

Article

Negative Impacts of Trace Metal Contamination on the Macrobenthic Communities along the Santos Port Complex—Brazil

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Abstract: Port sites represent one of the most impacted coastal areas; this impact is due to intensive anthropogenic pressures. In addition to the port complex itself, associated activities, such as indiscriminate disposal of pollutants, including trace metals, affect the local ecosystem. Macroinvertebrate benthic communities are one of the most effective bioindicators of environmental health because of their importance as a primary food source for many fish, birds, and mammals, as well as their influence on sediment stability and geochemical composition. This article evaluates the benthic macrofauna in the Santos Estuarine System (SES), the location of the Santos Port Complex (SPC), linking trace metal levels to differences in microbenthic community structure and pollutant bioavailability. The distribution of Cd, Ni, and Pb was directly related to organic matter deposits, while Cu and Zn appeared to result from port activities. The SES contained a poor benthic macroinvertebrate community, resulting from the contaminated muddy sediments. A significant negative correlation was found between the macrobenthic diversity and concentrations of Cu in the soluble phase; this implied the pollution-induced degradation of the macrobenthos in SES.

Keywords: macrobenthos; heavy metals; sediments; bioindicators



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1. Introduction

Harbors significantly impact the surrounding environments, including their biota and the pre-established ecosystem balance. They are generally located in geomorphologically protected water bodies, such as bays or estuaries, with low hydrodynamic conditions and restricted water exchange with the adjacent sea; they also frequently present lower oxygen levels in the water column, accumulating highly contaminated sediments [1]. As a result, many harbors are classified as critically contaminated spots, with low pollutant removal in the sediments resulting in high bioavailability and toxicity to the local trophic net [2,3].

The low hydrodynamic flow conditions result in the accumulation of fine-grained sediments, turning these sites into potential sinks for pollutants, especially trace metals. This may result in negative impacts on the marine ecosystem and environmental health [4]. Trace metals usually enter aquatic environments as a result of human activities linked to agriculture, fuel burning, metal corrosion, industrial plant residues, domestic sewage, and vehicular transit [5]. The bioavailability of metal to the trophic net may result in

several negative health disturbances [6]. According to Alomary and Belhadj (2007) [7], these disorders include cancer, sensorial problems, intelligence anomalies, kidney damage, and miscarriage or stillbirth in humans. Additionally, trace metals represent persistent elements not easily degraded in the ecosystem. This means that once they are deposited in sediment, these trace metals tend to be stored in this pool for a long time. Natural levels in aquatic sites are usually low [8], with high concentrations of trace metals in sediments acting as a potential indicator of anthropogenic sources [9,10].

Trace metals are insoluble in the water column, being predominantly attracted to suspended material. Under low current conditions, these pollutants tend to decant to the subaquatic bottom [11]. The evaluation of trace metal contamination in aquatic sediments is thus important [12]. However, the isolated study of heavy metals may not indicate their bioavailability; special laboratory extraction methods, together with study of specific bioindicators in the sediments, is crucial. Despite this, there are less data on port macrofauna available in the scientific literature than from other shallow water environments [13]. Human disturbances negatively influence aquatic macrobenthic communities [14–16], and so these organisms can be effective monitoring tools [17].

Marine benthic macroinvertebrates play a fundamental ecological role and, as a result, can function as effective indicators of the biotic health of marine sites, especially the intertidal areas [18]. The macrobenthos is fundamental in the maintenance of ecosystem ecology, involving material reworking and resulting nutrient recycling in sediments; this allows the function of the bottom-up mechanism through food webs. Additionally, as non-migrant species, macrobenthic organisms can monitor the local environmental conditions in sediments, where many contaminants such as heavy metals and organics are ultimately deposited [19,20].

As an example of a bioindicator, the polychaete *Laeonereis acuta* may be cited. This worm is largely found along South American Atlantic estuaries and coastal geomorphologically protected areas [21,22]. Several researchers have suggested *L. acuta* as an effective domestic pollutant bioindicator; due to its reduced lifecycle, it is able to rapidly recolonize areas of impacted land [23]. Other taxa (e.g., the polychaete *Magelona dakini* and the amphipod *Periculodes longimanus*) are more sensitive to ecosystem alterations and are unable to survive in highly polluted areas [24,25]. These characteristics show that these organisms have adapted to survive under different environmental conditions, making macrobenthic communities an invaluable tool for environmental monitoring [19].

The main object of the present study was to diagnose the status of the macrobenthic community in Santos Estuarine System (SES) and to evaluate its relationship with the concentrations of heavy metals in the estuary sediments.

