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Abbreviations

Abbreviation	Description
BQE	Biological Quality Elements
COD	Chemical Oxygen Demand
EQR	Ecological Quality Ratio
ERL	Effects Range Low
ERM	Effects Range Median
HCMR	Hellenic Centre for Marine Research
HES	Hellenic Evaluation Score
HTCO	High Temperature Catalytic Oxidation
IBI	Index of Biotic Integrity
ICES	International Council for the Exploration of the Sea
NCS	Nutrient Classification System
WCO	Wet Chemical Oxidation
WFD	Water Framework Directive

External cooperation

This document was drafted with the cooperation of:

- HCMR

ANNEX 8 D ECOLOGICAL STATUS OF MAIN INLAND WATER BODIES (INCL. ABIOTIC AND BIOTIC CHARACTERISTICS)

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8 D.2 OBJECT OF CONTRACTOR WORK

For the needs of the Environmental Impact Study of the EAST MED Project, a study for recording the terrestrial water fauna in specific proposed river sites is required.

Specifically, the project concerns the sampling and analysis of surface waters and river sediments, to assess the chemical/physicochemical condition as well as the hydromorphological and hydrobiological conditions, at the most significant points of intersection between the East Med pipeline and surface water bodies.

The sampling stations are located at the Peloponnese and Western Greece (Table D-1).

Table D-1 Surface water bodies where sampling stations are located.

Sequence number	CODE	NAME	LAT WGS84	LONG WGS84
1	EL0331R000700004N	MARIOREMA R._4	36.925070°	22.717398°
2	EL0333R000211040N	EVROTAS R. 11	37.117692°	22.413417°
3	EL0129R000221056N	ALFIOS R. 12	37.355609°	22.156010°
4	EL0228R000204007N	LADON PINIAIOS R. 2	37.807095°	21.559288°
5	EL0228R000203009N	PINIOS R. 4	37.914911°	21.548539°
6	EL0420R000201069N	EVINOS R. 1	38.340426°	21.531663°
7	EL0415R000202007H	ENOTIKI TAFROS	38.551190°	21.435545°
8	EL0415R000200011H	ACHELOOS R. 5	38.642749°	21.301891°
9	EL0514R000100048N	DIPOTAMOS R.	39.068784°	21.071405°
10	EL0514R000201050N	ARACHTHOS R. 1	39.082830°	21.022853°

Sequence number	CODE	NAME	LAT WGS84	LONG WGS84
11	EL0546R000201077N	LOUROS R.	39.158706°	20.800802°
12	EL0513R000202044N	ACHERON R. (MAVROPOTAMOS) – TRIBUTARY KOKTOS (VOUVOS)	39.261166°	20.546773°

