



Black Sea SIERRA

Project 101124670 – Black Sea SIERRA
Handbook on Marine Pollution Assessment Methodology and Sources

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This handbook is a comprehensive synthesis of the findings obtained within the scope of the Black Sea SIERRA Project. Peer-reviewed articles produced within the project (Tunca et al., 2026; Burduhos Nergis et al., 2025) were also utilized in the creation of some raw data, specific datasets, ecotoxicological models, and visual representations included in the document. These studies, which form the foundation of the project, have been appropriately cited throughout the text, and the inclusion of these data in the handbook is an inevitable necessity for the integrity of the project.

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1. Introduction and Project Context

The Black Sea SIERRA Project aims to reveal the different pollution loads on the Black Sea by sampling the north, west, and south of the Black Sea. Within this framework, it is designed to investigate the pollution pressure created on the ecosystem by civil and recently highly significant military conflicts by taking water, sediment, and biota samples from the sea, river mouths, and lagoons. Sharing the results with decision-makers and providing necessary trainings are also included within the scope of the project. The aims and objectives of the project can be grouped under the following headings.

1.1. The Objective of the Black Sea SIERRA Project

1.1.1. Identification of pollution elements and pollution-intensive regions (hotspots)

In this context, the primary objective is mainly to reveal the elements responsible for the pollution load. The determination of the current status of numerous toxic agents such as heavy metals, nutrient pollution, microplastic in the Black Sea is also among the objectives of the project. Along with these, the identification of the regions where these elements are concentrated, namely hotspots, also holds great importance.

1.1.2. Samplings belonging to different compartments of the ecosystem

In order to reveal the current pollution status of the ecosystem with a holistic approach, both abiotic (water and sediment) and biotic (plant, mollusk, and fish for elements) compartments are included in the sampling scope. In order to reveal the transfer from water and sediment to living tissues, namely the bioaccumulation profile, sampling from organisms at different trophic levels was targeted. This approach allows the transport and accumulation profiles of pollutants (elements) along the food chain to be evaluated as a whole.

1.1.3. Methodological harmonization among countries

Determining a common field sampling and laboratory methodology among the countries participating in the project was targeted. With this target, minimizing the differences that may arise due to different methodologies among the countries was aimed. Additionally, with these standardized protocols, it is

also aimed that the findings obtained across the Black Sea basin are fully comparable with each other.

1.1.4. Innovative technologies and remediation

Cleaning an already polluted environment is a highly difficult process that creates an intense stress load for the ecosystem. Additionally, its success rate is also low. With an innovative approach, developing a prototype device for remediation trials within the scope of the project was targeted. With this device, conducting remediation trials at the experimental level for specific pollutant types on the coasts of the Black Sea possessing different characteristics was targeted. Additionally, the identification of the best available techniques (BAT) to intervene in the current pollution status in the Black Sea is also among the aims of the project.

1.1.5. Sustainable management, capacity building, and cross-border cooperation

One of the most important aims of the project is not only producing scientific results but also presenting results that will ensure competent authorities and decision-makers take the right actions. Moreover, it is to realize this with a multinational integration between European Union member countries (Italy, Bulgaria, and Romania) and non-European Union member countries (Türkiye and Ukraine). This joint working protocol starting to build a common conservation culture in the Black Sea on the axis of science and policy is also among the most important objectives of the project.

1.2. Brief results of some recent studies indicating the pollution pressure on the Black Sea

In the study conducted by Önel and colleagues in 2025 in 8 cities bordering the Black Sea in Türkiye, they examined element accumulation levels in *Mytilus galloprovincialis* and *Rapana venosa*. The average quantity ranking of the elements was found as $\text{Fe} > \text{Al} > \text{Zn} > \text{Cu} > \text{As} > \text{Mn} > \text{B} > \text{Cd} > \text{Se} > \text{Ni} > \text{Cr} > \text{Pb} > \text{Co} > \text{Hg}$. The average quantities, on the other hand, were determined respectively as $114.8 > 79.7 > 39.49 > 9.75 > 5.74 > 3.22 > 2.97 > 2.74 > 0.72 > 0.40 > 0.24 > 0.22 > 0.11 > 0.015 \mu\text{g g}^{-1}$. While the MPI in *Mytilus galloprovincialis* was determined as 1.25, the MPI in *Rapana venosa* was determined as 2.51. Additionally, the THQ value for Cd was determined to be above 1, and its consumption was reported to be inappropriate in terms of public health (Onel et al., 2025).

In the study conducted by Terzi and colleagues on the Black Sea coasts in Türkiye in 2025, they conducted microplastic research over 29 rivers. Accordingly, the microplastic quantity was determined as $9.63 \pm 1.27 \text{ MP m}^{-3}$ and its range as 1.03 to 29.8 MP m^{-3} . The annual discharge capacity is 1.49×10^{11} . The most frequently encountered microplastics were Polyethylene terephthalate $59.3\% \pm 2.66\%$, polyethylene $20.8\% \pm 2.04\%$, and polypropylene $14.1\% \pm 2.36\%$ (Terzi et al., 2025).

Akçay and Kobya investigated some radionuclide concentrations in brown algae on the eastern coasts of the Black Sea Türkiye. Accordingly, the results were determined as follows: ^{226}Ra 11.2, ^{232}Th 7.61, ^{137}Cs 2.74, and ^{40}K 690.7 Bq/kg. The annual effective dose, on the other hand, was calculated as 1.37–18.83 $\mu\text{Sv/y}$. Accordingly, the results do not carry any risk potential (Akçay and Kobya, 2024).

In their study published in 2025, Dağtekin and colleagues investigated the quantity of benthic litter dispersed in the sediment. The study was conducted in the eastern section of the Türkiye coasts of the Black Sea. According to the results, the average litter density is $48,744.69 \pm 7543.02 \text{ items/km}^2$ and $151.35 \pm 14.04 \text{ kg/km}^2$. Plastics constituted more than 50% of the litters, and bags (36.4%) and bottles (18.97%) were predominant (Dağtekin et al., 2025).

In the study conducted by Pramatarov and colleagues on 4 different bivalvia species on the Black Sea coasts of Bulgaria, they examined both microplastics and, together with microplastics, the elements Cd, Cu, Cr, Ni, Pb, Zn, and Fe. They showed that 94.7% of the microplastics encountered in the species consisted of pellets, and specifically Pb significantly triggered protein oxidation and glutathione levels (Pramatarov et al., 2025).

In the study conducted by Bobchev and colleagues on the Black Sea coasts of Bulgaria in 2024, microplastic quantities and types in the sediment were investigated. The results are as follows: $464.44 \pm 272.50 \text{ MP/kg}$ in beach samples, $825.93 \pm 396.30 \text{ MP/kg}$ in seabed samples. In coastal areas without anthropogenic activity: it is $84.44 \pm 147.57 \text{ MP/kg}$ in beach samples, $550.62 \pm 245.71 \text{ MP/kg}$ in seabed samples. It is thought that the pollution created by the litter on the seabed is the source of the microplastics (Bobchev et al., 2024).

In the 2024 study by Nemirovskaya and colleagues, aliphatic and PAHs were studied in suspended particulate matter in water and in seabed sediment. In the offshore surface waters of the Gelendzhik and Golubaya bays in Russia, the average aliphatic hydrocarbon was determined as $\approx 20 \mu\text{g/L}$ and PAH as $\approx 130 \text{ ng/L}$ (Nemirovskaya et al., 2024).

In their study dated 2024, Zakhariikhina and colleagues studied trace element levels and rare earth element levels in marine macroalgae. In the study, in the study at the mouth of the Sochi River in Russia, a new index named "Total Pollution Index (Zc)" was developed. Additionally, it was determined that the ele-

ment accumulation profile in the macroalgae in the region, particularly the Rare Earth Elements, directly reflects the regional geochemical profile transported from the Sochi River valley (Zakharikhina et al., 2024).

In the study by Nemirovskaya and colleagues in 2025, the status of fuel oil pollution originating from the spill of the Volgoneft-212 and Volgoneft-239 tankers was investigated. The composition of chloroformed bitumen, aliphatic hydrocarbons, and PAH was compared with the compositions on the beaches, in the suspension of bottom waters, and in the bottom sediment. The results show that the vicinity of Anapa is the most critical region in terms of pollution (Nemirovskaya et al., 2025).

In the study conducted by Shoman and colleagues in 2026 on the Sevastopol coasts of the Black Sea, the toxic effects of zinc oxide (ZnO) and copper oxide (CuO) nanoparticles, their effects on the natural picophytoplankton community of the Black Sea, were examined. According to the results, CuO nanoparticles suppressed the photosynthetic activity of the algae in a dose-dependent manner ($EC_{50} = 18.3 \mu\text{g/L}$). Additionally, it changed the community structure in favor of picoeukaryotes at a rate of 90–98% at the $20 \mu\text{g/L}$ concentration level. As for ZnO nanoparticles, it was observed that they selectively inhibited the growth of phycoerythrin-containing cyanobacteria in the region (Shoman et al., 2026).

In their 6-year multi-matrix study published in 2025, Oros and colleagues reported the quantities of Cd, Pb, Ni, Cr, Cu, and Co in the surface waters of the Romanian Black Sea. The highest average concentration was measured in Cu with $10.80 \mu\text{g/L}$. Legal pollution limits were exceeded at a rate of 13% for Pb, 10% for Cd, 7% for Cu, and 4% for Ni. In 277 analyzed sediment samples, while the average Ni concentration ($40.20 \mu\text{g/g}$) surpassed the pollution threshold ($ERL = 20.9 \mu\text{g/g}$) in more than 75% of the samples; Cu and Pb were determined to have exceeded this threshold at rates of 21% and 4%, respectively (Oros et al., 2025).

In the study by Ciucă and colleagues in 2025, the initial assessment of the quantity, color, shape, and size of microplastics in the gills and digestive tract of the *Engraulis encrasicolus* (European anchovy) species collected from different locations along the Romanian coast of the Black Sea was conducted. Plastic was detected in the digestive system and gills of 88% of the anchovies. In the examinations, 98.5% of the particles were classified as microplastics, and 1.5% as mesoplastics. An average of 3.03 plastics per individual was detected. Additionally, the most common pollutant type in the study was determined as transparent fibers in the size range of $330 \mu\text{m}$ to 1 mm (Ciucă et al., 2025).

In the joint study by Barbulescu and Barbeș dated 2026, water quality was studied from a microbial perspective (*E. coli* and Intestinal Enterococci) at 20 different

beaches on the Romanian Black Sea coasts. While European Union water quality standards were met in the majority of the locations, bacterial contamination was detected in Năvodari, Vadu, and Mamaia (Bărbulescu and Barbeș, 2026).

In the study conducted by Damir and colleagues on the Romanian Black Sea coasts in 2026, they studied the presence and trophic transfer of heavy metals, POPs (OCPs and PCBs), and PAHs in the food chain. According to the research results, while the trophic transfer of heavy metals showed variability, organic pollutants (especially PCBs) exhibited a more pronounced biomagnification in benthic prey-predator relationships. Generally, it was determined within the scope of the study that organic pollutants exhibit stronger biomagnification in the food web compared to heavy metals (Damir et al., 2026).

In the study conducted by Snigirova and colleagues on the Black Sea Coasts of Ukraine in 2024, microplastic density and its relationship with ichthyoplankton were examined. According to the obtained results, it was revealed that 75% of the microplastics are in the form of fibers (predominantly black and red), and 25% are in fragment form, and they consist of polyethylene, polyester, polyurethane, and polypropylene polymers. Additionally, they were detected at a density of 136 ± 74 in the neuston layer, and 46 ± 30 particles/m³ in the hyponeuston layer (Snigirova et al., 2024).

In the 2025-dated study by Kvach and colleagues, the impact of the war between Russia and Ukraine on the biodiversity in the region was studied. The sudden and high-volume freshwater impact mixing into the Black Sea with the detonation of the Kakhovka dam was dwelled upon in detail. In the study, it is stated that military activities (oil spills, explosions, fires) and the destruction of the Kakhovka Dam taking place on June 6, 2023, are the greatest ecological disaster leading to habitat destruction and population loss. Against this, it was emphasized that human-induced pressures decreasing due to the war, such as marine traffic, fishing, and tourism, created a temporary recovery on the ecosystem (Kvach et al., 2025).

In the study conducted by Boitsov et al., (2026) on the Black Sea coasts of Bulgaria, some of both organic and inorganic toxic agents were studied. This study aimed to investigate the effects of the ongoing war between Ukraine and Russia on the Bulgarian coasts as well. In the study published in 2026, 32 surface sediments and 4 sediment core samples were used. In the study, along with metals, PAHs, PCBs, PBDEs, DDT, HCHs, HCB, and PFAS were also studied. According to the results, pollutant levels in most locations are low. However, high pollution was detected in the Varna-Burgas ports and the mouth of the Kamchia River. PBDEs and PFASs were reported for the first time in the region. While an increase in PAHs was observed in core samples, an anthropogenic effect was not detected in metals (Boitsov et al., 2026).

The study conducted by Komorin and colleagues on the Odessa coasts of Ukraine was published in 2026. The study covers the years between 2019-2025. In the period under the effect of war from 2023-2025, a decrease in dissolved oxygen minimum levels and sharp nutrient peaks were detected in the study. Additionally, intense heavy metal and organic pollutant fluctuations were also determined along the river-sea line. Oil residues washing ashore in 2025 were analyzed, and it was revealed within the scope of the study that they spread from multiple sources. War conditions created hotspots in the region that clustered on the near coastline, short-term but shaking the ecosystem, rather than a general and homogeneous pollution (Komorin et al., 2026).

In the 2025-dated study by Jiang and colleagues, different parameters were studied in the Dnieper-Bug Estuary and Odessa coastal waters in the period after the destruction of the Kakhovka Dam, and the short-term pressure it created on the ecosystem was tracked. Data were collected with both in situ measurements and satellite photographs. According to the result of this study, water quality dramatically deteriorated, total suspended solids and Chlorophyll-a densities in the water rose to more than 50 times their average levels, and the Secchi disk distance decreased substantially. This situation triggered severe algal blooms continuing for 20 days (Jiang et al., 2025).

1.3. Purpose of the Handbook

This handbook aims to strengthen the collaboration of science and policy-makers as well as understanding the current pollution status across the region and investigating the impact of the war on the region. The primary objectives can be listed as follows:

- **Determination of the current pollution status:** To establish the general pollution status of the study region holistically through abiotic and biotic compartments.
- **The Ecological dimension of the war:** To reveal with its dimensions the pollution stress that the Russia-Ukraine war has started to create on the region.
- **Testing the region-specific remediation (cleaning/reclamation) solution:** To simulate whether the quantity of pollutants can be reduced specifically for the region with the prototype developed within the scope of the project.
- **Methodological Standardization:** To develop a common working methodology throughout the Black Sea Basin.

- **Regional policies produced upon evidence:** To establish a scientific infrastructure for decision-makers.
- **Capacity building and joint action:** To develop a culture of acting jointly by establishing cross-border cooperation.

1.4. Methodology of the Handbook's Preparation

The preparation methodology of this handbook relies on addressing the reports generated within the scope of the project with a multi-layered levels approach of a whole, instead of taking them as pieces separate from each other. The establishment of the "common methodology" that unites the 5 countries in a common consensus lies at the foundation of these layers. In the handbook, the first layer of its methodology is located just above the foundation formed by this common methodology. The abiotic (water and sediment) compartments collected synchronously within the scope of the project from the North, West, and South of the Black Sea Basin constitute this first layer. Additionally, this first layer reveals temporal differences as much as spatial distribution. Along with this, historical comparison via sediment core samples is important in terms of this layer in the handbook. The biotic compartment constitutes the second layer of the methodology. As one of the important features of this layer, the bioaccumulation in different tissues of organisms at different trophic levels having been demonstrated in the handbook. The third layer in the handbook encompasses the investigation of whether the bioaccumulation in organisms consumed by humans is dangerous in terms of human health. The fourth layer, on the other hand, is dedicated to the management of these detected risks. In this context, remediation methods customized to the Black Sea Basin were determined through the literature, and the innovative SIERRA prototype of the project stood out in the handbook.

While creating all these layers, simultaneously simplifying the complex ecotoxicological methods, evaluation methods, and multiple statistical approaches inherently contained by the nature of the work packages, and transferring them with a simple structure, was adopted fundamentally in the handbook. The reason for this is the necessity of the Handbook to appeal to all segments of society, especially lawmakers. Along with all these, this handbook was not limited only to the information produced by this project. The new findings obtained within the scope of the project were blended with past scientific findings in necessary places, and a document integrated with the literature was created. Thus, the similarities and differences of the new findings with the past findings were also revealed.

2. Consortium Methodological Guide

Agreeing on a common methodology carries great importance for the study that 5 different countries will conduct in such a vast basin. In parallel with this; samplings in abiotic (water and sediment) and biotic (bioindicator species at different trophic levels) compartments were conducted synchronously on close dates. Thus, the entire basin was integrated on the scale of a single field study. Through this methodology, examining the North, West, and South Black Sea coasts as a whole in a spatial and temporal context was made possible. Subsequently, the samples brought to the laboratory were analyzed with standardized methods again within the scope of the common methodology, and again standardized quality control methods (QA/QC) and intervals were applied.

All data obtained from the work packages led by different countries were passed through a multidisciplinary filter in the reporting process. These integrated data obtained with the common methodology and different research teams were blended with the historical records in the sediment cores and the literature history of the basin, and a holistic basin profile was formed. In this way, just as methodological consistency was ensured, the step-by-step progression of the project on scientific foundations was guaranteed. In the final stage, on the other hand, it was targeted that the jointly produced detailed scientific contents are clearly comprehensible by regional authorities through this handbook and easily convertible into action.

2.1. Project-Wide Sampling Strategies: Common field protocols established for water, sediment, and biota matrices across the North, West, and South Black Sea regions

2.1.1. Sampling strategy

The Black Sea SIERRA consortium operates under a common sampling framework in the Black Sea Basin. In this comprehensive study conducted jointly by institutions from different countries, ensuring field integrity was of major importance. In this context, all partners are required to comply with the same ISO standards (for example EN ISO 5667-1:2020; ISO 5667-3:2024; ISO 5667-12:2017; ISO 5667-19:2004). All samples must meet the representativeness, chemical stability, and physical integrity criteria from collection to analysis, within the jointly defined procedure. In this context, polyethylene, polypropylene, or glass materials were preferred depending on the sample type. The field campaign was repeated 4 times, 2 in the wet season and 2 in the dry

season (Figure 1 and Annex Figure 1-5). It was carried out extensively along the coasts of Türkiye, Bulgaria, Romania, and Ukraine, at major river mouths (the Danube, Dniester, Southern Bug, Dnieper, Kızılırmak), in lagoons, and along the Black Sea coast.

Within the Black Sea SIERRA Project, 2 key components carrying pollutant footprints were studied in particular. A) persistent pollutants (heavy metals, total petroleum hydrocarbons, microplastics) B) standard set of physicochemical parameters — pH, salinity, temperature, dissolved oxygen, chlorophyll- α , dissolved inorganic carbon, dissolved inorganic nitrogen species (NO_3^- , NO_2^- , NH_4^+) and dissolved reactive phosphate. This comprehensive research methodology was designed to reveal the current pollution status and the pollution pressure generated by war.

Within the field campaigns, staff protection was unquestionably the highest priority. Each team member had the authority to stop work when they judged conditions to be unsafe due to sea conditions, chemical exposure, or low visibility. Although no such situation occurred, the field coordinator had the authority to require the use of life jackets and safety lines whenever deemed necessary. For all chemicals used in the field, container integrity was checked before the field campaign, and type and concentration information was labelled. Multiparameter meters were used for in situ measurements. Before the field campaign, all instrument calibrations were checked, and calibrations were performed when necessary.