2. Study Site

The SES (Figure 1) consists of a coastal region severely modified by countless human activities of the most diverse natures, resulting in several potential pollution sources, including a huge industrial complex, highly populated areas, and agricultural and port activities. One of the most significant sources is Cubatão Industrial Park, located in the Santos watershed basin [26]. This industrial zone concentrates petrochemical, steel, chemicals, fertilizers, and logistics industries, as well as energy production and services; these activities are both local and diffuse sources of pollutants. The region is also polluted by irregular deposits of solid industrial waste, port activities, sewage treatment stations, submarine outfall, and clandestine discharges of domestic sewage and sanitary landfills. Finally, the SES is still subject to constant impacts generated by the maintenance of the Port of Santos, the largest port complex in Latin America, where periodic dredging activities are conducted (Figure 1). The coastal region, on the other hand, receives many tourists during the summer [26] and traditional fisheries are an important economic activity in the region [27].

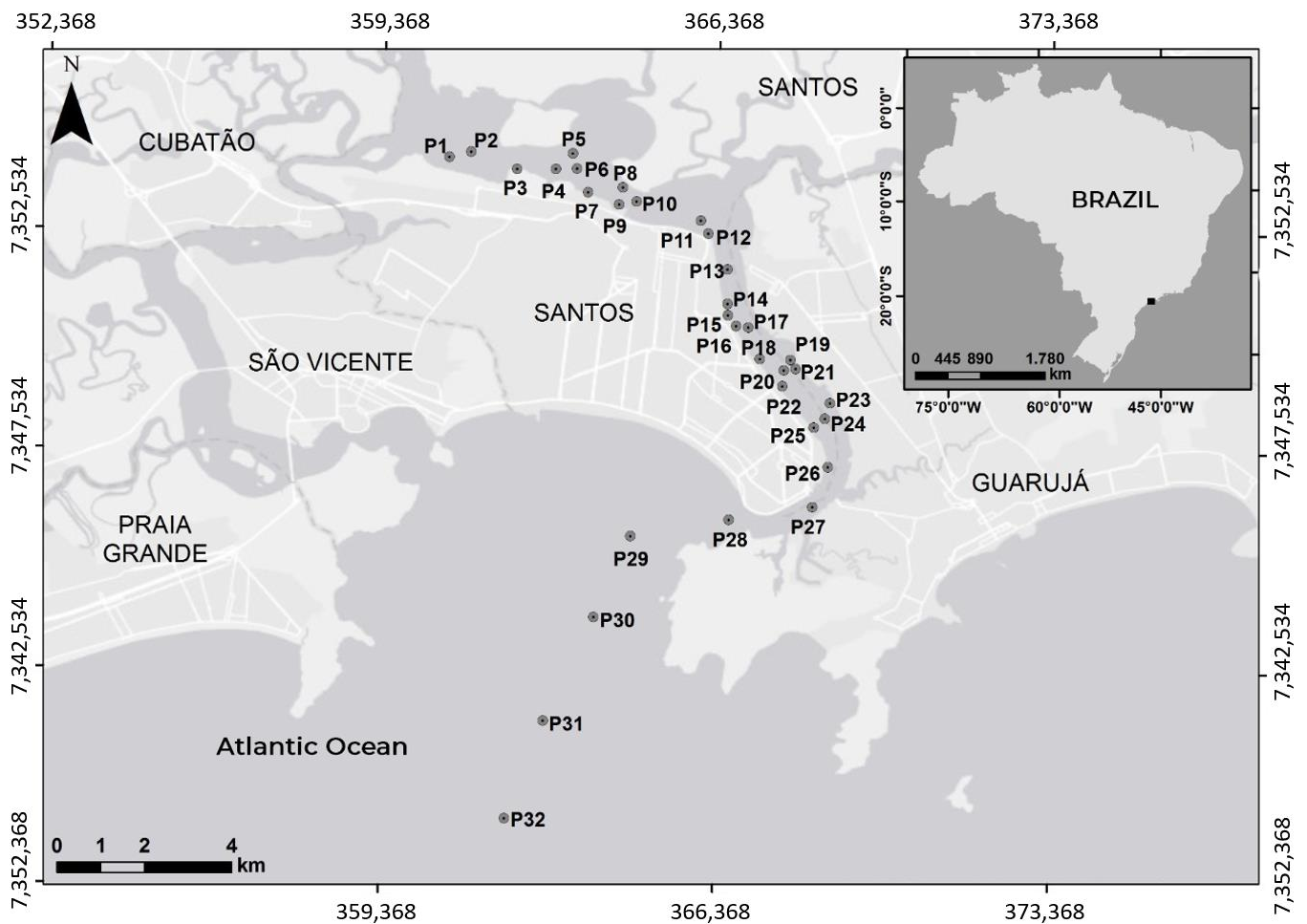


Figure 1. Study area and sampling stations.

