INTEGRATIVE ASSESSMENT OF SEDIMENT CONTAMINATION IN PORTMÁN BAY (SOUTHEAST-SPAIN)

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ABSTRACT – **Portmán Bay, southeast Spain, contains the most seriously heavy metal-contaminated sediments of the Mediterranean Sea. The objective of our study was to characterize the nature and extent of metal contamination and the responses of natural communities to it and to assess the toxicity of the sediment deposits ten years after mining had ceased. We studied the physical and chemical characteristics of the sediments, and the toxicity of the porewater and sediment-water interface using two sea urchin species. Metal bioavailability and patterns of macroinvertebrate community composition along the contamination gradient were also studied. Uni- and multivariate analyses indicate a strong relationship between sediment metal concentration, toxicity and community structure.**

KEYWORDS – **AVS/SEM; Benthos; Sea urchins; Sediment-water interface; Porewater; Sediment quality triad.**

INTRODUCTION

The mining of metals in the area of Portmán (Murcia, SE/Spain) has a long history. The name Portmán derives from the Latin ¨Portus Magnus¨, because it was a natural harbor from which lead was embarked for use throughout the Roman Empire. From 1958 to 1991, the Peñarroya mine pumped 3-10,000 tons of tailings per day, first directly into the bay and later, when the bay was filled up. In the extraction process 2 m^3 of water were used per ton of mineral and a ton of sodium cyanide and some 10 tons of sulfuric acid, as well as, copper sulfate, were used per day. All this material was poured into the sea including the remains of heavy metals know to be toxic and bioaccumulative, such as cadmium, copper, lead and zinc. A study carried out by the Spanish Institute of Oceanography found that the mineral wastes were distributed along the continental platform and continental slope to a depth of about 150m [1]. Before the mine closed, these wastes represented more than 50% of the contribution of metals to the Mediterranean Sea and more than 90% of the pollution caused by solid residues [2].

The spatial distribution of metal contamination (Cd, Pb, and Zn) in the water column and sediments was characterized during the 1980's [1,3,4]. However, the toxicity of these sediments and the interactive effects on benthic communities has not been addressed by previous studies. The objective of our study was to characterize the nature and extent of metal contamination, sediment toxicity and the responses of natural communities to assess the environmental quality of the sediment deposits ten years after mining ceased.

MATERIAL AND METHODS

SAMPLE COLLECTION AND FIELD MEASUREMENTS

Samples were collected synoptically along a spatial gradient at the same depth (10-20 m) in March 2002. The spatial sampling design followed previous studies (September and December 1999, October 2000) [5,6]. Sampling stations were selected at regular distances between the old mine discharge and Cabo de Palos (West-East; Fig.1), while the control station was located on Fraile Island, approximately 60 km from the old emission point.

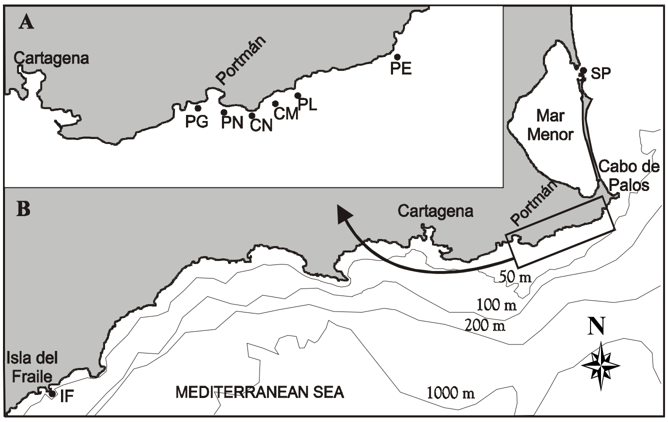

FIG. 1. A. MAP OF THE STUDY AREA AND B. LOCATION OF SEDIMENT SAMPLING STATIONS, IF- ISLA DEL FRAILE; PG - PUNTA GALERA; PN - PUNTA NEGRA; CN – CABO NEGRETE; CM – CANTO DE LA MANCEBA; PL – PUNTA DE LA LOMA LARGA AND PE – PUNTA ESPADA.

