WP3 - Activity 3.3
Distribution and diversity of benthic macroinvertebrates

Deliverable 3.3.1. *Macrobenthos classification in sediments: Sedimentological characterization (granulometry) in relation to the diversity and abundance of Macrobenthos.*

Deliverable 3.3.2. *Total organic carbon analysis.*

Deliverable 3.3.3. *Total Nitrogen Analysis.*

Deliverable 3.3.4. *Report on benthos biomonitoring.*

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Partner in Charge	PP8 – University of Ferrara
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AUTHORS

Tamara Cibic, Federica Nasi

Contents

1. Introduction

In semi-enclosed basins, such as ports and small marinas, the effects of point source and synergistic forms of contamination are emphasized. The effects of human pressure on benthic macrofaunal assemblages inhabiting marinas and tourist ports are seldom studied, especially in the western part of the Adriatic Sea. In the framework of the international European project ECOMAP (ECOsustainable management of MArine and tourist Ports), within WP 3.3, we investigated the macrofaunal communities in three tourist ports, two in Croatia, namely Špinut and Strožanac, and one in Italy, namely Marina Dorica in Ancona. The macrofaunal abundance, diversity indices, species composition, together with the physical-chemical features of the surface sediments and their contamination levels, were used to evaluate the ecological status of the three marinas. The macrofaunal communities, along with other physical-chemical parameters in the water column and sediments, were sampled in July 2019 and April 2021 in the two Croatian marinas, and in September 2020 in Marina Dorica. Here are reported the main results obtained.

2. The investigated marinas

Figure 1. Map of the investigated areas in Croatia

From 1st to 5th July 2019 and from 26th to 29th April 2021, OGS (PP7) performed the first and the second sampling campaign in the marinas of Špinut and Strožanac (municipality of Podstrana) (Figure 1), respectively. Sampling activities were done in collaboration with IOF (PP4) on board on their boat "Navicula". The aim of this investigation was to use benthic communities inhabiting the port sediments as bioindicators of the environmental status of these port areas. The sampling sites were located considering the confinement gradient (i.e. distance from the main entrance of the port, the time required for renewal of marine water) and the major sources of contamination in these basins. For this purpose, in the Marina of Špinut, sediments were collected at 5 sites, as follows: (i) SP1 and SP4 in front of the boathouse areas; (ii) SP3 at the main entrance, (iii) SP2 and SP5 at intermediate distance from the entrance and along the confinement gradient (Figure 2a).

Similarly, in the Marina of Strožanac, sediments for macrofaunal community were collected at 5 sites: PD1, PD3 and PD4 were positioned in the inner part of the basins, PD5 was located at the wide entrance of the basin, and PD2 was placed in front of the boathouse area (Figure 2b).

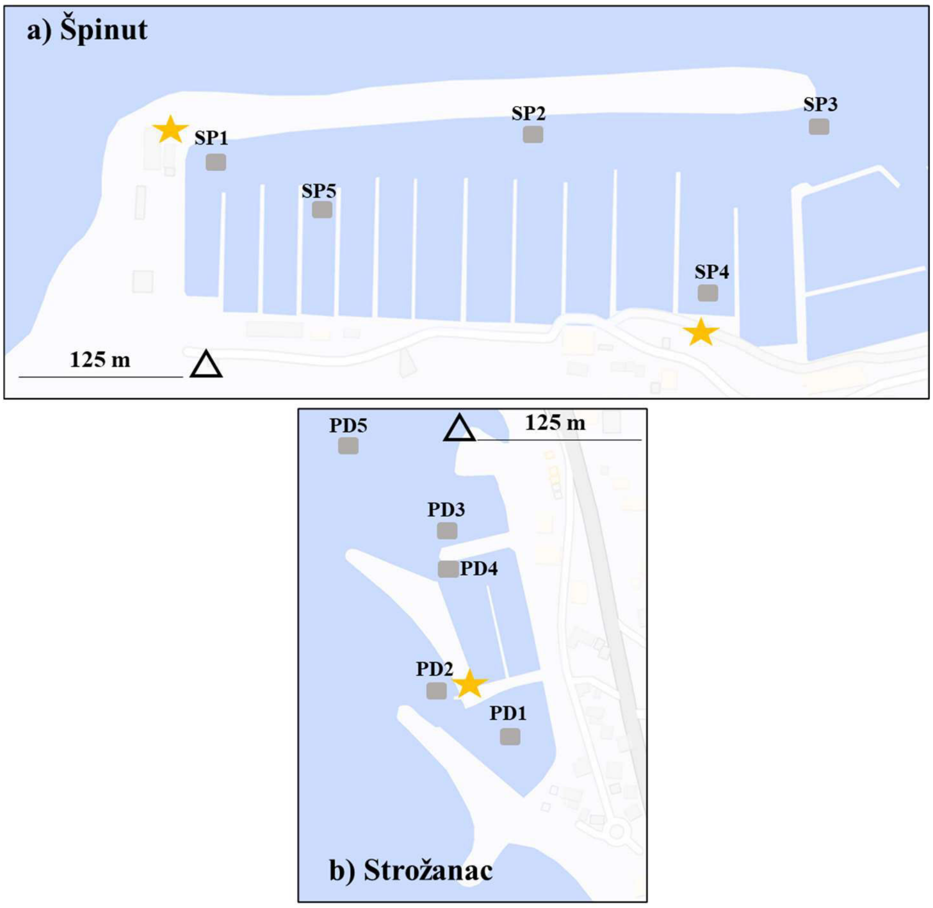

Figure 2. Sampling stations in Špinut (SP) and Strožanac (PD). Stars indicate the station nearby the anthropogenic activities, the triangle indicates the north.

Regarding the Italian coast, the Maritime Sport Society "Marina Dorica" nearby the main harbour in Ancona (Figure 3) was selected. On 22nd and 23rd September 2020, OGS (PP7) performed the sampling campaign in "Marina Dorica" in collaboration with Ancona Municipality (PP5).

Figure 3. Map of the investigated area "Marina Dorica"- Ancona

This investigation aimed to use benthic communities inhabiting the port sediments as bioindicators of the environmental status of the marina. Similarly to the approach already applied in the two Croatian marinas, the sampling sites were located considering the confinement gradient (i.e. far from the main entrance of the port, the time required for renewal of marine water), and the major sources of contamination persistent in this basin. For this purpose, sediments were collected at 5 stations, as follows: (i) MD1 at the main marine entrance; (ii) MD2 and MD3 placed at an intermediate distance from the entrance and along the confinement gradient (iii) MD4 nearby the oil station; (iv) MD5 in front of the boathouse area. Also, to compare the environmental features of the marina with the surrounding conditions of the investigated area, a reference site with similar depth was chosen outside the marina (MD_RS) (Figure 4).

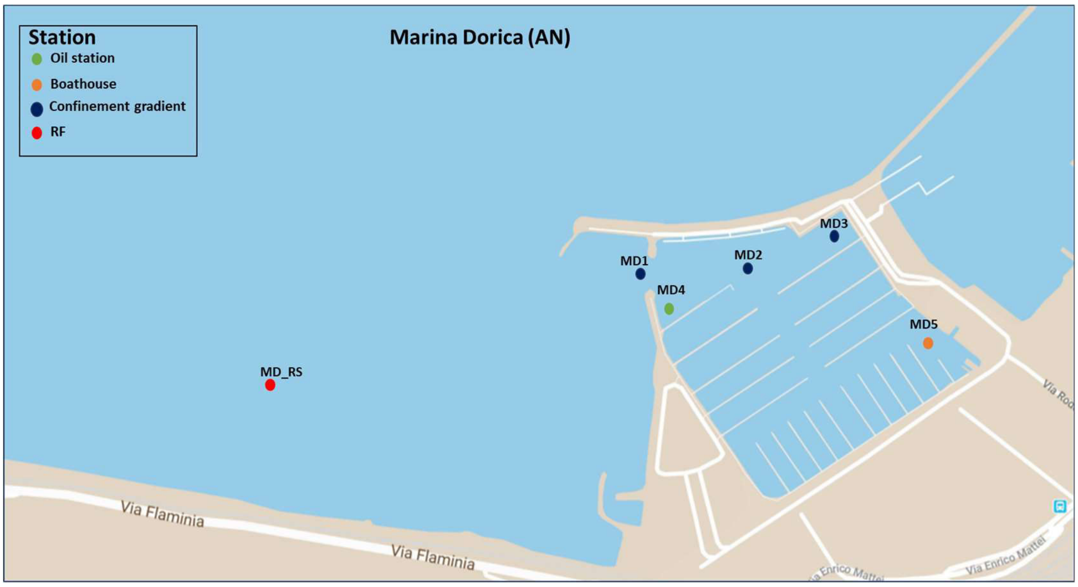

Figure 4. Activity location in "Marina Dorica" and sampling stations

3. Sampling activities

During each sampling campaign, the macrofaunal communities were collected using a van Veen grab (0.1 m^2) (Figure 5), and three replicates were taken at each station. One additional replicate was collected for the sediment physical-chemical characterization, (i.e. grain-size that was analysed by OGS). On the same sediment samples, the University of Ferrara analysed Total Organic Carbon-TOC, Total Nitrogen and heavy metals (i.e., Sb, As, Al, Bi, Cd, Cr, Cu, Pb, Mn, Ni, Se, Tl, V, Ag, Be, Ba, Co, Fe, Mo, Sn, Zn and Li. The latter were sampled to have an indication of the contamination levels in the marina. At each sampling station, salinity, temperature and dissolved oxygen were registered by a multiparameter probe YSI ECO2 EXP7 20014.