3. Methodology

3.1. Field Campaign

Sampling was carried out during May 2021. Thirty-two sampling spots were distributed along the main estuarine channel, as shown in Figure 1. Bottom water samples were taken during the ebb tide. Bottom water physico-chemical parameters were evaluated using a multiparameter Horiba U10 probe. All data groups were compared with trace metal concentrations and other environmental and biological parameters (pH, Eh, dissolved oxygen, and salinity).

A stainless Van Veen grab was used to maintain sediment composition integrity and avoid contamination during sediment sampling. The samples were properly packaged and stored under refrigeration (ca. -20°C) until analysis. Sediment grain size, total organic carbon (TOC), and trace metals (Ni, Cu, Zn, Cr, and Pb) were determined. Triplicate sampling was used for evaluation of benthic macroinvertebrates. The sediment was washed in sieves (0.5 mm mesh) and preserved in 4% buffered formalin.

3.2. Laboratory Analysis

Trace metal samples were maintained in pre-acidified plastic bowls and transported to the laboratory for analysis. For grain size evaluation, organic matter was first degraded with hydrogen peroxide (30%) and grain size was then measured using a Microtrac S3500 grain size analyzer. The results were classified into sand, silt, and clay.

The fine material (below 0.063 mm) was used for trace metal extraction. Sequential extraction was performed using the BCR approach. After centrifugation and further dilution, Pb, Cu, Ni, Zn, and Cd were analyzed by Inductively Coupled Plasma-Atomic

Emission Spectrometry (ICP-AES). The detection limits are: Pb, 1.5 mg/kg; Cu, 1.5 mg·kg⁻¹; Ni, 0.6 mg·kg⁻¹; Zn, 1.5 mg·kg⁻¹; Cd, 0.8 mg·kg⁻¹. The recovery rates for the trace metals varied between 87.8 and 102.5%. All utensils, tools, and equipment were acid-washed with 10% HNO₃ and rinsed with distilled water to avoid potential procedure contamination. Certified standard material was used to assure the accuracy of the analytical procedures (SPEX-QC-21-16-85AS-traceable to NIST). The maximum value for precision data was 5%.

The influence of environmental differences was removed from the picture and the effective pollution status of the local site (the extent of metal accumulation relative to background value/concentration) was determined using the geoaccumulation index (I_{geo}) Equation (1). I_{geo} is grouped into seven categories as recommended by Müller (1979) [28]: $I_{geo} < 0$ —uncontaminated; $0 < I_{geo} \leq 1$ —lightly to moderately contaminated; $1 < I_{geo} \leq 2$ —moderately contaminated; $2 < I_{geo} \leq 3$ —moderately to strongly contaminated; $3 < I_{geo} \leq 4$ —strongly contaminated; $4 < I_{geo} \leq 5$ —strongly to extremely contaminated; $5 < I_{geo} \leq 10$ —extremely contaminated.

$$I_{geo} = \ln(Cn/1.5 \times Bn) \quad (1)$$

where Cn = mean levels of the trace metal in soil and Bn = background value of the trace metal.

Finally, the macroinvertebrates were sorted, identified to the lowest inclusive taxonomic group, and counted with microscope.

3.3. Statistical Analysis

The normal distribution of the data was tested using the Shapiro–Wilk test. Spearman’s correlation was used to evaluate the relationship between macrobenthic data and the environmental parameters. Principal component analysis (PCA) was used to evaluate any synergy between all the parameters. Multivariate analysis was carried out using Past v.3 software. The macrofauna results were evaluated to find the total number of taxa, abundance, evenness, and Shannon Diversity Index using neperian logarithms. Spatial differences for univariate variables were analyzed by ANOVA test, after normality evaluation (Kolmogorov–Smirnov test).

4. Results and Discussion

Estuaries represent extremely important ecosystems. They not only provide nursery and feeding grounds for several living organisms, both aquatic and terrestrial [29–32], but are also one of the most productive ecosystems in the world [33]. Human communities benefit from this, with large cities and industries located nearby; hence, these geomorphological protected and low hydrodynamic areas suffer from increased anthropogenic pressures [34]. The control of environmental health is of extreme relevance for the maintenance of ecological balance and the sustainability of the activities undertaken in estuaries.