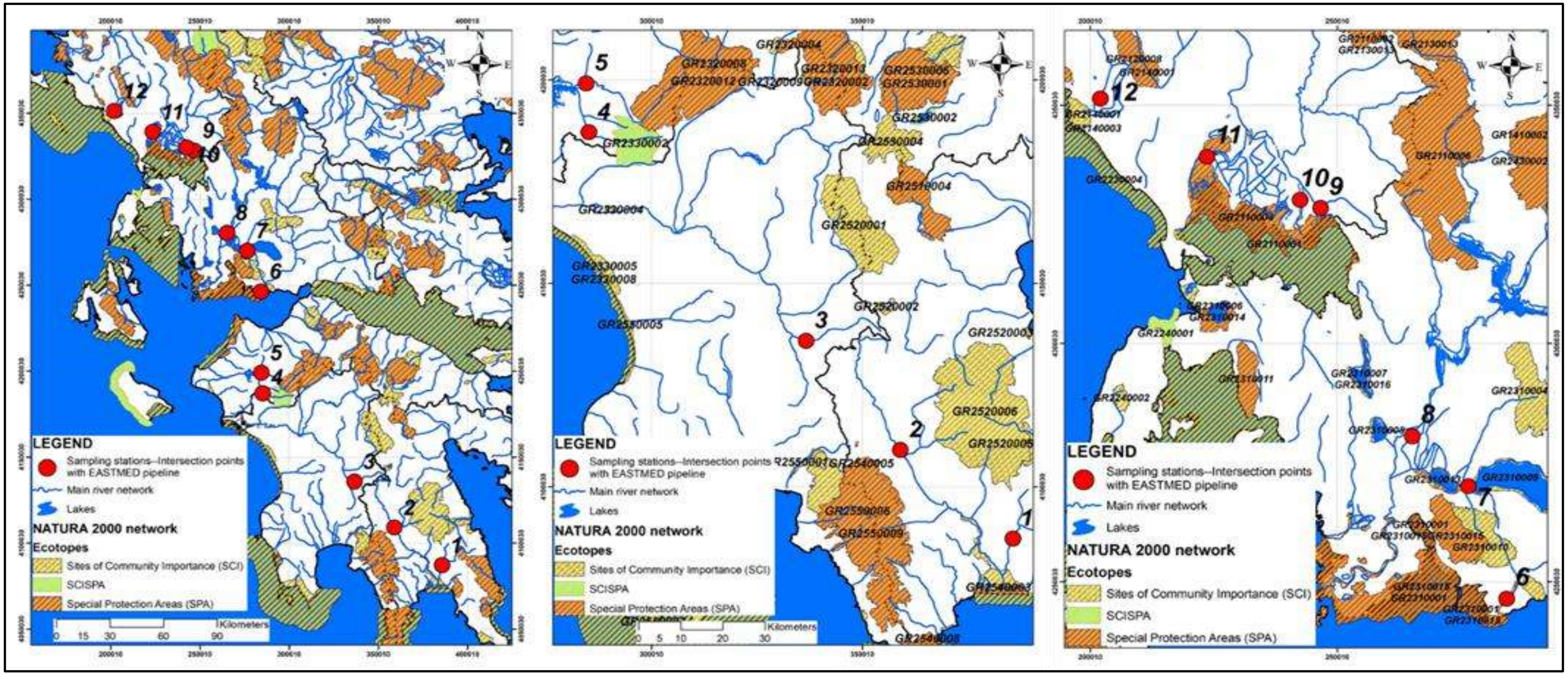

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Figure D-1 Illustration map of sampling stations / River intersections with the EASTMED pipeline. (The numbers coincide with the stations shown in Table D-1)

The sampling, analysis and treatment of all water quality parameters was carried out in accordance with the specifications and requirements of the Water Framework Directive (WFD, EU / 2000/60). More specifically, the following tasks were realized.

8 D.2.1 Surface waters

Surface water sampling has been performed at the crossing areas of the onshore section of East Med pipeline with the relevant water bodies. A total of 24 water samples was collected, one sample from the intersection point and one downstream of the crossing for each water body. The following physicochemical parameters were measured and/or determined in the laboratory:

- Temperature
- PH
- Conductivity (μS/cm)
- Dissolved Oxygen (mg/l)
- Turbidity (NTU)
- BOD5 (mg/l)
- COD (mg/l)
- TDS (mg/l)
- TSS (mg/l)
- N-NO3 (μg/l)
- N-NH4 (μg/l)
- N-NO2 (μg/l)
- Ptotal (μg/l)
- TOC (μg/l)
- Total hydrocarbons comp. as n-hexane (μg/l)
- SF cfu/100ml

8 D.2.2 Sediments

The sediment sampling was carried out in sites (Table D-1) that present obvious signs of pollution (strong presence of algae, unpleasant odor, dark color of sediment, presence of foam, rubbish). A total number of 12 samples were collected, one for each intersection point. The following sediment properties were determined:

- AOX (mg/kg)

- PAH-s (μg/kg)
- Nitrogen total (weight %)
- Phosphorus total (mg/kg)
- TOC (weight %)
- Metals (mg/kg): As, Cd, Cr total, Cr VI, Hg, Ni, Pb, Cu, Se, Zn
- Heavy HCs c12-c40 (mg/kg)
- Light HCs < C12 (mg/kg)
- PCB total (μg/kg)
- Granulometry

8 D.2.3 Benthic macroinvertebrates

There are several biological quality elements (BQEs) available for monitoring and assessment of the quality of running waters. Several methods have been proposed but one BQE that is most commonly used and recommended throughout Europe is based on the use of benthic macroinvertebrates. Understanding the changes that occur in macroinvertebrate communities in relation to pollution is a key issue for impact assessment and forms the basis of many biomonitoring methodologies in aquatic environments. Different groups of macroinvertebrates have a different behavior with respect to alterations of water quality and thus lists of tolerant or non-tolerant species can be found. Therefore, macroinvertebrates can be used as bioindicators in order to assess any change in freshwater quality due to pollution.

