The Italian National Recovery and Resilience Plan (NRRP) RETURN Project: proposal of new methodology for combined sampling and detection of traditional and emerging pollutants in the pilot site of the Port of Genoa

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Abstract

Purpose The RETURN (multi-Risk sciEnce for resilienT commUnities undeR a changiNg climate) Project aims to improve understanding of environmental, natural and anthropogenic risks. RETURN includes the creation of a new monitoring protocol for traditional and emerging contaminants (metals, asbestos fibers, organic microcontaminants, drugs, pesticides) and ecotoxicological effect in sea environment. We present the joint methodologies created by the collaboration of different project partners and the preliminary results obtained in the Port of Genoa (NW Italy).

Methods Dynamics and physical parameters of the water column were measured with ADCP and multiparametric probe. Emerging contaminants and metals were analyzed by direct measurements in seawater and using passive samplers by chromatographic techniques coupled with MS and ICP-MS. Sediments were chemically characterized by ICP-MS after microwave-assisted acid dissolution, by FESEM with EDX Spectroscopy and XR powder diffraction for qualitative and quantitative phase characterization. Ecotoxicology of water and sediments was determined by tests on *Dunaliella tertiolecta* and *Artemia salina*.

Results The first application of the monitoring protocol revealed some differences between the two port sites, including different dynamics (lower dynamic in Site 1 than in Site 2), metal concentrations in both seawater and sediments (e.g Cu in Site 1 > Site 2), and in ecotoxicological response.

Conclusions The proposed protocol proved to be valid for the characterization of the port basin and the further sampling and measurement campaigns in the framework of the project will allow us to refine the chosen methodology and better understand the results obtained.

Keywords: Italian NRRP RETURN project, asbestos fibers, passive samplers, ecotoxicology, dynamics, Port of Genoa.

1. Introduction

Ports are very complex areas which can have high impact on the community, economy and environment of a country or a region [1]. From the environmental point of view, impacts on the terrestrial, aerial, and marine environments inside ports are mixed with those deriving from the cities bordering the port basins and those inland, where many different activities can take place (from agricultural to industrial) and can produce multiple types of contaminants [2]. Regarding the marine environment, the water masses within the port basins receive various types of discharges (civil and industrial, such as wastewater, ship paint, stormwater runoff, nitrogen, oil spills, etc.) and also collect solid materials, from plastics dispersed in the environment to vegetables residues carried into the sea by streams and rivers [3,4]. With regard to discharges (sewer overflows, storm water drainage, farms and city runoff, boats and vessels discharges or accidental spills, and also atmospheric deposition of aerosols), they bring to the port water masses countless different types of contaminants: from those studied for decades such as metals and hydrocarbons [5-9], to the more recent (so-called emerging

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contaminants) drugs, pesticides, etc., [10-14] and other such as bacteria and fecal pathogens [15]. Water masses and marine sediments also receive and accumulate materials and elements resulting from both the runoff and erosion of rocks that geologically characterize the geographic area in which the port is located (sedimentary material of different sizes composed of different minerals such as quartz, feldspar, calcite, amphibole, etc.) [16,17], as well as products resulting from erosion and treatment of man-made materials, such as asbestos fibers [18,19].

The complex of contaminants that are input into port basins can lead to consequences on organisms living both in the water column and on the sea bottom, the site of ultimate deposition and stocking of chemicals [20,21]. Therefore, ports can represent a sink of ecotoxic substances and sediments can favor their dissolution and transfer into the water column, due to the sediment resuspension by the action of propellers or during activities such as dredging, which is often carried out in port basins to maintain navigability depth for vessels [22]. Some potentially toxic elements (PTEs) (e.g. metals or metalloids) can be toxic even at low concentrations due to bioconcentration and biomagnification in the food chain, and affect marine biodiversity not only in ports, but also in neighboring areas. Therefore, implementing effective monitoring plans and maintaining good quality standards of port waters is not only important for a good environmental status in the modern green port concept [23], but is also necessary for the survival of biodiversity and the protection of marine ecosystems [24].

These aspects are even more critical considering climate change with sea temperatures gradually rising [25], frequency of extreme sea storms rising, and changes in the frequency and intensity of rainfall (less frequent in the course of the year, but with more rain concentrated in a few hours - flashflood or extreme rainfall events) [26,27]. The identification of new methodologies or new combined protocols for sampling and analyzing multiple contaminants becomes necessary to identify and quantify the impacts produced by new contaminants and to understand the mechanisms at work in the marine system in relation to climate change.