Water quality and resulting pollutant dynamics in the estuarine water column is impacted mainly by parameters such as pH [35], dissolved oxygen, salinity [36,37], and redox [38]. In the present research, pH levels did not vary much over the whole area. Values ranged between 8.17 and 8.66, influenced by the natural buffer system that maintains ocean pH between 8.1 and 8.3 [39]. Salinity also varied little, remaining at around 28. Dissolved oxygen levels were similarly stable, remaining above 5.7 mg/L. All water physico-chemical parameters reflected the clear influence of water exchange with the open sea, which contributes to the relative environmental health balance. In the case of oxygen, benthic organisms begin to succumb to lack of oxygen from 5 mg/L and below [40,41], causing negative effects on the structure of marine ecosystems and their biodiversity [42–45]. Additionally, oxygen levels influence the redox potential (Eh) of the water environment, impacting the balance of nutrient cycles [46,47]. In the present study, all our samples had positive Eh values, confirming the oxygenated water status.

Sediment particle size is one of the most important features influencing the trace metal deposition dynamic. Usually, fine particles have a higher capacity for attracting trace metals

because of their greater specific surface area and the associated increased presence of clay minerals, organic matter, and oxides [48–51]. In the present study, finer grains (silt and clay) constituted the primary part of the sediment at each internal sampling spot (p1–p15) (Figure 2). The outer sampling stations (p16–p32) showed a slightly increased grain size, probably reflecting the stronger fluxes resulting from channel narrowing and increased hydrodynamics typical of open areas.

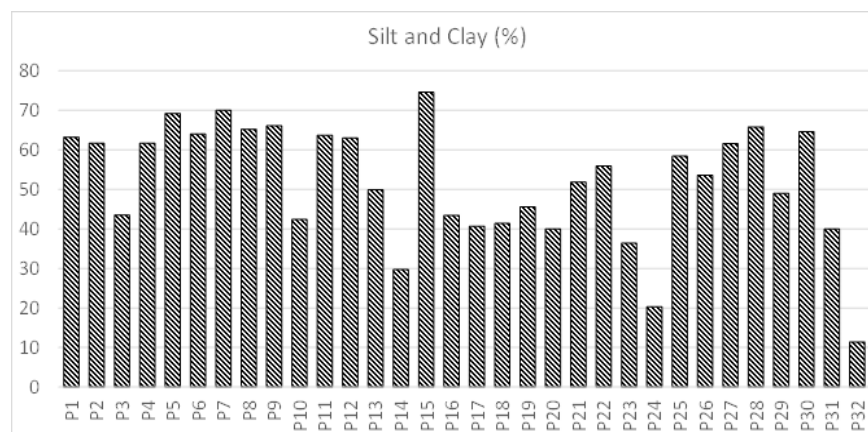


Figure 2. Fine particle distribution ($y = \%/x =$ sampling stations).

Significant quantities of organic matter may decant and accumulate in sediments due to physical, chemical, or biological processes in the water [52,53]. After sedimentation, they may be degraded and continuously mixed with other deposited compounds [44]. Thus, the organic matter accumulated in aquatic ecosystems can be allochthonous or autochthonous, originating from local primary production, or transported by tidal fluxes, agricultural runoff, and industrial and municipal wastes [54–57]. In the present study, sediment organic matter showed a similar pattern to that of small sediment particles (Spearman correlation = 0.392). However, it showed a slight tendency to increase in the direction of the open sea. This trend goes against expectations, since more sheltered estuarine waters normally have higher levels of organic matter due to the higher productivity and low hydrodynamics of the area, which allows its accumulation. On the other hand, the physicochemical conditions of the water can contribute to the accumulation of organic matter in the sedimentary stratum. These conditions affect the surface charges on the particle surfaces, leading to their flocculation, and this, in turn, can increase their overall size and weight, leading them to deposit on the estuarine floor [58,59]. This could explain why samples collected in the estuarine entrance channel revealed higher organic matter content. Another potential cause of the high concentrations of organic matter in the external sector is the presence of an underwater outfall very close to collection points p30, p31, and p32. Thus, both the flocculation dynamics and the presence of this source of sewage explains the higher values at the outermost sampling stations. It seems that the flows in the channel were slow enough to allow the decantation of fines; these varied, in this area, between 30 and 50%, despite the morphological tapering of the bed. This established hydrodynamic regime permitted the maintenance of organic matter content together with the settlement of relatively high numbers of fine-sized grain particles.