The sediment samples devoted to macrozoobenthos qualitative and quantitative determination were washed using a sieve of 0.5-mm mesh size to recover invertebrates. All material retained on the mesh was fixed in ethanol (80% final concentration) and stored.

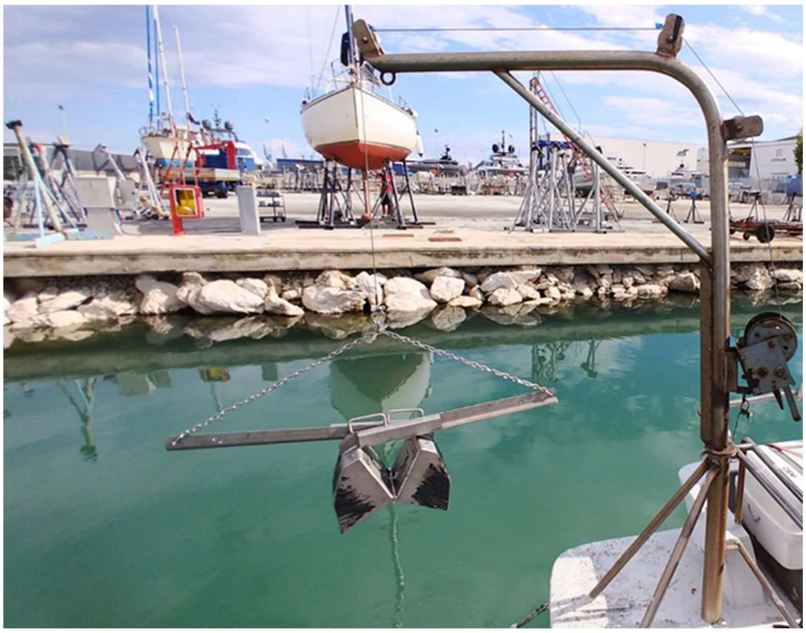

Figure 5. van Veen grab (0.1 m^2) for the sampling of macrofaunal communities

4. Sample processing

4.1 Sediment grain-size, organic matter and contaminants

For grain-size analysis, sediments were sieved at 2 mm and pre-treated with 10% hydrogen peroxide before being analysed with a BECKMAN COULTER LS 13 320 Laser Diffraction Particle Size Analyzer. Data are expressed as percentages of sand, silt and clay following the Udden-Wentworth grain-size classification (Wentworth, 1922).

To analyse Total Organic Carbon-TOC and Total Nitrogen-TN present in the samples collected in July 2019 and September 2020 the Soli TOC cube analyser by Elementar was used. Samples collected in April 2021 (Croatian marinas) were analysed using the analyser vario MAX cube by Elementar. Both instrument outputs were TOC (or TN) % value. The heavy metals in the collected samples were analysed with ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) techniques. For specific methodological details see the deliverable n. WP3_Activity 3.3_D 3.3.3.

4.2 Macrofauna

In the OGS laboratory, we performed the analyses of the macrofaunal community collected in Croatian and Italian marinas. After washing, we sorted out and separated the organisms according to their main taxonomical groups and preserved them in ethanol 60°. We identified all the animals to the highest possible taxonomical level using a stereomicroscope at 7-80X final magnification and counted them. For organism identification, we used specific taxonomical keys (Morri et al., 2004). Names of identified taxa were checked using the World Register of Marine Species database (WoRMS; http://www.marinespecies.org).

4.3 Data analyses

We performed the following univariate and multivariate analyses on abiotic (i.e., grain-size, TOC, TN, and heavy metals) and biotic (i.e., macrofaunal taxa abundances) data for each sampled marina. We assessed benthic community diversity and its spatial components, calculating the (i) species richness, (ii) Pielou equitability-J', and (iii) Shannon–Weaver biodiversity-H' (Clarke et al., 2014).

Multivariate analysis of macrobenthic community structure was performed on the species abundance matrix applying a non-metric multidimensional scaling analysis (nMDS) ordination model. In addition, the Principal Component Analysis (PCA) was carried out on physical-chemical sediment data to visualize spatial distribution of samples for each marina. Similarity Profiles (SIMPROF) analysis was used to test whether abiotic variables significantly ($p < 0.05$) co-varied coherently, or rather to determine whether parameters were associated with each other in terms of numerical variation through space. The matrix for PCA and SIMPROF tests was previously normalized and Euclidean distance was applied. BIO-ENV analysis was used to assess which environmental variables correlate best with the pattern of species composition at both areas, using the average value of each abiotic variable. The BIO-ENV routine was calculated using Spearman's coefficient. Abiotic data were normalized before entering the analysis. The biotic matrix for multivariate analyses (i.e. nMDS and BIO-ENV) was square root transformed, and the Bray-Curtis similarity was applied.

All univariate and multivariate analyses were performed using PRIMER 7 (PRIMER-E Ltd., Plymouth, UK) software (Clarke et al., 2014).

5. Results

5.1 Špinut and Strožanac marinas

5.1.1 Results on the physical-chemical features and heavy metals

					July 2019		April 2021	
Marina	**Station**	**Depth** m	**Coordinates**		°C	**T**	**Salinity** °C	**Salinity**
Špinut	SP1	5.3	43° 30.950' N	16°24'.942 E	19.9	35.6	15.2	36.4
	SP2	6.0	43° 30.951'N	16° 25.072'E	17.8	36.1	15.0	36.0
	SP3	5.0	43° 30.950'N	16° 25.218'E	17.6	36.2	14.7	36.9
	SP4	3.5	43° 30.899'N	16° 25.176'E	19.9	35.4	15.8	36.6
	SP5	4.5	43° 30.940'N	16° 24.988'E	18.5	36.0	15.6	36.5
Strožanac	PD1	3.0	43° 30.009'N	16° 32.041'E	20.7	35.5	15.7	34.2
	PD2	6.0	43° 30.033'N	16° 31.990'E	16.0	37.3	15.3	30.1
	PD3	3.5	43° 30.107'N	16° 32.000'E	20.4	35.7	15.6	34.5
	PD4	3.1	43° 30.081'N	16° 31.997'E	16.7	36.9	15.2	36.3
	PD5	4.2	43° 30.118'N	16° 31.944'E	19.7	35.8	15.0	27.30

Table 1. Depth, coordinates of sampling area and physical-chemical features in the water in July 2019.

The depth and the physical features of the water column (temperature and salinity) at the moment of sampling for both campaigns are reported in Table 1.

