofaunal abundance, biomass and diversity*

161 Each sediment sample was treated with ultrasounds (for 1 min 3 times, with 30 s intervals) to
162 detach organisms from the grain particle surface and, then, sieved through a 1000-µm and a 30-
163 µm mesh net to retain the smallest organisms (Danovaro, 2010). The fraction remaining on the
164 latter sieve was re-suspended and centrifuged three times with Ludox HS40 diluted with water to
165 a final density of 1.18 gcm⁻³ (Heip et al., 1985). All specimens from three independent replicates
166 per station were counted and sorted by taxa, under a stereomicroscope and after staining with
167 Rose Bengal (0.5 gL⁻¹). Meiofaunal taxa representing <1% of the total abundance were defined
168 as rare taxa (Bianchelli et al., 2010). Meiofaunal biomass was assessed by bio-volumetric
169 measurements of all retrieved specimens. Nematode biomass was calculated from their
170 biovolume, using the Andrassy (1956) formula ($V=L \times W^2 \times 0.063 \times 10^{-5}$, in which body length,

171 L, and width, W, are expressed in μm). Body volumes of all other taxa were derived from
172 measurements of body length (L, in mm) and width (W, in mm), using the formula $V=L \times W^2 \times$
173 C, where C is a dimensionless factor (specific for each meiofaunal taxon) used to convert $L \times W^2$
174 to body volume, according to models relating body dimensions and volume (Feller and Warwick,
175 1988). Each body volume was multiplied by an average density of 1.13 g cm^{-3} to obtain the
176 biomass (μg dry weight: μg wet weight = 0.25; Wieser, 1953) and the carbon content was
177 considered to be 40% of the dry weight (Feller and Warwick, 1988).

178

179 2.5 Nematode biodiversity

180 One-hundred and twenty specimens were randomly picked up from the three replicates per
181 station and were mounted on slides using the formalin-ethanol-glycerol technique (Seinhorst,
182 1959). Nematodes were identified to the species level or morphotypes (*sensu* De Mesel et al.,
183 2006) according to Platt and Warwick (1983; -1988), Warwick et al. (1998) and the NeMys
184 database (Bezerra et al., 2020). Nematode diversity was estimated as the expected species
185 number (Sanders, 1968, as modified by Hurlbert, 1971) for a theoretical sample of 51 specimens,
186 ES(51) (Gambi et al., 2014). Species evenness was measured using the Pielou's index (Pielou,
187 1975).

188 The trophic group of nematodes was determined according to the classification of Wieser (1953).
189 Each nematode species was assigned to one of the following 4 trophic groups, based on the
190 buccal morphology: (1A) selective (bacterial) feeders: no buccal cavity or a fine tubular one;
191 (1B) non-selective deposit feeders: large but unarmed buccal cavity; (2A) epistrate or epi-growth
192 (diatom) feeders: buccal cavity with scraping tooth or teeth and (2B) predators/omnivores:
193 buccal cavity with large jaws. The index of trophic diversity (ITD) was calculated as $\text{ITD} = g_1^2 +$
194 $g_2^2 + g_3^2 \dots + g_n^2$, where g is the relative contribution of each trophic group to the total number of
195 individuals and n is the number of trophic groups (n=4 in the present study) (Heip et al., 1985;
196 Gambi et al., 2003).

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

202

203 *2.6 Statistical analyses*

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

219 To visualize differences in the meiofaunal taxonomic composition among stations, bi-plots after
220 a Canonical Analysis of Principal Coordinates (CAP) were also prepared (Anderson and Willis,
221 2003).

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

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

246 dbRDA (Distance-based redundancy analysis) ordination after DISTLM forward analysis, was
247 carried out to fitted model of meiofaunal taxa and nematode species composition (based on

248 Bray-Curtis similarities of no transformed data) versus metals, and environmental and trophic
249 variables.

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

254

255 **3. Results**

256 *3.1 Environmental characteristics and contamination by heavy metals*

257 Grain size analyses revealed that silt-clay dominated in Sts 1 and 4 at 43m, and Sts 5 and 6 at ca
258 60m depth (Table 1). The silt-clay fraction generally increased significantly with increasing
259 water depth, except for St7 (Table 2).