With the aim of addressing these issues, the RETURN (multi-Risk sciEnce for resilienT commUnities undeR a changiNg climate) Project was born (https://www.fondazionereturn.it/en/). By enhancing basic knowledge, targeting the application and exploitation of technology, the RETURN Project contributes to strengthening key competences, technology and knowledge transfer, as well as Italian governance in disaster risk management, with the involvement of public administrations, stakeholders and private companies. This study is part of the RETURN Project and is concerned with creating a new protocol for measuring physical-chemical parameters of water masses and sampling multiple contaminants simultaneously, examining both traditional contaminants, such as metals and hydrocarbons, and emerging contaminants, such as pesticides, organic micro-contaminants, drugs and asbestos fibers. Here we present the monitoring strategy and the first application to the pilot site of the Port of Genoa (NW Italy).

2. Materials and method

2.1. Study area

The Port of Genoa (Liguria – NW Italy; **Fig. 1**) has characteristics of a complex basin in which commercial, industrial, and recreational functions coexist; in fact, the Port of Genoa is equipped to accommodate all types of ships for all types of goods (containers, miscellaneous goods, perishable products, metals, forestry, solid and liquid bulk, petroleum products) and also hosts a ferry terminal and a cruise terminal, as well as several marinas of different extensions.

The Port of Genoa is geographically located on the northern side of one of the most active areas of cyclogenesis in Europe (Ligurian Sea), in which climate changes have highlighted new trends and new phenomena that can negatively impact the port basin and the surrounding environment. Examples of this are the floods that occurred in recent years [28,29] and the destructive sea storms of 2018 [30] and 2023 which also damaged the current breakwater of the port.

The port basin collects the waters of two important streams (Bisagno and Polcevera) and numerous minor streams which develop on lithotypes peculiar for their chemistry from ophiolite geologic units (basalts, gabbros and serpentinites) and from sedimentary rocks (mostly lime schists and shales) [31], making port waters and sediments a scientifically interesting and continuously evolving area.

The Port of Genoa was chosen as the pilot site because, starting from May 2023, it is involved in several major activities of construction of maritime and land infrastructures and materials reuse or storage (Fig. 1). The first project is the construction of new breakwater of the port (Fig. 1 in red) which will rest on seabed up to 50 m

deep and will allow the entrance of latest generation ships inside the port (the breakwater construction is controlled by the Environmental Monitoring Plan drawn up by the DISTAV of the University of Genoa and based on the Descriptors of the Marine Strategy Framework Directive); the project involves dredging sediment from the port and destroying part of the existing breakwater with the reuse of the dredged sediment and material from the old breakwater to fill caissons of the new breakwater. The second project is the construction of the highway bypass "Gronda" that will avoid transit across the city of Genoa; in this case, the project includes the dumping of drilling mud and sediment from green rocks directly into a purpose-built fill within the Port of Genoa via a slurry pipeline (Fig. 1 in purple), which could therefore discharge a significant amount of chrysotile (more generally, asbestos fibers) into the marine port environment. The third project is the construction of the sub-port tunnel which will connect the east to the west of the city, bypassing the center; the project involves filling some of the port quays (Fig. 1 in green) with excavated material to create new docks for cargo and container handling.
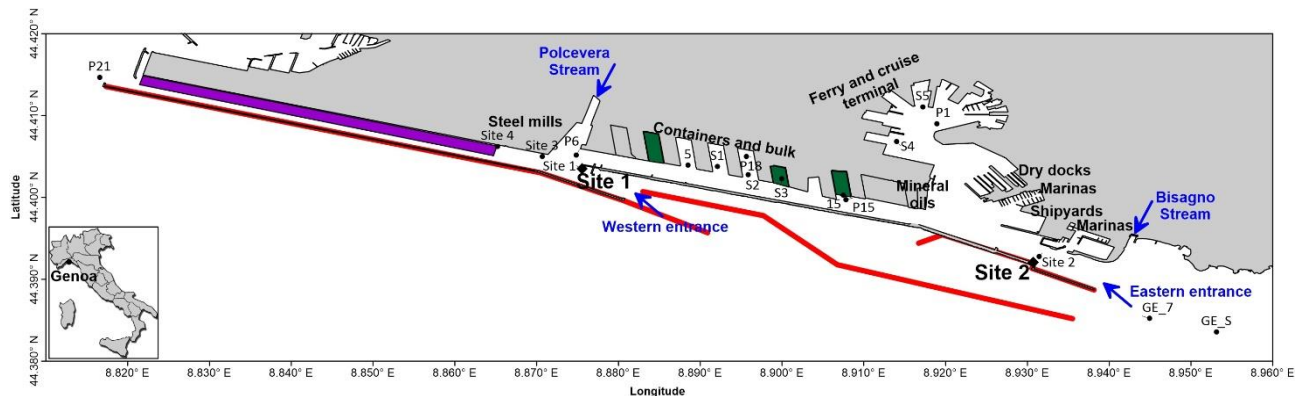