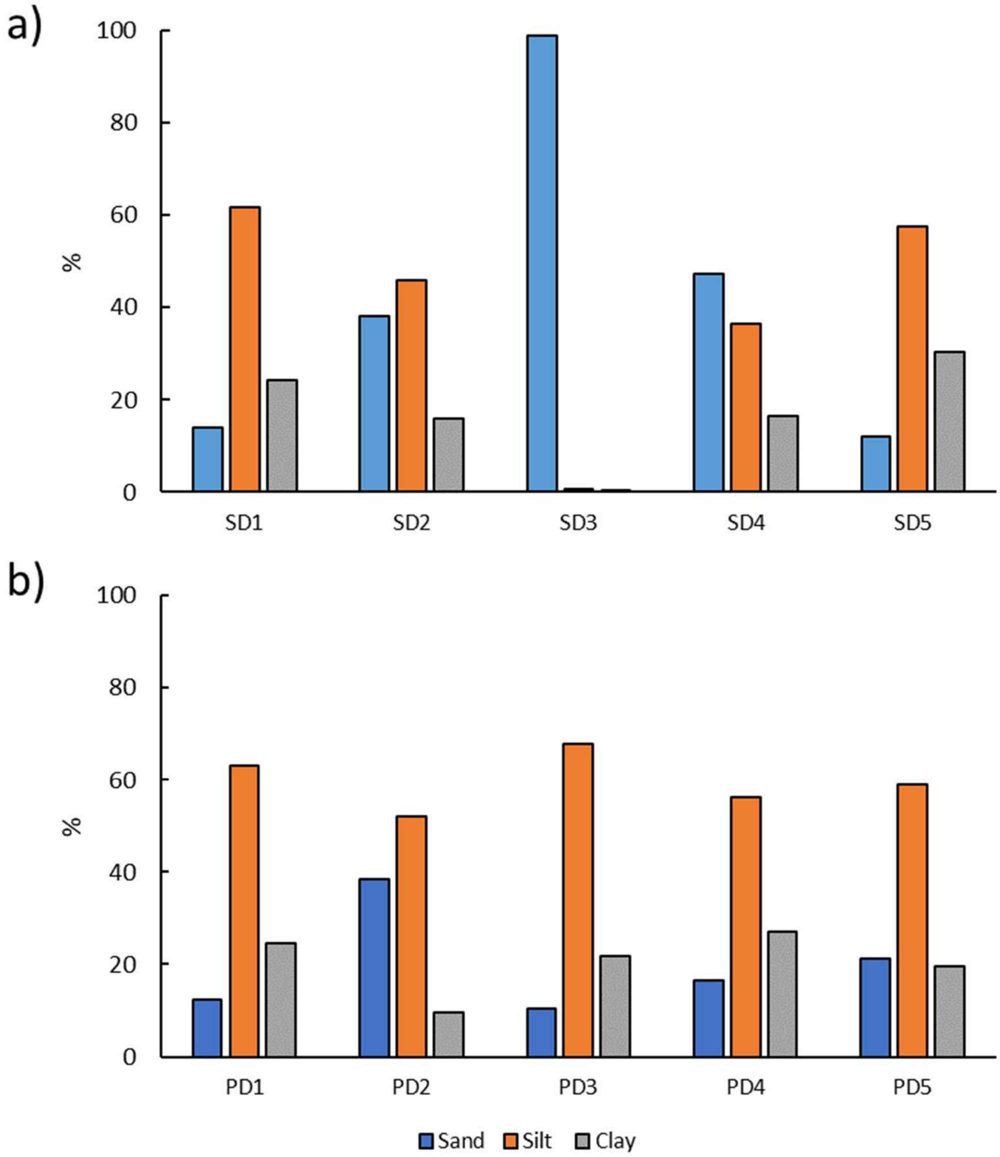

Figure 6. Percentages of grain-size at sampling stations in Špinut (a) and Strožanac (b) marinas in July 2019

Špinut marina was characterized by muddy sediments in the inner part, and higher sand percentage at the port entrance (SP3, Figure 6a). A similar grain-size distribution pattern was noticed in Strožanac, where the highest percentage of sand was observed at the main entrance (P2, Figure 6b). Overall, we observed the same grain-size distribution pattern in Špinut and Strožanac marinas also in the second sampling period (April 2021; Figure 8a and b). However, the grain-size distribution was quite different at some sampling stations, particularly PD3-4-5 and SP1-2-5, compared to the first sampling, due to a much higher percentage of sand obtained in the second sampling.

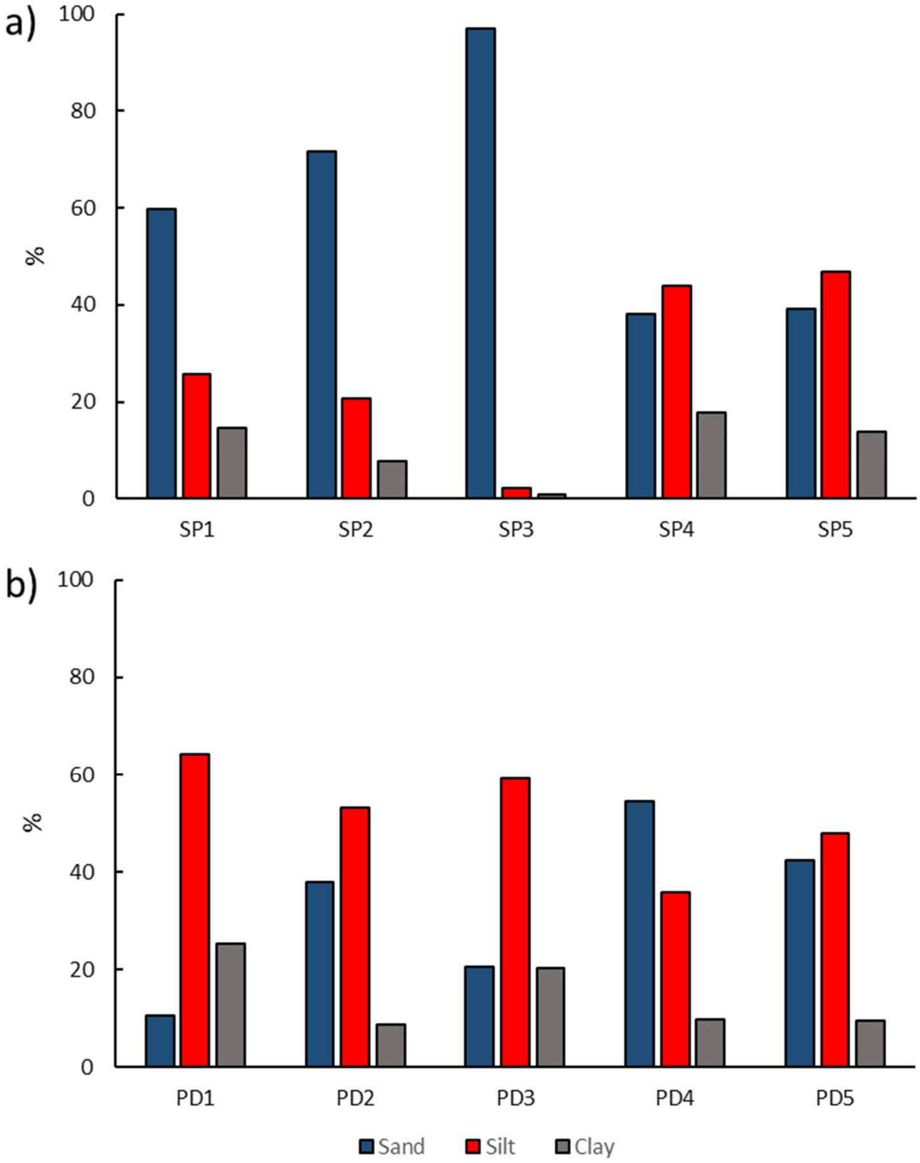

Figure 8. Percentages of grain-size at sampling stations in Špinut (a) and Strožanac (b) marinas in April 2021

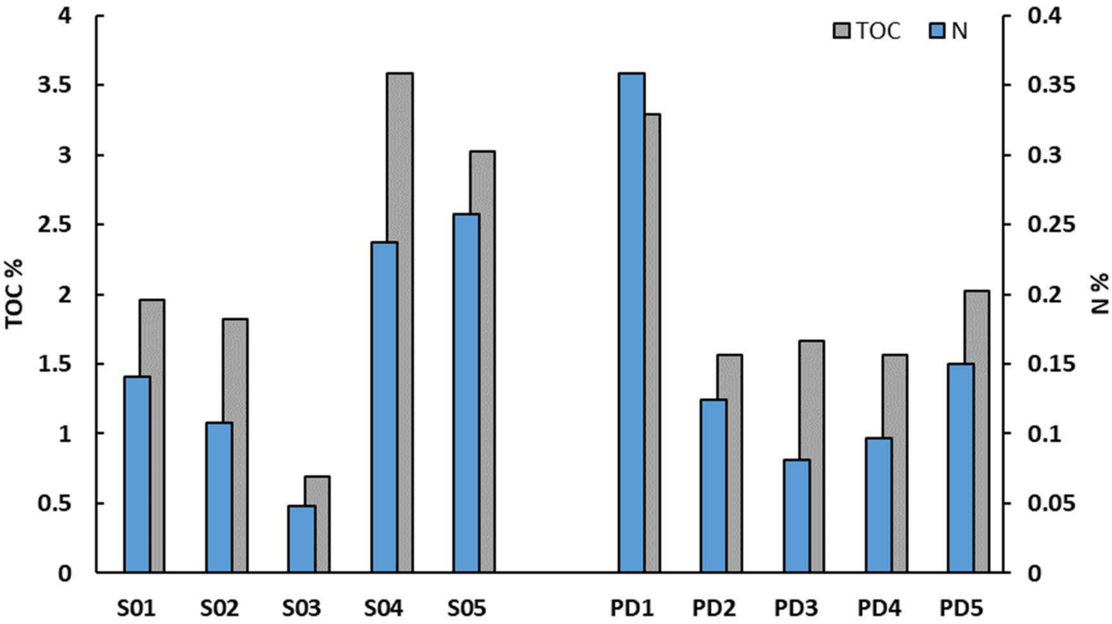

Figure 9. Percentages of Total Organic Carbon and Nitrogen at sampling stations in Špinut and Strožanac marinas in July 2019

In July 2019, sediments in Strožanac displayed slightly higher contents of organic carbon compared to Špinut (2.02±0.73 and 2.21±0.22 %, respectively) in July 2019. In Špinut, the major contents of organic carbon were noticed at SP4 and SP5 (3.53 and 3.02 %, respectively). In Strožanac, carbon content ranged from 1.56 % (PD2) to 3.29 % (PD1), at the inner stations (Figure 9). In both marinas, at stations characterized by macroalgal and seagrass coverage, the highest total nitrogen values were noticed (0.26% at SP5 and 0.36% at PD1; Figure 9).