Trace metals are frequent contaminants in aquatic environments. They arise from natural and anthropogenic sources, as well as industrial, agricultural, and domestic loads [60–62]. When released into the water body, they tend to decant and deposit in the subaquatic sediments, where they may liberate their contaminants [63,64]. The total concentrations of Cu, Zn, Ni, Cd, and Pb in the SES are presented in Figure 3. Two pattern groups can be seen. The first (Ni, Cd, and Pb) shows a slight increase in level toward the open sea, while the second (Cu and Zn) shows no such variability. The likely reason for this is that the source of pollution by group two metals is the harbor itself. Thus, deposition

and accumulation are directly impacted by inputs from the harbor area. The statistical correlation between the harbor and contamination by the second group of metals confirms this hypothesis. Harbor activity seems to be a significant source of Cu and Zn. According to Bighiu et al. (2017) [65], antifouling paints used in port sites represent important sources of Cu and Zn in the environment. Additionally, according to Karbassi and Marefat (2017) [38], Cu and Zn behave differently from other metals through the estuarine flocculation process, especially in response to oxygen variations.



Figure 3. Total Trace Metal concentrations.

The variability of the other metals was shown to be directly linked to the organic carbon content of the sediment.

According to the I_{geo} results presented in this study, four of the five trace metals can be classified as above normal environmental levels (Figure 4). Only Cu I_{geo} values (level 2–3) were classified as “light to moderate” contamination, while Zn, Ni, and Pb values were classified as “moderate to strong”, suggesting the influence of human activities in the area. To determine whether these contaminant levels could represent a threat to the local biota, the extracted soluble phase of the metal concentrations was compared with the macrobenthic fauna for each sampling station; this will be discussed later in the present article.

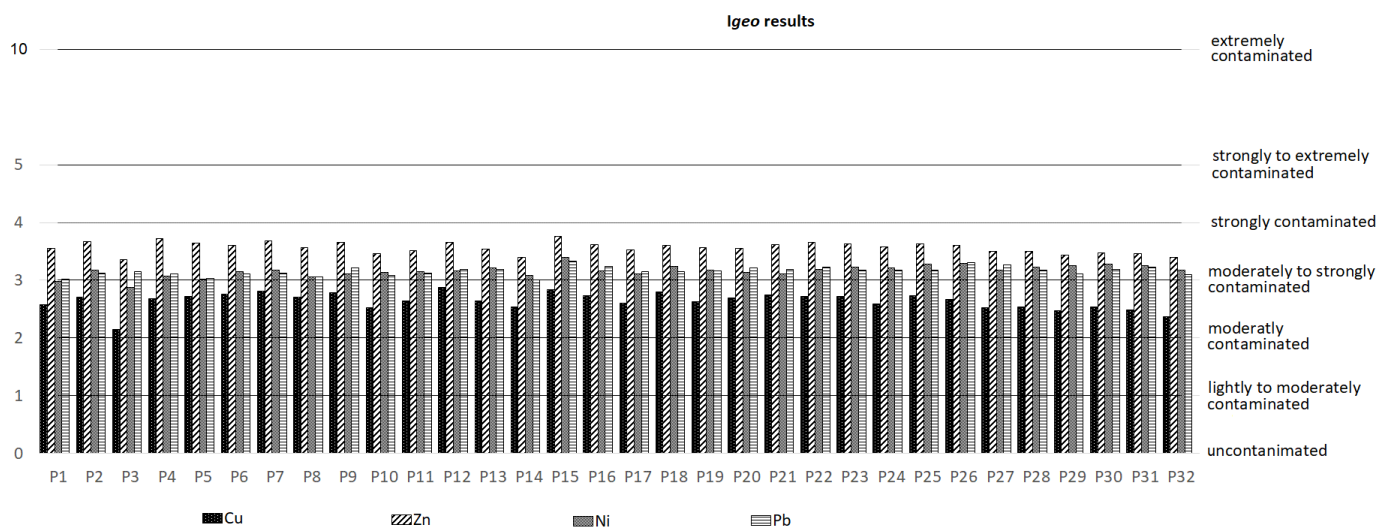


Figure 4. I_{geo} results for trace metals at the sampling stations.

Macrobenthic fauna are diverse and represent a fundamental constituent of soft-bottom estuarine environments, exhibiting a high adaptability to different environments [66]. They colonize a wide range of ecosystems, from intertidal to hadal systems, in which the sediment conditions play a key factor in controlling the structure and function [67,68]. The present campaign, in May 2021, recorded five groups of benthic organisms (Molluscs, Echinoderms, Crustaceans, Annelids and Others (Figure 5)), totaling 294 organisms in 32 taxa (Table 1). Organisms were identified to the lowest possible taxonomic class; a large number of unidentifiable fragments were found and placed into the “Others” group. Monitoring of the benthic community revealed a poor profile in terms of ecological diversity; this was likely a response to the local human activities and resulting contamination. According to the ANOVA, differences in benthic community composition did not depend on the monitoring point.