Fig. 1 Genoa port basin, main characteristics (anthropic activities in black and natural features in blue) and localization of the monitoring sites (black rhombus – Site 1 and Site 2 - are the fixed stations, and black points are the sediment sampling points for asbestos fibers). The layout of the new breakwater of the port is shown in red; the fill area for the material of the Gronda project is shown in purple; and the fill area for the material of the sub-port tunnel is in green

2.2 Sampling and measurement protocol

Physical-chemical parameters of the water column and dynamics were measured by fixed and mobile instruments. Fixed monitoring stations (Site 1 and Site 2) are deployed on the internal part of the port breakwater at 7 m-depth and continuously measure. Fixed stations are equipped with a multiparametric probe (CTD, Idromarambiente) with temperature, turbidity and dissolved oxygen sensors, and a horizontal acoustic Doppler current profiler (H-ADCP WorkHorse 300 kHz, RD Instruments) for current velocity and direction measurement. A portable CTD (Idromarambiente) and vertical current meter (V-ADCP WorkHorse 300 kHz, RD Instruments) were used by monitoring vessel for measurements of temperature, salinity, turbidity, dissolved oxygen and chlorophyll-a and dynamics in the water column (from the sea surface to the bottom) near the fixed station.

Bottom sediments were sampled with a steel Van Veen grab and surface water was sampled with a 5-L Niskin bottle.

Seawater samples were filtered by 0.45 μm membrane filters (Millex-HA, Merck) and acidified with 1 % HNO_3 during the sampling and then, analyzed in laboratory by ICP-MS (Agilent 7800).

DGTs (Diffusive Gradients in Thin-films) for the measurements of metals (Cd, Cr, Cu, Fe, Ni, Pb and Zn), As and Hg were deployed for 7 days (from 15 to 22 December). Immediately after the retrieval from seawater, the devices were rinsed with Milli-Q water; successively, in laboratory, the metals were eluted from the binding gel in 1 ml of 2M HNO_3. The elution extracts were diluted with Milli-Q water and trace metal analysis was carried out by using ICP-MS (Agilent 7800). A blank resin was always considered. DGT performance was evaluated according to the method used by Zhang and Davison [32]. The concentrations of metals measured by DGT, in two replicates, were calculated using equations and parameters provided by [33]. Analysis of total mercury concentrations in DGT units was performed using AMA 254 mercury analyzer: the chelating resin

was removed from the device and directly analyzed without further preparations. A blank resin was analyzed in the same conditions and its mercury content was always subtracted to the mercury concentrations measured for the samples.

Sediments aliquots used for metal analyses (except Hg) were oven-dried (40°C) and then, sieved to 2 mm and finely ground. The determinations of trace metal content (except Hg) in sediments were obtained by a microwave-assisted acid digestion procedure, using an acid mixture of HNO_3, HF and H_2O_2, followed by the chemical analyses performed by an ICP-MS instrument (Agilent 7800). Sample sediments for Hg analyses were air-dried in a fume hood and Hg determination in sediments was carried out by the automated Hg analyzer FKV AMA-254.

Organic contaminants are sampled by two different passive samplers, Semi-Permeable Membrane Device (SPMD) and Polar Organic Chemical Integrative Sampler (POCIS) for lipophilic and hydrophilic compounds, respectively; they are deployed for 21 days. Passive sampling (PS) is based on mass transfer due to the different chemical potentials of analytes between water and the collection medium inside the sampler [34]. The synergy between active and passive sampling systems will allow the detection of contaminant at levels below the ng/L. For this purpose, alongside the PS, sediment and water sampling has been conducted at the beginning and at the end of the PS deployment.

Organic chemicals are analyzed through high sensitivity instrumentations, mainly based on the coupling of chromatography to mass spectrometry, in particular: GC-MS (Agilent 7890A-5975C), LC-MS/MS (Agilent 1200-6430 LC-QQQ) and UPLC-MS/MS (Xevo G2-XS-QTOF, Waters).

Regarding SPMD, the membranes are extracted twice for 24 h with 300 mL n-hexane containing internal standards. The two extracts are dried passing through anhydrous sodium sulfate. The n-hexane is reduced in volume using a vacuum rotary evaporator. The 1 mL sample is eluted in a 3 g silica column with firstly 10 mL of n-hexane and then 20 mL of n-hexane:dichloromethane (1:1). The two different eluted are analyzed for polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs).

POCIS are well suited for semi-polar compounds, belonging to different classes of emerging contaminants (pharmaceuticals, estrogens, UV-filters, PFAS, stimulants, artificial sweeteners, etc.). In this case, the sampler is opened and the sorbent is transferred into a glass cartridge which is eluted using 20 mL of MeOH and 5 mL of DCM:IPA (80:20 v/v). The obtained eluate is then reduced to dryness on a rotary evaporator (Rotavapor® R-100, BUCHI, Switzerland), reconstituted in 1 mL of methanol and filtered through a 0.2 µm hydrophilic PTFE filter [35]. Appropriate dilutions are performed before LC-MS/MS analysis, in order to detect and possibly quantify the highest possible number of emerging contaminants.