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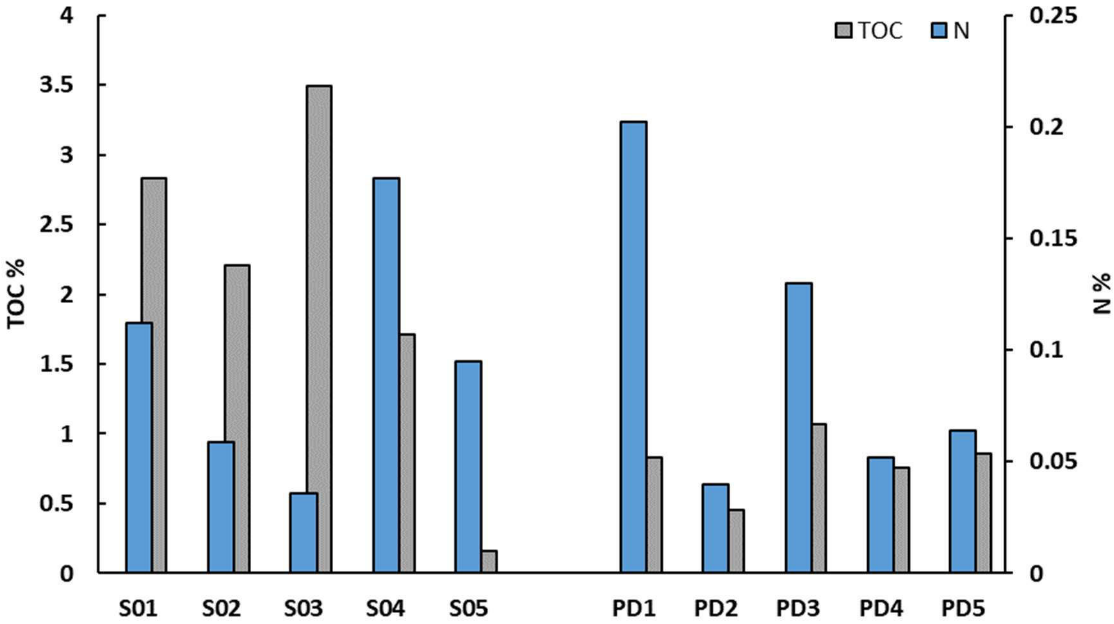


Figure 10. Percentages of Total Organic Carbon and Nitrogen at sampling stations in Špinut and Strožanac marinas in April 2021

In Špinut marina, the TOC values observed in April 2021 ranged from 0.16% to 3.50 % at SP5 and SP3, respectively. In Strožanac marina, the lowest value was observed at PD2 (0.45%), whereas the maximum was noticed at PD3 (1.07%). In Špinut marina, the highest N % was observed at SP4 (0.18%), nearby the boathouse area. In Strožanac, major content of N was noticed at station characterized by seagrass coverage (0.20%; Figure 10)

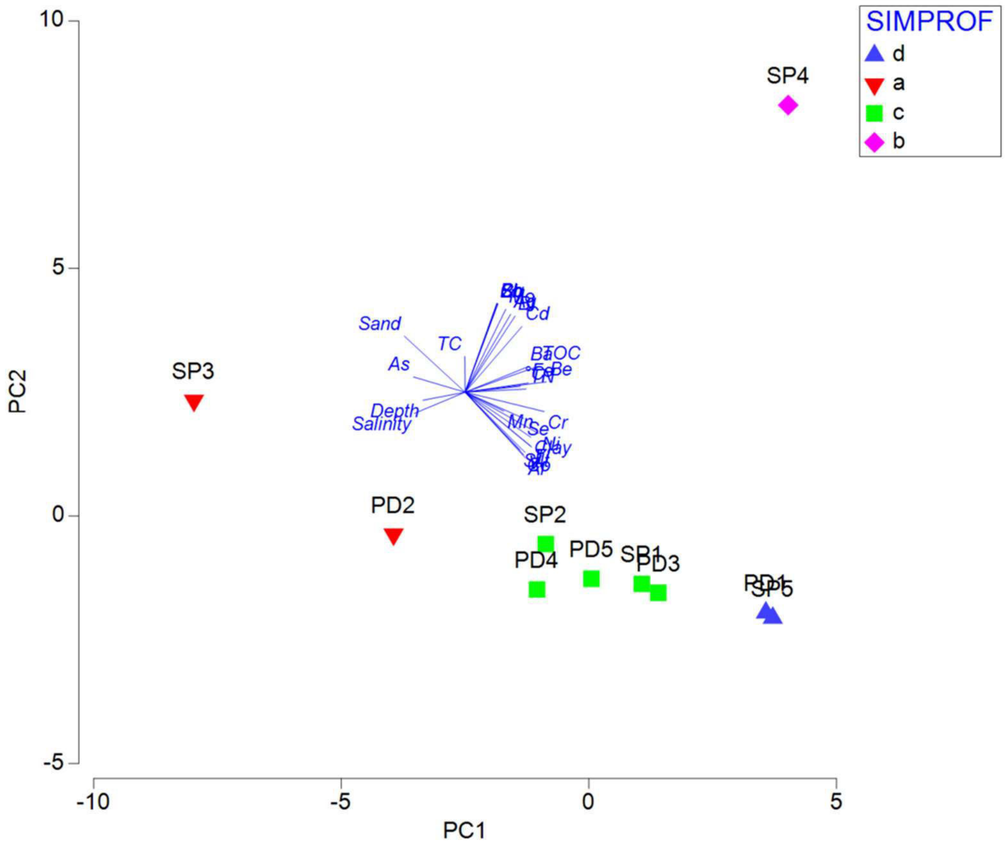


Figure 11. Principal component analysis (PCA) of physical-chemical features of sediments in both marinas during July 2019. Stations were gathered based on the SIMPROF outputs. The sum of the two PCA axes is equal to 78.2%.

Regarding the multivariate analysis, the SIMPROF test significantly divided the stations into four groups that were also gathered in the PCA plot (Figure 11). The stations of group" a" were present in both areas at the main entrance with higher depths. At these stations, high values of salinity, sand, and As among heavy metals were also noticed. Group "b" was represented by only one station. The SP4, located near the boathouse, was characterized by higher temperature values than other stations. In addition, increasing contents of TOC and TC were measured, as well as heavy metals like Cd, Pb, Ag at SP4. The group "d" was characterized by two stations, and both were sampled in the sheltered zones of the two marinas. At those stations, we observed a higher % of silt, clay, TOC and other heavy metals like Al, Cd, Ni. In group "c" the analysis gathered the stations sampled in marinas with intermediate concentrations of heavy metals and sand, silt and clay, and carbon content. In fact, the latter stations were plotted in the PCA analysis along the first axis between the two groups of stations "a" and "d" (Figure 11).

5.1.2 Macroscopic results

We detected a high amount of plastic debris at all stations in the first macroscopic observation, mainly plastic fibres. The latter could derive from fishing nets since the Marina of Špinut hosts many boats. At SP5 (July 2019), a bulk of fishnet pieces was observed (Figure 12a). Also, in Marina of Špinut at SP4, we noticed much debris like dead shells pieces attached to the antifouling coating in both sampling periods (Figure 12b)

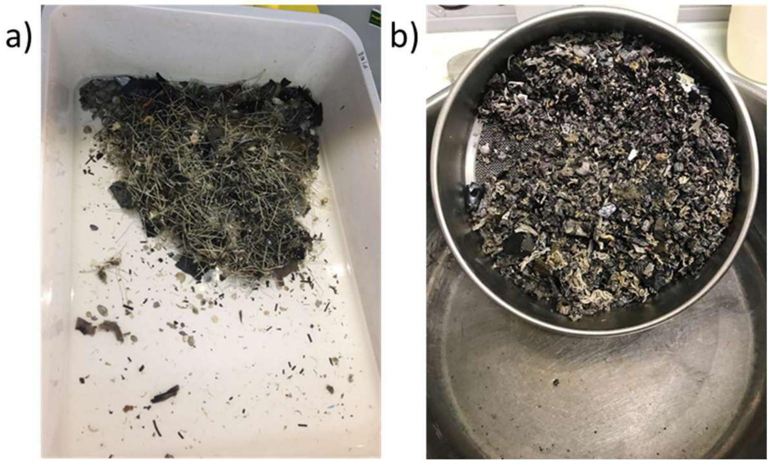


Figure 12. Sediments collected at SP5 site in July 2019 (a) and at SP4 in April 2021 (b)

5.1.3 Macrofaunal abundance and diversity

The macrofaunal abundance in Špinut marina varied from 76.6±124.2 (SP4) to 2356.6±1438.5 (SP2) ind. m^{-2}. Higher densities were noticed at stations nearby the main entrance (SP3 and SP2), whereas lower abundances were observed in the inner sites (SP1 and SP5). Also, the presence of the boathouse area directly influenced the abundance at station SP4, where an extremely low number of organisms was observed (Figure 13a). A clear confinement gradient pattern was mirrored in the number of species (Figure 14a). The highest species number (90) was obtained at the station nearby the main entrance (SP3) whereas decreasing numbers were observed toward the inner ones. Despite a few species were observed at SP4, the lowest value of H' (Shannon-Wiener biodiversity) was obtained at SP1. This was due to the highest dominance of a single species, i.e. the polychaete *Pseudoleiocapitella fauveli* (1370±199.7 ind m^{-2}).

In Strožanac marina, the lowest macrofaunal abundance was observed at PD3 (146.7±136.1 ind m^{-2}) whereas the highest value was recorded at PD2 (2573±830.3 ind m^{-2}) (Figure 13b). In this marina, the macrofaunal densities did not highlight a clear confinement gradient. However, the higher densities were observed at stations nearby the main entrances (PD2 and PD4). Regarding the number of species, the two inner stations were less biodiverse compared to the outer ones. In fact, at PD5 and PD2 the higher values of H' were obtained (4.81 and 4.68, respectively) (Figure 14b).