260 The concentration of the investigated heavy metals is reported in Table 3. The results of
261 PERMANOVA analysis revealed that the concentration of heavy metals in Sts 2 and 3 (at ca
262 30m depth) was significantly higher than in all other investigated stations, whereas Sts 1 and 4
263 (at ca 43m) showed concentrations significantly higher than in the deepest stations (Sts 5, 6 and
264 7; ca 60m-depth) (Tables 2 and 3). The results of PERMANOVA carried out separately for Ni,
265 Zn, Fe, Cu, Pb, Cd, As and Hg concentrations revealed that the values were significantly higher
266 in the shallowest stations (Sts 2 and 3) than in the deepest ones (Sts 5, 6 and 7; Supplemental
267 online material Table S1). Among the investigated metals, Zn, Fe, Pb, Cd and As generally
268 showed decreasing concentrations with increasing water depth (Supplemental online material
269 Table S1).

270

271

272

273

274 3.2 *Total phytopigments and sedimentary organic matter*

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

278

279 3.3. *Meiofaunal abundance, biomass and diversity*

280 Meiofaunal abundance varied significantly among stations, with values in Sts 2 and 3 (30m-
281 depth) lower than those in all other stations (except St5 at 50m-depth; Fig. 2a and Table 4) while
282 total meiofaunal biomass did not vary significantly among sampling stations (Fig. 2b and Table
283 4).

284 The richness of meiofaunal taxa ranged from 6 to 12, with values in St5 significantly higher than
285 in Sts 1-4 (Fig. 2c and Table 4). Overall, 13 taxa were identified: nematodes, copepods,
286 polychaetes, ostracods, kinorhynchs, oligochaetes, cumaceans, amphipods, isopods,
287 tanaidaceans, acarians, nemerteans and decapods larvae. Nematodes were the dominant taxon
288 (77-91% of total meiofaunal abundance) at all sampling stations, followed by copepods
289 (including their nauplii 4-18%) and polychaetes (1-5%). All other taxa accounted each for <1%
290 of the total meiofaunal abundance and were here reported as “rare taxa”. The composition of
291 either higher and rare taxa assemblages varied significantly among stations (Table 4). The CAP
292 analyses on higher meiofaunal taxa highlighted the presence of assemblages in Sts 2 and 3
293 clearly different from those in all other stations (Fig 3a). The same analysis carried out on
294 meiofaunal rare taxa identified again three distinct groups of stations (Fig. 3b).

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

298

299

300

301 3.4 Nematode biodiversity

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

309 The output of the CAP analyses revealed the presence of different nematode assemblages
310 segregating according to the contamination gradient present at different depths (Fig. 3c). The
311 SIMPER analysis showed that nematode turnover diversity was high as dissimilarity in species
312 composition between couples of stations varied from 63% (between Sts 2 and 3 and between Sts
313 6 and 7) to 88% (between Sts 3 and 5).

314 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
315 turnover component of dissimilarity ranged from 0.59 (Sts 1-4 vs Sts 5-7) to 0.65 (Sts 2-3 vs Sts
316 5-7) while the nestedness-resultant component varied between 0.04 (Sts 2-3 vs Sts 1-4) and 0.14
317 (Sts 2-3 and Sts 1-4 vs Sts 5-7).

318 The trophic structure of nematode assemblages is reported in Fig. 4b. Deposit feeders (selective
319 plus non-selective trophic groups) represented from 64 to 82% of the entire nematode
320 assemblages dominated in all stations at 43m and 60m, whereas epistrate feeders provided a
321 prominent contribution (48-65%) at the shallowest stations located at 30m. Predators accounted
322 for from 1 to 11% of nematode assemblages. The contribution of the different groups to the
323 nematodes trophic structure varied significantly among the shallowest stations (dominated by
324 epistrate feeders) and all other sampling sites (dominated by deposit feeders; Fig. 4b and Table
325 4).