Sediments are extracted using an Accelerated Solvent Extractor (ASE 200) with acetone:n-hexane (1:1). Internal standards are added to the sample before the extraction. The extract is transferred with 20 mL of water in a separator funnel and shaken to remove the acetone; successively the extract is dehydrated on anhydrous sodium sulphate and then it undergoes to the same method of PS, so after volume reduction, it is fractionated through the silica column. Seawater samples are transferred into the separator funnel, shaken, volume reduced and fractionated, as already explained above.

The ecotoxicological assays were performed on seawater samples and sediment elutriate. For the preparation of sediment elutriate, synthetic seawater [36] was added to the sediment in the ratio 4:1 (volume/dry weight) and placed in a shaker for 1 h at room temperature [36]. Then, the mixture was centrifuged at 3,000 rpm (1,000×g) for 20 min and filtered (Ø 0.45 µm). Pore water was prepared by centrifugation of sediments at 13,000 rpm (2.2× 104 g) for 45 min at room temperature in an Eppendorf centrifuge (5810 R; r=11.5) using polycarbonate bottles. For aqueous samples, a bioassay battery, consisting of four different species representing different trophic levels, algae *Dunaliella tertiolecta*, crustacean *Artemia salina*, was chosen.

D. tertiolecta test - The chronic test was carried out according to ISO 10253:2016. Bioassays were performed using dilutions of elutriate and seawater sample. Artificial seawater [36] was used for the dilution of samples. The samples were placed in sterilized glass flasks, in triplicate. An algal suspension at a concentration of 1×10^6 cells/mL was prepared. Then, an aliquot of algal suspension was added to each replicate to reach the final concentration of 1×10^4 cells/mL. Culture medium has been utilized as negative control (six replicates). The test flasks were placed in a thermostatic chamber at 20 °C with a light source in the 7,000– 8,000-lux range for 72 h. The cell density of each sample was measured after 72 h by the Burker chamber. EC50 was calculated

for each sample, and the maximum growth inhibition percentage was estimated in the case EC50 was not computable.

Artemia salina test - *A. salina* cysts were hatched by using the procedure described in [37]. The encysted organisms were first hydrated in a volume of artificial seawater (Instant Ocean 3 % m/v) for 1 h at 25 °C at 3,000–4,000 lx. Then, the cysts were incubated for 24 h in the dark at the same temperature. Acute toxicity test (96 h) was conducted according to [37]. Ten nauplii were transferred in a beaker with 40 mL of sample. Each sample was tested in triplicate. The negative control consisted of six replicates of artificial seawater. The treatments were incubated at 25 °C with a light regime of 14:10 h light/dark. No food was provided during the exposure. Every 24 h, the number of the live individuals was recorded. The effect percentage for each sample was calculated with respect to the control.

The sediment sampling addressed at investigation for fibrous minerals was carried out at the two fixed stations together with the other samplings, but also at other points distributed inside and outside the harbor in order to characterize the sediments and obtain information on the entire basin. A total of 18 samples were taken between 10 November 2023 and 19 March 2024. The sediments were prepared for the quantitative analysis of asbestos to discriminate the natural vs. anthropic contribution to the harbor environment.

The preparation of samples followed the prescription of MD 120/2017. The analytical determinations are carried out on the powder with a grain size spectrum of <0.100 mm obtained by milling of a representative subsample with a grain size of ≤20 mm. The preparation is detailed and discussed in [38].

The mineral characterization on fine-grained sediments was based on reflected optical microscopy (OM), SEM/EDS, to identify the fibrous and EPM minerals and distinguish the phase morphology, composition and microtextures. The microscopic mineral identification was carried out on gold-coated dusts dispersed on membranes was conducted at 2500× magnification, 20 kV of acceleration voltage, by SEM-EDS methodology using a Vega 3 XML TESCAN (Brno, Czech Republic) equipped with an energy-dispersive X-ray spectroscopy AZtec 2.4 by Oxford Instruments, installed at the Department of Earth, Environment and Life Sciences, University of Genoa. The elemental analysis of minerals was acquired with a counting time of 30 seconds. The phase identification by XR Powder Diffraction was carried out by the Rigaku Miniflex diffractometer equipped with a HPAD (HyPix-400 MF 2D) hybrid pixel array detector and a 600W X-ray source at the DCCI, University of Genova.