Because of the large number of samples and the field work carried out by different researchers, a correct labelling strategy was of great importance for the project to yield sound results (Annex Table 1-2). In this context, an alphanumeric coding system (XYDDMMYYYYAA) was used. The first character denotes the matrix type (1 = water, 2 = sediment, 3 = biota), the second character the water body category (1 = tributary, 2 = lagoon, 3 = coastal area), the following eight digits the sampling date, and the last two digits the sequential sample number within that day. GPS coordinates, sampling depth, time, and matrix type were recorded for each sampling event.

2.1.2. Matrix-specific protocols

Samples taken from each station were placed in glass containers and plastic containers. Water samples collected for chlorophyll- α were filled into 5 L light-proof plastic canisters. Samples were transported to the laboratory as quickly as possible (+4 °C).

Bottom sediments collected according to ISO 5667-2:1991 and ISO 5667-12:2017 were taken from the upper 5 cm layer, homogenized, and subjected to dry sieving through a 63 μm sieve. The resulting fraction was divided in two with a spatula. The first of these fractions was transferred to a plastic container for trace metal analyses, while the other was wrapped in aluminum foil for microplastic analyses. At least 100–200 g of sample from the <63- μm fraction is required for each analytical study.

$\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and %C and %N ratios were used as indicators of trophic history. In addition, depth-related trace element profiles were also investigated. In this context sediment cores were collected from Balık Lagoon in Türkiye. Two replicate cores located several tens of metres apart were taken from each site, and a total of four cores were obtained. Each core was obtained by driving 60–70 cm long and 8–10 cm diameter PVC tubes into the sediment by hand or with the aid of a sledgehammer, and obtaining at least 30–40 cm of sediment.

- A (compression height): Inside (core height from the top of the core to the sediment surface inside the core) minus Outside (core height from the top of the core to the sediment surface outside the core);
- B (core penetration): Tube length – Inside;
- Compression % = $A \times 100 / B$.

Cores showing more than 20% compaction were not accepted and resampled. Accepted cores were sealed at both ends, assigned a unique identification number, positioned by GPS, and transported in an upright position throughout transport.

2.1.3. Preservation and transport

All samples were kept at 2–4°C in insulated cooling boxes during transport. In the laboratory, they were stored in deep freezers at –20°C in accordance with ISO 5667-3:2024. Sediment samples were stored in aluminium foil bags or zip-lock plastic bags; water samples were kept in sealed glass or plastic containers depending on the target analyte. Sediment cores were kept upright throughout transport and were frozen upright immediately after arrival at the laboratory. This practice is essential to prevent interlayer mixing and to suppress microbial activity after sampling.



Figure 1. Study area overview map.

2.2. Standard Laboratory Analytical Procedures: Sample preparation processes.

2.2.1. Sample preservation, reagents and transport

Care was taken that all reagents used in the field studies comply with analytical reagent quality (ISO 5667-3:2024). Additionally, reagents were labeled, their shelf lives were not exceeded, and they were stored separately from sample containers between field studies. Immediately after the preservative was added, each sample was labeled and field blanks were used for periodic verification. In water samples, the analytical importance of the container material was considered. Metals were acidified with HNO_3 to $\text{pH} < 2$ in polyethylene or Teflon containers, chlorophyll- α filters were stored frozen at -20°C in the dark (Annex Table 3). Sediment preservation varies according to the target parameter.

2.2.2. Physicochemical parameters

pH was determined potentiometrically (EN ISO 10523:2012); while dissolved oxygen was determined by Winkler titration (EN 25813:2004). Oxygen saturation, on the other hand, was calculated from the equilibrium value at ambient temperature and salinity. Nutrient species were measured spectrophotometrically. The values are as follows: N-NH_4 630 nm, N-NO_2 540 nm, N-NO_3 , 540 nm after Cu-

Cd column reduction or alternatively with sodium salicylate 415 nm SR ISO 7890-3:2000; while total nitrogen was determined with Cu-Cd reduction 540 nm following alkaline persulfate oxidation. Phosphate was measured as the reduced antimony-phosphomolybdate complex 885 nm. For total phosphorus, pre-persulfate mineralization was applied before the same colorimetric reading. Dissolved silicon was determined as the reduced silicomolybdate complex 810 nm with the addition of oxalic acid to suppress phosphate interference. TOC (Total Organic Carbon) was measured by catalytic oxidation at 680°C with non-dispersive infrared (NDIR) detection after carbonate removal, TSM (Total Suspended Matter) was determined gravimetrically on pre-dried 0.7 µm glass fiber filters. In-situ salinity, conductivity, TDS (Total Dissolved Solids) and pH were recorded simultaneously at sea, lagoon and river stations with a portable multiparameter meter whose calibration was checked/performed prior to the field-work (Nedelcu et al. 2026).

2.2.3. Microplastics

Sediment samples obtained in the field studies were washed with deionized water, dried at 60°C for 3 days and passed through a 5 mm sieve. One-kilogram aliquots were subjected to ZnCl₂ density separation (1.5 L, 2 min magnetic stirring, overnight settling) and this process was repeated three times; the supernatant was collected with the help of a 20 µm metal sieve using Milli-Q water. After being dried at 60°C for 24 hours, the organic matter was digested with H₂O₂. The extract was filtered with the help of a Whatman filter and dried in glass Petri dishes at 60°C for 24 hours. The particles were examined under a microscope with image analysis (Alexandrova et al. 2025). Subsequently, they were classified according to color, shape (pellet, foam, fragment, fiber, film) and their sizes across five different classes (from <1000 to 4001–5000 µm). FTIR spectroscopy was applied for chemical identification and three representative pieces were analyzed for each visual group.

2.2.4. Heavy metals in water and sediment

River and lagoon water (10 mL acidified with 0.1 mL of 65% HNO₃) was analyzed by ICP-MS according to EPA 200.8 (TDS < 0.1%); while seawater (0.1–0.2 mL diluted to 10 mL in 1% suprapur HNO₃) was analyzed in accordance with the EPA 6020 (TDS up to 0.2%) procedure. In both methods, triplicate measurements were made using Agilent 27-element calibration standards (8500-6940 2A and 8500-6940 Hg), CRM UME CRM 1201 and Agilent 5188-6525 internal

standard; LOD (Limit of Detection) and LOQ (Limit of Quantification) were reported for each element (Annex Table 4).

Algal samples were prepared using a modified EPA 200.2 method, while fish and mollusk tissues were processed according to EPA 200.3. Algal samples (0.2–0.5 g) were digested in Teflon vessels in a microwave digestion system at 180 °C using concentrated nitric acid (%65 HNO₃). Fish and mollusk tissue samples (1.5 g) were digested at 200 °C in a microwave digestion system using a mixture of concentrated nitric acid (%65 HNO₃) and hydrogen peroxide (%30 H₂O₂). After the digestion process was completed, all solutions were brought to a final volume of 50 mL with ultrapure water and prepared for elemental analyses.

Sediment samples were dried at 50–55°C for ≥72 hours, crushed in an agate mortar and sieved at <63 µm size. 0.5–1 g subsamples were digested in aqua regia (HCl:HNO₃, 3:1; 9 mL 30% HCl + 3 mL 65% HNO₃) in closed Teflon containers by microwave at 200°C for 15 + 15 minutes (ISO 11466, ISO 12914). Subsequently, they were transferred to 50 mL polypropylene tubes and diluted with ultra-pure water. In calibration, the Agilent 8500-6940-2A-Hg standard and the 5188-6525 internal standard were used, and quality control was checked in triplicate against CRM NIST 2702 (13 certified elements: As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, V, Zn). LOD, recovery, %RSD and CRM results were tabulated. Twenty additional elements (Li, Na, K, Rb, Cs, Be, Mg, Ca, Sr, Ba, Ti, Mo, Ag, B, Al, Si, P, Se, Tl, U) were measured for informational purposes without performing recovery calculation.

2.2.5. Sediment core processing and geochemical analysis

Frozen cores were bisected longitudinally, and photographed for stratigraphic documentation. Later, they were separated into 1 cm slices with a cleaned serrated knife. Each slice was divided into two; one half was processed at the University of Palermo for trace metals and stable isotopes, while the other half was archived. For each half slice, the volume was measured by syringe displacement (60 or 100 mL; 1 cc = 1 cm³), the wet weight by emptying into pre-weighed Petri dishes, while the dry weight was determined after being dried at 60°C until constant weight (24–48 hours) and cooled in a desiccator for ≥1 hour. Subsequently, after shells, stones and debris were removed, they were ground to a fine powder with a mortar. A minimum dry weight of 15 g was standardized for each subsample.

Total phosphorus and heavy metals (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, V, Zn) were determined quantitatively by ICP-OES (Optima 8000, PerkinElmer; USEPA 6010C/2007) following microwave acid digestion in an HNO₃:H-

F:H₂O₂:Milli-Q (6:2:0.4:1.6) mixture using the Mars 5 CEM system (USEPA 3051/2007). In the analysis of elements with high volatility such as As and Hg, a hydride generator was integrated into the ICP-OES device. CRM NIST 2702 was used in quality control. LOQ was measured as 0.003 mg kg⁻¹ dw (dry weight) for most metals, 0.01% for Fe and Al and 0.005% for TP.

Stable isotope ratios and element contents ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, TN, TOC) were determined by an IRMS (Thermo Scientific Delta XP) coupled to an elemental analyzer (Flash EA1112): ~20 mg ground sediment in tin capsules (5x9 mm) for $\delta^{15}\text{N}$ and TN; while ~10 mg ground sediment in silver capsules treated with HCl and dried at 50°C for 30 minutes was used for $\delta^{13}\text{C}$ and TOC. The values were expressed in delta notation (‰) relative to atmospheric N₂ and Pee Dee Belemnite limestone: $\delta\text{X}_{\text{sample}}\text{‰} = ((\text{R}_{\text{sample}}/\text{R}_{\text{standard}}) - 1) \times 1000$. The LOQ was recorded as 0.005% for TN and 0.01% for TOC.

2.3. Statistical Methods and Data Evaluation Methodology

To make the dense dataset obtained within the scope of the Black Sea SIERRA Project comprehensible, processing it with a common methodology as statistical, visual, and evaluation methods has been of utmost importance. In this context, SPSS and R (RStudio) software were utilized for statistical analyses and visualizations. Prior to the correlation analysis, whether the data exhibited a normal distribution was examined using the Shapiro-Wilk test. Upon the data not exhibiting a normal distribution (or the number of samples being small), Spearman correlation analysis was used for the correlation, and non-parametric tests were used for the comparison analysis. In the study, Hierarchical Cluster Analysis was preferred among the clustering analyses (CA heatmap). Z-Score correction was applied to the analysis, Ward's method was used, and Euclidean distance was preferred (Tunca et al. 2026). Additionally, within the scope of the executed project, bioaccumulation and physiological accumulation indices, and human health risk assessment indices were also utilized. Detailed explanations of all these indices can be accessed in the Annex Table 5. The statistical significance of differences in microplastic characteristics (colors, shapes, and polymer types) across study sites was evaluated using one-way permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity indices, employing 9,999 permutations to ensure robust testing (Anderson, 2001).

3. Pollution Sources, Distributions, and the Impact of Armed Conflicts

In this section, river discharges, industrial pressures, urban wastes, and the war between Russia and Ukraine, which are the main pollutant sources shaping the element distribution in the Black Sea basin, are evaluated. Spatial patterns and statistical clusterings on a basin scale are demonstrated, and current data and pre- and post-conflict records are compared. The traces of the war on water and sediment quality, possible risk changes, and their meaning in terms of environmental monitoring are discussed through the obtained results. In addition to this, the literature is also utilized for the better understanding of the effects of the Russia and Ukraine impacts on the Black Sea Basin. Additionally, a risk-based monitoring framework for the detection, monitoring, and reporting of conflict-induced pollution is presented.

3.1. Basin-Wide Pollution Source Analysis: Regional examination and comparison of the impact of river discharges, industrial activities, and urban waste on element distribution in water and sediment

3.1.1. Relationships between regions and sediment elements

The hierarchical clustering analysis applied to determine the heavy metal distribution in the study area and similarities among stations revealed clear groupings on the basis of element and location (Figure 2). When the element-based clustering results are examined, Cd is clearly separated from the other three elements (Cu, Zn and Ni). Cd formed an independent primary group. This separation indicates that the input of Cd within the study area originates from a source different from that of the other metals and/or that it exhibits a distinct geochemical behavior mechanism. Within the other main cluster, Cu and Zn showed the highest similarity, forming a separate sub-group, while Ni was more loosely associated with this pair. This strong clustering between Cu and Zn suggests that these two metals may have entered the region through similar industrial or urban-derived activities. Such co-occurrence is also consistent with the proximity of these metals to similar industrial or urban-derived activities, such as port operations, antifouling coatings, galvanized infrastructure, and surface runoff — sources that are well documented in the literature as principal drivers of Cu-Zn co-occurrence in coastal sediments. While brass alloy (approximately 70% Cu, 30% Zn) is also the principal raw material used in

munitions casings, Cu-Zn co-occurrence alone does not constitute a reliable diagnostic signature of military origin, since it is equally consistent with multiple common anthropogenic sources unrelated to conflict. Attribution to a conflict-related source would require corroborating evidence, such as the co-occurrence of explosive residues (e.g., TNT, RDX) at the same stations, isotopic signatures specific to ammunition-grade alloys, or spatial proximity to documented munitions deposition sites. In the absence of such evidence, the Cu-Zn association observed in this dataset should be interpreted primarily within the framework of conventional industrial and urban pollution sources, while remaining a hypothesis worth testing in future sampling campaigns specifically designed to capture co-located explosive markers.

When location-based clustering and regional distribution are considered, metal accumulation appears to be closely associated with river discharges and coastal morphology.

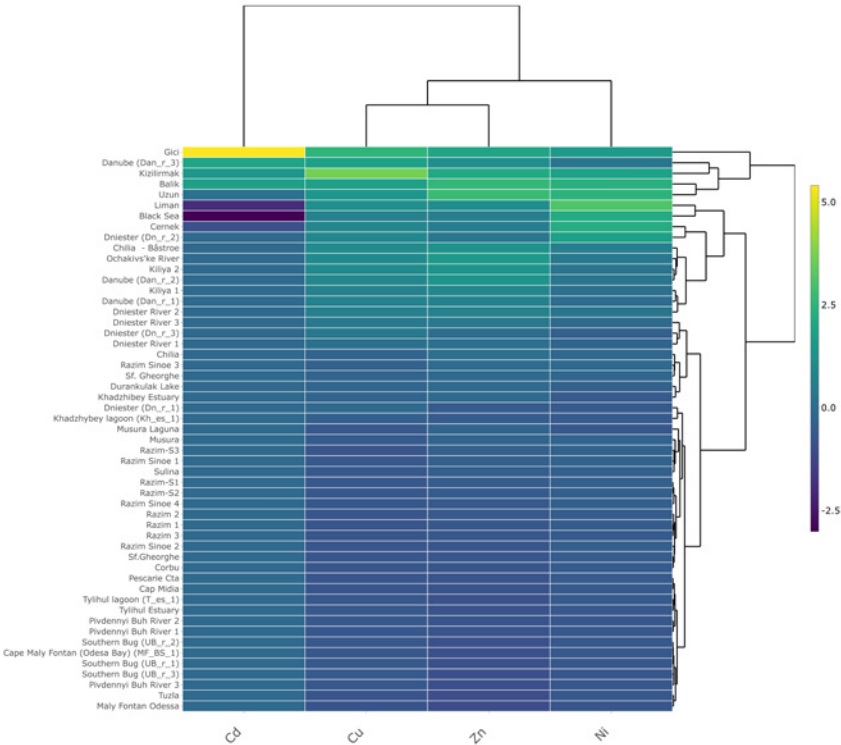


Figure 2. Hierarchical clustering and heatmap array illustrating the geochemical relationships and regional distribution patterns of heavy metals (Z-score normalized) across the investigated coastal and riverine locations.

In the analysis, the Gıdı Lagoon station stood out as the most distinctive location. Gıdı Lagoon has the character of a “hotspot” representing the highest metal accumulation within the entire data set. In particular, this station, which shows an extreme value in terms of Cd concentration ($Z \approx 5$), may be under intense anthropogenic pressure. Such an extreme value may increase the standard deviation of the data set in Z-score normalization. This, as a result, may bring the Cd Z-scores of the other stations close to zero and visually mask the real differences among some stations.

In the secondary group consisting of the Danube (Dan_r_3), Kızılırmak River, Balık Lagoon and Uzun Lagoon locations, it is noteworthy that Cu and Zn are within an above-average accumulation tendency. However, it is also highly striking that these stations exhibit distinct dark tones on the Cd column in the heatmap (negative Z-score). In other words, this group is high in terms of Cu and Zn, while below the data set average in terms of Cd. This Cu/Zn – Cd anti-correlation constitutes additional geochemical evidence supporting the Cd separation in the element dendrogram. It strengthens the thesis that Cd and Cu-Zn at these stations are linked to different source dynamics. Within the group, Kızılırmak River was observed to separate positively from the other stations, particularly in terms of Cu concentration.

In contrast to the pollution pressure in the main channel of the Danube River (Dan_r_3), the stations representing the distal branches of the Danube Delta (Chilia, Sulina, Musura and Sf. Gheorghe) were located in the clusters in the lower part of the heatmap. These clusters are jointly more homogeneous, with low-to-moderate concentrations. This pattern indicates that the metal deposition dynamics across the broader delta area differ and that the pollution pressure diminishes by dilution toward the delta outlets. Similarly, the stations of Ukrainian origin (Dniester [Dn_r_1, 2, 3], Pivdennyi Buh, Tylihul and the Odessa region [Maly Fontan]) showed low metal loads and were included in the homogeneous “background” cluster.

The Razim-Sinoe lagoon complex representing the Romanian coast and the stations near Cap Midia, Corbu, Pescarie Cta formed the widest and most homogeneous sub-cluster in the analysis. The fact that metal concentrations in these areas remained at “background” levels without peaks indicates that basin-derived metal input remained limited.

Liman Lagoon, Black Sea (Kızılırmak Delta coast) and Cernek Lagoon stations stand out with their different profiles. At these locations, Cd values remained at the lowest levels of the data set, whereas Ni concentrations were above average. This separation means that Ni in the region is largely different from Cd sources.

As a general synthesis, Cd appears to be the dominant pollutant among the studied elements within the study area and to concentrate at specific local hotspot points. In terms of overall distribution, apart from the main channel of the Black Sea Basin Danube River or specific points such as Gıcı Lagoon, the analyzed metals show a relatively clean/slightly polluted profile.

3.1.2. Regional comparisons of sediment elements

Accumulation differences were statistically investigated at a 95% significance level and the comparison of countries was made accordingly (Annex Table 6). Bulgaria was excluded from the “comparison of means analysis” due to the scarcity of data.

Copper (Cu) Analysis: It was observed that Cu concentrations in Türkiye were significantly higher than those recorded in the Romania 1 (INCDPM), Romania 2 (MBNAR), and Ukraine locations ($p < 0.001$). Cu levels in Ukraine were found to be significantly higher than both Romanian study areas (INCDPM and MBNAR) ($p < 0.05$) and in the intra-country comparison conducted for Romania, a statistically significant difference could not be detected between the Romania 1 and Romania 2 study areas in terms of Cu accumulation ($p=0.198$).

Nickel (Ni) Analysis: In terms of Ni, Türkiye, as in Cu, was determined as the location with the highest accumulation among all examined areas and the difference is statistically significant ($p < 0.001$). Specifically for Romania, Romania 1 is significantly higher than Romania 2 ($p=0.003$). No statistical difference was observed between the Ukraine data and Romania 1 data ($p=0.984$). Ukraine also exhibited higher values compared to the Romania 2 region, but this difference did not reach statistical significance at the 0.05 level ($p=0.069$).

Lead (Pb) Analysis: The high variation and standard error (40.29) of the Pb concentration in Türkiye caused the differences between Türkiye and other countries to turn out statistically insignificant ($p > 0.05$). While Ukraine possesses significantly higher Pb concentrations compared to both the Romania 1 ($p=0.042$) and Romania 2 ($p < 0.001$) regions, when the Romania datasets were compared, it was determined that the Romania 1 study contained distinctly higher Pb levels than the Romania 2 study ($p=0.044$).