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

328

329 *3.5 Drivers of meiofaunal assemblages and nematode diversity*

330 The results of the DISTLM analyses revealed that depth (49%), Cr (19%), Ni (10%) Cd (6%)
331 and Biopolymeric C (2%) accounted for 86% of total variance of meiofaunal abundance (Table
332 5). Depth (41%), Cr (25%), Pb (12%), Zn (4%) and total phytopigments (3%) accounted for 85%
333 of total variance of meiofaunal higher taxa composition while Biopolymeric C and Cd (both
334 18%), grain size (13%), total phytopigments (11%) and Fe, Pb and Zn (6-4%) accounted for 75%
335 of the total variance of rare taxa composition. Cd (54%), grain size (11%), depth (9%), Cu (8%),
336 total phytopigments and Hg and Ni (2-3%) accounted for 91% of the total variance of nematode
337 diversity while Zn (22%), Cd (10%), total phytopigments (9%), Biopolymeric C (7%), Fe and
338 Pb(5-6%) accounted for 59% of the total variance of species composition. The effect of metal
339 contamination on meiofaunal abundance and (nematode) diversity and composition accounted
340 for 91% of variance after the removal of the covariables (depth, grain size and food source)
341 (Supplemental online material Table S2).

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

350

351

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 Dyngadjupet (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 (Trannum 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

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

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

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

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

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

429 Heavy metal contamination has a prominent role in driving the spatial distribution of meiofaunal
430 composition since three different assemblages can be identified according to the metal
431 concentration at increasing distance from the shoreline along the bathymetric gradient. We can
432 explain this pattern as the result of the spatial distribution of taxa, especially rare groups, with
433 different levels of tolerance to the metals contamination along the increasing distance from the
434 shoreline and the increasing of food sources availability.

435

436 *4.2 Impact of mine tailings on nematode diversity*

437 The effect of heavy metal contamination due to mine tailing discharge on benthic fauna is
438 evident also at the nematode species level and, in particular, nematode diversity is highest in
439 deepest sediments (Sts 5 and 7), where metals contamination is the lowest. The tolerance to
440 metal pollutants has been shown to vary widely among nematode species (Hagerbaumer et al.,
441 2015 and references therein), and it has been observed that a long exposure to metals can lead to
442 the selection of tolerant nematode species (Millward and Grant 1995; Bastami et al., 2017;
443 Gambi et al., 2020). Accordingly, our results reveal that highly contaminated sediments host
444 specific nematode assemblages, characterized by a few epistrate feeders species. As for the
445 whole meiofaunal community, heavy metal sedimentary content is the main factor influencing
446 the spatial distribution of nematode species in Portmán Bay. Changes in nematode species
447 composition, indeed, allow discriminating again three groups whose dissimilarity in species
448 composition is high and increases when assemblages from stations with high and low metals
449 concentrations are compared. This is also confirmed by the average pairwise dissimilarity
450 estimated according to Balsega (2010, -2013). Our results indeed suggest that the variation of the
451 nematode species composition is mainly due to species replacement among assemblages at
452 increasing distance from the shoreline and thus at decreasing metals contamination while the
453 variation in species composition derived from nested patterns is negligible.

454 The dominant nematode species in the most contaminated sediments belong to the genera
455 *Sabatieria*, *Spilophorella*, *Desmodora* and *Pseudochromadora*, which are known to tolerate
456 different impacts, including sediments affected by long-term heavy metals' contamination (e.g.,
457 Tietjen, 1980; Hendelberg and Jensen, 1993; Steyaert et al., 1999; Heip et al., 1990; Vanreusel,
458 1990; Montagna and Harper, 1996; Schratzberger et al., 2006; Moreno et al., 2009; Frascchetti et
459 al., 2016). On the other hand nematode assemblages in the less contaminated sediments are
460 dominated by species belonging to the genera *Richtersia* and *Desmoscolex*, sensitive to different
461 sources of impact (Moreno et al., 2011). Our investigation reveals that changes in species
462 composition are also related to changes in the nematode trophic guilds composition (deposit vs
463 epistrate feeders) suggesting that the high levels of contamination and the low food availability
464 can act as a physical barrier in the shallower stations at 30m-depth. These patterns reflect
465 changes also in the Maturity Index, thus suggesting the presence of nematode assemblages with
466 different trophic and life strategies. Indeed highly contaminated sediments with lower food
467 availability show a large contribution of "colonisers" (as opportunistic species), while sediments
468 with low metals concentration and higher food availability host "persister" species (Bongers et
469 al., 1991; Bongers and Ferris, 1999). Food availability and heavy metals content play an
470 important role in influencing nematode assemblages suggesting a similar response observed for
471 macrofauna inhabiting contaminated sediments where the competition for food sources is an
472 important driver to define species composition (Ryu et al., 2011).