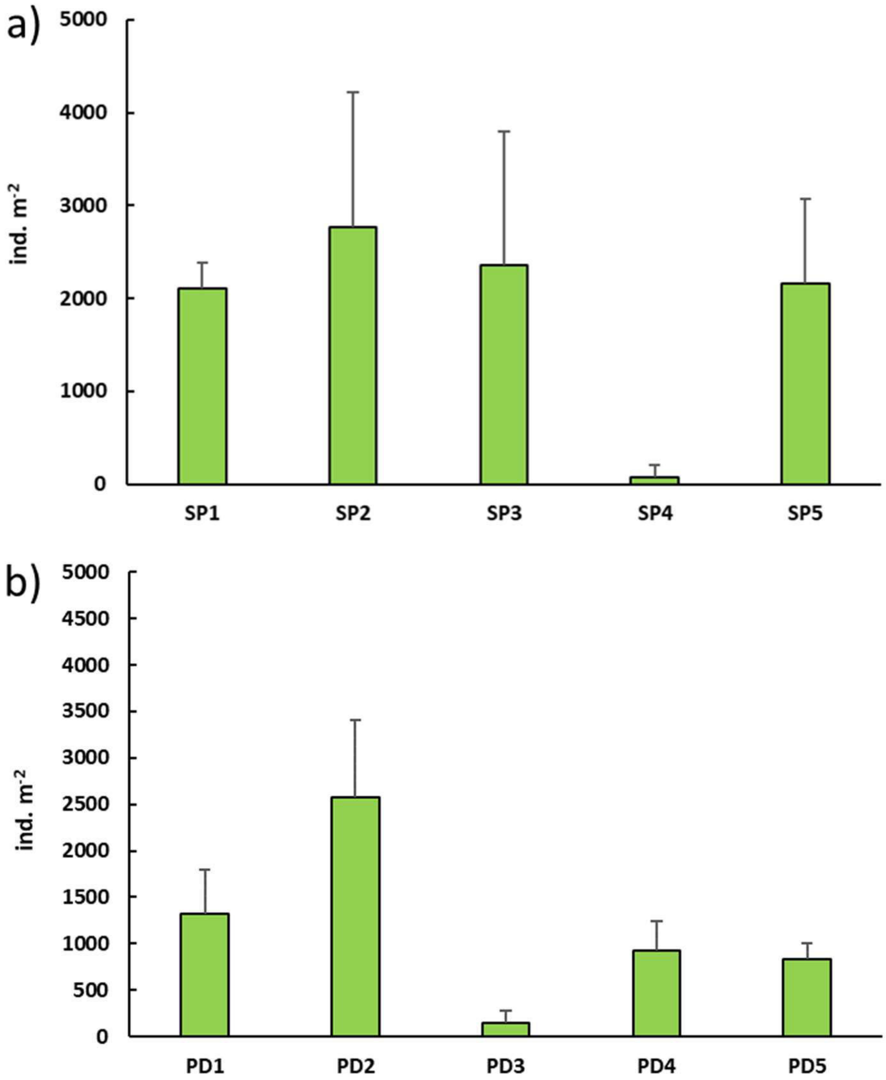


Figure 13. Abundances of macrofaunal invertebrates at sampling sites in Špinut (a) and Strožanac (b) marinas in July 2019

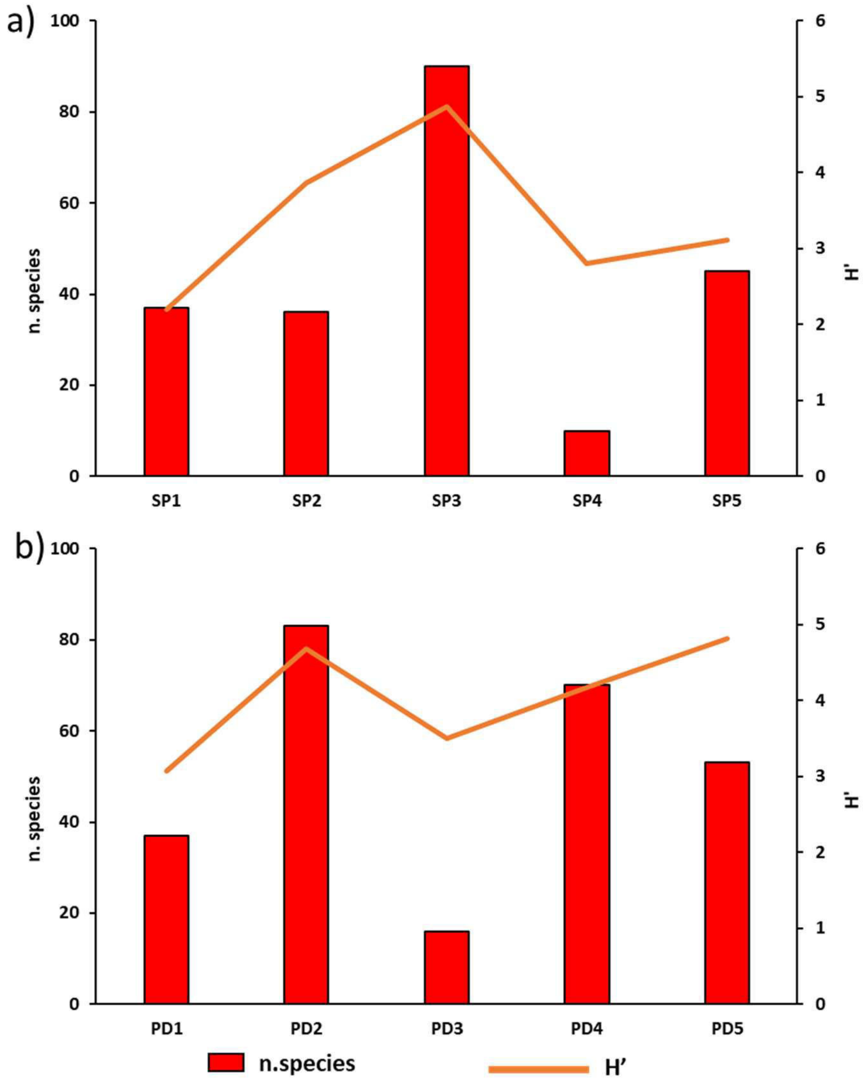


Figure 14. Number of species and diversity values (Shannon-Wiener-H, log_2) of macrofaunal communities at sampling stations in Špinut (a) and Strožanac (b) marinas in July 2019

Overall, the macrofaunal community in both marinas was characterized by the dominance of marine/estuarine species (e.g. the polychaetes *Heteromastus filiformis* and the bivalve *Abra prismatica*). In Špinut marina, the differences among stations were principally due to grain-size distribution and anthropogenic influence in the basin (i.e. boathouse area) (Figure 15). The highest diversity observed at SP3 was due to the presence of mixed environmental conditions (i.e. major seawater renewal and major sand content). In fact, at the latter station, common marine species, rare species (the polychaete *Paragoniadides* sp.) and alien taxa (the polychaete *Neopseudocapitella brasiliensis*) were all co-occurring (Figure 16). Conversely, we observed an impoverished community nearby the boathouse area (SP4). This long-lasting anthropic activity deeply modified the sediment characteristics (we observed e.g. pieces of antifouling coating), leading to poor environmental conditions.

In Strožanac marina, the confinement gradient and grain-size distribution did not directly influence the macrofaunal community distribution. This is likely due to the morphology of the basin. In this marina, the basin is a more open system compared to Špinut. Principally, the presence of seagrass and seaweed coverage at the bottom enhanced the difference among stations and between the two sampling areas. In particular, we observed higher differences in species composition at site PD1 and PD3, which are located in the inner part of the marinas but with high seagrass coverage. Lastly, the macrofaunal community collected at the station nearby the boathouse (PD2) did not seem to be influenced by this activity. PD2 was located toward the outer part of the basin, thus the sediments are directly influenced by a high water renewal improving the quality of the sea bottom (Figure 115).