Zinc (Zn) Analysis: When Zn levels are examined, Türkiye, again, showed the highest values compared to all other locations and the difference is large enough to turn out statistically significant. ($p < 0.001$). While Zn levels in Ukraine were significantly higher than the Romania 2 region ($p < 0.001$), a statistical difference was not observed between Ukraine and the Romania 1 region

($p=0.637$). Among the Romania locations, Romania 1 showed significantly higher Zn accumulation than Romania 2 ($p=0.003$).

Iron (Fe) Analysis: Türkiye showed the highest accumulation in terms of Fe as well ($p < 0.001$). Between Ukraine and Romania 1, a significant difference was not observed ($p=0.433$). Similarly, a statistically significant difference in terms of Fe concentrations was not detected between the two Romania studies either ($p=0.194$). However, the Ukraine region exhibited significantly higher Fe concentrations compared to the Romania 2 region ($p=0.001$).

In summary: The statistical comparisons clearly put forward a regional pollution gradient. Accordingly, while the Türkiye coastal region consistently exhibits the highest accumulation for almost all of the analyzed metals (Cu, Ni, Zn, Fe), the Romania 2 (MBNAR) location stands out as the relatively cleanest environment by showing mostly the lowest metal concentrations across the dataset.

3.1.3. Relationships and Clustering of Water Parameters and Elements

The parameter dendrogram has formed two different sub-clusters separating from each other with distinct, almost absolute lines (Figure 3).

Cluster I: This cluster covers Cd, TPH, conductivity, temperature, chlorides, $N-NH_4$, $P-PO_4$, $N-NO_2$, $N-NO_3$. The strong correlation among conductivity, temperature, and chlorides reflects the basic mineralization and salinity gradient of the water masses. Cd forms the closest sub-cluster directly with TPH in the dendrogram. This situation carries the meaning of a strong bond and a common source between Cd and TPH. This strong primary bond with TPH indicates specific anthropogenic sources such as port activities and ship traffic. Cd is connected to the conductivity-temperature-chloride group at an upper level.

Cluster II: This cluster, on the other hand, covers Fe, chlorophyll, $N-NO_2$, Ni, $N-NO_3$, Zn, Cu, turbidity, and pH. The close clustering of Ni, Zn, and Cu with each other and with turbidity indicates that these metals are associated with suspended particulate matter. Again, based on this finding, the probability of Ni, Zn, and Cu being transported in the particulate phase must also be taken into consideration. This pattern at the same time indicates that terrestrial-originated surface runoffs play an important role as a carrier mechanism as well. Another important finding is also the presence of chlorophyll and Fe within the same sub-cluster. This situation suggests that Fe levels in the region might be a limiting factor contributing to primary production.

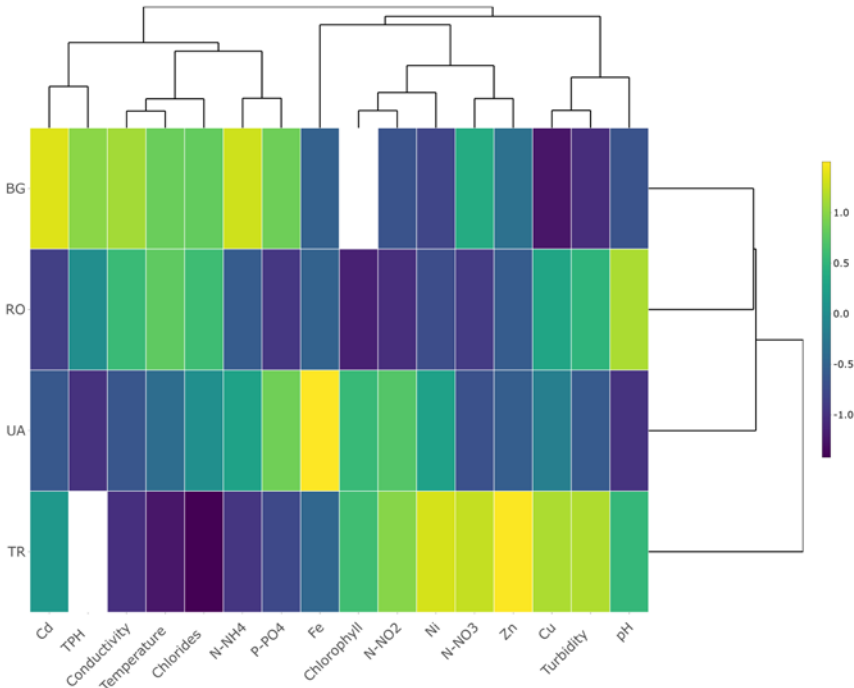


Figure 3. Heatmap and hierarchical clustering analysis of water quality parameters and trace elements across the Black Sea.

3.1.4. Spatial Clustering and Regional Characteristics

According to the dendrogram, the similarity hierarchy is as follows: (Bulgaria – Romania) > Ukraine > Türkiye. Here, Romania 1 (INCDPM), Romania 2 (MB-NAR) are handled as a single cluster.

Bulgaria and Romania: These two regions exhibit the highest similarity in terms of mineralization parameters. Bulgaria stands out distinctly among all regions with the yellow color in the Cd column on the heatmap (the highest Z-score of the dataset). Along with this, there are also remarkable different separations. In spite of high Cd, Zn and Cu values in Bulgaria are among the lowest levels of the dataset. This Cd/Cu-Zn separation is a highly important finding regarding that the Cd load in Bulgaria may be independent of industrial metal sources (which typically carry Zn and Cu together) and exhibits specific point-source dispersion. In Romania, on the other hand, it is highly remarkable that while the Cd value remains at the lowest levels of the dataset, the pH value reaches the highest Z-score.

Ukraine: The most prominent feature of the Ukraine profile is the high value in P-PO₄ concentrations clearly visible with the yellow color on the heatmap. This parameter reaches its peak in Ukraine among all regions. This finding is additionally also important in the context of armed conflict, which is the main focus of the project. Since the dysfunction or shutdown of wastewater treatment infrastructure under conflict conditions (or the destruction of the Kak-hovka Dam) can significantly increase the nutrient load, the P-PO₄ anomaly in question must be handled not only as a hydrological process but also as a potential traceable signal of conflict-induced infrastructure degradation.

Türkiye: Türkiye is completely separated from the other countries in the dendrogram and is positioned as a distinct outlier. Türkiye samples put forward seriously high values compared to all other countries in terms of Ni, Zn, Cu, N-NO₃ (which, contrary to N-NO₂ in Ukraine, indicates agricultural surface runoffs), and Turbidity. The yellow tones on the heatmap show these high values clearly. In contrast to this, Türkiye carries the lowest values of the dataset for Temperature, Chlorides, and Conductivity (similarly to Ukraine, as a result of river-originated freshwater dilution). Observing low salinity together with high metal and turbidity values in this way strengthens the probability of the input being terrestrial through rivers.

3.1.5. Microplastic Distribution, Morphological Characteristics and Polymer Composition in the Sediment

To reveal the pollution transport mechanisms within the basin and assess the chronic pressure of terrestrial inputs on the marine ecosystem using a holistic approach, a microplastic (MP) study was also conducted as part of the project. The study was carried out on samples obtained from Türkiye. Along the continuum extending from the riverine line to the lagoon systems and from there to the coastal seabed, microplastic abundance, size distribution, color profile, morphological structures and polymer compositions in sediment samples were investigated. The conducted analyses revealed that there are variations in the spatial distribution and physicochemical character of microplastics throughout the basin.

Microplastic Abundance and Terrestrial Transport Gradient: The quantitative presence of microplastics in the sediment exhibited a statistically highly significant variation among the sampling locations (ANOVA, $F = 11.76$, $p < 0.001$). Riverine sediments, harboring the highest microplastic load of the basin, stood out as the primary pollution source and carrier vector with an average abundance value of 70 items/kg⁻¹ (± 26.94 items/kg⁻¹). On the discharge line moving away from the input focus and advancing towards the marine environ-

ment, a distinct decrease was recorded in MP density as a result of hydrodynamic dispersion, sedimentation dynamics and dilution mechanisms. Sediment samples taken from the coastal seabed constituted the global minimum pollution level of the basin with 6.8 ± 4.14 items/kg-1. Lagoon systems positioned between riverine and marine compartments, on the other hand, exhibited intermediate values as transition zones filtering the plasticity load of the terrestrial runoff. The river seems to be a carrier (a source for the receiving environment), the sea a receiving environment, and lagoons transition matrices.

Size Distribution and Fragmentation Dynamics: The statistical difference observed in the abundance data was not seen in the distribution of microplastic particles among the sampling areas according to their sizes, and a statistically significant difference was not detected (ANOVA, $F = 0.775$, $p = 0.59$). The size of the particles is homogeneous independent of the matrices.

Color Profile and Localized Tracer Signals: Within the scope of the study, microplastic presence was detected in a total of 7 different color categories, being white, black, transparent, blue, red, green and pink in the sediment matrix. For the color results, a one-way PERMANOVA analysis was performed using the Bray-Curtis coefficient. In contrast to the homogeneity seen in MP sizes among the environments, it confirmed that there is a very strong variation in the distribution of colors among the stations (One-Way PERMANOVA, $F = 1.973$, $p = 0.0031$). In all analyzed sediment samples, the "white" color reached the highest abundance rate and gained the dominant category quality. In contrast, "pink" colored microplastics stand out as the ones having the lowest discharge rate.

Morphological Classification and Shape Distribution profile: As a result of the morphological characterization studies conducted in the sediment matrix, 4 main microplastic shapes were defined, being foam (foam), fiber (fiber), fragment (fragment) and film. However, the pellet form was not encountered. The spatial distribution of microplastic morphologies exhibited a statistically significant heterogeneity on a station basis (One-Way PERMANOVA, $F = 3.124$, $p = 0.0008$).

FTIR Spectroscopy and Polymer Matrix Verification: Fourier Transform Infrared (FTIR) Spectroscopy analyses, performed for the purpose of analytical verification of visual identification, showed that the microplastics consisted of 4 main polymer types. These are Polystyrene (PS), Polypropylene (PP), Polyethylene (PE) and High-Density Polyethylene (HDPE). The spatial distribution of these polymer types among the sampling stations revealed a statistical variation just as in the shape distribution and color distribution (One-Way PERMANOVA, $F = 3.383$, $p = 0.0001$).

3.2. Pre- and Post-Armed Conflict Data Comparison: Risk variations revealed by comparing current data obtained from conflict zones with historical data

3.2.1. Comparison of historical data and current data using core samples

A total of 4 core samples were retrieved from Balık Lagoon of the Samsun Kızılırmak Delta, comprising 2 from reference (Ref R1 and Ref R2) and 2 from impacted zones (Imp R1 and Imp R2). The reason for studying these core samples is to compare past period and present-day data and to look for evidence in assessing war impacts, if any. For this reason, the surface and bottom layers of the sediment cores retrieved from Balık Lagoon and the samples were compared with each other. Within the findings, geochemical patterns distinctly differentiating from one another in the impacted and reference areas draw attention. In the impacted zone, the vertical distribution of organic carbon (Corg) and total nitrogen (Ntot) exhibits a difference between the two cores. In the Imp-R2 core, it was observed that these parameters reached their highest values not in the surface layers, but in the 8–19 cm depth interval, and by decreasing distinctly towards the surface they regressed to reference zone levels (Figure 4). Corg drops to 0.2% DW at the surface, and Ntot to 0.020%. In contrast, in the Imp-R1 core, Corg and Ntot concentrations trend higher in the bottom part. Ptot also followed a similar pattern, and high values were observed in the middle part in Imp-R2, and in both the upper and lower parts in Imp-R1.

When isotopic data are examined, it is seen that $\delta^{13}\text{C}$ values in the impacted zone generally trend low despite exhibiting a slight increase (up to -26.1‰) towards the surface. This situation indicates that allochthonous (external-source) organic matter input and the ongoing eutrophication pressure still maintain their dominance in the region. $\delta^{15}\text{N}$ values, on the other hand, decrease near the surface in the Imp-R2 core, and this pattern can be interpreted as the anthropogenic nitrogen input having partially relaxed.

When trace element profiles are evaluated, it is seen that As exhibits a distinct increase towards the surface in the impacted zone (Figure 5). In the Imp-R1 core, the concentration rose from 6.19 mg kg^{-1} to 14.51 mg kg^{-1} . Co, on the other hand, showed distinct enrichment in the upper half of the profile in both impacted cores. In contrast, while peak concentrations of Pb, Cu, Fe, Zn, V, Ni and Hg were observed only in the 8–20 cm depth interval in the Imp-R2 core, in the Imp-R1 core, Cu, Fe and Zn exhibit lower values in the middle part. Cr does not exhibit a distinct vertical trend in either zone.

Reference samples (Ref R1 and Ref R2), in contrast to the impacted zone, present a largely smooth and homogeneous vertical profile. Nevertheless, two im-

portant exceptions catch the eye. $\delta^{13}\text{C}$ values increase from -27.6‰ up to -21.3‰ towards the surface. Together with the accompanying $\delta^{15}\text{N}$ decrease, this pattern indicates a shift towards autochthonous organic matter sources in the recent period and that the trophic status has relatively weakened. Mn, meanwhile, shows a gradual increase towards the surface in the reference zone, whereas As decreases towards the surface. This pattern exhibits an exact opposite trend to the impacted zone.

In the overall evaluation, two findings particularly stand out. The most intense pollution period in the impacted zone corresponds not to the surface layers, but to the 8–20 cm horizon in the Imp-R2 core and the bottom part in the Imp-R1 core. In the reference zone, on the other hand, the increase in $\delta^{13}\text{C}$ and the decrease in $\delta^{15}\text{N}$ towards the surface stand out as the indicator of a trophic load that manifests itself not through trace element enrichment but through an isotopic shift, and which has weakened in the recent period.

Findings obtained from the core study did not encounter element accumulation that could be war-sourced in the Samsun Kızılırmak Delta. On the contrary, it detected a declining trend in most element concentrations in recent periods. However, the risk of the impacts from non-war, especially agricultural, industrial and domestic discharges in the region masking the war impacts maintains its importance.

In addition, the section below contains some important studies investigating the impacts of the Russia-Ukraine war in the region.

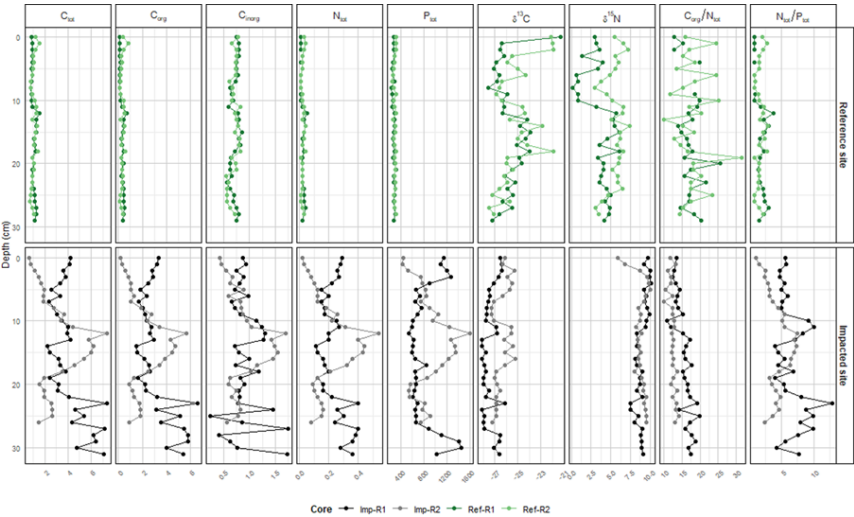


Figure 4. Vertical profiles of C_{tot} (%), C_{org} (% DW), C_{inorg} (%), N_{tot} (%), P_{tot} (mg kg^{-1}), $\delta^{13}\text{C}$ (‰), $\delta^{15}\text{N}$ (‰), $C_{\text{org}}/N_{\text{tot}}$ (ratio), $N_{\text{tot}}/P_{\text{tot}}$ (ratio).

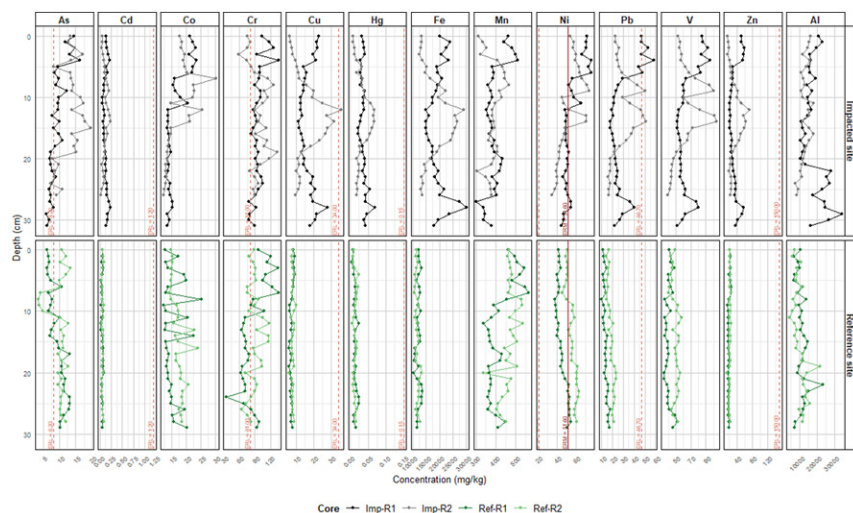


Figure 5. Vertical profiles of elements with the reference to effect range low (ERL)/effect range median (ERM) limits (Long and Morgan 1991).

In their study published in 2025, Kvach and colleagues investigated the impacts created on the Black Sea ecosystem by the Kakhovka Dam, which was destroyed during the war between Russia and Ukraine. According to the results, in the first 6 months of the War (2022), a total of 31 massive oil spills covering 16.2% (4711.6 km²) of the Northwestern Black Sea were detected via satellite imagery. These spills severely hit the “Marine Protected Areas” (MPA) in the region. It was detected in the study that 21% of “Zernov’s Phyllophora”, which is an important seabed habitat, and 67% of Beloberezhzia Sviatoslava National Park were exposed to oil pollution. According to the study, with the collapse of the Kakhovka Dam, decades of industrial wastes of the Zaporizhzhia region, bottom muds harboring heavy metals, more than 150 tons of machine oil, organic wastes and mines were swept into the sea by a massive flood. Consequently, salinity suddenly dropped to as low as 4-5‰ in places, and ammonium nitrogen, which is an indicator of wastewater pollution, skyrocketed to 13.8 times the legal limits. Massive nutrient load (nitrogen/phosphorus) entering the ecosystem, an increase at the rate of 170% to 280% in chlorophyll-a levels was recorded, and a record bloom of +1400% was experienced in potentially toxic cyanobacteria (blue-green algae). Due to the sudden freshwater shock and pollution, massive carp and freshwater fish mortalities were observed on the Black Sea coast. With the collapse of the dam, 11400 tons (worth approximately 267 million dollars) of fish stock were lost in the fisheries sector. With the environment completely turning into freshwater in the Dnieper Estuary, it was

determined that species such as shrimp, ctenophore and jellyfish completely disappeared, whereas freshwater species such as rudd and pike became dominant. With the increase of freshwater flow, the marine seagrasses in the estuary were uprooted and thrown away, and in their place, the freshwater plant named *Ceratophyllum demersum* (hornwort) became intensely dominant on the shores up to 200 meters offshore (Kvach et al., 2025).