Biological indices for the assessment of water quality offer great advantages over other organisms, since macroinvertebrates are easy to sample, good identification keys are available for most orders and for many orders there is sufficient information regarding pollution tolerance. In addition, many are sedentary so they are less able to escape the effects of sediment and other pollutants that diminish water quality and hence assist in detecting the precise location of pollutant sources. Some of them have relatively long-life histories, thus allowing detection of past pollution events such as fertilizer spills and illegal dumping and providing a facility for examining both temporal changes and prolonged or variable exposure to pollutant concentrations. Finally, and perhaps the most useful feature of this group of animals, is that many methods of data analysis, including pollution indices and diversity indices have been formulated.

8 D.2.4 Benthic diatoms

The hydrobiological study complies with the provisions of the EU Water Directive (WFD) regarding water quality assessment. Benthic diatoms are widely used as indicators for assessing the quality of river waters. They are an important biological element of freshwater ecosystems that responds to rapid environmental changes. In particular, benthic diatoms are considered an excellent indicator of water quality that measures the effects of pollution on rivers. In this study, benthic diatoms were used to assess the effects of nutrient and organic pollution on the ecology of the under-study stations, since there is a developed methodology and relevant indicators for assessing the ecological status of this biological element.

8 D.2.5 Icthyofauna

The major stressor in lowland Greek riverine systems are the excessive water abstractions usually for agricultural or other uses. The selected biological element of the used index for the purposes of this specific study is fish since it is considered to be the most appropriate to identify any diversions from the reference conditions caused by the dominant stresses impacting the Greek rivers. In this work the index that is applied is the Hellenic Fish Index- HeFI (Zogaris et al. 2018), this index has been intercallibrated with other EU indices and is now the official index used in Greece for the application of the WFD. This index is based on using a standardized sampling effort per river site. Briefly, a stream portion of about 100 m (ranging from 50 to 200 m) was sampled, a single electrofishing pass was conducted and no stop nets were used. In streams and small rivers, the entire river channel was surveyed.

8 D.2.6 Hydromorphology

Hydro morphology has been assessed through visual estimation and measurement of hydromorphological features such as channel width, flow type, river bed substrate and bank substrate. The 'QBR' index (Munné et al. 2003) has been used for this purpose and the study is supported with photographic material

8 D.3 METHODS

8 D.3.1 Surface waters analyses

8 D.3.1.1 Introduction

In this study, field measurements of physicochemical, hydrologic, chemical and microbiological parameters were carried out from a network of 12 sampling stations covering water bodies that will be crossed by the onshore section of the East Med pipeline (Table D-1). The selection of the sampling stations was driven by the objective of conducting precise assessments of physicochemical, hydromorphological and hydrobiological conditions in the most important water bodies that will be crossed by the Onshore section of the East Med pipeline. During field samplings, water physicochemistry (pH, electrical conductivity, dissolved oxygen, total dissolved solids - TDS, temperature and turbidity) was measured in situ with a portable multi-parameter probe Horiba U-50 Multiparameter Water Quality Checker. Prior the measurements, the probe is calibrated according to the scientific standards. Water samples were collected from the same sites for the analytical determination of nitrates (NO_3^-), nitrites (NO_2^-), ammonium (NH_4^+), phosphates ($P\text{-}PO_4^{3-}$), total phosphorus (P total), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total coliforms, total organic carbon (TOC) and total hydrocarbons.