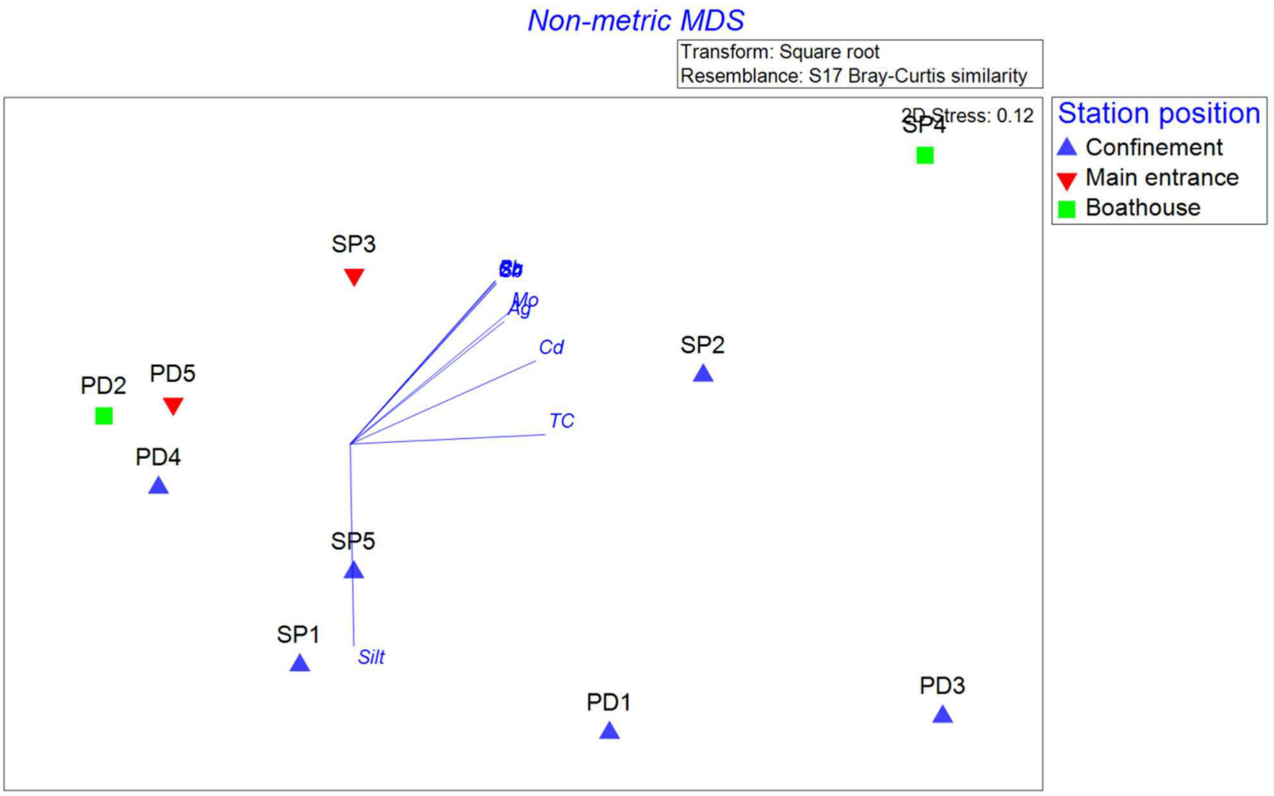


Figure 15. Nonmetric multidimensional scaling (nMDS) ordination plot of species at sampling stations. The main environmental drivers (from BIOENV analysis) are overlaid. Stations are gathered based on their position.

Overall, what was stated above was confirmed by BIOENV analysis. Silt, TN, Sb, Cd, Cr, Pb, Ag, Mo, Sn, Zn were the principal drivers for the macrofauna community (BIOENV analysis: r=0.578, $p<0.05$).

In April 2021, we observed the same variation patterns of macrofaunal density, species richness, and composition as in July 2019. The community did not seem to be influenced by seasonal variation and preserved its conservative features. On the contrary, the macrofauna at SP4 was severely influenced by anthropogenic pressure. We again observed a drastic reduction of species richness and abundance, similarly to what we already noticed in July 2019.

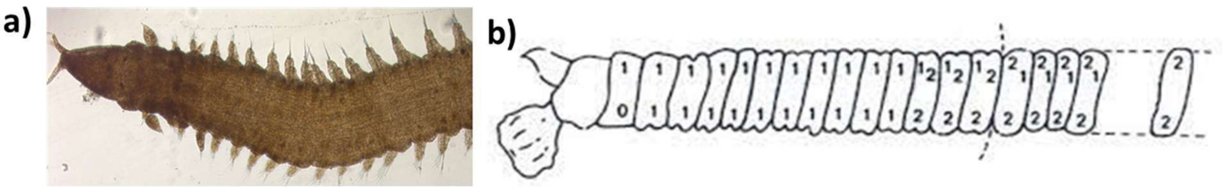


Figure 16. The polychaetes Paragoniadides sp. (a) and the non -indigenous species Neopseudocapitella brasiliensis, both discovered in Špinut marina.

5.2 Marina Dorica-Ancona

5.2.1 Results on the physical-chemical features and heavy metals

In Table 2 are reported the physical-chemical data measured in the water column at the sampling stations. No differences were noticed among stations inside the marina or the reference site. On the contrary, it is evident a clear decrease of DO (mg/l) from the station located at the main entrance to the inner ones. This result confirms the confinement gradient present inside the marina.

Station	**Depth**	**Coordinates**		**Temperature (°C)**		**Salinity**		**DO (mg/L)**		**pH**	
	(m)			Sup	Bottom	Sup	Bottom	Sup	Bottom	Sup	Bottom
MD1	6.0	43°36'38.916''N	13°28'53.724''E	24.6	24.4	35.2	35.6	5.3	5.5	8.1	8.1
MD2	3.0	43°36'39.132''N	13°29'0.779''E	24.1	24.4	35.8	36.6	5.1	4.8	8.0	8.0
MD3	2.5	43°36'41.076''N	13°29'6.756''E	24.3	24.8	35.8	36.8	3.8	3.7	7.9	7.9
MD4	3.4	43°36'36.972''N	13°28'55.019''E	24.7	24.5	34.5	35.9	5.8	5.9	8.1	8.1
MD5	2.5	43°36'35.028''N	13°29'14.567''E	23.7	24.8	35.6	37.0	5.0	4.2	8.0	8.0
MD_RS	3.3	43°36'32.148''N	13°28'31.33''E	24.2	24.1	35.7	35.7	4.7	4.9	8.1	8.2

Table 2. Depth, coordinates, temperature, salinity, Dissolved Oxygen (DO), and pH measured in "Marina Dorica"

The investigated area was characterized by sandy sediments, in particular at the reference site where the sand fraction reached 92.2%. The confinement gradient was observed even in the grain size percentage. Indeed, high fractions of sand were observed at stations nearby the main entrance (MD1 and MD4), whereas increasing percentages of silt and clay were detected at stations located in the inner part of the basin (MD2, MD3, and MD5) (Figure 17).

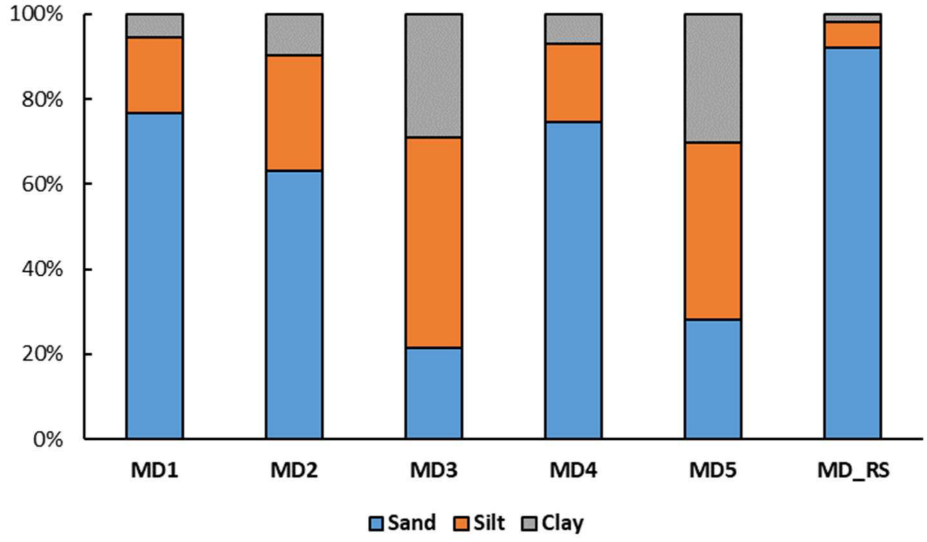


Figure 17. Sand, silt, and clay percentages of sediments at sampling stations

For the Total Nitrogen (N), Total Organic Carbon (TOC) and Total Carbon (TC) contents, a clear confinement gradient was not evidenced in the sampled marina. At MD2 the highest values of N and TOC were observed (0.42 and 7.28%, respectively), whereas the lowest values were detected at MD5 (0.01 and 0.45%, respectively; Figure 18).