473 Huge progress has been made in the last years toward technologically feasible and economically
474 viable seafloor mining (Jones et al., 2017). While mining techniques differ depending on the
475 resources to be exploited and the ecosystems hosting them, many of the potential impacts on the
476 ecosystem are similar (Levin et al., 2016; Van Dover et al., 2017). Upcoming industrial seabed
477 mining is expected to determine habitat modifications and species mortality due to mechanical
478 disturbances (Ellis, 2001; Van Dover, 2014; Jones et al., 2017) and impacts due to the release
479 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.

500

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507

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819 **Caption of figures**

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

823 **Figure 2.** Meiofaunal assemblages in Portmán Bay. Reported are meiofaunal: a) abundance (ind
824 10 cm^{-2}), b) biomass ($\mu\text{gC } 10\text{ cm}^{-2}$) and c) richness of taxa (as minimum and maximum number
825 of taxa among three replicates) in all investigated stations. Reported are average value $\pm$ standard
826 deviation for abundance and biomass.

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

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

834 **Figure 5.** Nematode functional diversity in Portmán bay. Reported are a) Index of Trophic
835 Diversity and b) Maturity Index in all investigated stations. Reported are average value $\pm$
836 standard deviation.

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

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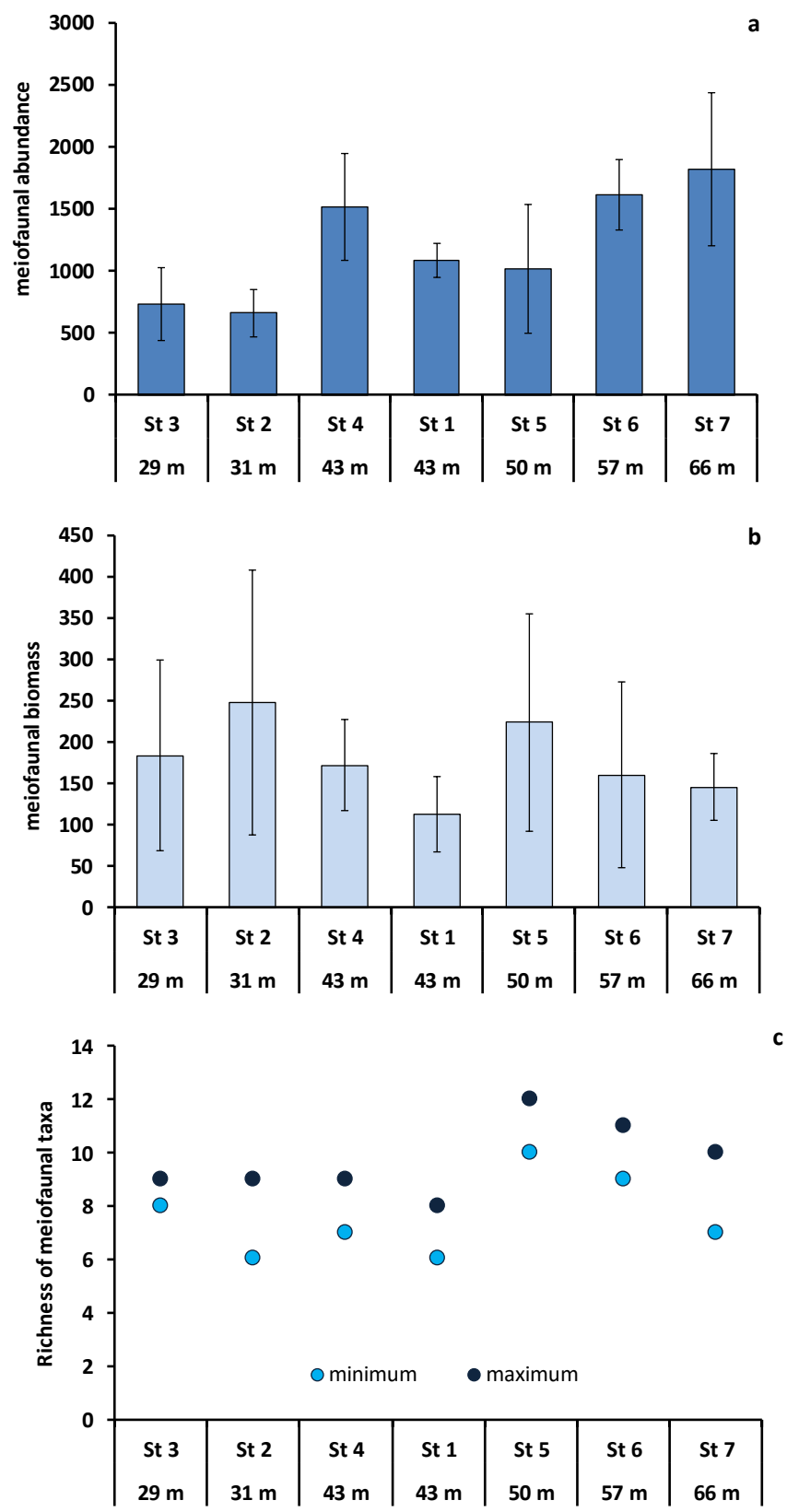
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Figure 1