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Replicate samples (n=4) were collected from all points (n=7) along the contamination gradient on a spatial scale considered appropriate for examining differences (km) along the gradient. Intact sediment cores were collected by SCUBA divers, capped and sealed carefully underwater and retained in the tubes (10 cm diameter) throughout storage (4ºC in the dark). Sediment samples were divided into subsamples for the chemical analyses and toxicity testing to maximize the potential for data integration. Only the top 3-5 cm of the superficial sediment was used. Sediments were stored at 4ºC in the dark for no longer than 7 days, prior to toxicity testing. Sediment porewater was extracted by centrifugation (2,500 G) for 10 min at 4ºC. The supernatant was decanted and the process was repeated to remove any remaining particles. Porewater extracts were kept at 4ºC for no longer than 24h prior to toxicity testing. The control and dilution water used in the experiments consisted of natural seawater (38 psu) collected in unpolluted areas (where the sea urchins were also collected) and filtered through a GFC Watman® filter. Laboratory subsampling took place under strictly anaerobic conditions for acid-volatile sulfide and simultaneously extract metals (AVS/SEM), and were stored frozen (-20ºC) to prevent sulfide oxidation.

Four replicate samples were collected from each sampling station for benthic analysis using a 0.04 m^2 hand grab by SCUBA divers and sieved through a 500 µm mesh bag. The macroinvertebrates retained on the screen were fixed with 4% buffered formalin, and later washed and transferred to 70% isopropyl alcohol prior to sorting and identifying the macrofauna. Each sieved sample were individual taxa identified and enumerated in the laboratory by stereoscope microscopy to assess species richness and abundance. All the organisms were sorted and identified to the lowest possible taxon level and their abundance was counted.

SEDIMENT CHEMICAL AND PHYSICAL ANALYSIS

The sediment water content was measured as a percentage of wet weight lost by drying until constant weight at 60ºC for 24h. The dried sediments were finely ground and carefully sieved in a 63 µm stainless steel mesh. The total organic carbon (TOC) content was determined with a Carlo Erba Instruments (EA1108) elemental analyser following sample preparation with 1 N HCL to decompose the carbonate [7]. The percentage of organic matter in samples was estimated by the loss of weight on ignition at 600ºC for 8h of dried sediment from which the carbonates had previously been removed by acid treatment [8].

The concentration of ammonia (NH_3) was determined from the total ammonium (NH_4) concentration, pH, temperature and salinity of each sample.

Grain size was determined by standard mechanical dry sieve-shaker techniques to determine the sand, silt and clay fractions [8].

The moisture content of the sediment subsamples necessary to calculate AVS and SEM concentrations on a dry weight basis was determined by measuring the weight loss after freeze drying. The basic procedures for extracting AVS and selected metals that are solubilized during the acidification step (SEM) have been described by Allen *et al.* [9]. Five grams of wet sediment was weighed exactly on a 2 cm^2 piece of Parafilm® and introduced into the standard taper round-bottom flask with 100 ml deionized water (Milli-Q®) and a magnetic stirring bar. The sediment samples were acidified for 30 min by 20 ml of 6 N HCl solution added to the digestion flask to form H_2S, which was subsequently trapped in a 80 ml sulfide antioxidant buffer (SAOB II) trap solution. The apparatus was deaerated by bubbling nitrogen for 10 min at a flow rate of 100 cm^3/min, and then the flow was reduced to 40 cm^3/min during the digestion period. The sulfide ion concentration in the trap solutions was measured with a combined sure-flow silver/sulfide ion selective electrode (ISE-Orion model 9616), which offers the additional benefit of not requiring a separate reference electrode. Following digestion, the samples were analysed for aluminium, arsenic, cadmium, copper, iron, mercury, nickel, lead and zinc. These simultaneously extracted metals (SEM) were collected by filtration of the acid-sediment slurry and measured with an optical emission spectrometer (Optima 2000 DV– Perquin Elmer).

TOXICITY TESTING

The sea urchins used in this study were obtained by SCUBA divers off Fraile Island (IF). The toxicity of the sediment porewater (PW) and sediment-water interface (SWI) was determined using embryo larval development tests with two sea urchin species, *Arbacia lixula* and *Paracentrotus lividus*, following the procedures described previously [6]. Simultaneous with the porewater and sediment-water interface toxicity test, each lot of embryos was tested with four reference toxicants: ammonium chloride (NH_4Cl), cadmium chloride ($CdCl_2$), sodium dodecyl sulfate ($C_{12}H_{25}NaSO_4$) and zinc sulfate ($ZnSO_4$), in accordance with accepted guidelines) [10-12]. The results of these reference toxicants and comparisons between amphipods and sea urchins species were reported in a previous [13].