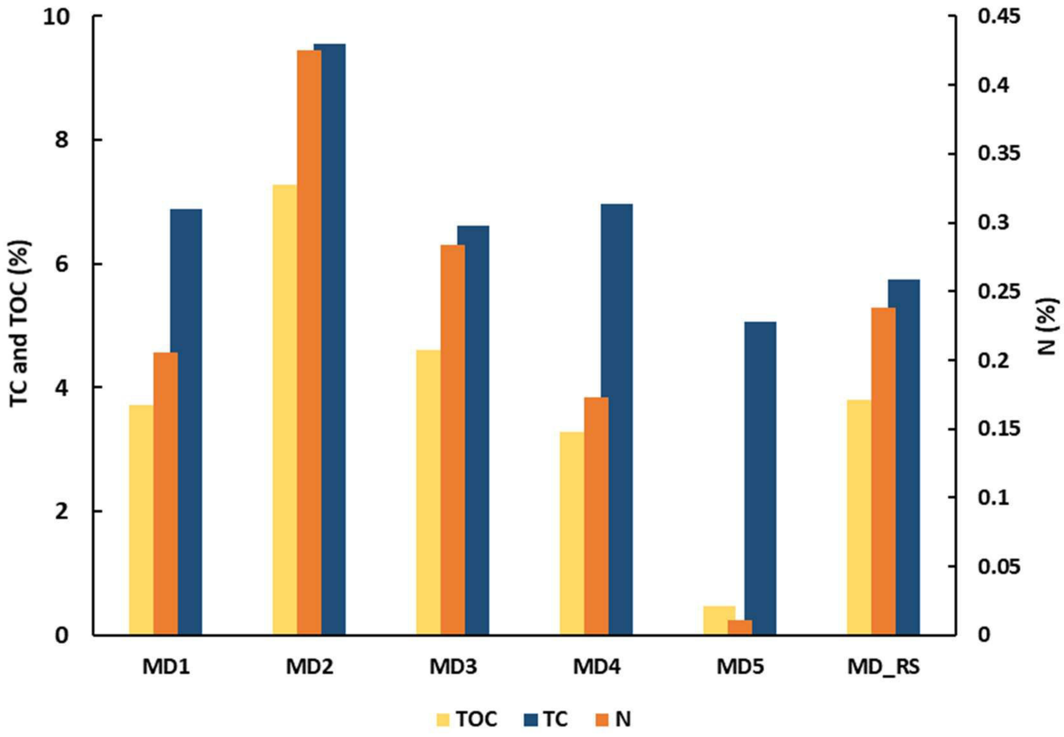
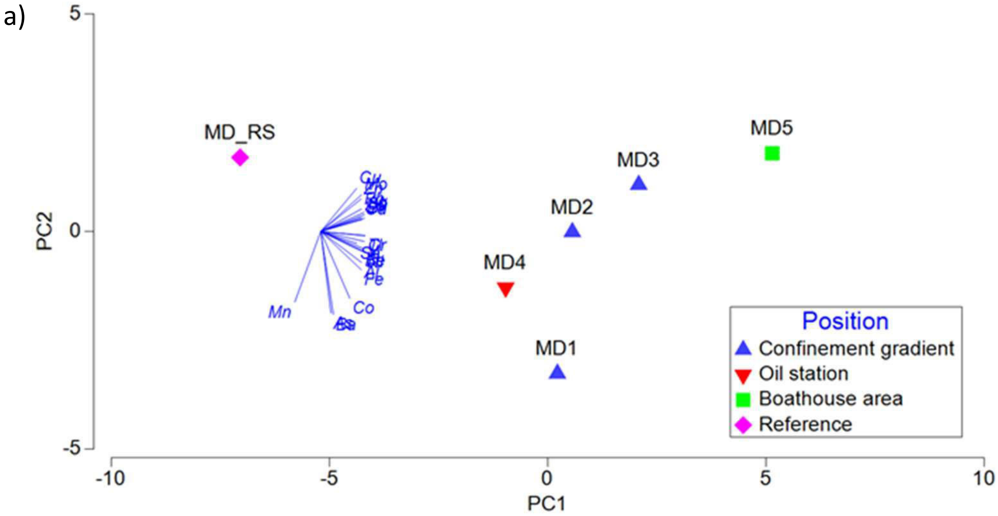


Figure 18. Total Nitrogen (N), Total Organic Carbon (TOC) and Total Carbon (TC) percentages at sampling stations

The PCA plot evidences a significant spatial variation between sediments sampled inside the marina and at the reference station (Figure 19a). Higher values of heavy metals concentrations were measured inside the marina compared to the reference station. Inside the basin (Figure 19b), higher values of As, Mn, Ba, Co were observed at the main entrance of the marina (MD1). In contrast, higher concentrations of Cr, Pb, Cu, Ag, and Zn, which could derive from antifouling paints (Singh and Turner, 2009), were measured at MD5. The latter station was placed nearby the principal boathouse area of the "Marina Dorica".



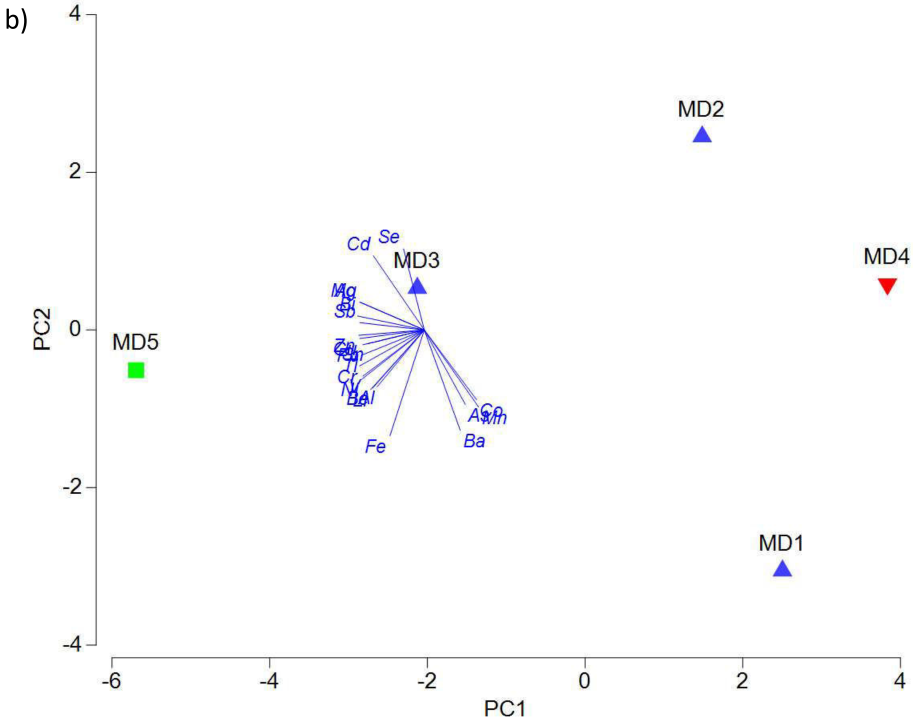


Figure 19. Principal component analysis (PCA) of heavy metals in the sampling area with the reference site (a) and only the stations inside the marina (b).

5.2.2 Macrofaunal abundance and diversity

The macrofaunal abundance in "Marina Dorica" varied from 340 (MD2) to 14500 (MD_RS) ind. m^{-2}. The highest density was obtained inside the basin at the station nearby the main entrance, whereas decreasing numbers were observed toward the inner sites. However, a clear confinement gradient was not mirrored in the macrofaunal density (Figure 20).