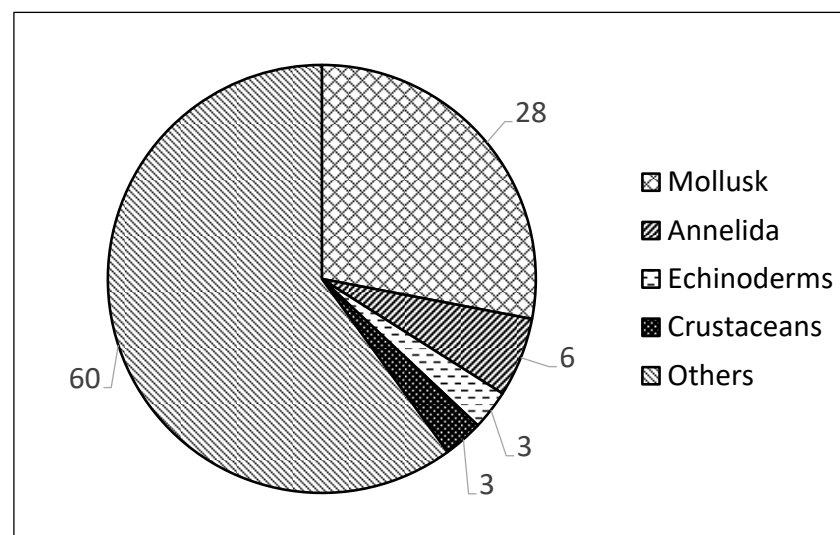


Figure 5. Relative abundance (%) of species by taxonomic group.

The statistical analyses of the populations are shown in Table 2. The Shannon Diversity Index (H') and the Pielou Equability parameter (J') were used to assess the ecological makeup of the communities. The higher the value of H' , the greater the diversity. J' varies from 0 to 1; the maximum value indicates a situation where all species have the same number of individuals, (i.e., no ecological dominance).

Table 2. Pielou evenness index and Shannon test for each sampling spot.

Sampling Station	Pielou Equability	Shannon Test	Diversity
	J'	H'	
P1	0.00000	0.00000	Very low
P2	0.43841	1.15699	Low
P3	0.54075	1.42706	Low
P4	0.57713	1.52308	Low
P5	0.00000	0.00000	Very low
P6	0.59003	1.55711	Low
P7	0.53964	1.42413	Low
P8	0.36008	0.95027	Very low
P9	0.00000	0.00000	Very low
P10	0.64256	1.69574	Low
P11	0.00000	0.00000	Very low
P12	0.00000	0.00000	Very low
P13	0.75463	1.99151	Low
P14	0.69372	1.83077	Low
P15	0.48497	1.27985	Low
P16	0.81901	2.16142	Low
P17	0.00000	0.00000	Very low
P18	0.00000	0.00000	Very low
P19	0.26265	0.69315	Very low
P20	0.00000	0.00000	Very low
P21	0.00000	0.00000	Very low
P22	0.00000	0.00000	Very low
P23	0.39397	1.03972	Low
P24	0.50673	1.33729	Low
P25	0.24119	0.63651	Low
P26	0.50384	1.32966	Low
P27	0.51457	1.35798	Low
P28	0.39966	1.05472	Low
P29	0.00000	0.00000	Very low
P30	0.00000	0.00000	Very low
P31	0.36008	0.95027	Very low
P32	0.00000	0.00000	Very low

Table 3 shows that the number of organisms found does not depend on the monitoring point ($p < 5\%$).

Table 3. ANOVA test for the hypothesis that the collection site influences the number of species.

Variation Source	SQ	gl	MQ	F	Value- p	F
Inter group test	464.9196429	31	14.99740783	2.0878927	0.00073392	-
Intra group test	2988.142857	416	7.183035714			-
Total	3453.0625	447				

Consisting of several different species, macrobenthic organisms have various tolerances to environmental pressures. These differences make them function as effective indicators of environmental health [69]. Bivalves have been regarded as suitable bioindicators of metal pollution in the marine and estuarine environments. For instance,

Anomalocardia brasiliiana (Gmelin 1791) (Bivalvia-Veneridae), occurring in the upper layers of sediment in marine intertidal regions from the West Indies to Southern Brazil, has been frequently used as an indicator for trace metal pollution. In the SES area, this organism was one of the most common, despite low richness and benthic faunal abundance; this indicates its resistance to environments in which other more sensitive benthic organisms cannot survive (Table 1). According to Lima et al. (2017) [70], it represents one of the main benthic bioindicators, particularly of domestic, industrial, and agricultural wastes.

The impacts of heavy metal pollution on macrobenthic communities have been studied by several authors [71–75]. Hall et al. (1996) [72] evaluated the effects of trace metal pollution on the macrobenthos in two North Sea estuaries; they recorded that macrobenthic communities in both ecosystems responded positively to reduced contamination. Warwick (2001) [72] found that trace metal levels in sediments were the main parameter influencing the structure of the macrobenthic populations of the Fal Estuary.