2.3 First application

The first application of the presented monitoring plan was carried out for the first time between 15 December 2023 and 8 January 2024 inside the Port of Genoa (**Fig. 1**). Fixed stations were chosen at the two entrances to the port (the western entrance - site 1, and the eastern entrance - site 2) for their peculiarities. Site 1 is at the western entrance to the port and is characterized by the presence of the mouth of the Polcevera Stream and a steel mill to the north, and the Airport channel to the west. The western entrance is not used for the transit of ships or vessels, but only for service boats and tugs. The area receives the sewage discharge of the western part of the city of Genoa and is subject to strong dynamics due to the proximity of the port entrance and the open sea. Site 2 is located at the eastern entrance to the port that is the only entrance to the port used for the access of commercial ships, cruise ships, and pleasure boats. In front of the site, there are marinas, shipyards, dry docks for vessels. The entrance channel is characterized by high dynamics and is affected by the influence of the Bisagno Stream which flows through the city and receives water from minor urban streams and city discharges. After heavy rainfall, the turbidity plume of Bisagno enters the port at the eastern entrance, especially in SE wind and sea conditions.

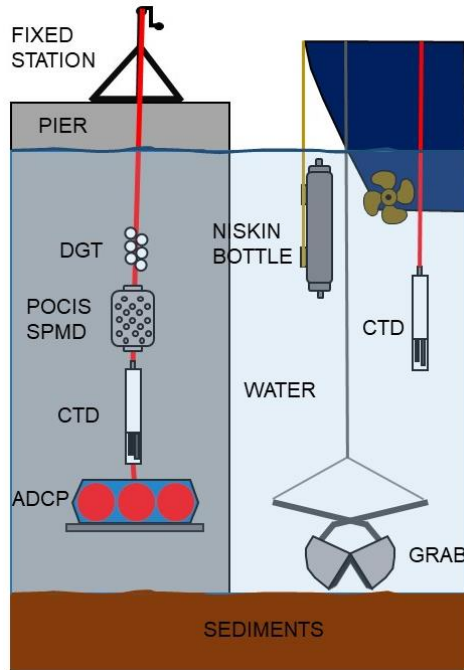**Fig. 2** shows the positioning of all instruments used at the fixed stations.

Fig. 2 Diagram summarizing all the instruments used in monitoring and their respective locations. The drawing is not to scale

4. Preliminary results and discussion

Some preliminary results of the first sampling are reported below.

Meteorologically speaking, the sampling period, after the first few days of good weather, was characterized by cloudy skies with even heavy rainfall, winds mostly from the southern quadrants, and shallow to rough seas (especially between December 18 and 25 and in early January), which are associated with increased current velocities (maximum values 0.8 m s^{-1} at Site 2). During the period (**Fig. 3**), water temperature fluctuated between 14.2°C and 16.3°C, turbidity showed some increases due to solid inputs from the streams (maximum 11.5 FTU at Site 2), dissolved oxygen showed values between 54 and 83%, and the direction of the currents was mostly outgoing from the port at both Site 1 and Site 2, making the two sites more affected by water coming from the inner part of the port.
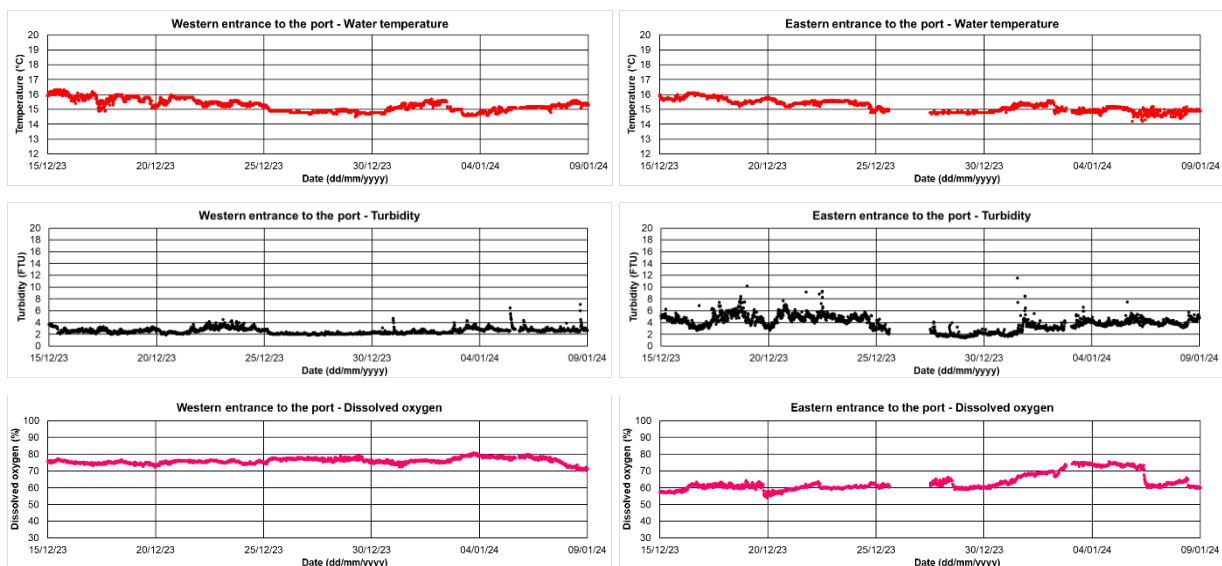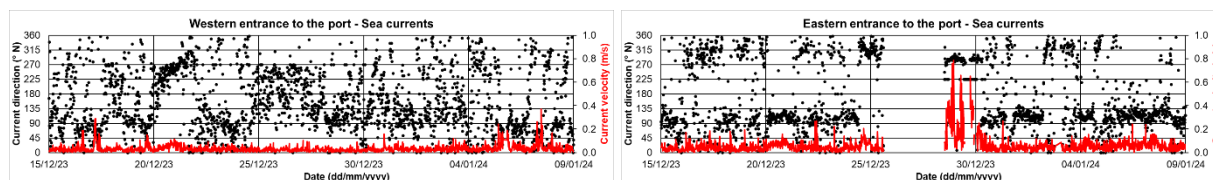
Fig. 3 Water temperature (in °C, red), turbidity (in FTU, black), dissolved oxygen (in %, purple), and direction (in °N, red) and velocity (in m s^{-1}, black) of currents measured by fixed stations at Site 1 (western entrance, on the left) and Site 2 (eastern entrance, on the right) during the period from December 15, 2023 to January 8, 2024. Between 25 and 28 December, there was a malfunction of the fixed station of Site 2 and thus loss of data