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Figure 2

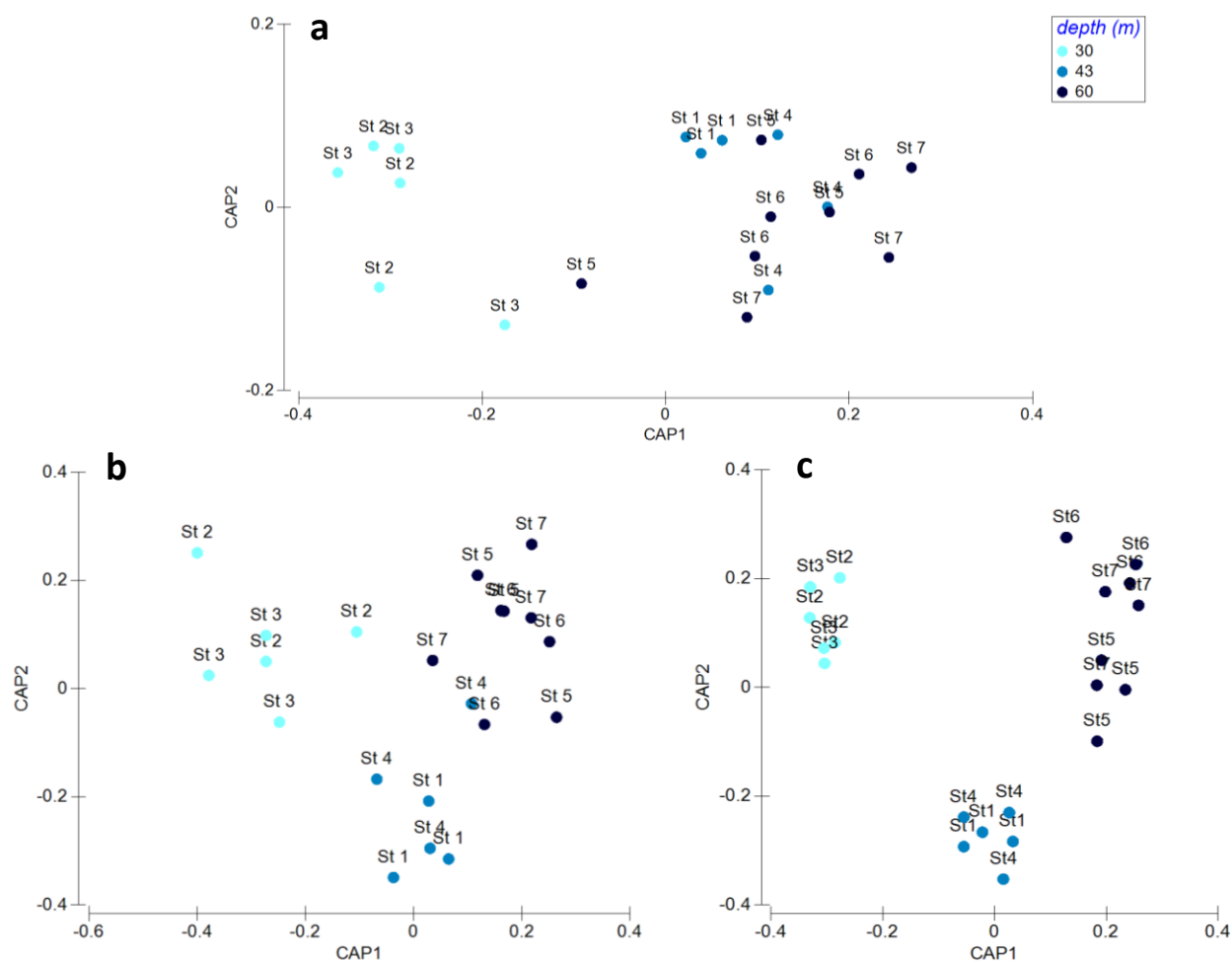


Figure 3

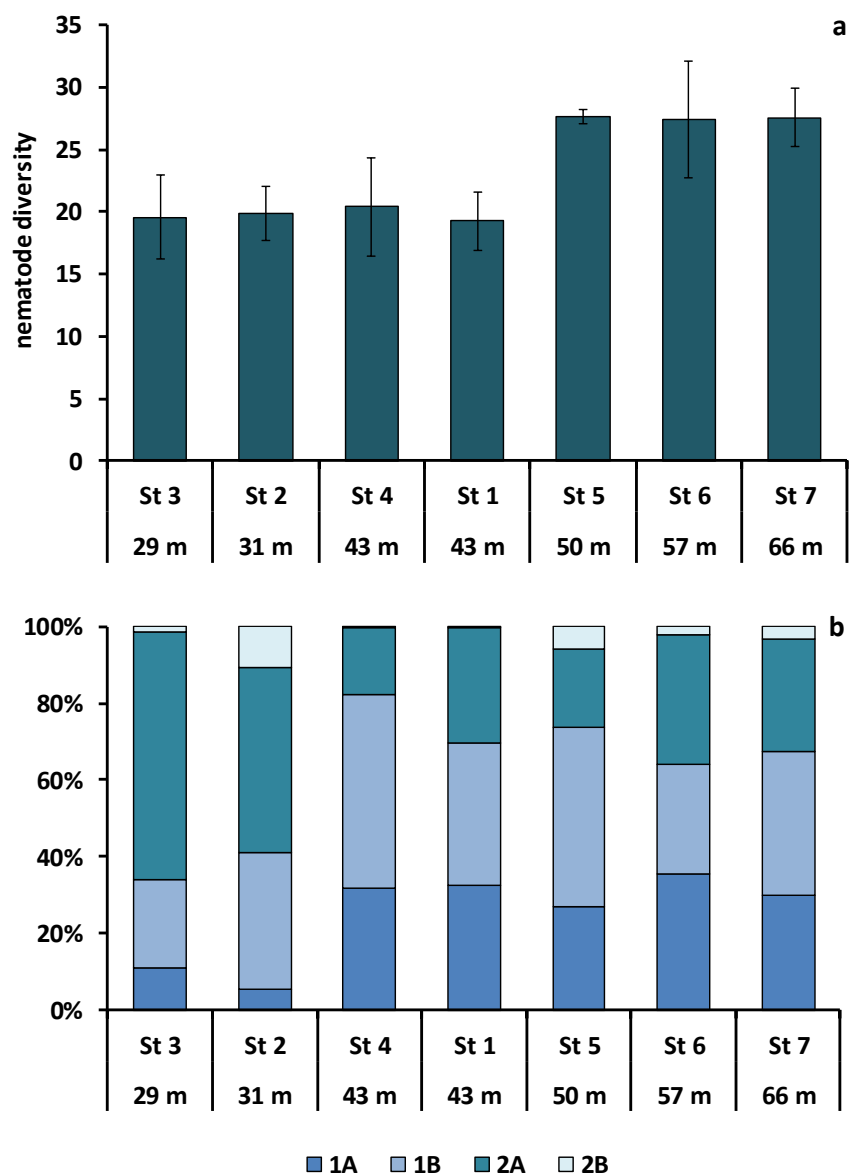


Figure 4

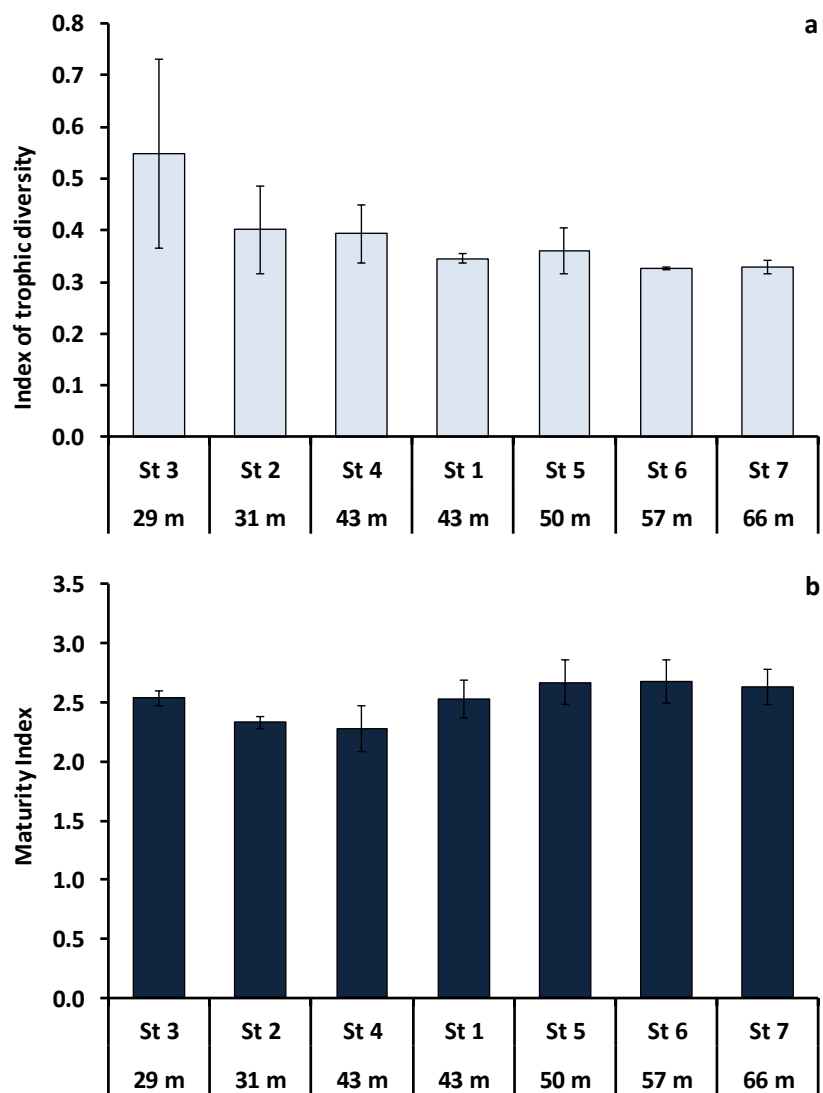


Figure 5