In the 2025 study of Jiang and colleagues, the short-term biogeochemical responses occurring in the Northwestern Black Sea following the collapse of the Kakhovka Dam were analyzed with satellite data (Sentinel-3 OLCI), hydrological modeling and in situ data. According to the results, the Dam collapse affected more than 50% of the Northwestern Black Sea area. In 57% of the region, Total Suspended Solid (TSS) level rose above 5 times the pre-disaster average, and TSS concentration reached 100 times that of the pre-disaster in two areas, being the Fontanka coasts (from 0.5 g/m^3 to $>55 \text{ g/m}^3$) and the central Northwestern Black Sea (from 0.6 g/m^3 to 65 g/m^3). In terms of nutrients, distinct increases in nitrate and phosphate levels were observed around the Odesa coast. This environmental shock triggered severe algal blooms within a week, and these blooms continued for 20 days. In 66% of the Northwestern Black Sea, chlorophyll-a level exceeded 5 times its pre-collapse average. While the average Chl-a/TSS ratio inside the TSS plume was 0.6 on June 9, it rose to a maximum of 1.8 on June 16, which caused the color of the water to turn from brown/gray to green. Also, with the explosion of the dam, it transported high concentrations of heavy metals (Fe, Mn, Cu, Zn, Cr, Cd) to the Northwestern Black Sea as well. In water analysis, it was determined that some metals (especially Zn) exceeded environmental quality standards (EQS) (Jiang et al., 2025).

In the study conducted by Komorin and colleagues published in 2026 in the Northwestern Black Sea region, specifically in the Odesa coastal waters and the Dnieper river-estuary-sea transect feeding these waters, they detected and modeled the pollution signals formed on the Black Sea coasts in the context of the Russia-Ukraine war, with three different approaches (routine hydrochemistry, priority pollutants and the chemical fingerprint of beached oil). According to the findings of the article, although long-term routine measurements show that seawater has a clear seasonal cycle, short-term yet very severe anomalies started to be recorded in the chemistry of the water from the second half of 2024 onwards. These anomalies manifested themselves in the form of sudden drops (dangerous minimums) in dissolved oxygen levels and sharp spikes in nutrients such as nitrogen/phosphorus. Priority pollutants (heavy metals, polycyclic aromatic hydrocarbons - PAH, polychlorinated biphenyls etc.) measured along the river, estuary and sea transect (2023–2025) exhibited strong spatial variations and "hot spot" behaviors as advancing towards the sea. Oil residues washing ashore in clusters on the Odesa coastline between January 24 and March 18, 2025 (and in the later months

of the year) were examined. “Chemical fingerprint” analyses conducted with advanced GC-MS conclusively proved that the pollution did not originate from a single shipwreck or an isolated spill. Accordingly, the beached materials were divided into 3 different classes: 1-Weathered heavy fuel oil, 2-Mixed fuel oil with distillate components, 3-Diesel/gas oil-like materials. This structure with different characteristics indicates that multiple types of petroleum products continuously mixed into the region through different pathways (Komorin et al., 2026).

The study published by Boitsov and colleagues in 2026 encompasses surface sediment samples retrieved from 32 different locations and sediment cores retrieved from 4 locations on the Bulgarian coasts in the Black Sea. Organic and inorganic pollutants were investigated in the sediment samples. Metals, polycyclic aromatic hydrocarbons (PAHs), legacy chlorinated polychlorinated biphenyls (PCBs) and brominated pollutants polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane (DDT) and degradation products, hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), along with new generation contaminants of concern per- and polyfluoroalkyl substances (PFAS) were included in the scope of the investigation. PBDEs and PFAS were reported for the first time with this study for the marine sediments in this region. According to the results, no trace of the ongoing war between Ukraine and Russia was encountered (Boitsov et al., 2026). A monitoring program has been designed within the scope of the project to track war-induced pollution.

3.2.2. Objective of the War Related Pollution Monitoring Program

The primary objective of the monitoring program is to measure the direct (munitions, sunken vessels, bombardment residues) and indirect (infrastructure collapses such as the destruction of the Kakhovka Dam, wastewater treatment plant interruptions) ecological degradation caused by the Russia-Ukraine armed conflict on the Black Sea ecosystem. Additionally, classifying these impacts and placing them on a legal basis is also extremely important for the program. The program, instead of establishing a system from scratch, presents a risk-based and adaptive monitoring architecture built upon existing international directives (MSFD, WFD) and regional infrastructures (BSIMAP). The most critical priority is to ensure uninterrupted and safe data flow by protecting field personnel from mine and active conflict risks within the framework of the “Do No Harm” principle.

Contaminants, Matrix and Sampling Frequency

The analytical framework of the program manages all specific contaminant sub-groups originated from conflict and infrastructure destruction, the matri-

ces to be investigated, and the temporal frequency of field operations through the following comprehensive matrices (Annex Table 7-8).

Physical access constraints and security risks created by war conditions have made the execution of the monitoring program mandatory for technologically innovative methods.

Innovative Tools:

- Unmanned Surface Vehicles (USVs) and Unmanned Aerial Vehicles (UAVs/ Drones) and autonomous underwater vehicles (AUVs/ROVs) will be utilized for the purpose of physical sampling and data collection in risky zones where manned vessels cannot enter due to mine hazard or active conflict.
- Earth Observation data based on satellites have been integrated into the system for the macro-level tracking of large-scale oil spills, river plumes, suspended solid matter distributions, and algal blooms.

Data Handling and Reporting:

- All data obtained from the field and remote sensing tools will be stored by being centralized in the Black Sea e-Platform and Water Quality Database (WQDB) operated by UkrSCES (Ukrainian Scientific Centre of Ecology of the Sea).
- The most fundamental element constituting the legal and international weight of the monitoring program is that the collected data will not remain local but will be directly integrated into Europe-wide institutional data networks such as EMODnet Chemistry and WISE Marine. This integration will ensure that the conflict-induced ecological degradation in the Black Sea officially remains on the radar of the European Union's environmental policies, reporting cycles, and future environmental accountability/reparation mechanisms.

4. Bioaccumulation and Human Health Risk Management

One of the most important issues emphasized within the scope of the study is in what way the current element status in the Black Sea Basin spreads to living organisms. For this, living organism samples with different trophic levels and exhibiting bioindicator characteristics were collected especially from different

points in Türkiye and Romania, and element profiles were extracted by performing tissue differentiation.

4.1. Element Accumulation in Bioindicator Organisms: Observed element accumulation capacities and bio-transfer processes in fish, macroalgae, and mollusk species

In this project the Metal Pollution Index (MPI) was used to compare the amount of elemental accumulation among tissues and species (Figure 6). Fe was intentionally excluded from the MPI calculations. The reason is to prevent its elevated baseline concentrations from masking the low toxicological signals of trace elements such as Cd and As. This approach avoids data distortion and establishes this adjustment as a deliberate methodological choice. MPI values across the Romanian and Turkish sampling stations ranged from 0.043 (*R. venosa* outer shell, Razim 3) to 29.620 (Algae, Balık Lagoon). Algae constituted the highest MPI matrix in Türkiye. Accordingly, Balık Lagoon (29.620), Kızılırmak River (13.261), and the Black Sea (10.846). In Romania, on the other hand, the highest values belong to *Rapana venosa*, and Razim 3 whole body (22.513) and the egg value in Pescarie (20.616) stand out. Romanian algae values remained between 1.043 (Pescarie Cons.) and 3.428 (Pescarie). Whole-body values of *R. venosa* reached the highest level at Razim 3 (22.513) and Sulina (8.750). In Türkiye, on the other hand, it declined to 4.230 at the Black Sea station. The egg MPI value at Pescarie (20.616) approaches the Razim 3 whole-body maximum. Within Cap Midia, tissue-level values formed a 10.5-fold range from the outer shell (0.554) to the soft tissue (5.795). Inner shell (1.396) remained between these two extremes. Shell fractions progressed consistently low across the entirety of the Romanian stations. Additionally, outer shell values were measured as 0.043 at Razim 3, 0.535 at Corbu, and 0.554 at Cap Midia, while inner shell values were measured as 1.396 at Cap Midia and 0.353 at Corbu. Among Romanian bivalve, *Anadara inaequalis* collected from Cap Midia, with a soft tissue MPI value of 8.360, exhibited a value approximately 12 times higher than its inner shell (0.688) and approximately 25 times higher than its outer shell (0.330). While the inner shell value (0.856) in the same species at Corbu was found higher compared to Cap Midia (0.688), the outer shell (4.701) reached a remarkably high value. Plants at Razim 1 gave two values close to each other (6.825 and 7.509) and the MPI value in *Idotea balthica* at Pescarie Cons. reached 4.453.

MPI Variation Across Organisms and Tissues (Romanian & Turkish Coasts)

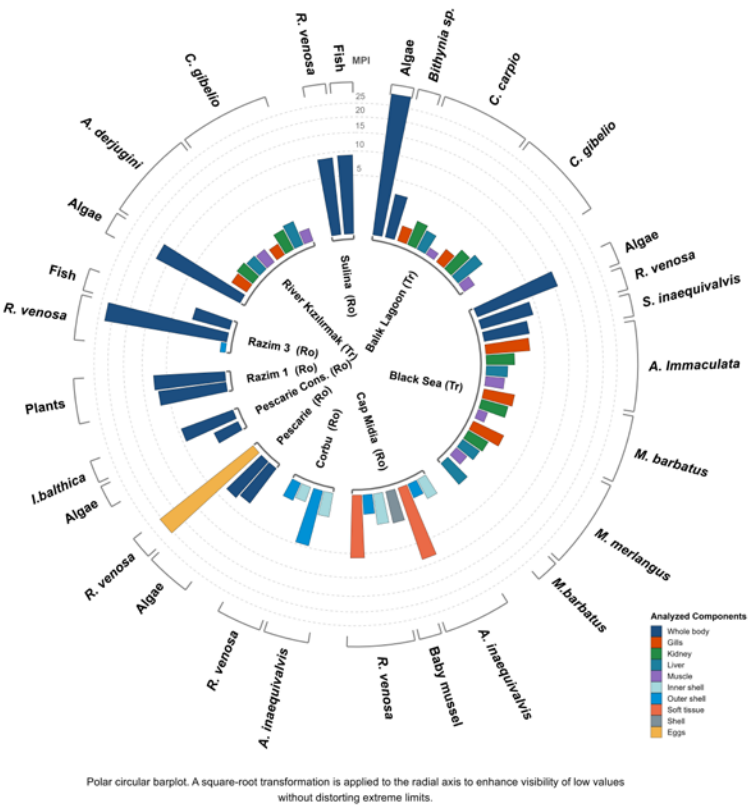


Figure 6. Variation of the Metal Pollution Index (MPI) across different taxa and tissues from the Romanian and Turkish coasts.

In three marine fish species sampled from the Turkish Black Sea, gills recorded the highest tissue MPI values. *Alosa A. Immaculata*, (2.858), *Merlangius merlangus* (1.670), and *Mullus barbatus* (1.431). Muscle tissue gave the lowest values in all three species (0.533, 0.283, and 0.147, respectively). Kidney and liver values progressed close in *M. merlangus* (0.773 and 0.627), while they were found nearly identical in *M. barbatus* (1.120 and 1.132).

In Türkiye, the tissue ranking in two cyprinid species showed differences. While liver (1.423) and kidney (0.952) exceeded the gill value (0.371) in *Carassius gibelio* at Balık Lagoon, muscle tissue remained at the lowest value (0.230). In *Cypripinus carpio*, kidney (0.952) and liver (0.645) exceeded the gill (0.359) and, similarly to *C. gibelio*, muscle tissue remained at the lowest value again (0.087). At Kızılırmak River, *C. gibelio* maintained the liver-weighted pattern (liver (0.948) >

kidney (0.737) > muscle (0.255) > gill (0.239)). *Alburnus derjugini*, on the other hand, became the species exhibiting the narrowest inter-tissue distribution among the sampled species (kidney: (0.601) > gill (0.472) > liver (0.460) > muscle (0.385)).

The bioaccumulation factor (BAF), which is the ratio of element accumulation in tissue to element accumulation in water, and the biota-sediment accumulation factor (BSAF), which is the ratio of element accumulation in tissue to element accumulation in sediment in the captured species, are also important.

The BAF and BSAF data encompass four organism groups (except fish) from three locations. These organisms are *R. venosa* and *S. inaequalvis* from the Black Sea, *Bithynia* sp. and algae from Balık lagoon, and algae from the Kızılırmak. Eleven elements were analyzed, and Cr remained below the limit value for ICP-MS in the Black Sea organisms (*R. venosa*, *S. inaequalvis*, algae) and in Kızılırmak algae, whereas As remained below the limit value for all three organisms in the Black Sea (BDL).

For interpretation, BAF values were classified as follows: BAF < 1000 indicated no bioaccumulation potential, 1000 < BAF < 5000 indicated bioaccumulation potential, and BAF > 5000 indicated high bioaccumulation potential (Arnot & Gobas, 2006). Across the BAF values, algae ranked ahead of the animal species (Figure 7). At the element level, location-based differentiation is clear. In Balık Lagoon algae, it exhibited the highest BAF values for Al (264157), Cr (180028), As (2056) and Pb (23421), whereas in Black Sea algae the highest BAF values were for Mn (356575) and Ni (13111). The highest values for Fe (178301), Cu (5868) and Zn (2398) were recorded in Kızılırmak algae. For Hg, the highest BAF value was measured in Balık Lake algae (385.71), and the lowest in Black Sea algae (34.33).

In animal species, the dominant element rankings differ by species. In *R. venosa*, Mn (36917) and Cd (25614) formed the first two ranks, while in *S. inaequalvis* Mn reached a much higher value (96636), followed by Fe (14996) and Cd (14540). In *Bithynia* sp., Cr (29657) emerged as the dominant element, and *Bithynia* sp. was the only organism among the animal species in which Cr was detected outside BDL.

The BSAF analysis generally clusters under 3 classes: BSAF <1: non-concentrators, 1 < BSAF < 2: low concentrators, and BSAF >2: high concentrators (Jolao-sho et al., 2024). Accordingly, the most extensive exceedance in the BSAF analysis was observed in *R. venosa*. This species exceeded the threshold for five elements Cd (74.07), Hg (4.29), Cu (2.83), As (1.82) and Zn (1.61). *S. inaequalvis* exceeded the threshold only for Cd (42.05) and Zn (1.27). The BSAF values of the other elements remained between 0.805 (Hg) and 0.003 (Cr). All BSAF val-

ues of *Bithynia* sp. ranged from 0.407 (Cd) to 0.001 (Pb), and no element reached the threshold. Among the algae, only Mn in Balık Lake algae (5.42), and in Black Sea algae Cd (2.53) and As (1.78) exceeded the bioaccumulation threshold. In Kızılırmak algae, the highest BSAF value remained in Zn (0.77).

“BAF and BSAF calculations were also performed to cover four specific tissues (muscle, kidney, liver, gill) of six fish species from three different locations (Figure 8). These species are *A. immaculata*, *M. merlangus* and *M. barbatus* from the Black Sea, *C. carpio* and *C. gibelio* from Balık Lagoon, and *A. derjugini* and *C. gibelio* from the Kızılırmak River. As with invertebrates and algae, eleven elements were analyzed, and in Black Sea water samples Cr and As remained below the ICP-MS detection limit (BDL) and could not be detected. Accordingly, BAF analysis could not be carried out for Cr and As in the Black Sea samples. Similarly, in the data from the Kızılırmak River location, Cr flow could not be tracked in the BAF metrics.

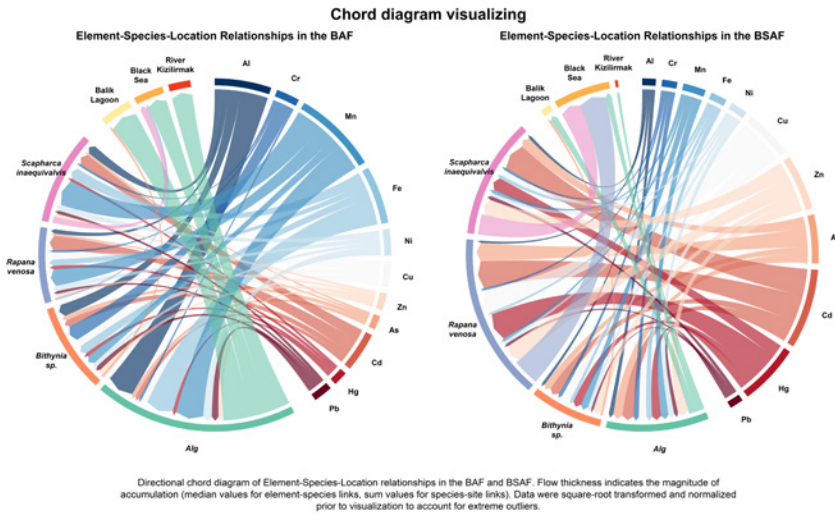


Figure 7. Chord diagram visualization of 11 elements BAF and BSAF interrelationships for non-fish taxa.

Across the BAF values, location-based differentiation at the element and species level is highly pronounced. In the Black Sea ecosystem, the gill tissue of *A. immaculata*, constituted the global maxima of the dataset for Mn (417268), Al (27377) and Ni (10988). For the Fe element, the highest BAF value was observed in the liver of *C. gibelio* at the Kızılırmak River location (63144). In addition, the highest BAF value for Cd was again in the kidney of *C. gibelio* in Kızılırmak River (14063), for Cu in the liver of *C. gibelio* (24312), for Cr in the liver of *C. carpio* in Lake lagoon (7495), and for As in the liver of *A. derjugini* (455). Again at the Kızılırmak location, Zn (1893.94) and Pb (690.55) reached striking accumulations in the gills of *A. derjugini*, while Hg accumulation in the muscle tissue of the same species (10331) surpassed all other tissues and formed the Hg peak of the dataset. The sediment-derived BSAF analysis remained within a much narrower range compared with water-derived BAF and the global maximum of the dataset could reach only the level of (10.48). The noteworthy point for BSAF values was the relatively high values of highly toxic elements such as As, Cd and Hg. Hg again yielded the global maximum, and its intense accumulation in muscle tissue also drew attention. Among all species, the greatest accumulation for Hg was again observed in *A. derjugini*. BAF and BSAF patterns exhibit marked divergence in terms of the uptake dynamics of elements from environmental compartments (water and sediment). While it was seen that macro elements that were volumetrically dominant in BAF (Mn, Fe, Al) were heavily accumulated from the water column into the gills (e.g. *A. immaculata*, Mn BAF: 417268), their BSAF counterparts in the same tissues remained far from exceeding the high concentrator threshold (2.0). In contrast, the highly toxic trace elements Cd and Hg showed a similar tendency within their own data ranges in both metrics, revealing transfer to biota from both sediment and water compartments.

4.2. Human Health Risk Assessment Models: Carcinogenic (CR) and non-carcinogenic (THQ, HI) health risks calculated based on seafood consumption

Within the scope of the study, individual carcinogenic risk (CR) and total carcinogenic risk (TCR) indices indicating the cumulative effect based on heavy metal concentrations in fish species (*C. carpio*, *C. gibelio*, *M. barbatus*, *A. immaculata*, *M. merlangus*, *A. derjugini*) obtained from Balık Lagoon, Black Sea, and Kızılırmak River locations were calculated (Table 1). When the obtained findings are evaluated in the context of the reference values of 10^{-6} (lower limit) to 10^{-4} (upper limit), which is the acceptable risk range, it is seen that the cumulative risk draws a much more critical picture compared to individual element risks.