Analyses of metals showed higher contamination at Site 1 than at Site 2, in both seawater and bottom sediment. In water samples, concentrations were similar between the two sites and between the two sampling dates, with only Fe being higher at Site 1 with 1.19 µg/L, but metals accumulated by DGT were higher in Site 1 with 1.33 µg/L of Cu compared to 0.01 µg/L in Site 2, 4.58 of Zn compared to 0.28 µg/L in Site 2, 0.63 µg/L of Pb compared to 0.05 µg/L of Site2, and 29 ng/L of Hg compared to 0.30 ng/L. In the sediments of Site 1, V, Cr, Co, Ni, Cu and Zn show values even twice as high as those of Site 2. The different concentrations between the two sites could be due to the different activities that affect the two port areas; in fact, some metals are typical of the steelworks such as Cr, Cu, Ni, and V [39]. Moreover, differences could also be to the different contributions of the two main streams that cross valleys with different vocations: the Polcevera Valley with an industrial vocation and that of the Bisagno with a more residential vocation.

Table 1 Seawater metal concentrations (values in µg/L) in the two sites (Site 1 and Site 2) and on the two sampling dates (15 and 22 of December 2023)

Site and date	V (µg/L)	Cr (µg/L)	Mn (µg/L)	Fe (µg/L)	Co (µg/L)	Ni (µg/L)	Cu (µg/L)	Zn (µg/L)	As (µg/L)	Mo (µg/L)	Cd (µg/L)	Pb (µg/L)	U (µg/L)
Site 1 15/12/2023	1.87	<1.0	1.58	1.19	0.034	0.484	0.688	14.7	1.49	11.9	<0.050	0.530	4.03
Site 1 22/12/2023	1.77	<1.0	1.75	1.19	0.041	0.483	0.681	4.58	1.50	12.1	<0.050	0.600	4.08
Site 2 15/12/2023	1.77	<1.0	1.59	0.172	0.046	0.378	0.345	8.90	1.51	11.9	<0.050	0.893	3.99
Site 2 22/12/2023	1.81	<1.0	1.48	<1.0	0.039	0.300	0.580	3.48	1.52	11.8	<0.050	0.628	4.04

Table 2 DGT-labile metal concentrations in the seawater of the two sites

Site	Cr (µg/L)	Fe (µg/L)	Ni (µg/L)	Cu (µg/L)	Zn (µg/L)	As (µg/L)	Cd (µg/L)	Pb (µg/L)	Hg (ng/L)
Site 1	0.09	0.39	0.80	1.33	4.58	0.02	0.01	0.63	29
Site 2	0.09	0.47	0.10	0.01	0.28	0.01	0.01	0.05	0.30

Table 3 Total metal concentrations in the sediments sampled in the two sampling sites

Site	Be (mg/kg)	V (mg/kg)	Cr (mg/kg)	Mn (mg/kg)	Fe (%)	Co (mg/kg)	Ni (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	As (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Hg (mg/kg)
Site 1	1.79	102	162	845	3.5	15.9	109	86.6	192	11.1	0.253	73.7	0.704
Site 2	1.20	60.2	80.9	905	2.0	9.30	48.6	37.3	105	9.14	0.216	59.1	0.357