Guerra-García and García-Gómez (2004) [1] evaluated polychaete communities and sediment contamination in the surroundings of Ceuta Harbor. They suggested that contamination gradient was the main factor impacting polychaete structure, richness, and diversity. Dauvin (2008) [75] suggested that the effects of trace metals varied among species, with trace metals being responsible for only part of the disturbances in benthic community structure. In the present study, the extracted Cu solution demonstrated a significant inverse correlation between Cu and the biological community characteristics (Table 4).

Table 4. Spearman correlation test results.

	Cu (FI)	Zn (FI)	Ni (FI)	Pb (FI)	Cd (FI)	Fine Grainsize	Richness	Abundance	Shannon	Mo (%)	Cu (Total)	Zn (Total)	Ni (Total)	Pb (Total)	Cd (Total)
Cu (Soluble)		−0.172	0.343	0.050	−0.198	0.133	−0.387	−0.337	−0.354	0.356	0.174	0.060	0.625	0.555	0.182
Zn (Soluble)	−0.172		−0.114	0.201	0.144	0.340	0.012	−0.017	−0.017	−0.038	0.723	0.790	−0.099	0.044	−0.170
Ni (Soluble)	0.343	−0.114		0.064	0.059	−0.197	−0.062	−0.093	−0.073	0.002	−0.110	−0.156	0.488	0.181	0.156
Pb (Soluble)	0.050	0.201	0.064		0.203	−0.265	0.170	0.100	0.203	0.091	0.309	0.279	0.164	0.617	0.456
Cd (Soluble)	−0.198	0.144	0.059	0.203		0.089	−0.155	−0.221	−0.157	0.222	0.174	0.072	−0.019	0.026	0.549
Fine Grainsize	0.133	0.340	−0.197	−0.265	0.089		−0.171	−0.127	−0.091	0.392	0.453	0.470	−0.062	0.004	−0.213
Richness	−0.387	0.012	−0.062	0.170	−0.155	−0.171		0.946	0.953	−0.444	−0.135	−0.009	0.004	−0.024	−0.163
Abundance	−0.337	−0.017	−0.093	0.100	−0.221	−0.127	0.946		0.865	−0.403	−0.132	0.029	0.089	−0.008	−0.208
Shannon	−0.354	−0.017	−0.073	0.203	−0.157	−0.091	0.953	0.865		−0.375	−0.112	−0.013	−0.011	0.017	−0.106
Mo (%)	0.356	−0.038	0.002	0.091	0.222	0.392	−0.444	−0.403	−0.375		0.217	0.160	0.288	0.361	0.399
Cu (Total)	0.174	0.723	−0.110	0.309	0.174	0.453	−0.135	−0.132	−0.112	0.217		0.868	0.051	0.262	−0.020
Zn (Total)	0.060	0.790	−0.156	0.279	0.072	0.470	−0.009	0.029	−0.013	0.160	0.868		0.051	0.257	−0.117
Ni (Total)	0.625	−0.099	0.488	0.164	−0.019	−0.062	0.004	0.089	−0.011	0.288	0.051	0.051		0.546	0.384
Pb (Total)	0.555	0.044	0.181	0.617	0.026	0.004	−0.024	−0.008	0.017	0.361	0.262	0.257	0.546		0.407
Cd (Total)	0.182	−0.170	0.156	0.456	0.549	−0.213	−0.163	−0.208	−0.106	0.399	−0.020	−0.117	0.384	0.407	

The PCA results revealed the opposite relation between the benthic community features and site parameters such as organic matter and grain size (Figure 6). In addition, the PCA confirmed the greater bioavailability of Cu indicated by the first correlation result. The organic matter content was shown to be strongly negatively correlated with macrobenthic community health features and index.