BENTHIC COMMUNITY ANALYSIS

The macroinvertebrate taxonomic data was quantified using the relative benthic index (RBI) adapted to Mediterranean fauna, developed by Anderson *et al.*, [14,15] and the IBI (Integrative Benthic Index), a new index calculated for this study that integrated different ecological community parameters was based on the same methodology. The original RBI was based on six categories, including total number of species, number of crustacean species, number of mollusk species, number of crustacean individuals, and the presence or absence of positive and negative species. The IBI was based on ten categories, including the total number of species, the number of crustacean species, number of mollusk species, number of polychaetes families, total number of individuals, and presence or absence of positive and negative species obtained from multidimensional scaling (MDS) analysis and other classical ecological indices (Shannon-Wiener, Pielou and Margalef). The positive indicator species were *Phoxocephalus aquosus, Urothoe grimaldii* (Amphipoda) and *Branchiostoma lanceolatum* (Branchiostomida); the negative indicator was *Apseudes latreilli* (Tanaidacea). Positive indicators are not generally found in polluted habitats and are characteristic of regions where anthropogenic and other severe disturbances do not play a major role in structuring communities, while the negative indicators are highly distributed in polluted stations and are not found in unpolluted points [16].

The threshold value for a degraded benthic community was set at 0.30 since 0.00 to 0.30 was considered indicative of a degraded benthic community, 0.31 to 0.60 was considered transitional, and 0.61 to 1.00 was considered undegraded.

Statistical analysis

Toxicity data were checked for normality and homoscedasticity assumptions with Shapiro-Wilk's and Bartlett's test, respectively. Larval development data were arc sine square root transformed prior to statistical analysis. Differences were evaluated with a parametric analysis of variance (ANOVA), followed by Dunnet's test. These analysis were carried out with the statistical package Toxstat$^{®}$ V.3.3 [17]. The Newman-Keuls test was also applied to compare the means of normally developed larvae obtained in the sea urchin toxicity tests.

Univariate measures included the Shannon-Wiener diversity indices calculated using natural logarithms (H'), species richness (Margalef´s d), evenness (Pielou´s J), total abundance (A), and abundance of taxa (S). The significance of differences between points was tested using one-way ANOVA.

Interrelationships between components

Multivariate relationships between chemical concentrations and biological effects were evaluated using correlation-based principal components analysis (PCA). Associations were evaluated among the 6 biological effects and 16 physical-chemical variables. In all cases, only significant negative associations were reported (i.e. increase chemical concentrations associated with decreased biological structure). The analysis was conducted with a correlation matrix and varimax rotation and included any factors that accounted for greater than 10% of the total variance. A component loading cutoff of 0.40 was used in selecting variables for inclusion in factors [18] that a cutoff of at least 0.32 be used and that component loading of greater than 0.45 be considered fair or better.

For most of the analyses nonparametric multivariate techniques were used, as described in the PRIMER (Plymouth Routines In Multivariate Ecological Research, v5) [21-22] suite of computer programs developed at the Plymouth Marine Laboratory, UK. Ranked lower triangular similarity matrices were constructed using a range of data transformations, the Bray-Curtis similarity measure and group- average sorting. Transformations (fourth root) were used to reduce contributions to similarity by abundant species, and therefore to increase the importance of the less-abundant species in the analysis.

Community structure was examined using multidimensional scaling (MDS) and the significance of differences between stations was assessed using the analysis of similarities (ANOSIM- randomization/permutation test) using the Euclidian distance matrix underlying the ordination [19, 23].

Results

Sediment chemical and physical analysis

Concentrations of un-ionized ammonium (NH_3), total organic carbon, acid volatile sulfides (AVS) and simultaneously extracted metals (SEM), AVS/SEM ratio and grain size, are provided in Cesar et al. [24]. The metal concentrations in sediments showed a strong gradient despite the cessation of mining activity in Portmán Bay twelve years ago. Sediment concentrations of heavy metals, including Zn, Al, Pb and Fe, were low in the control station (IF) and progressively increased towards the emission point (PG), where the highest levels were recorded. Zn, Al, Pb and Fe were found in high concentrations near the emission point and were statistically associated with the toxicity of sea urchin larvae and benthic community structures.