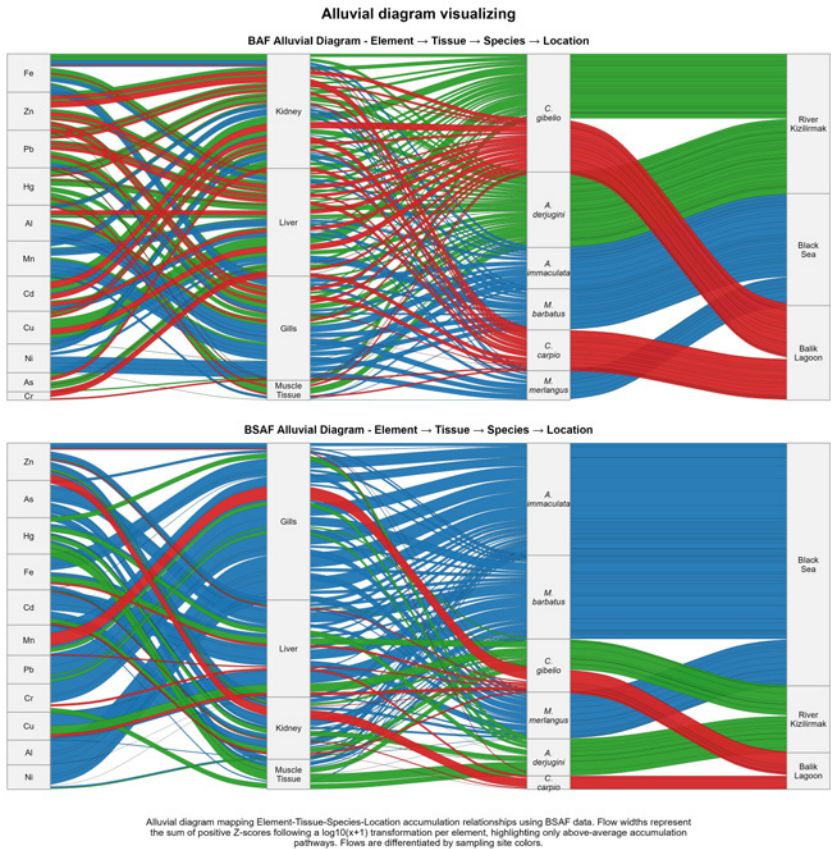


Figure 8. Alluvial diagram visualizing of 11 elements BAF and BSAF interrelationships for fish taxa.

CR		Cr	Ni	As	Cd	Pb	TCR
Black Sea	<i>A. immaculata</i>	9.96E-07	8.20E-06	8.15E-05	1.52E-04	5.51E-08	2.42E-04
	<i>M. merlangus</i>	2.09E-07	0	1.20E-05	8.75E-05	4.06E-08	9.97E-05
	<i>M. barbatus</i>	4.33E-07	1.25E-07	8.55E-05	4.77E-05	3.72E-08	1.34E-04
Balık Lagoon	<i>C. gibelio</i>	2.04E-07	0	4.86E-07	1.70E-05	5.06E-08	1.77E-05
	<i>C. carpio</i>	2.85E-09	0	8.87E-07	8.19E-06	3.73E-08	9.12E-06
Kızılırmak River	<i>C. gibelio</i>	3.46E-07	0	1.31E-06	1.70E-05	3.77E-08	1.86E-05
	<i>A. derjugini</i>	4.69E-06	2.52E-05	9.21E-07	9.54E-06	3.84E-08	4.04E-05
CSF: Carcinogenic slope factor (mg/kg/day)		0.42	0.91	1.5	15	0.0085	10 ⁻⁴

Table 1. Estimated carcinogenic risks (CR and TCR) associated with heavy metal exposure via consumption of fish from three aquatic ecosystems (Tunca et al. 2026).

4.2.1. Critical Findings Exceeding the Acceptable Upper Risk Limit (10⁻⁴)

When examined on an individual element (CR) basis, the only measurement exceeding the 10⁻⁴ upper risk limit is the Cd concentration in the *A. İmmaculata* species sampled from the Black Sea location (1.52 × 10⁻⁴). However, when the analysis is evaluated from a total risk (TCR) perspective, the severity of the picture increases. The unacceptable upper risk limit, which is 10⁻⁴, is exceeded by two different species in the Black Sea location. Accordingly, the *A. İmmaculata* species constitutes the highest cumulative carcinogenic risk level within the dataset with a TCR value of 2.42 × 10⁻⁴. The TCR value of the *M. barbatus* species sampled from the same location was also calculated as 1.34 × 10⁻⁴ and clearly exceeded the upper risk limit (on a CR basis, no single element of this species had exceeded this limit alone). Additionally, the cumulative risk value of the *M. merlangus* species belonging to the Black Sea location was determined as 9.97 × 10⁻⁵. This value is extremely close to the unacceptable risk threshold (1.00 × 10⁻⁴).

4.2.2. Findings in the Risk Range Requiring Monitoring (10⁻⁶ - 10⁻⁴)

The measurements exceeding 10⁻⁶, which is the lower risk limit, but remaining below the upper limit are as follows on an element basis. The element Cd took

place in this monitoring band in all species except *A. immaculata*, which has the highest cumulative value. Risk originating from As, on the other hand, is dominant in the Black Sea location (*M. barbatus*: 8.55×10^{-5} ; *A. immaculata*: 8.15×10^{-5} ; *M. merlangus*: 1.20×10^{-5}) and reached the lower risk limit level only in the *C. gibelio* (1.31×10^{-6}) species in Kızılırmak among the samples outside the Black Sea. While the findings exceeding the 10^{-6} limit for Ni remained limited to *A. derjugini* (2.52×10^{-5}) in Kızılırmak River and *M. barbatus* (8.20×10^{-6}) in the Black Sea, the only record passing the threshold in question for Cr was found in the *A. derjugini* (4.69×10^{-6}) species in Kızılırmak River. In the context of cumulative effect (TCR), on the other hand, the TCR values of all samples other than the species exceeding the limit in the Black Sea are in this monitoring band range. *A. derjugini* (4.04×10^{-5}) and *C. gibelio* (1.86×10^{-5}) in the Kızılırmak location; *C. gibelio* (1.77×10^{-5}) and *C. carpio* (9.12×10^{-6}) in the Balık Lagoon location.

4.2.3. Analyses Remaining Below the Risk Limit ($< 10^{-6}$) and TCR Contrast

When evaluated on a CR element basis, Pb remained well below the 10^{-6} reference value in all species and locations (Highest Pb value Black Sea *A. immaculata*: 5.51×10^{-8}). However, the most striking finding of the study is this: When Total Carcinogenic Risk (TCR) data are evaluated holistically, no fish species included in the study took place below the 10^{-6} lower limit (in the risk-free zone). Even if the risk levels of individual elements are low, cumulative loadings transported all species at least to the "risk range requiring monitoring". Even *C. carpio* (9.12×10^{-6}) in Balık Lagoon, which is located at the lowest step of the monitoring range, is above the risk-free area.

4.2.4. Conclusion

When individual and total risk data are evaluated together, it has been proven that the Black Sea location is the cumulatively dominant risk source in the context of Cd and As accumulation. *A. immaculata* and *M. barbatus* species broke the carcinogenic upper limit (10^{-4}) due to multi-metal exposure. The fact that many situations that appear individually risk-free ($< 10^{-6}$) on an element basis leap into the range requiring monitoring when the elements are calculated collectively reveals numerically how critical the cumulative approach (TCR) is in risk assessments.

Within the scope of the study, Target Hazard Quotient (THQ) and Hazard Index (HI) values were also calculated to evaluate non-carcinogenic health risks. Unlike the carcinogenic risk profiles, the findings obtained for THQ and HI did not exceed the acceptable threshold limit (>1) in any of the analyzed fish species

across all sampling locations. This indicates that the non-carcinogenic systemic health risks associated with the consumption of the examined species remain within safe boundaries under the current environmental conditions.

5. Innovative Remedial Solutions

In the last decade technological developments have offered effective solutions against pollution problems in marine environments. However no treatment method can produce a solution to all pollutant types alone. The methods to be applied for known pollutants such as plastics, heavy metals, oil products and industrial wastes are determined entirely according to the type of the pollutant and the conditions of the contaminated environment.

5.1. Technological Remedial Solutions Database: Presentation of existing global and regional “state-of-the-art” solution methods used in combating marine pollution

5.1.1. Treatment Methods for Oil Spills

Oil pollution is one of the most destructive forms of pollution in marine environments. Its source can be based on extraction, transportation or ship explosions due to armed conflicts. In the current literature three main methods stand out for remediation, namely mechanical, chemical and biological.

- In mechanical methods skimmer and boom systems, due to their simplicity and affordable costs, effectively collect the oil on the water surface.
- In chemical methods dispersants (dispersant) and solidifiers break down the oil products and facilitate natural degradation. However strict control is mandatory so that the substances used do not themselves become pollutants.
- Bioremediation on the other hand is considered as the most “bio-compatible” approach that ensures the degradation of oil products in a more environmentally friendly manner with the introduction of microparticles and microorganisms to the environment.

5.1.2. Treatment Methods for Plastic Wastes

Despite reduction programs marine plastic pollution continues to be a growing problem (Raykov et al. 2020). While the degradation products of plastics exert

toxic effects on ecosystems some plastic materials turn into direct death traps for marine life. Methods differ according to the plastic type and its location.

- Floating barriers and booms: They can collect surface plastics without requiring energy however the transportation of the collected material to land is mandatory.
- Mechanical and manual cleaning: It relies on the active participation of communities and local governments. Intervention to the environment is minimal. But its dependence on human power creates a significant limitation.
- Mycoremediation and microbial degradation: Within its scope *Aspergillus* species fungi can effectively break down polyethylene and similar plastics through hydrolase enzymes.
- Chemical methods: Among chemical methods pyrolysis stands out. Thermal degradation carried out in an oxygen-free environment transforms waste plastics into raw materials without releasing toxic by-products. Besides these, new technologies such as the dual-layer mesh roller method and smart material integration yield promising results in the separation of micro plastics from the aquatic environment.

5.1.3. Treatment Methods for Eutrophication and Nutrient Removal

Nitrogen and phosphorus loading originating from agricultural drainage and wastewater discharge causes rapid algae growth, namely eutrophication, in marine environments. This situation leads to dissolved oxygen depletion and toxin release originating from algae degradation, creating hypoxic conditions. Three main approaches are applied.

- Physical methods (aeration and sediment cleaning): provide minimum intervention to marine life. Along with this low efficiency and high operational cost constitute a disadvantage.
- Chemical methods (nutrient precipitation with flocculants and coagulants): must be supported by physical removal. This situation limits the efficiency.
- Biological methods (microorganisms and aquatic plants): while supporting natural restoration processes may carry the risk of incompatibility with the local ecosystem. It should be known that none of the available methods are sufficient alone. Developing integrated approaches combining all techniques is seen as mandatory for the solution of the problem.

5.1.4. Treatment Methods for Heavy Metals and Chemical Pollution

Heavy metal pollution constitutes one of the most persistent problems in marine environments.

- Among physical methods sediment dredging and the use of sorbent material are common. However the management of the extracted pollutant material stands out as a critical problem.
- Within the scope of chemical methods phosphate application and electrocoagulation transform heavy metals into complex compounds that are less harmful to aquatic life.
- Among biological methods while biostimulation and bioaugmentation (bioaugmentation) include the introduction of microbial strains that facilitate target metal removal into the environment, within the scope of phytoremediation aquatic plants, primarily the water hyacinth (*Eichhornia crassipes*), have exhibited remarkable efficiency in Cd and Pb removal.
- In the context of nanotechnology applications Fenton reagent and nZVI nanoparticles stand out with the advantage of high reactivity and large surface area in transforming both organic pollutants and heavy metals into non-toxic compounds.

5.1.5. Conclusion

While technologies specific to each waste type exist, the most effective approach in reducing future pollution is the raising of awareness and the inclusion of the society and policymakers into the process. The encouragement of community participation and the establishment of international legal regulations will assume a determining role, especially in the protection of marine environments. Educational programs targeting young generations on the other hand are evaluated as the most sustainable long-term investment in terms of the dissemination of treatment and waste reduction methods.

5.2. SIERRA Pollution Remedial Solution Prototype: Detailed description of the design, working mechanism, and application potential of the innovative prototype developed within the scope of the project

This section includes the introduction of the innovative prototype produced within the scope of the project aimed at the simultaneous reduction of pollutants in marine environments. This section presents an innovative multi-stage and multi-layer filtration method (mechanical and chemical) based on geopolymer matrices.

5.2.1. Geopolymers as Green Intelligent Materials

Geopolymers are inorganic binders obtained by the alkaline activation of aluminosilicate precursors such as fly ash or metakaolin. Because they do not require calcination at high temperature, energy consumption and CO₂ emissions are significantly low. Studies also confirm that these materials substantially reduce greenhouse gas emissions compared to traditional cement-based systems. Their porous structures, which can be customized with techniques such as direct foaming offer a massive surface area for the adsorption and permanent encapsulation of heavy metal ions such as Pb²⁺, Cd²⁺, Cr⁶⁺ and Cu²⁺. The heavy metal removal efficiencies of various geopolymers are in the range of 90% to 100%.

5.2.2. Designing the Marine Pollution Remedial Solution Prototype

A two-stage system requiring no external power source (pump etc.) has been designed. Although life cycle assessment (LCA) studies prove greenhouse gas advantages current laboratory experiments remain insufficient in reflecting the complexity of “real marine chemistry” such as fluctuating ionic strength and competing species (Figure 9).

- First Stage: Coarse pollutants are removed with low-cost sand and gravel. While impurities are encapsulated in the geopolymeric matrix, the materials can subsequently be converted into aggregates. The 50-micron washable filter downstream of the sand prevents the passage of large particles.
- Second Stage: The “intelligent cartridge” consisting of multi-layer geopolymers with customized porosity removes heavy metals. The 50-micron

second filter at the outlet both prevents particle leakage and reduces the water flow rate, increasing the contact/adsorption time.

5.2.3. Production and Characterization of Intelligent Filters

Geopolymerization technology produces products capable of replacing ordinary Portland cement (OPC) by converting industrial solid wastes into valuable materials. The geopolymer system consists of two main components. The first component is a material rich in aluminum and silica oxides, including red mud, metakaolin, rice husk ash, calcined clays, fly ash, waste glass, copper mine tailings, and zeolite. The second component is an activator, which may be either an alkaline or an acidic liquid. When alkaline activation is employed, the activator is generally composed of sodium hydroxide, sodium silicate, potassium hydroxide, or potassium silicate. In contrast, acidic activation is typically achieved using phosphorous acid-based activators.

5.2.4. Identification and Characterization of Suitable Raw Materials

The raw materials used in this study comprised metakaolin (MK), sodium silicate (Na_2SiO_3), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), and zeolite aggregates. The synthesis procedure of these materials was carried out in accordance with Burduhos Nergis et al. (2025).

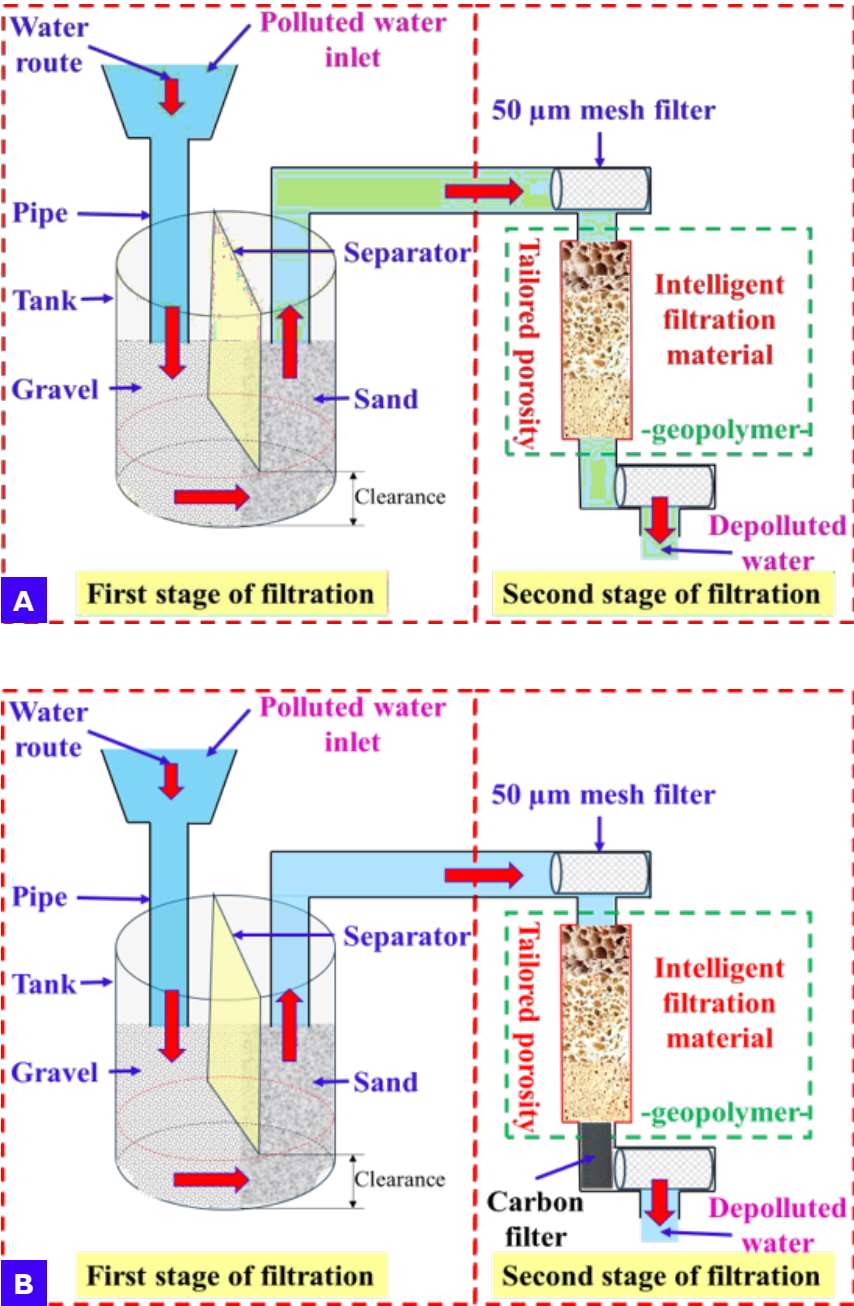


Figure 9. The first (A) and the second (B) design of the marine pollution remedial solution prototype (Burduhos Nergis et al. 2025).

The natural zeolite aggregates used in the production of geopolymers for the second type of prototype are mainly composed of clinoptilolite (87–90%). They are thermally stable up to 450°C. The zeolite sample consists primarily of Si and Al, which account for 26% and 5.5% of its total weight, respectively. These elements form the characteristic aluminosilicate framework typical of zeolites. The sample also contains a notable amount of C, at 2.8%. This is followed by alkali and alkaline earth metals that likely act as extra-framework cations, including Ca at 2.2%, K at 1.8%, Na at 0.8%, and Mg at 0.8%. The transition metal content is dominated by Fe at 1.4%, followed by Cu at 0.9%, and trace amounts of Mn and Ti, both at 0.1%. Other minor elements contribute less than 0.1% of the total mass, while O accounts for the remaining proportion and stabilizes the tetrahedral structure of the mineral. The Si/Al molar ratio is around 4.54, which is typical of clinoptilolite-type zeolite.

5.2.5. Identification of the Most Suitable Manufacturing Method

Four basic methods used in the production of porous geopolymers were compared:

- Self-forming method (SFM): Produces small, closed pores: not suitable for water filtration.
- Filler addition method (AFM): Suitable for insulation applications: not favorable for water filtration since its outer surfaces remain compact.
- Particle stacking method (PSM): Porosity control is difficult: carries the risk of low mechanical strength due to incomplete sintering.
- Direct foaming method (DFM): Offers customized porosity depending on the type and amount of the foaming agent: selected as the most suitable method for water filtration.

The methodology is described in detail by Burduhos Nergis et al. (2025).

5.2.6. Characterization of Geopolymers

- Pre-use SEM analysis: Revealed a homogeneous aluminosilicate matrix resulting from the effective geopolymerization reaction. Connected macropores provide water permeability while mesopores maximize the contact surface (Figure 10 and Figure 11).
- Pre-use EDS analysis: Confirmed the presence of high Si and Al (polymer skeleton) and free Na⁺ ions in the structural channels. These ions are capable of carrying out ion exchange with heavy metal ions (Pb²⁺, Cd²⁺, Cu²⁺)

- Post-use SEM analysis: Fine precipitates and slight clogging were observed in small pores. This situation stands as direct evidence of mechanical retention. Initially sharp pore edges have become rounded. Alongside this, no cracks formed in the main structure. This confirms the mechanical strength of the matrix under saltwater conditions (Figure 10 and Figure 11).
- Post-use EDS: Showed that Na^+ density decreased significantly (evidence of the ion exchange mechanism), and elements originating from seawater such as Cl and Mg and contaminant traces such as Cu, P appeared in the structure. The Si/Al ratio remaining constant indicates that the filtration process is a superficial and controlled ion exchange operation, and that the material has not chemically degraded.