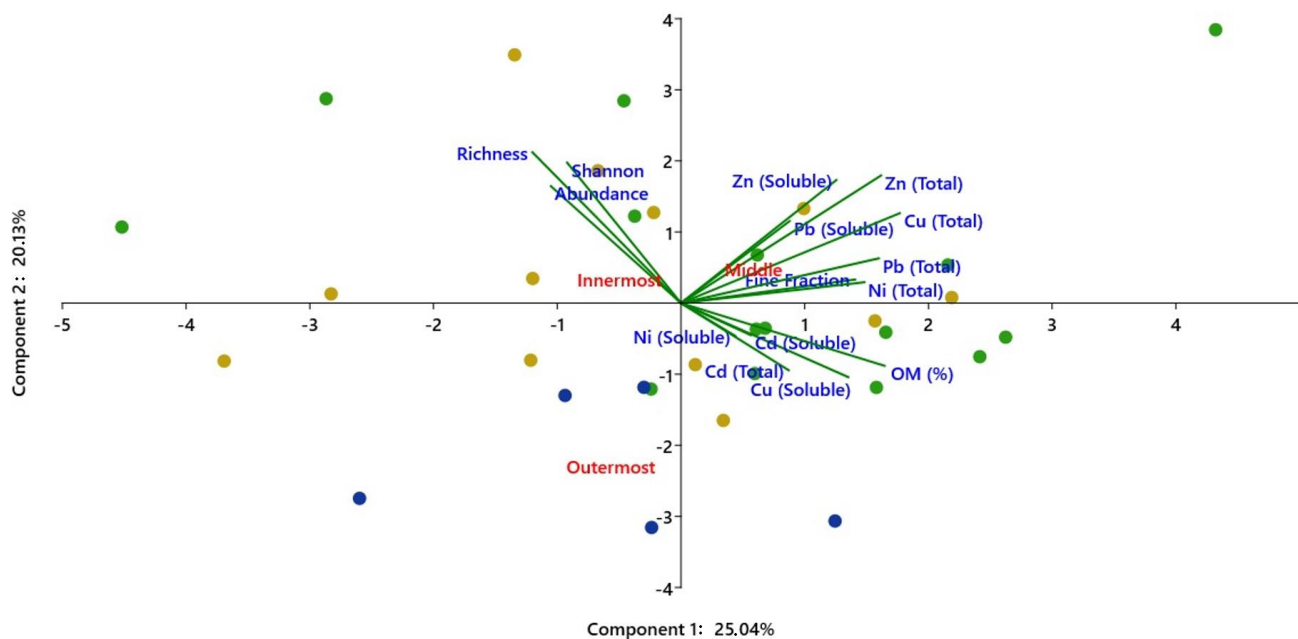


Figure 6. PCA results.

According to the literature, environmental characteristics are one of the main factors controlling macrobenthic invertebrates. Sediment particle size [75–79], salinity [17,80–82], organic matter content [83], and dissolved oxygen concentrations [84] are the main variables that influence the abundance, diversity, and distribution dynamic of macrobenthic populations [68]. In the SES area, the fine particles and organic matter in the sediments act synergistically with trace metals and represent the main environmental factors determining biotic health at the benthic level.

Rygg (1985) [71] found similar results in his research. He evaluated the relationship between macrobenthic community structure, trace metal levels, and organic matter in sediments from Norwegian fjords. There was a strong negative correlation between benthic community richness and Cu levels, with higher Cu levels in subaquatic sediments proving to be toxic to several species, including *Glycera rousii*, *Phylo norvegica*, *Sosane gracilis*, *Terebellides stroemi*, *Eriopisa elongate*, and *Ennucula tenuis*. The result was impoverished diversity.

5. Conclusions and Recommendations

In harbor sites, constant ship transit, dredging processes, waste discharge, and other port activities may result in loss or degradation of the adjacent habitat areas and impact marine organisms. The infrastructure linked to port activities, including the installation of industrial parks and high population density, makes port sites one of the most severely impacted environments. Data from the SES area presented in this article suggest that Cd, Ni, and Pb levels are directly related to the deposition of organic matter, while Cu and Zn levels may be increased because of port activities. Organic enrichment influences the pollutant geochemical dynamic directly, impacting microbenthic community composition and reducing diversity by excluding species with low tolerance. The results highlight the importance of environmental parameters such as grain size and organic matter content on macrobenthic structure. Correlation with the Cu soluble phase from sediments indicated a significant impact on the local macrobenthic fauna, contributing to their impoverishment.

Based on the results obtained in the present study, it is understood that measures to control the chronic impacts resulting from the activities undertaken in port areas must be constantly implemented in order to allow adequate environmental management of these areas. Chemical monitoring procedures must always be accompanied by evaluation of bioindicators since these determine the effectiveness of toxicology on the local biota.

Author Contributions: J.d.F.D.: conception; development of the theory; discussion of results and contribution to final manuscript; R.M.A.: conception; development of the theory; discussion of results and contribution to final manuscript; L.d.S.L.: discussion of results and contribution to final manuscript; C.C.G.: conception; development of the theory; discussion of results and contribution to final manuscript; J.A.B.N.: discussion of results and contribution to final manuscript; S.C.d.S.P.: discussion of results; B.F.d.S.G.: discussion of results; E.M.d.F.: conception; development of the theory; discussion of results and contribution to final manuscript. All authors have read and agreed to the published version of the manuscript.

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