Toxicity testing

The percentage of normally developed larvae in PW and the SWI tests were reported in a previous work [6]. The results indicated adverse effects in SWI and PW tests in stations PG, PN, CN, CM and PL, which showed statistical differences from the control station, IF. However, station PE was not significantly different from the control IF and can also be considered as a reference station. Toxicity tests with PW and SWI presented a similar pattern of response although the normally developed larvae were lower in the former. Results of the analysis of variance and post hoc tests pointed to significant differences in normally developed larvae between the PW and SWI tests (table 1).

TABLE 5. ANALYSES OF VARIANCES AND POST HOC TEST FOR TOXICITY TESTS AND RELATIVE BENTHIC INDEX.

	Univariant measures	F	p-level	Post hoc test Tukey HSD
Benthic Index	RBI	12.5583	***	IF PE PL CM CN PN PG
	IBI	23.5174	***	IF PE PL CM CN PN PG
Toxicity Tests	*A. lixula* (PW)	676.451	***	IF PE PL CM CN PN PG
	A. lixula (SWI)	410.292	***	IF PE PL CM CN PN PG
	P. lividus (PW)	325.814	***	IF PE PL CM CN PN PG
	P. lividus (SWI)	320.624	***	IF PE PL CM CN PN PG

Benthic community analysis

Univariate metrics of the community structure for all the sampling stations are provided in Cesar et al. [24]. where they point to a significant variation along the metal contamination gradient. Stations PG, PN and CN (with a degraded benthos) had fewer species, lower abundance, lower diversity, and lower benthic index scores than stations with a healthy benthos (PE, IF). A similar pattern of disturbance was indicated by the RBI and IBI values. In both indices, all the samples from PG, PN and CN showed signs of disturbed assemblages and the lowest values. However, a more effective assessment of the gradual changes in the benthic community structure along the Portmán gradient was obtained with the IBI values than with the original RBI (Table).

INTERRELATIONSHIPS BETWEEN COMPONENTS

Metal concentrations in sediments were associated by PCA with indicators of biological effects: toxicity to sea urchin larvae in PW and SWI, RBI and IBI. The chemical or biological variables which had mutual correlations averaging more than 0.95 were reduced to a single representative. The variables, Zn, Al, Pb, and Fe, were reduced to Zn because of their high degree of correlation. *A. lixula* SWI was retained and the other three variables were excluded (*A. lixula* and *P. lividus* PW and *P. lividus* SWI), leaving 12 abiotic variables and 3 biotic variables in the PCA search. PCA ordination of the stations (Fig.2) based on these 15 new variables showed an environmental gradient of biological and chemical variables. The application of factor analysis to the 15 variables (chemical concentration in sediments and biological effects) identified three new variables or principal factors. These new factors explained 72.8% of the variance in the original data set. The analysis also indicated that Zn, Al, Pb, Fe and organic matter were associated with biological effects along the contamination gradients. The first principal factor, PC1, was predominant and accounted for 42.6% of the variance. This factor combines the chemical concentrations of Zn, Pb, Al, Fe and organic matter and the biological variables (larval toxicity tests, RBI and IBI). The second factor, PC2, accounts for 17.4% of the variance and combines NH_4 and Ni. This may be taken to represent the toxicity of ammonium in porewater. The third factor accounts for 12.8% of the variance and is a combination of AVS and AVS/SEM. and represents the heavy metals that are associated with the structure of the community. Factors PC4 and PC5, with the lowest eigenvalues, accounted for the lowest variances (8.2 and 5.6%). The two-dimensional PCA plot represented in figure 2, with the stations ordered along the first axis according to biological effects and heavy metal concentrations. All samples from PG and PN had PC1 scores greater than 2, suggesting that they are severely polluted. However, samples from the control and reference stations (IF and PE) or and these taken from the points furthest from Portmán Bay (PL, CM and CN) had PC1 scores of between -3 and 0, pointing to undisturbed or moderate levels of disturbance (according to this interpretation).

MDS ordinations (Fig.3) at species level showed a gradation in the benthic community structure. PE and IF stations (at the top of the plot) were clearly separated from impacted stations PG, PN and CN (at the bottom of the plot), and the transitional station PL end CM (near the top of the plot). The stations closest to the disposal points PG, PN and CN were located in the lowest edge, suggesting greater disturbance, especially at station PG close to the old effluent discharge.