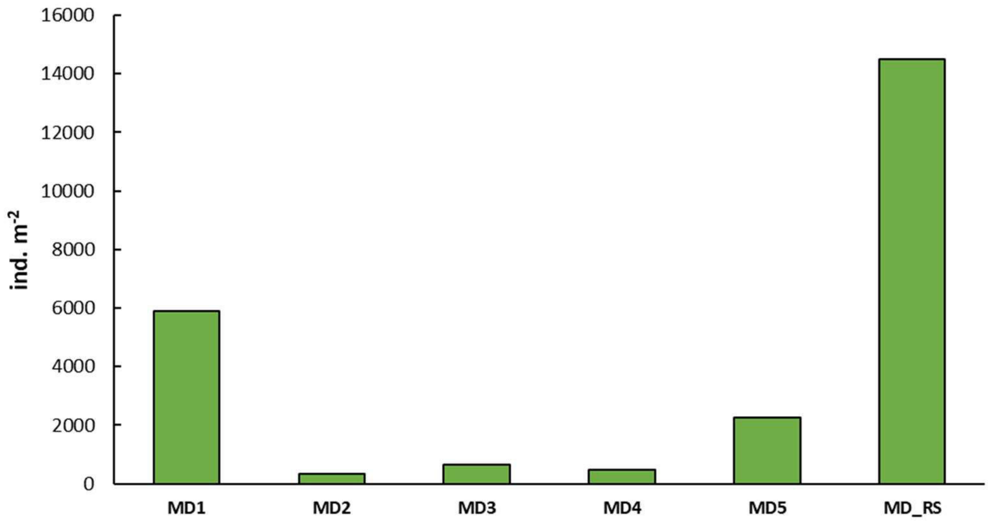


Figure 20. Abundances of macrofaunal invertebrates at sampling sites

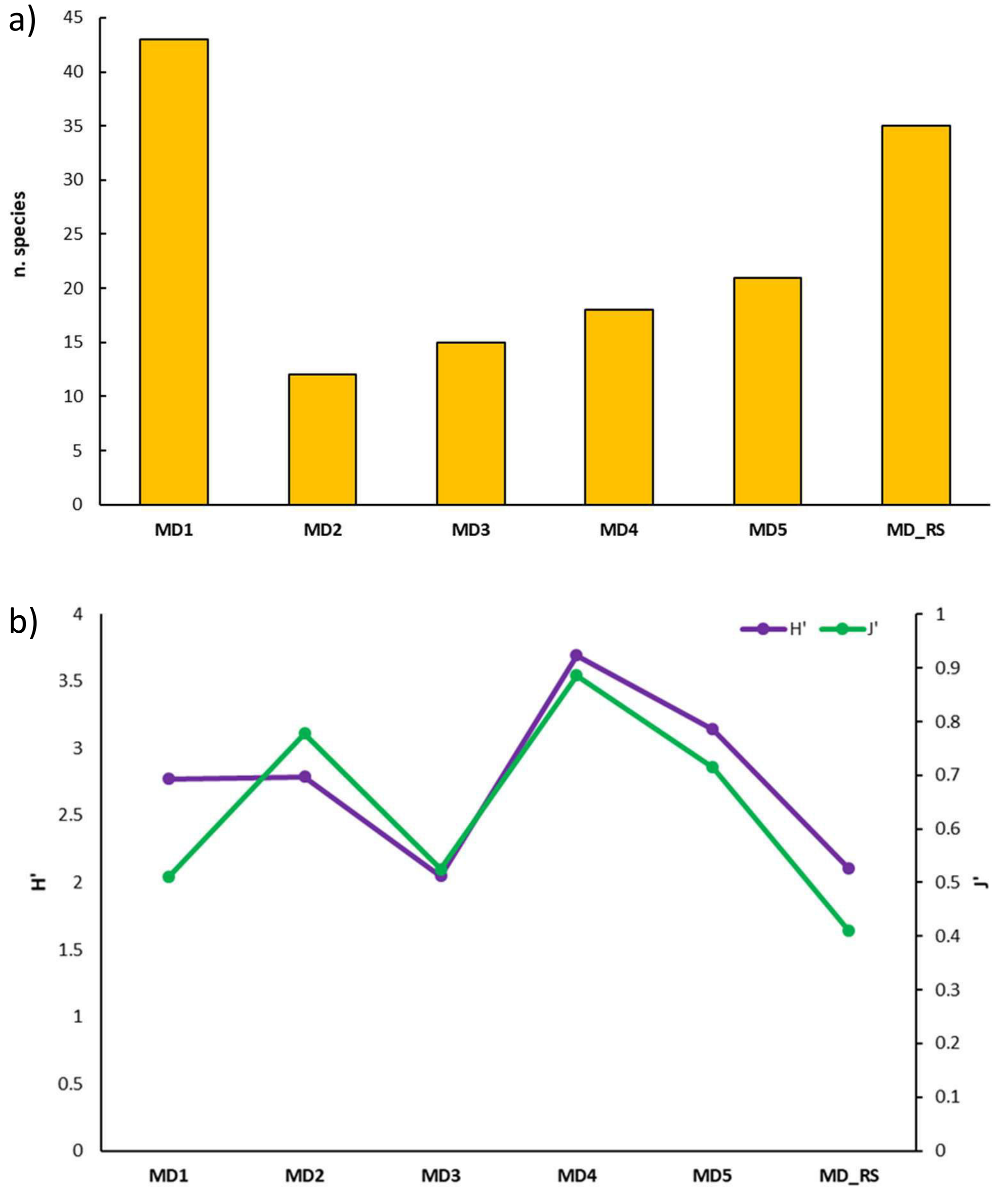


Figure 21. Number of species (a) and diversity values (Shannon-Wiener-H, log_2 and Pielou's evenness-J) of macrofaunal communities at sampling stations

More species were noticed at stations near the main entrance (43 at MD1) compared to the reference site (35 at MD_RF). The lowest value was observed at MD2 (12). Overall, increasing species richness values were noticed along the confinement gradient (Figure 21a). Regarding diversity values (H' and J'), they followed the same variation pattern among sampling stations (Figure 21b). Although a higher number of species was observed at MD_RF and MD1, the lowest value of H' and J' at MD1, MD3 and MD_RS were due to the highest abundance of few species. These species were the bivalve *Donax trunculus* (at MD_RS and MD1) and the polychaete *Streblospio shrubsolii* (at MD3).

The principal environmental parameters driving the species distribution in the sampling area were Sand, Silt, N, TOC, TC, Al, Se, Be, Co, and Li (BIOENV analysis: $r=0.507$, $p<0.05$; Figure 22). The macrofaunal community was characterized by the dominance of marine species at MD_RS and MD1 (like *Donax trunculus,* Figure 23a) that are strictly linked to the higher sand percentages at those stations. Furthermore, at MD1, the highest diversity was due to mixed environmental conditions. We observed common marine and estuarine/euryhaline species. Overall, inside the marina at MD3 and MD5, the macrofaunal community was deeply influenced by fine-grained sediments (higher silt values) at MD3 and MD5 (Figure 21). Many estuarine/euryhaline species were noticed at the stations farther from the main entrance, particularly at MD5 (e.g., the polychaetes *Streblospio shrubsolii* (Figure 23b) and *Neanthes succinea*).

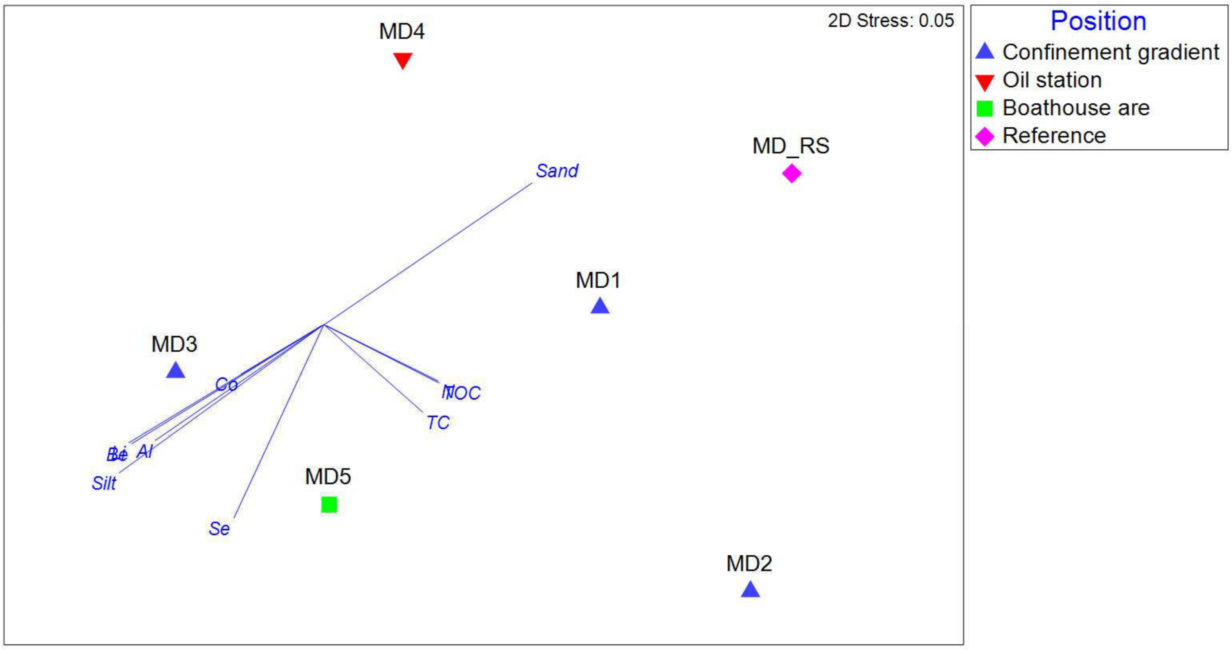


Figure 22. Nonmetric multidimensional scaling (nMDS) ordination plot of taxa composition at sampling stations. The main environmental drivers (from BIOENV analysis) are overlaid. Stations are gathered based on their position

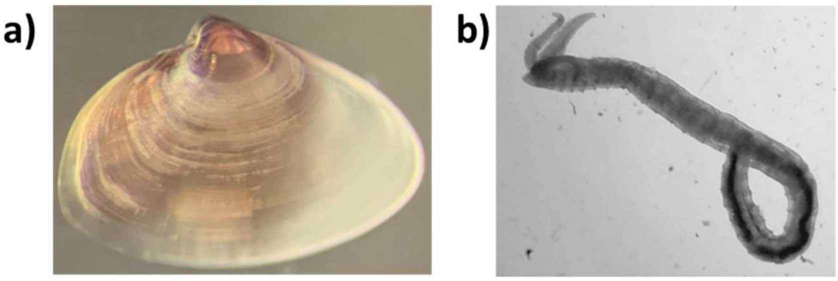


Figure 23. The bivalve Donax trunculus (a) and the polychaete Streblospio shrubsolii (b), observed in Marina Dorica

6. Conclusions

- In Strožanac marina the macrofaunal community was not directly influenced by the confinement gradient and grain size distribution. This is likely due to the morphology of the basin. In this marina, the basin is a more open system compared to Špinut.
- In Špinut the morphology of the basin, as well as the largest catchment area, contribute to the accumulation of contaminants in the sediments.
- In “Marina Dorica” the macrofaunal community is not directly influenced by the confinement gradient. Despite the presence of boathouse activities, the macrofaunal community, in terms of abundance and diversity, does not seem to be directly affected by this anthropogenic activity. Overall, the grain-size distribution plays a crucial role in the variation of the macrofaunal community among stations.
- This study indicates that by including the macrofaunal community features in monitoring plans could help local managers of ports and marinas design site specific environmental interventions to mitigate anthropogenic disturbances.

7. References

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