5.2.7 On-site Validation of the Marine Pollution Remedial Solution Prototype

The prototype model is described by Burduhos Nergis et al. (2025). It was tested on water samples collected during two separate on-site campaigns from four different regions of the Black Sea (Corbu, Midia Cap, Pescărie Constanța and Tuzla). Samples were taken as both untreated and filtered water.

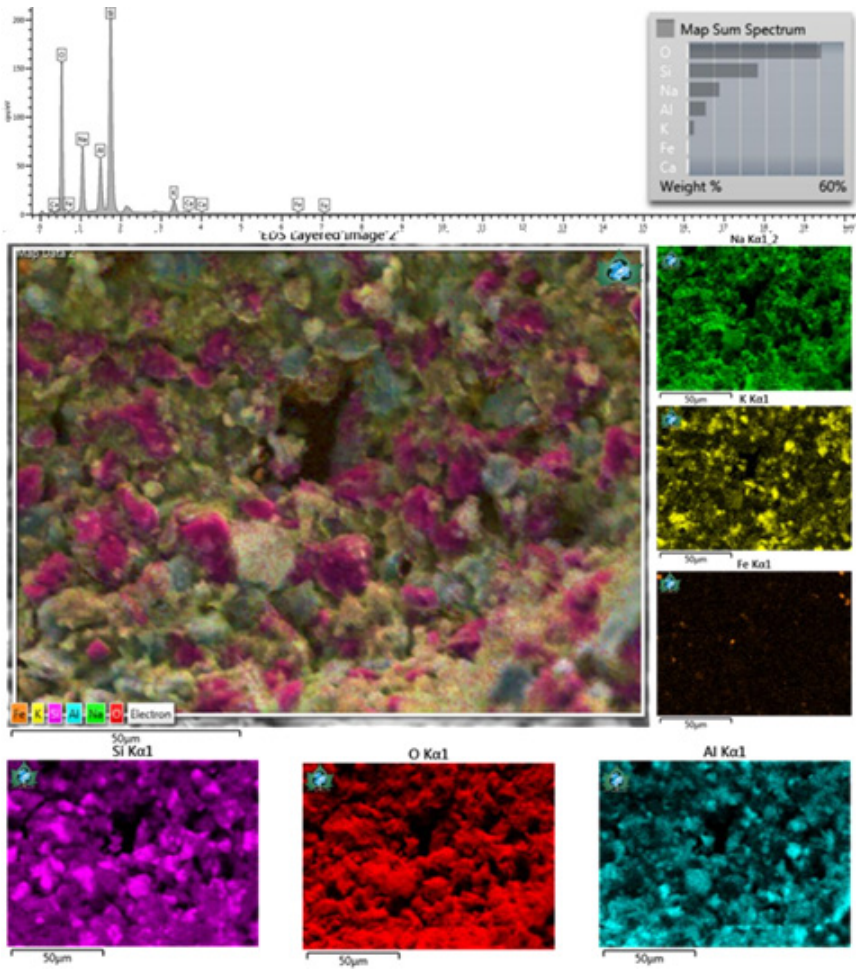
Heavy metal removal results

- Cu removal: 99.91% at Location 1, 42% at Location 2, 58.31% at Location 3, 49% at Location 4.
- Zn removal: 54.36% at Location 1, remained stable at Location 2, 49% at Location 3, 13% at Location 4.

The fundamental adsorption mechanisms are in the form of ion exchange ($\text{Na}^+ \rightarrow \text{HM}^{2+}$), surface complexation, surface precipitation, electrostatic interactions and pore diffusion. A small iron increase was observed in all locations and it was suggested that this problem would be eliminated with the use of low iron content raw material.

It was determined that metakaolin-based geopolymers remained insufficient in the removal of nitrogen compounds (nitrites, nitrates, ammonium), phosphate and chlorite. For this reason, a composite geopolymer containing 20% zeolite aggregate was subsequently developed.

- Phosphate removal: Reduced to undetectable levels at Location 1 and 2, 91.37% at Location 3, 89.36% at Location 4.
- Total petroleum hydrocarbons: 45% decrease in the first three locations, no change was observed at Location 4 since the initial concentration was very low (0.03 mg/L).



A

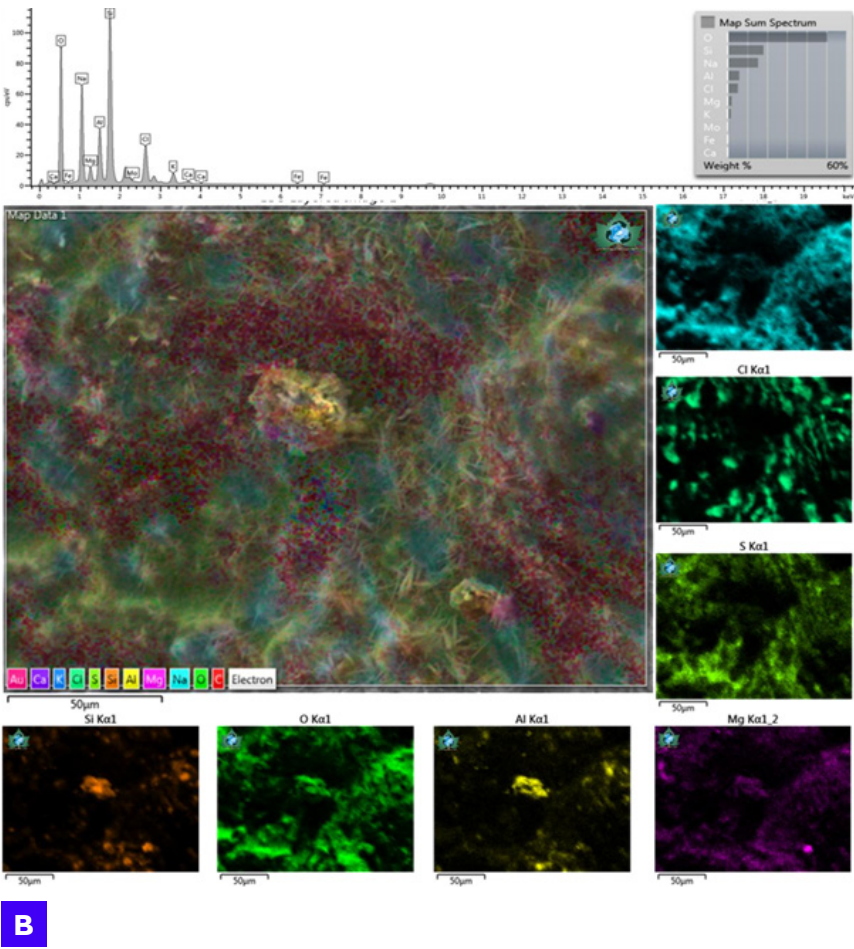
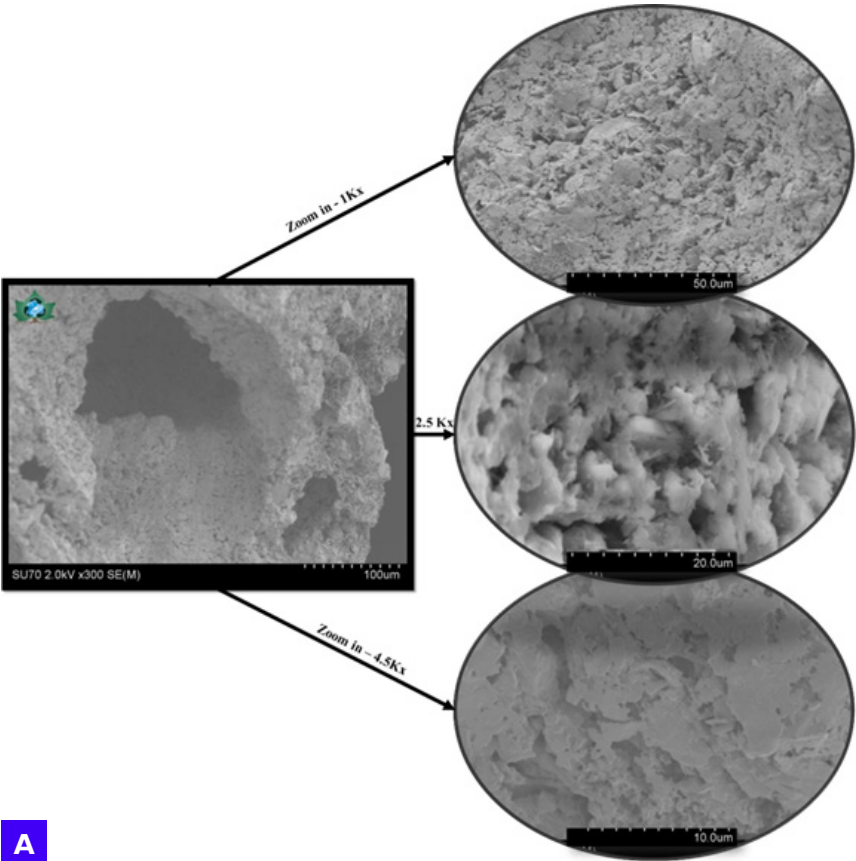
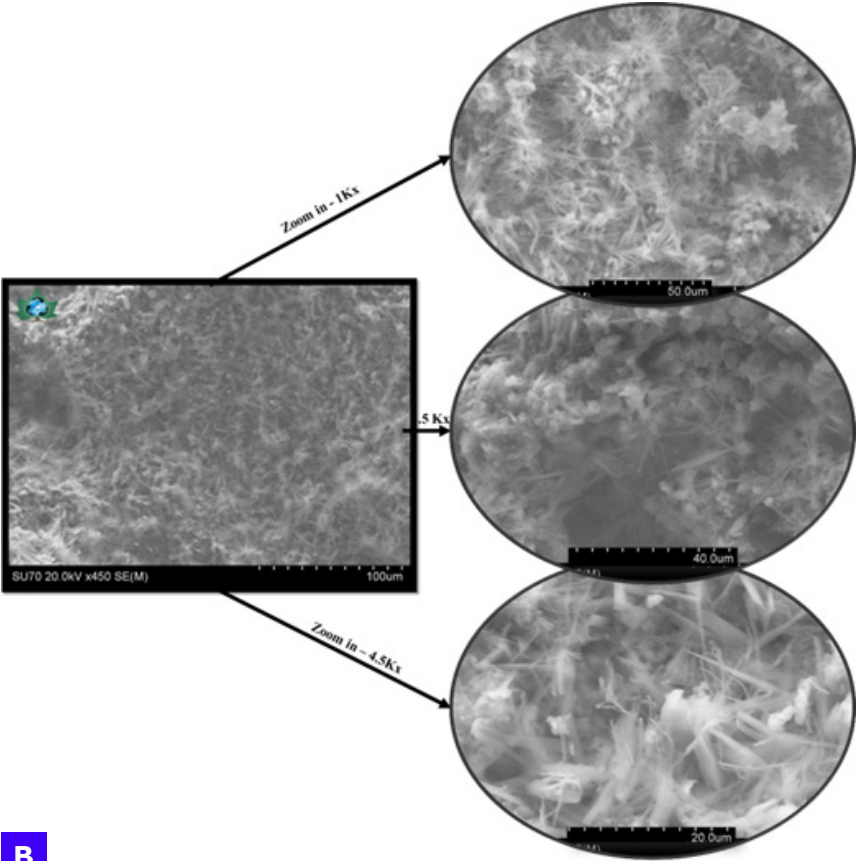


Figure 10. The elemental mapping of the geopolymers before (A) and after (B) use in saltwater filtration.





B

Figure 11. *The morphology of the geopolymers before (A) and after (B) use in saltwater filtration.*

The turbidity increase problem of the water exiting the filtration was solved by adding an active carbon filter downstream of the geopolymer filter (Figure 9). This second design creates a synergistic treatment system by combining the mechanical barrier and molecular membrane function of the geopolymer filter with the organic matter and turbidity removal function of the active carbon.

The system does not require an external power source (does not contain a pump or solenoid valve) and with its compact and multi-layer structure, it can be deployed to any pollution point via small automatic boats or unmanned underwater vehicles. Post-use filter materials can be re-evaluated as raw materials in concrete or mortar production.

5.2.8. Conclusion

This section presents a multi-layer, passive remediation solution against marine pollution of warfare and industrial origin. The developed two-stage filtration system stands out with varying efficiencies depending on the inlet concentration in heavy metal removal. Cu removal reaches 99.91% in some locations. Phosphate removal, on the other hand, reaches more than 99% under certain conditions with the composite geopolymer containing zeolite aggregate. The results obtained for nitrogen compounds and hydrocarbons remain more limited.

It is recommended that future research concentrates on batch adsorption experiments covering microplastics and pharmaceutical residues, and adsorption isotherm, kinetic, and thermodynamic analyses under different pH conditions. Remediation solutions must be made operational directly in the field.

6. General Evaluation and Conclusion

6.1. Synthesis of Project Findings: A holistic evaluation of the pollution, risk, and remediation data obtained across the Black Sea basin.

The Black Sea SIERRA Project has brought together research institutions from Türkiye, Romania, Ukraine, Bulgaria, and Italy within a shared methodological framework. In this context, the study covering the entire Black Sea basin was carried out as a single field study with common ISO protocols, sample collection, storage and laboratory analytical procedures, and standard QA/QC practices. The data obtained from abiotic compartments, blended with tissue data from organisms at different trophic levels, show that the Black Sea basin is trapped between chronic industrial/agricultural loads and acute environmental shocks resulting from armed conflict.

Statistical approaches covering the entire basin have captured specific patterns across the region. Comparative analyses showed that the Turkish coasts had statistically significantly higher concentrations of Cu, Ni, Zn, and Fe than the other Black Sea countries included in the project. Romania, by contrast, was identified as having the cleanest coasts relatively. Among the elements studied, Cd separated itself from the other elements in terms of pattern. This increased the likelihood that Cd originated from a different source and enabled it to stand out as an independent hotspot pollutant across the basin. In water analyses, common clustering was identified as a result of highly correlated movements between Cd and TPH. This suggests a point-source pressure specific to port activities and ship traffic.

The impact of the war on the Black Sea is read through different parameters. On the Ukrainian coast, the collapse of wastewater treatment infrastructure under conflict conditions and the sudden dramatic changes in water parameters triggered by the destruction of the Kakhovka Dam stand out as important war-related findings. Sediment cores from Balık Lagoon in the Kızılırmak Delta reveal the temporal dimension. The most intense pollution profile was not localized at the surface but at a depth of 8–20 cm, and recent element concentrations showed a general downward trend. Although empirical evidence for direct war-induced element accumulation could not be obtained in this region, the potential of agricultural and industrial inputs to mask possible war signals should not be ignored. On the other hand, literature findings documented the ecological load on the basin measurably that the destruction of the Kakhovka Dam transported heavy metals to the northwestern Black Sea, increased ammonium nitrogen to 13.8 times the legal limits, and created a record burst of +1400% in cyanobacteria density.

The transfer of pollution pressure from abiotic compartments to the trophic web was mapped clearly with BAF and BSAF indices. It was determined that a high-volume macro element (Mn, Fe, Al) flux occurred from the water column towards the gills. It was also detected that sediment and water-derived highly toxic trace elements (Cd and Hg) exceeded serious bioaccumulation limits in benthic species such as *R. venosa*. The reflection of the pollution on human health showed mathematically how misleading single pollutant analyses can be. While on a Single Carcinogenic Risk (CR) basis only Cd pushed the upper limit in a single species, when the basin was viewed from a Total Carcinogenic Risk (TCR) perspective, the picture became dramatic. *A. immaculata* and *M. barbatus* species broke the unacceptable risk threshold (10^{-4}) due to multi-metal exposure. The fact that none of the examined fish species took place in the risk-free zone ($< 10^{-6}$) in a cumulative context reveals that the cumulative approach (TCR) is an indisputable necessity in risk management in the basin. In contrast, non-carcinogenic systemic risk indicators (THQ/HI) remained within safe limits across all species and locations.

Against this multi-layered pollution diagnosed by comprehensive monitoring and risk analyses, it was not settled with mere situation assessment, an innovative remediation prototype was produced as a direct intervention tool and tested in the field. The synthesized metakaolin-based and zeolite aggregate composite geopolymer filter, working as both a chemical molecular sieve and a mechanical barrier, successfully removed target heavy metals (e.g. Cu 99.91%) and phosphate (100%) from seawater. The active carbon module integrated downstream of this autonomous structure requiring no external power source transformed the system into a synergistic whole in organic matter and turbidity removal.

These obtained holistic data will be integrated with European networks such as EMODnet Chemistry and WISE Marine. All these data show the necessity of international policies exceeding national borders for the ecological restoration of the Black Sea. These evidence-based policies must be supported by common methodologies, and passive technological remediation solutions must be made operational directly in the field.

6.2. Roadmap for Future Monitoring Programs: Strategic steps to be taken in the future for sustainable marine management and combating pollution

The Black Sea SIERRA Project has revealed some multi-layered pollution profiles in the Black Sea Basin with measurable data and investigated the acute ecological destruction created by armed conflicts. Along with this, it has also determined methods for the remediation of the existing pollution, developed an innovative prototype. However, sustainable marine management is possible not only with situation assessment, but with the transformation of these obtained scientific outputs into forward-looking adaptive and proactive action plans. The strategic roadmap determined for future monitoring and remediation programs is built upon four main pillars:

6.2.1. Establishment of Technologically Integrated Adaptive Monitoring Networks

Physical access constraints and mine risks created by war conditions cause classical ship-based monitoring programs to be interrupted. Also, the issue of personnel safety is a major problem. Future programs must transition to hybrid monitoring networks where unmanned surface vehicles (USV), unmanned aerial vehicles (UAV), autonomous underwater vehicles (AUV/ROV), and high-resolution satellite data (Sentinel) are integrated. This transition is at the same time the operational equivalent of the “Do No Harm” principle protecting field personnel from active conflict and mine risks. These monitoring methods will carry personnel safety to the highest level, ensure the continuity of data flow.

These integrated monitoring systems must be usable for instant disaster scenarios as well. Autonomous sensor networks must be established at key river mouths in order to institutionalize “Rapid Response” protocols that can be immediately activated in sudden disasters such as the Kakhovka Dam destruction. Additionally, the analytical scope of the monitoring programs to be planned must not remain limited to conventional pollutants either. Conflict-derived ex-

plosive residues (e.g. TNT, RDX) and munition-derived specific heavy metals (Pb, Cd etc.) must be integrated into multi-matrix monitoring programs covering water, sediment, biota, and coastal habitats.

6.2.2. Institutionalization of the Science-Policy Bridge, Data Integration, and Capacity Building

Overcoming the fragmented data management in the basin is a critical step in the context of international environmental law and also a necessity for increasing scientific precision. The methodological harmonization (ISO methods, common QA/QC, common field studies etc...) provided in the Black Sea SIER-RA project must be made a binding standard for all future national monitoring programs. The produced abiotic, biotic, and risk assessment data must be centrally stored in the Black Sea e-Platform and Water Quality Database (WQDB) within UkrSCES. From here, they must be transferred simultaneously to European networks such as European Marine Observation and Data Network (EMODnet) and WISE Marine. This integration will guarantee the formulation of evidence-based regional policies in line with Marine Strategy Framework Directive (MSFD) and Water Framework Directive (WFD) targets and the conflict-induced ecological destruction gaining official status in EU environmental accountability mechanisms. Along with this, making investments in institutional capacity building programs and cross-border educational collaborations is mandatory in order for scientific outputs to be converted into action by policy makers and local authorities.