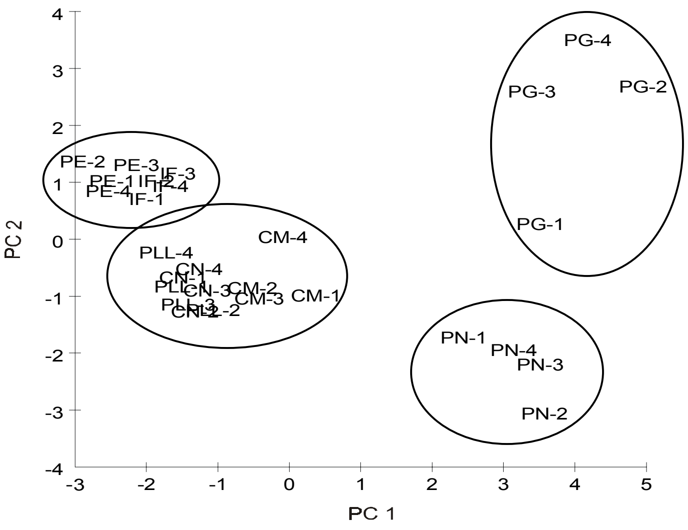

FIGURE 2. PCA ORDINATION OF ENVIRONMENTAL GRADIENT OF BIOLOGICAL AND CHEMICAL VARIABLES FROM SAMPLING STATIONS.

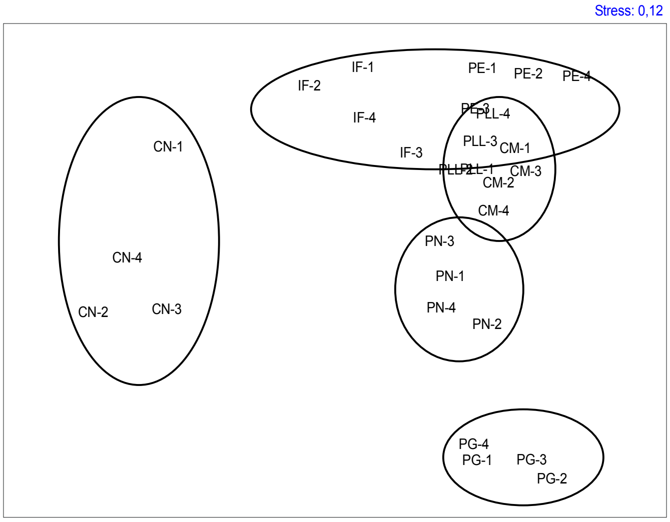

FIGURE 3. MULTIDIMENSIONAL SCALING ORDINATIONS FOR FOURTH ROOT TRANSFORMED TOTAL FAUNA ABUNDANCE (STRESS = 0.12) IN SAMPLING STATIONS.

DISCUSSION

Most investigations of mining-related sediment toxicity have concentrated on Cu, Zn, Pb and Cd [25] and the redistribution processes of these metals among the different sediment phases that effect their bioavailability and, therefore, the environmental quality of the system [26]. This is of special importance for littoral ecosystems because of their fragile nature and the high amounts of metals they generally receive.

In own study, multivariate analyses indicated a strong relationship between community structure and sediment metal concentrations because there were no other confounding factors such as organic matter, depth and freshwater runoff. Multivariate analyses suggest that the threshold levels for the benthic community were those prevailing between CM and PL stations, where the sediment metal concentration ranged from 0.21-1.35 μM/g dry weight for Zn and 0.07-0.32 μM/g dry weight for Pb.

Sediment quality values need to be a developed to help protect public health and the environment [27]. A multivariate statistical analysis, based on PCA as the extraction method during the evaluation of data using factor analysis, has been proposed to establish the concentration ranges of the chemicals associated with adverse effects [27].

Sediment toxicity tests are technically well developed [28, 29] and are widely accepted as useful tools for a wide variety of research and regulatory purposes [30-32]. For example, they are used to determine the sediment toxicity of single chemicals and mixtures, chemical bioavailability, the potential adverse effects of dredged material on benthic marine organisms, and the magnitude and spatial and temporal distribution of pollution impacts in the field [33]. The present study contributes toward the standardization of biological assessments of sediments using embryolarval sediment-water interface. These bioassays were conducted with the Mediterranean sea urchin species *Arbacia lixula* and *Paracentrotus lividus,* which showed similar degrees of sensitivity to sediment metal concentrations. The test methodology is relatively simple and can be conducted using a minimum of space and time. To determine the extent and the environmental significance of sediment contamination in Portmán bay, sediment toxicity, benthic community structure and sediment chemistry need to be assessed in an integrated, weight of evidence approach. For this reason we analyzed the interactions of sediment toxicity, sediment chemical concentration and infaunal community structure.