Analyzing the results reported in **Fig. 3**, it is noteworthy that seawater samples consistently exhibited the highest toxicity for algae, whereas the effect on crustaceans was not relevant. Only at one sampling point (Site 1) was an effect percentage of 30% recorded, indicating moderate contamination. Concerning sediment elutriates, very low toxicity was observed for both algae and crustaceans. The results indicate a notable disparity in toxicity levels between seawater and sediment elutriates, as well as differential impacts on algae and crustaceans. Seawater samples consistently demonstrated high toxicity levels for algae, suggesting a potentially harmful environment for these organisms. This could be indicative of pollutants or contaminants present in the seawater that adversely affect algal growth and health. The high toxicity levels in seawater samples may raise concerns about the overall health of marine ecosystems in the sampled area, as algae play a crucial role in marine food webs and ecosystem functioning. On the other hand, the effect of seawater samples on crustaceans was not found to be relevant. This could suggest either a lower sensitivity of crustaceans to the detected pollutants or a different mechanism of toxicity compared to algae. Further investigation into the specific contaminants present in seawater and their effects on different marine organisms could provide valuable insights into this disparity.

In contrast to seawater samples, sediment elutriates exhibited very low toxicity levels for both algae and crustaceans. This suggests that the sediments may act as a sink for contaminants, reducing their bioavailability and mitigating their potential impact on marine organisms. However, continued monitoring of sediment quality is essential to ensure that contamination levels remain low and do not pose a threat to benthic organisms or the wider marine ecosystem.
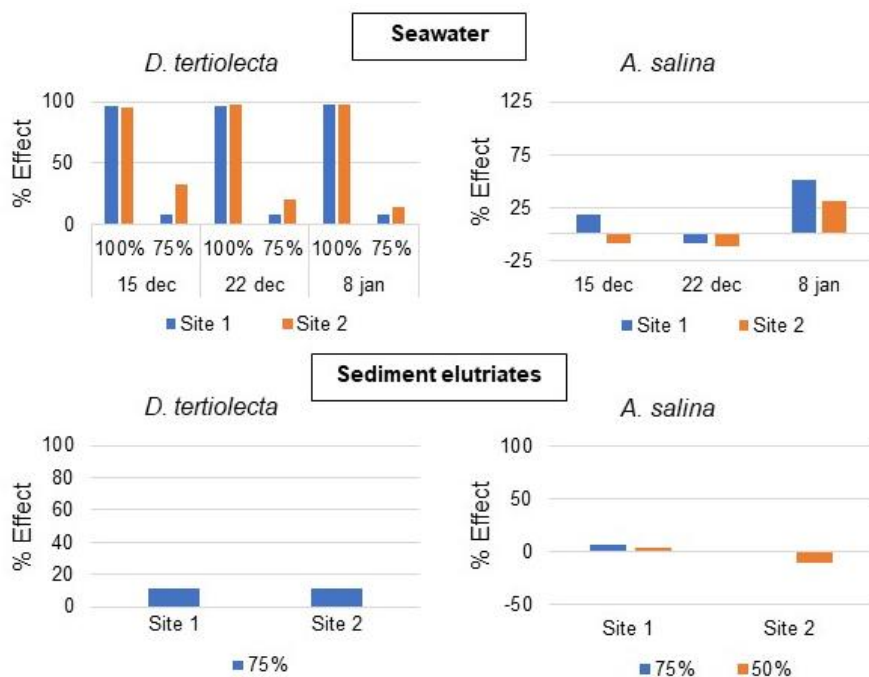

Fig. 3 Ecotoxicological results for seawater and sediment elutriates on *D. tertiolecta* and *A. salina*

The sampling addressed at investigation for fibrous minerals was increased since the first sampling in November 2023 (5 samples) to 9 samples at the end of March 2024. The overall composition (**Table 4**) includes carbonates (calcite and dolomite), metallic minerals (Fe- and Ti oxides), and silicates (antigorite, chlorite, mica, plagioclase, pumpellyite, quartz and tremolite with the habit of cleavage fragments). In the sample sets through the month of November 2023, a first occurrence at site P21 (top western entrance of the harbor) shows asbestos concentrations as high as 260 ± 130 mg/kg. The early winter sampling represents the contribution to the inner harbor deposits from urban streams and did not provide evidence for asbestos occurrence.

The sampling at the end of March 2024 yielded 430 ± 215 mg/kg from the sampling site S2 (Sampierdarena channel) between the wharfs and with possible contribution from the Polcevera Stream that runs across alpine

ophiolites. An unexpected concentration as high as 300 ± 150 mg/kg occurred at site GE_7 (eastern entrance of the harbor, that intercepts the sediments of the Bisagno Stream).