6.2.3. Scaling-Up of Passive Remediation Technologies in the Field (Scaling-Up)

The use of metakaolin-zeolite composite geopolymer filters proven successful at the pilot scale must be moved from laboratory and test stages to industrial field scaling. These passive treatment systems must be positioned as permanent barriers to potential hotspots such as river discharge points, port exits, and pollution accumulation zones. In the phase where the system completes its lifespan, the filter materials being convertible into concrete or mortar aggregate presents a massive circular economy potential and this situation must be evaluated as one of the main design parameters in scaling processes. Future research and development must focus on increasing the removal efficiency of the system on nitrogen compounds and petroleum hydrocarbons, and its adaptation against new generation pollutants (emerging contaminants) such as microplastics, pharmaceutical residues, and PFAS must be ensured, and focus must be placed on developing its adsorption capacity.

6.2.4. Cumulative Risk-Oriented Cross-Border Cooperation and Environmental Law

One of the most critical deductions of the project findings has also been that, despite single element concentrations appearing harmless, unacceptable thresholds (10^{-4}) are exceeded in the context of Total Carcinogenic Risk (TCR). For this reason, future environmental quality standards (EQS) and seafood consumption policies must be reorganized according to cumulative toxicological pressure parameters instead of single analyses. Additionally, new and detailed calculation methods must be developed, and synergistic effects must strictly be included in the calculation in these methods. The countries sharing the Black Sea Basin, by abandoning the reflex of acting isolated against environmental disasters, must develop a joint crisis management and ecological restoration legislation under the umbrella of BSIMAP (Black Sea Integrated Monitoring and Assessment Program).

Consequently, the re-establishment of the ecological resilience of the Black Sea will be possible not with static approaches trapped within national borders, but with the determined implementation of this integrated roadmap where science, advanced technology, and joint international political will meet at the same table.

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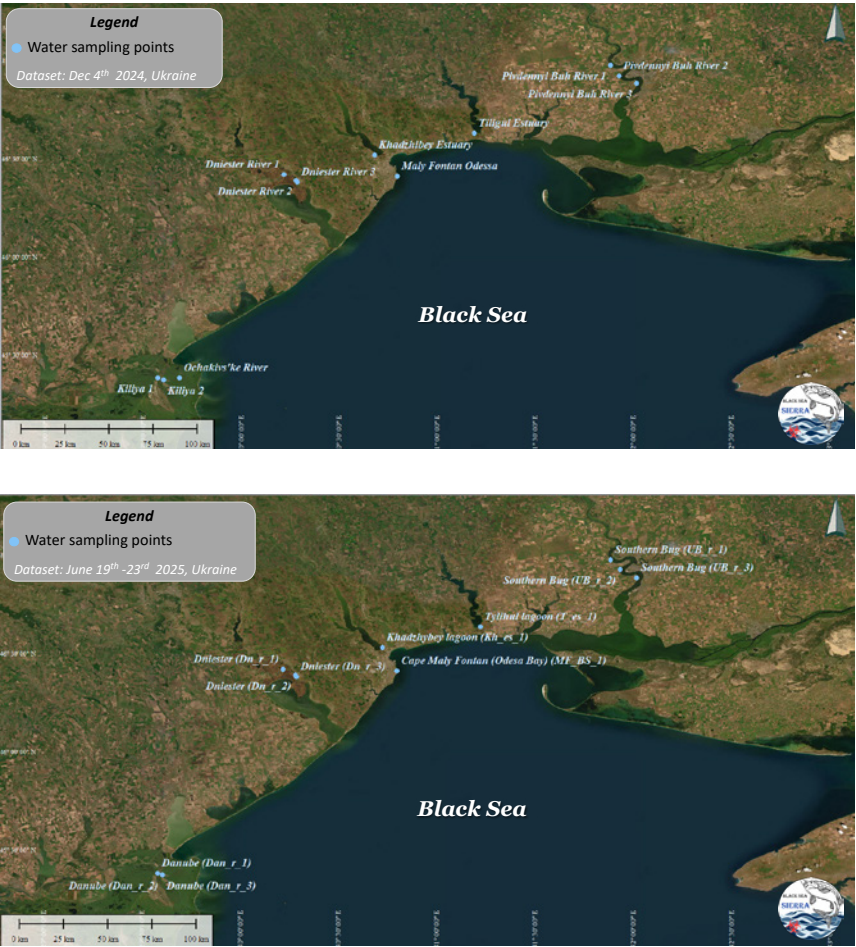
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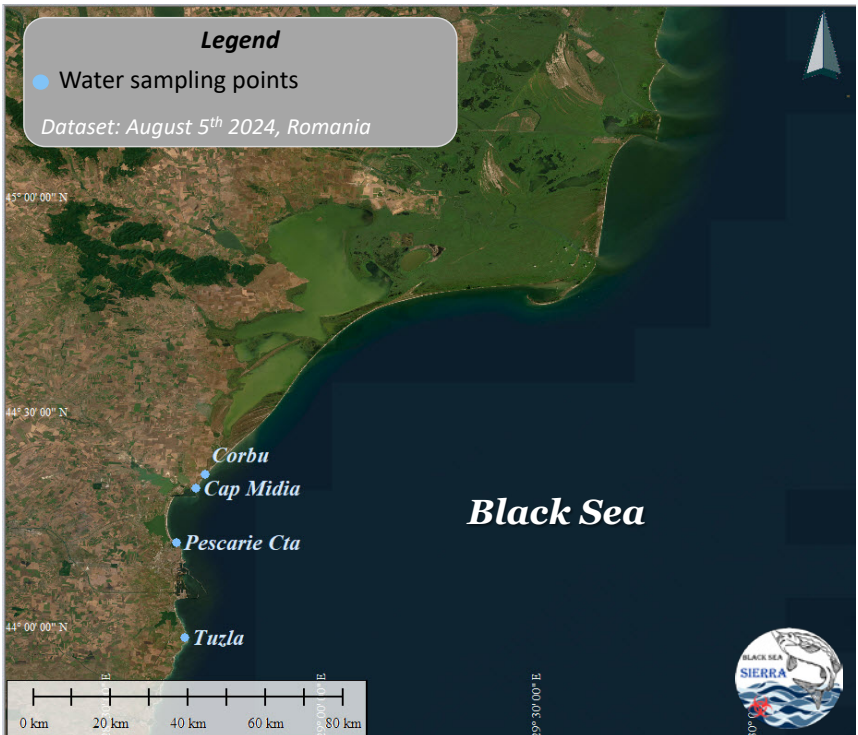
Location map Ukraine.

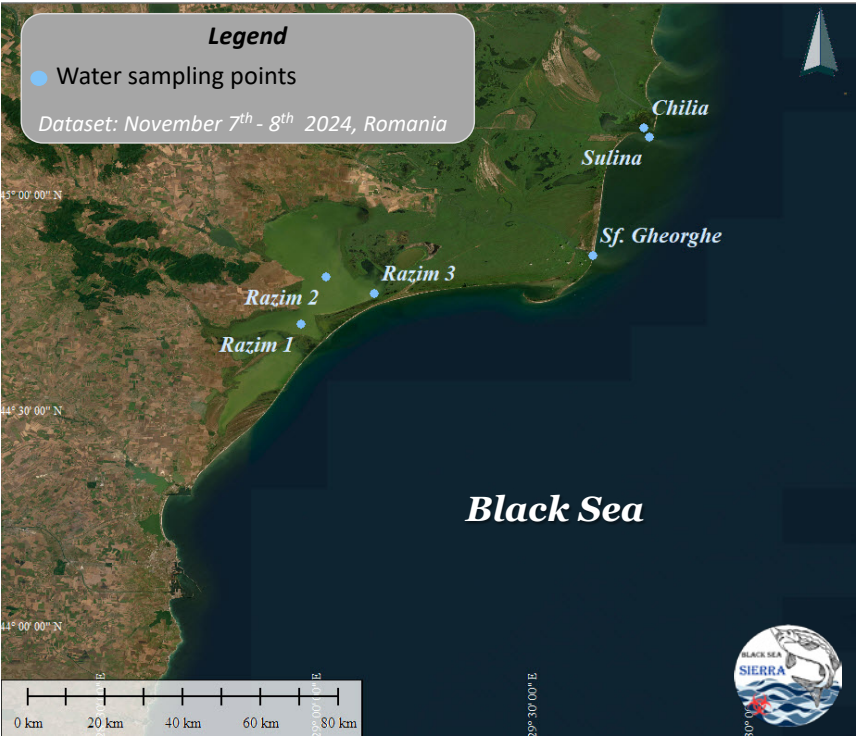


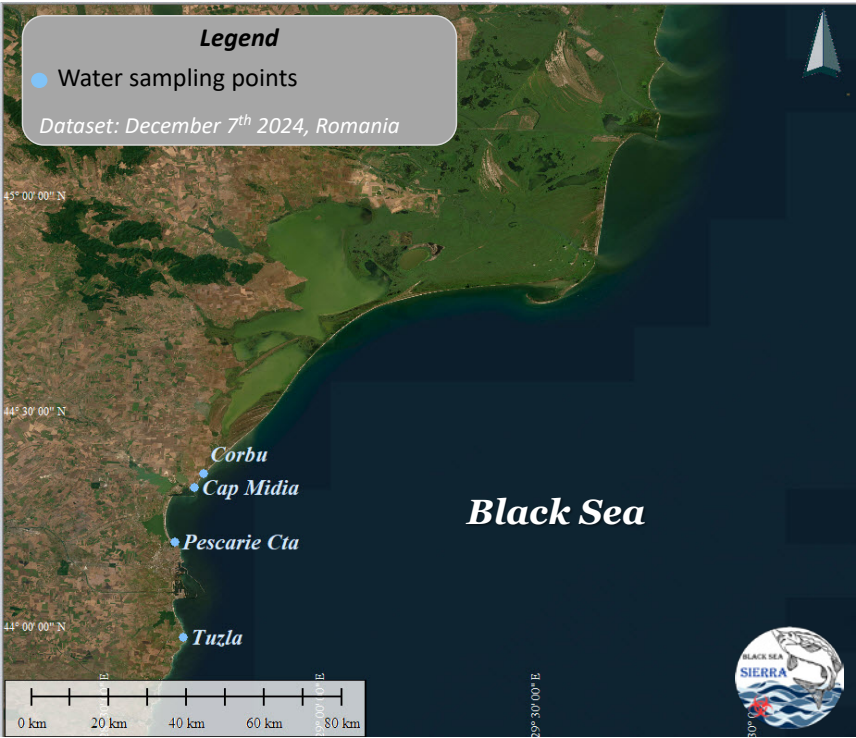


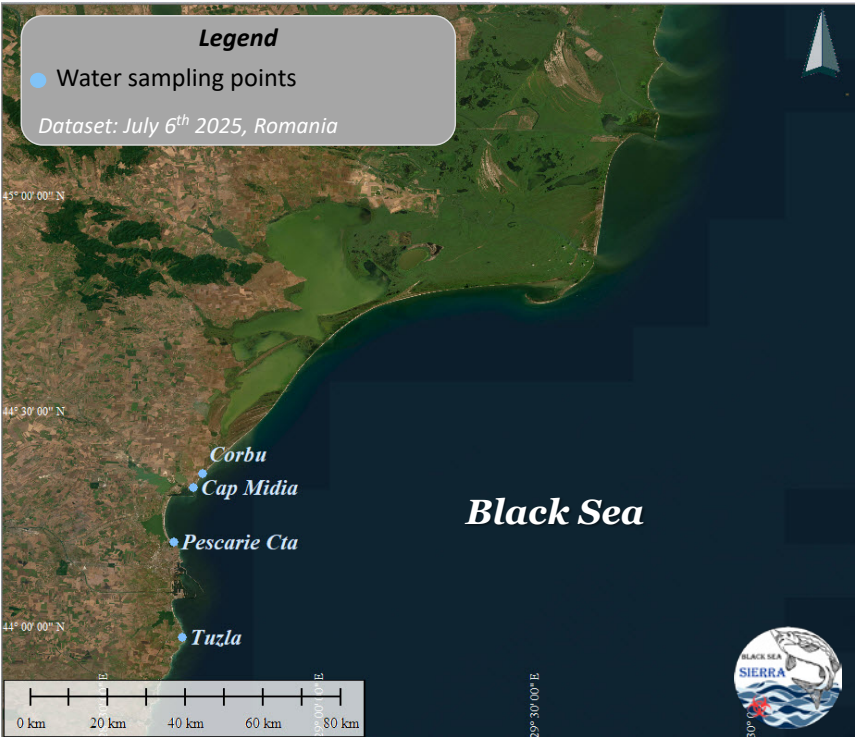
Annex Figure 2

Location map Romania.



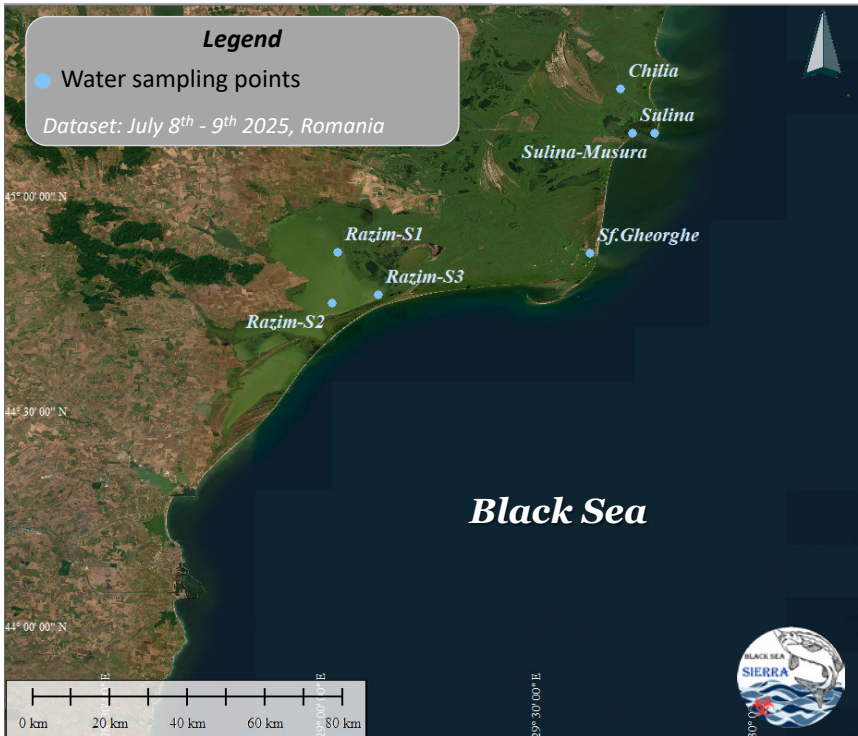


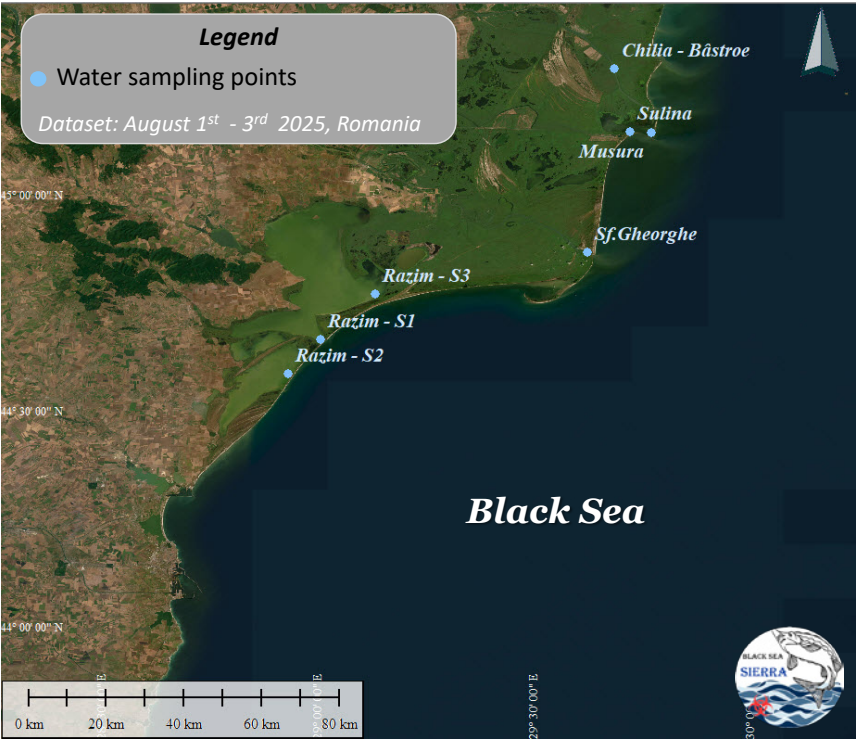


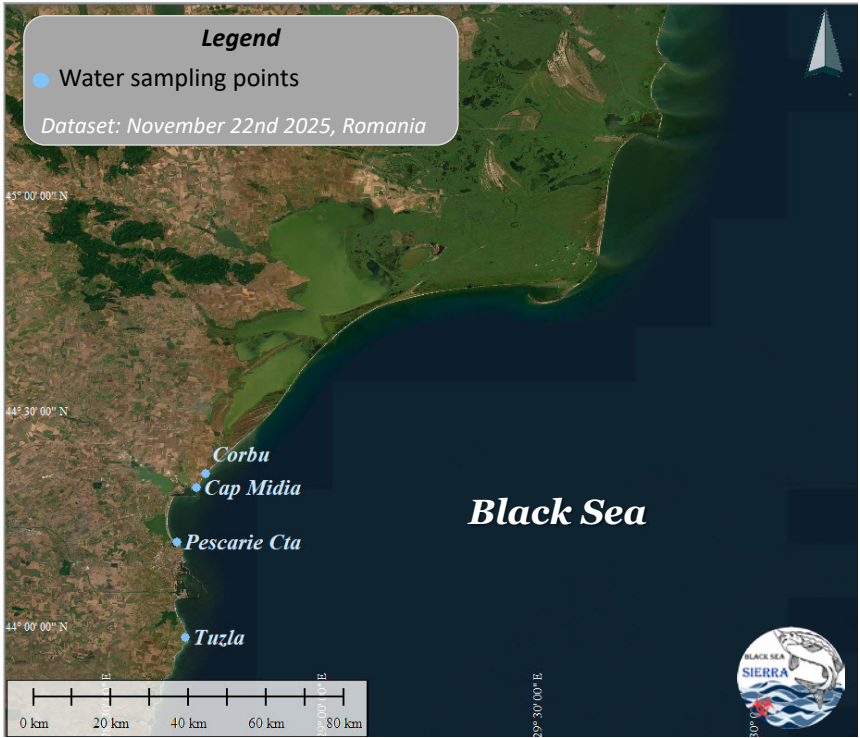


Annex Figure 3

Location map Romania (Continued).