The mine tailings dumped in Portmán Bay contained calcite, dolomite, pyrite, sulfides of Cd, Cu, Pb, and Zn, and some aluminum and silica minerals. The acidic wastes are evolved from reduced sulfur materials (e.g., pyrite or FeS_2 in eastern Spain) that have been oxidized on exposure to water and oxygen, a process often brought about by mining activities. Pyrite oxidation reactions produce sulfuric acid and ferric hydroxides and mobilize other trace metals, depending on the surrounding mineralogy. These toxic acids and metals flow in surface waters, where the acid is eventually neutralized, causing the precipitation of metals and metal oxides on coastal streambeds, impairing habitats and adversely affecting water quality. Heavy metals are important components of many marine discharges, including sewage, industrial wastes and mining wastes [34]. There is considerable uncertainty with regard to the concentration of heavy metals that may pose significant ecological risks because metal bioavailability is determined by the concentration of acid-volatile substances formed in anoxic conditions [35]. In marine sediments, sulfides are responsible for metal concentrations in pore and overlying water [10] and affect the distribution of benthic invertebrates. Thompson, *et al.,* [36], found that when the sea urchin *Lytechinus pictus* was exposed to marine sediments dosed with varying concentrations of sodium sulfide, toxic effects occurred at levels below 1 mg/l.

Predicting the bioavailability and toxicity of metals in aquatic sediments is a critical component in the development of sediment quality criteria. The use of total sediment metal concentration (μM/g dry weight) as a measure of the bioavailability concentrations is not supported by available data [37]. These differences have been reconciled by relating organism response to the chemical concentration of porewater [37,38]. In this study, toxicity of the sediment porewater (PW) and sediment-water interface (SWI) was determined using the embryo larval development tests with two sea urchin species *Arbacia lixula* and *Paracentrotus lividus.* The porewater tests were more sensitive to metal contamination than the toxicity of sediment-water interface tests, probably due to the presence of higher levels of ammonia, organic mater and AVS. Beiras [39], suggest that prior to porewater testing is necessary the aeration of water in order to avoid the toxicity caused by these natural conditions characteristic of highly reduced sediments. However, SWI results had a better concordance with chemical and the macroinvertebrate community changes observed along the contamination gradient.

The determination of acid-volatile sulfides (AVS) is widely used as a measure of reduced sulfur species in sediments. Di Toro *et al.* [37] proposed that if the simultaneously extracted metals SEM/AVS ratio is less than 1, there will be no toxic effect from Cd, Cu, Hg, Ni, Pb, or Zn. Short-term sediment bioassays showed that the molar ratio of AVS/SEM provided a first-order control on activities of at least some metals in porewater [40,41]. Activities were reduced to very low levels at ratios <1, because of the high stability of the metal sulfide. Toxicity and bioaccumulation were correlated with porewater metal activities in the toxicity tests [42].

Different dredging projects have been proposed to restore Portmán Bay, but since no project has contemplated the potential adverse effects on organisms, the debate over the convenience of dredging the Bay continues. In the present study we demonstrate that mine wastes are spread at least 7300 m along the Mediterranean coast of Murcia, Spain.

The PCA analysis indicated that the Zn, Al, Pb and Fe, and the organic matter are associated with biological effects along the contamination gradient. Spearman rank correlations indicated significant negative correlations between benthic community structure and the metals, Zn, Al, Pb, Fe, Hg and Cu. The effects of metal sediment contamination on the benthic community structure are visible in the sampling stations (PG, PN and CN). The marine sediments of Portmán Bay continue to show high toxicity due to the high heavy metal concentrations they contain and any sediment resuspension in this toxic hot spot of the Mediterranean Sea must be treated with caution. The sediment quality triad (SQT) tools were used in this study, and the combination of analytical chemistry, toxicity tests and benthic community structure, proved useful in providing a full picture of the extent of heavy metal contamination along the coast of Murcia.

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