The preliminary data of sediment compositions suggest that the solid stream contribution is representative of the crossed lithologies. A direct correlation arises e.g for most of the phases in samples S1 to S5, where the presence of calcite and micas has a correspondence with the marly-limestone formations drained. The occurrence of silicate and metallic minerals is only in part connected with the direct drainage from overlying formations: antigorite $\pm$ chlorite and Fe – oxide are phases derived from the Mt. Beigua ophiolitic formation, as well as the quite low concentrations of chrysotile and tremolite cleavage fragments. Chlorite + pumpellyite $\pm$ plagioclase are possibly derived from the low grade metabasalts outcropping inland of Sestri Ponente. The dolomite evidenced through all sampling could have either an origin in the Triassic dolostone north of Sestri Ponente, or in dispersion from bulk goods or from submarine weathering of walls.

Finally, Ti-oxide is a ubiquitous occurrence, particularly in the sampling of end March 2024. The spread of this phase should be considered in the follow up of measurements, as TiO2 has commonly a needle like aspect ratio, possible health implications and is a natural occurrence in most of the geological formations excavated by the "Terzo Valico" Railway is common [40]. However also an anthropic input cannot be excluded.

Table 4 Mineral composition by SEM-EDS, sampling sites with reference to Fig. 1, and asbestos concentration for the Port of Genoa case study. Minerals are listed in order of decreasing abundance. In samples where asbestos fibers were below the detection limit (<100 mg/kg) we indicated "not found"

Sampling site		P1	P6	P15	P18	P21
Month	Asbestos	not found	not found	not found	not found	260 ± 130 mg/kg
November 2023		quartz, chlorite, muscovite, dolomite, antigorite, tremolite (cleavage fragments)	chlorite, quartz, dolomite, muscovite, plagioclase, Fe-oxides, antigorite	quartz, dolomite, chlorite, muscovite, antigorite, tremolite (cleavage fragments)	quartz, calcite, chlorite, muscovite, plagioclase, antigorite, tremolite (cleavage fragments)	chlorite, antigorite, quartz, calcite, muscovite, dolomite, tremolite (cleavage fragments)

Sampling site		Site 1	Site 2	Site 3	Site 4
Month	Asbestos	not found	not found	not found	not found
January 24		quartz, calcite, chlorite, muscovite, antigorite, plagioclase, Ti-oxide, Fe-oxides, pyrite	quartz, calcite, dolomite, chlorite, muscovite, antigorite, plagioclase, epidote, Ti-oxide, Fe-oxides, pyrite	quartz, calcite, dolomite, chlorite, muscovite, plagioclase, antigorite, tremolite (cleavage fragments), Ti-oxide, Fe-oxides	quartz, calcite, chlorite, muscovite, plagioclase, antigorite, tremolite (cleavage fragments), Ti-oxide, Fe-oxides

Sampling site		S1	S2	S3	S4	S5	5	GE_S	GE_7	15
Month	Asbestos	not found	430 ± 215 mg/kg	not found	not found	not found	not found	not found	300 ± 150 mg/kg	not found
March 2024		chlorite, mica, quartz, calcite, antigorite, Ti-oxide, tremolite (cleavage fragments)	mica, quartz, Ti-oxide, calcite, chlorite	calcite, chlorite, mica, quartz, antigorite, calcite	calcite, quartz, pumpellyite, plagioclase, chlorite, antigorite, Fe-oxides, tremolite (cleavage fragments)	calcite, quartz, chlorite, dolomite, antigorite, mica, plagioclase, Ti-oxide, tremolite (cleavage fragments)	quartz, mica, antigorite, calcite, chlorite, dolomite	mica, plagioclase, dolomite, calcite, quartz, chlorite	calcite, quartz, antigorite, plagioclase, dolomite, chlorite, Ti-oxide, tremolite (cleavage fragments)	chlorite, calcite, quartz, Ti-oxide, antigorite, tremolite (cleavage fragments)

6. Conclusions

The proposed measurement and sampling protocol proved suitable for highlighting the differences that are present within the Port of Genoa even after just one application. Further campaigns are planned as part of the RETURN project and will allow the instrument positioning system to be refined to make it more efficient and to obtain additional information on the marine port environment so as to be able to explain in detail the differences found. As for sediment composition, most of the samples directly correlate with the geology of formations drained by streams of first and second order. However, the mineral diversity increases through sampling, and can be correlated with a seasonal meteorological trend characterized by several heavy rain and flood episodes during late winter that possibly dispersed the minerals originated in the western part of the harbor, towards its eastern part, this accounting for the asbestos content of 300 ± 150 mg/kg found downstream of the marly – limestone Antola formation. The results of the analyzes on organic pollutants ae in progress and they will allow us to obtain further information which give information on the contamination origin and confirm whether or not what was found for metals in the water and sediments.

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