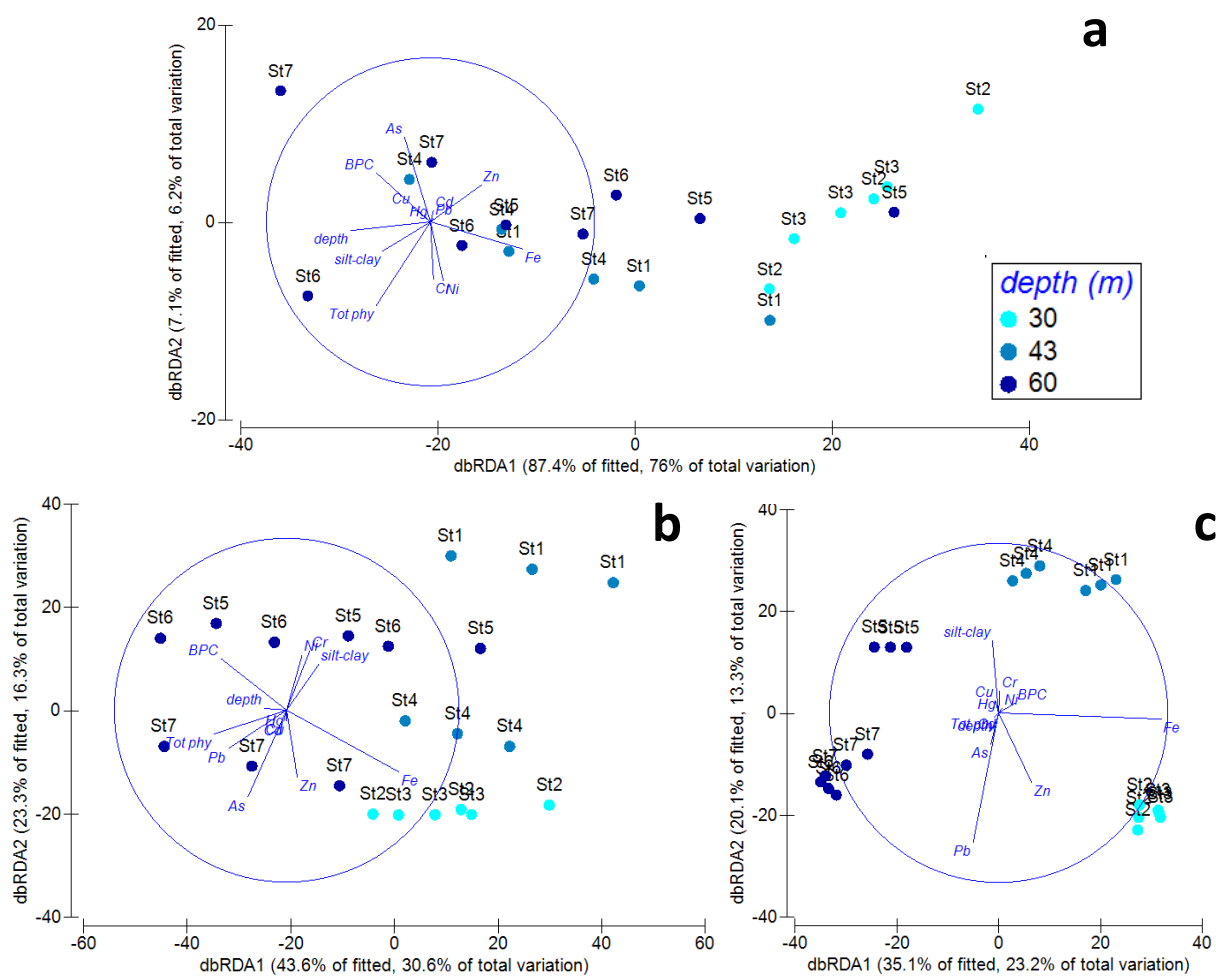


Figure 6

877 **Supplemental online material**

878 Table S1. Output of the PERMANOVA analysis carried out to test for differences in the selected
879 heavy metals concentrations in Portmán Bay (DF=degrees of freedom; MS=mean square; F=F
880 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				St2>St4 and St6; St3>StSt4, St6 and St7; St1>St4, St6
			4.69E-03	25.352	***	and St7; St4>ST5, St6 and St7; St5>St6 and St7
	Residual	14	1.85E-04			
	Total	20				

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

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	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	

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