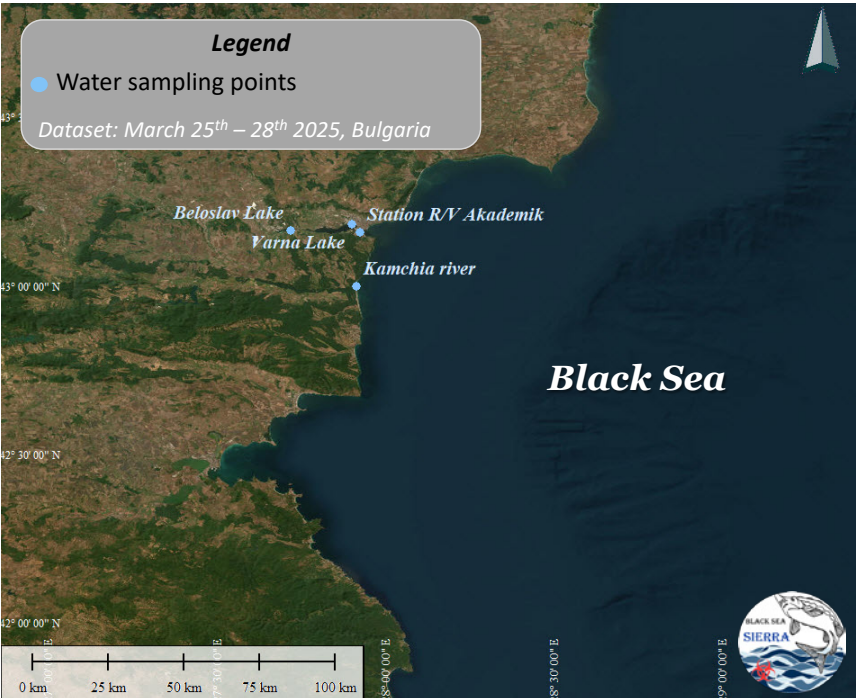


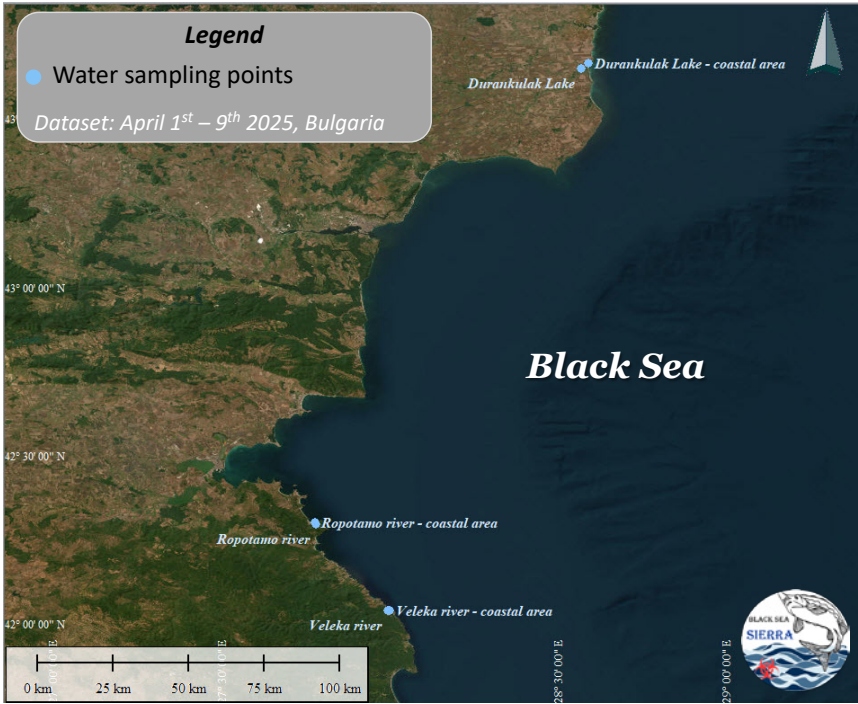


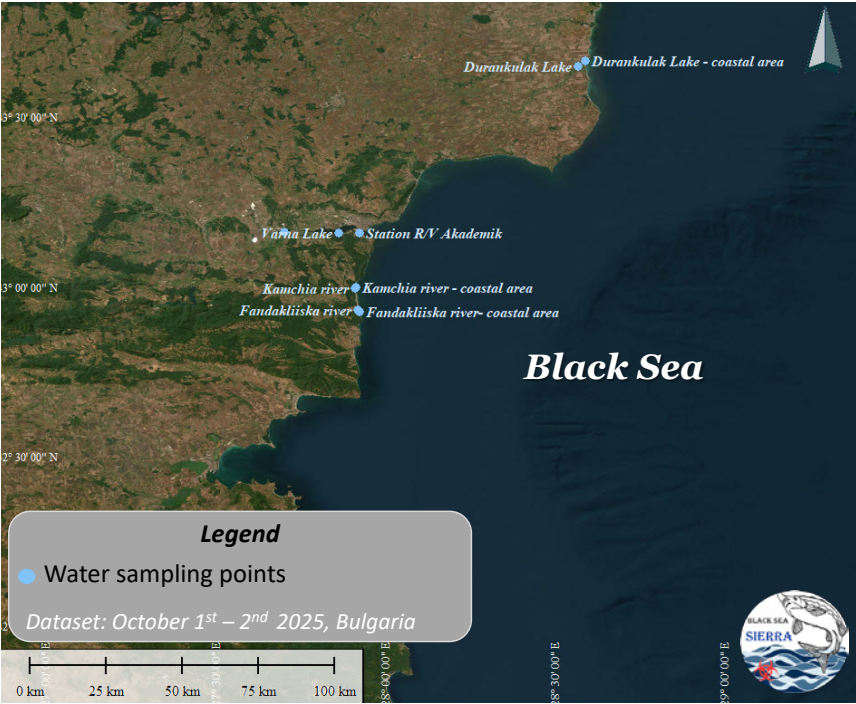


Annex Figure 4

Location map Bulgaria.

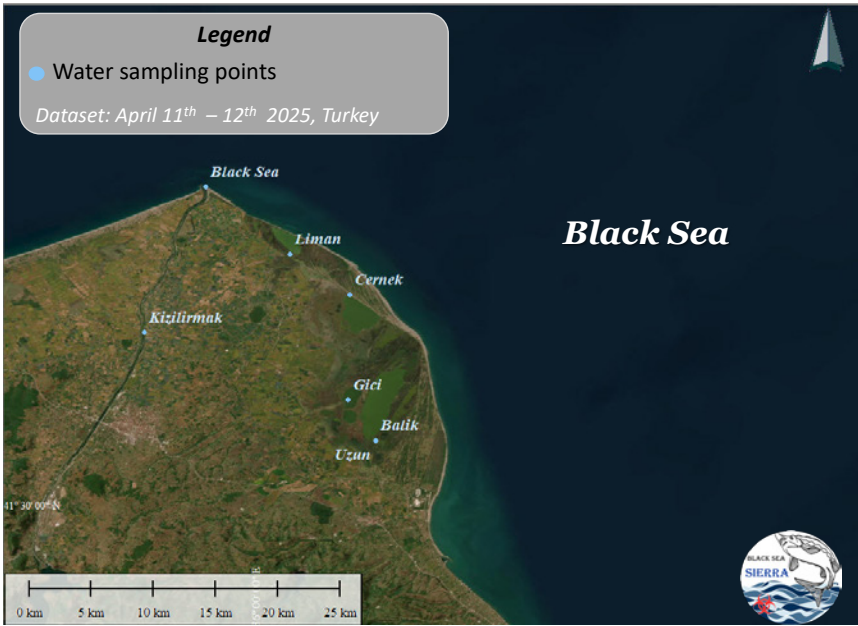






Annex Figure 5

Location map Türkiye.





Annex Table 1

Standardized data to be recorded for each sampling point.

Black Sea SIERRA project							
Collection date: DD/MM/YYYY				Collection time: HH:MM:SS			
Point location							
Latitude (N) DD.MM.SS or DD.DDDD				Longitude (E) DD.MM.SS or DD.DDDD			
Collection depth (m)							
Sample type W-water sample; S-sediment sample; B-bioaccumulation sample							
Sample ID XYDDMMYYYYAA X - Sample type (1=water sample; 2=sediment sample; 3=bioaccumulation sample) Y-Water body type (1=tributaries; 2=lagoon;3=coastal area) DDMMYYYY - date AA- sample number for the respective day from 00 to 99							

Annex Table 2

Model for sampling point information.

Black Sea SIERRA project							
Collection date:	25	09	2024	Collection time:	13	00	00
Point location							
Latitude (N)	44° 52' 48"			Longitude (E)	29° 37' 01"		
Collection depth {m}					0.5		
Sample type					W		
Sample ID					112509202404		

Annex Table 3

Recommendations for sample sizes, containers, preservation techniques, and storage times (King County Environmental Lab. 1997).

Parameter	Minimum sample size (g)	Container	Preservation technique	Storage time
Total Solids	50	Glass or polyethylene (LDPE)	Freeze (-18°C) Refrigerate (4°C)	6 months 14 days
Total Volatile Solids	50	Glass or Polyethylene (LDPE)	Freeze (-18°C) Refrigerate (4°C)	6 months 14 days
Total Organic Carbon	25	Glass or Polyethylene (LDPE)	Freeze (-18°C) Refrigerate (4°C)	6 months 14 days
Oil and Grease	100	Glass	Freeze (-18°C) Refrigerate (4°C)	6 months 28 days
Total Sulphides	50 (a 250 mL for 5 mL Zn Acetate)	Glass	Refrigerate (4°C) (+5 mL 2N sol. ZnAcetate)	7 days
Total Nitrogen	25	Glass or Polyethylene (LDPE)	Refrigerate (4°C)	28 days
Biochemical Oxygen Demand	50	Glass or Polyethylene (LDPE)	Refrigerate (4°C)	7 days
Chemical Oxygen Demand	50	Glass or Polyethylene (LDPE)	Refrigerate (4°C)	7 days
Volatile Organics	50	Glass (no headspace)	Refrigerate (4°C)	14 days
Semi volatile Organics	100	Glass	Freeze (-18°C) Refrigerate (4°C))	1 year 14 days

Note: This table outlines some general reference protocols (King County Environmental Lab. 1997) adopted for the fieldwork. Only the specific parameters relevant to the project objectives (e.g., metals, nutrients, TOC) were analyzed and reported.

Annex Table 4

Certified reference material (CRM) recovery rates and limits of detection (LOD) for all analyzed matrices (Tunca et al 2026).

Analyte	V	Sb	Al	Cr	Mn	Fe	
Unit	PPB	PPB	PPB	PPB	PPB	PPB	
Wet Season							
Alg							
IAEA 336 (Measured)	1457	73.88	695209	1065	63350	440731	
IAEA 336 (Expected)	1470	73	680000	1060	63000	430000	
Recovery Rate (%)	99.13	101.22	102.24	100.51	100.56	102.50	
LOD	0.00782	0.00726	0.5701	0.09758	0.04707	0.5894	
Tissues							
TORT-2 (Measured)	1687	77	23669	741	13572	109916	
TORT-2 (Expected)	1640	X	23500	770	13600	105000	
Recovery Rate (%)	102.87	X	100.72	96.23	99.79	104.68	
LOD	0.007517	0.01171	0.5244	0.08412	0.05308	0.5894	
Sediment							
UME EnvCRM 03 (Measured)	69109	1385	58645502	56594	515025	19121783	
UME EnvCRM 03 (Expected)	69500	1380	58864000	56400	520000	19189000	
Recovery Rate (%)	99.44	100.39	99.63	100.34	99.04	99.65	
LOD	0.01503	0.01405	1.824	0.1464	0.07011	0.8841	

	Co	Ni	Cu	Zn	As	Mo	Cd	Hg	Pb
	PPB	PPB	PPB	PPB	PPB	PPB	PPB	PPB	PPB
	277	1066	3609	30218	605	152	116	205	4905
	290	1070	3600	30400	630	153	117	200	4900
	95.72	99.66	100.26	99.40	96.06	99.88	99.89	102.59	100.11
	0.0164	0.1049	0.1055	0.3038	0.01842	0.04697	0.00171	0.00987	0.01541
	518	2506	106670	175712	21565	918	26957	267	350
	510	2500	106000	180000	21600	950	26700	270	350
	101.58	100.26	100.63	97.62	99.84	96.62	100.96	98.98	100.05
	0.01478	0.1066	0.09806	0.3232	0.02026	0.02285	0.00223	0.00808	0.01192
	41644	48843	67520	127624	70165	506	1303	322	63587
	41700	48900	67300	128500	70300	X	1290	310	63100
	99.86	99.88	100.33	99.32	99.81	X	101.03	103.92	100.77
	0.02704	0.1693	0.1998	0.5817	0.03574	0.02285	0.00223	0.00897	0.01657

Water							
UME CRM 1201 (Measured)	0	5.32	100.4	34.9	14.2	45.6	
UME CRM 1201 (Expected)	X	5.37	102	34.6	14.5	45.6	
Recovery Rate (%)	X	99.20	98.40	100.77	98.13	99.95	
LOD	0.007517	0.01405	0.5701	0.08916	0.05008	0.5423	
Dry season							
Sediment							
UME EnvCRM 03 (Measured)	68998	1361	57330318	56338	512750	19128318	
UME EnvCRM 03 (Expected)	69500	1380	58864000	56400	520000	19189000	
Recovery Rate (%)	99.28	98.65	97.39	99.89	98.61	99.68	
LOD	0.02389	0.01305	2.76	0.1039	0.07229	0.6151	
Water							
UME CRM 1201 (Measured)	<0.000	5.34	102.85	34.44	14.37	45.78	
UME CRM 1201 (Expected)	X	5.37	102	34.6	14.5	45.6	
Recovery Rate (%)	X	99.46	100.83	99.56	99.12	100.39	
LOD	0.01551	0.01283	1.455	0.0856	0.06747	0.5515	

	9.6	16.9	80.7	100.5	6.5	<0.000	3.9	0.1	14.4
	9.6	16.8	83.1	104	6.5	X	3.65	X	14.7
	99.64	100.49	97.16	96.59	99.66	X	99.17	X	97.97
	0.01463	0.09309	0.09251	0.3232	0.01916	0.02285	0.00237	0.00897	0.01454
	41233	48585	66653	127439	70040	717	1280	304	62279
	41700	48900	67300	128500	70300	X	1290	310	63100
	98.88	99.36	99.04	99.18	99.63	X	99.25	98.17	98.70
	0.0389	0.01204	0.4054	X	0.0486	0.1371	0.0024	0.00797	0.0526
	9.63	16.66	83.22	102.60	6.53	0.81	4.03	0.18	14.56
	9.6	16.8	83.1	104	6.5	X	3.65	X	14.7
	100.29	99.14	100.14	98.66	100.56	X	102.04	X	99.11
	0.02893	0.00880	0.2654	X	0.03564	0.1371	0.0032	0.0089	0.0462

Annex Table 5

Overview of the equations, classification criteria, and parameter definitions for BAF, BSAF, MPI, and human health risk assessment indices.

Indices	Formula
BAF : Bioaccumulation Factor	$BAF = \frac{C_i}{C_w}$
BSAF : Biota–Sediment Accumulation Factor	$BSAF = \frac{C_i}{C_s}$
MPI _{tissue} : Metal Pollution Index (tissue-based)	$MPI = (C_1 \times C_2 \times ... \times C_n)^{1/n}$
EDI : Estimated Daily Intake	$EDI = \frac{C \times FCR \times EF \times ED}{BW \times AT} = \frac{C \times FCR}{BW}$
THQ : Target Hazard Quotient	$THQ = \frac{C \times FCR \times EF \times ED}{RfD \times BW \times EF \times ED} = \frac{EDI}{RFD}$
HI : Hazard Index	$HI = \sum_{i=1}^n THQ_i$
CR : Carcinogenic Risk	$CR = \frac{C \times FCR \times EF \times ED \times C_{SF}}{BW \times AT} = EDI \times C_{SF}$
TCR : Total Carcinogenic Risk	$TCR = \sum_{i=1}^n CR_i$

C: Fish muscle tissue element concentration
FCR: fish consumption rate: 7.1 kg/year (AEPDI, July 2024)
EF : Exposure frequency: 365 days per year
ED: Exposure duration: 75.3 year <https://data.who.int/countries/792>
BW: Body weight: 73.7 Kg (TUIK, 2022)
AT: Total exposure time: (Exposure frequency (EF) x Exposure duration(ED)).
RfD: Reference dose factors (Öğretmen, Karsli, & Emanet, 2025; USEPA, 2024)
: Carcinogenic slope factor: (OEHHA, June 1 2009)

	Classification and Interpretation	Definitions
	BAF<1000: no bioaccumulation potential 1000<BAF<5000: bio accumulation potential BAF>5000: high bioaccumulation potential	C_i : Metal concentration C_w : Metal concentration in the water
	BSAF<1: non concentrators 1<BSAF<2: low concentrators BSAF>2: high concentrators	C_i : Metal concentration C_s : Metal concentration in sediment
	Comparative index; higher values indicate greater metal burden.	$C_{k1}...C_{kn}$: Metal concentrations in the specific tissue. n : Total number of elements.
	Assessed relative to RfD or TDI reference values.	(below the table)
	< 1: No significant risk ≥ 1: Potential non-carcinogenic risk.	EDI : Estimated Daily Intake.
	< 1: Acceptable exposure ≥ 1: Potential adverse effects.	THQ_i : Target Hazard Quotient Σ : Sum of all THQ values.
	CR< 10–6: Negligible risk 10–6< CR <10–4: Tolerable risk CR >10–4: Unacceptable carcinogenic risk	EDI : Estimated Daily Intake. C_{SF} : Cancer Slope Factor (mg/kg/day) ⁻¹ .
	TCR<10–6: Negligible 10–6<TCR<10–4: Tolerable risk >10–4: Unacceptable risk	CR_i : Carcinogenic Risk n : Number of carcinogenic metals

Annex Table 6

Pairwise Multiple Comparisons of Heavy Metal Concentrations (Cu, Ni, Pb, Zn, Fe) Between Countries.

Cu				Fe			
I	J	Mean Difference	Sig.	I	J	Mean Difference	Sig.
Ro1	Ro2	3.80783	0.198	Ro1	Ro2	3618.73795	0.194
Ro1	Tr	-36.46262*	0.000	Ro1	Tr	-24100.48643*	0.000
Ro1	Ua	-9.40759*	0.003	Ro1	Ua	-4142.61032	0.433
Ro2	Tr	-40.27045*	0.000	Ro2	Tr	-27719.22437*	0.000
Ro2	Ua	-13.21542*	0.000	Ro2	Ua	-7761.34826*	0.001
Tr	Ua	27.05503*	0.000	Tr	Ua	19957.87611*	0.000
Ni				Zn			
Ro1	Ro2	9.98249*	0.003	Ro1	Ro2	17.56733*	0.003
Ro1	Tr	-92.83667*	0.000	Ro1	Tr	-65.71143*	0.000
Ro1	Ua	-3.96283	0.984	Ro1	Ua	-9.89535	0.637
Ro2	Tr	-102.81915*	0.000	Ro2	Tr	-83.27876*	0.000
Ro2	Ua	-13.94531	0.069	Ro2	Ua	-27.46268*	0.000
Tr	Ua	88.87384*	0.000	Tr	Ua	55.81608*	0.000
Pb							
Ro1	Ro2	2.46207*	0.044				
Ro1	Tr	-64.43336	0.578				
Ro1	Ua	-3.96590*	0.042				
Ro2	Tr	-66.89543	0.538				
Ro2	Ua	-6.42797*	0.000				
Tr	Ua	60.46746	0.642				

*The mean difference is significant at the 0.05 level.

Annex Table 7

Priority Contaminant Groups and Analytical Methods.

Contaminant	Main Components	Analytical Method
Petroleum hydrocarbons and Lubricants	TPH (Total Petroleum Hydrocarbons), PAH (Polycyclic Aromatic Hydrocarbons), lubricants originated from vessel and port infrastructure destruction	Standard MSFD/WFD suite
Heavy metals and Trace elements	Pb, Cu, Zn, Ni, Cd, Hg, As	ICP-MS / ICP-OES (EPA)
Explosives & energetic compounds	TNT, RDX, HMX, nitroaromatics, nitramines and military munitions residues	HPLC/LC-MS (EPA)
POPs and Agricultural Contaminants	PCBs (Polychlorinated Biphenyls), organochlorine pesticides, industrial chemicals and flood-induced agricultural contaminants	Standard MSFD/ Stockholm suite
Debris, Plastic & Combustion Products	Macro-debris, micro/mesoplastics, combustion products released directly into the sea by bombardment and missile attacks	Physical counting / Imaging techniques

Annex Table 8

Matrices and Sampling Frequency.

Matrix	Rationale	Routine Frequency	Post-Event
Water column	Acute contaminant distribution, river plume, oil spills, short-lived chemical and nutrient pulses	Seasonal–monthly	Intensified campaign (Rapid response)
Bottom sediment	Long-term archive (sink); heavy metals, POPs, explosive residues and collapsed macro-debris	3–6 years (WFD/MSFD/OSPAR CEMP)	Corings (Core sampling) + intensified sampling
Biota	Bioaccumulation and biomagnification monitoring; region-specific bivalves (mussels) + demersal (bottom) fish	Annual–biennial	Additional campaign in critical areas
Coastal habitats and beaches	First recipient line; tarballs, plastic/macro-debris accumulations, river transport plumes	Event-driven + periodic	According to cleanup priorities and risk ranking