8 D.3.1.2 Nutrients in surface waters

For the determination of nitrates ($N\text{-}NO_3^-$), nitrites ($N\text{-}NO_2^-$), ammonium ions ($N\text{-}NH_4^+$), phosphates ($P\text{-}PO_4^{3-}$) and total phosphorus (P total) concentration, water samples were collected in polyethylene bottles that were pre-cleaned with 10% HCl solution for 24h. Samples were preserved with the addition of mercury chloride solution 1% (1ml/L of sample), stored in coolers at 4 ^oC and transported to the laboratory as soon as possible. Nitrates, nitrites, ammonium ions and phosphates were analyzed at Hydrochemistry Laboratory in HCMR with a Skalar automated continuous flow analyzer according to the following analytical standards:

Skalar continuous flow analyzer: Roger Kerouel & Alain Aminot (1997) for ammonium ions, Standard Methods for Examination of water and waste water, 15th edition 1980 APHA-WPCF pages 410-425 and Boltz & Mellon (1948) for phosphates, Methods for chemical analysis of water and wastes, EPA 1983, Standard Methods for the determination of water and waste water, 17th edition, 1989 & 15th edition 1980 and Navone (1964) for nitrate and nitrites.

Total phosphorus was determined according to the wet chemical oxidation method (WCO) described by Raimbault et al. (1999). For each sample, 40 ml of water are placed in 50 ml Pyrex vials (Duran Schott) with screw cap. The vials are pre-cleaned with solution HCl 10% for 24h, following oxidation for the removal of any organic residue. Right before the sampling the content of the vials is discarded and the vials are washed with the sampled water. This methodology achieves the oxidation of phosphorus compounds to inorganic phosphates enabling their quantification with the use of an automated analyzer SKALAR as described in the abovementioned method for the determination of phosphates.

The detection limits are 1μg/L for nitrites (N-NO_2^-), 2μg/L for nitrates (N-NO_3^-), 1 μg/L for phosphates (P-PO_4^{3-}) and 5 μg/L for ammonium ions (N-NH_4^+). Results beyond detection limits are referred to as <LOQ.

8 D.3.1.2.1 Physicochemical quality

The physicochemical water quality was assessed with the Nutrient Classification System (NCS) (Skoulikidis et al., 2006), modified to include the dissolved oxygen concentration parameter (Cardoso et al., 2001). According to this method the sites are classified into five quality classes (High, Good, Moderate, Poor, Bad) depending on the concentration of nitrates, nitrites, ammonium ions and phosphates (Table 2). The quality classification based on the dissolved oxygen concentration followed the boundaries listed in Table D- 3. The final physicochemical quality class for each site results from the average of all individual quality classes (including the dissolved oxygen) that are scored from 1 (for Bad quality class) to 5 (for High quality class) (Table D-2).

Table D-2 Classification scheme based on the concentrations of nutrients according to the Nutrient Classification System (NCS) (Skoulikidis et al., 2006).

		High	Good	Moderate	Poor	Bad
N-NO_3^-	μg/l	< 220	220-600	601 -1300	1301-1800	> 1800
N-NH_4^+	μg/l	< 24	24-60	61-200	201-500	>500
N-NO_2^-	μg/l	< 3	3–8	8.1–30	31-70	> 70
P-PO_4^3	μg/l	<70	70-105	106-165	166-340	> 340
TP	μg/l	<125	125-165	166-220	221-405	> 405

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Table D- 3 Quality classes based on dissolved oxygen according to the Norwegian classification (Cardoso et al., 2001)

	High	Good	Moderate	Poor	Bad
Dissolved oxygen (mg/l)	> 9	9–6.4	6.4-4	4-2	< 2

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8 D.3.1.3 Biochemical Oxygen Demand (BOD5)

Water samples were collected in polyethylene bottles that were pre-cleaned with HCl 10% solution for 24 h. Samples were maintained in a cooler (4 ^{o}C) and shipped to the laboratory within 24 h. The biochemical oxygen demand (BOD_5) was determined with the WTW OxiTop System as follows: Water sample was placed in dark bottles containing a small magnet. The bottles are sealed with electronic caps and are placed in incubator (at 20 ^{o}C), under continuous stirring, for 5 days. The electronic caps record changes in pressure due to oxygen consumption that are determined as mg/l of BOD. The classification into five (5) quality classes follows the following scheme (Table D-4):

Table D-4 Quality classes based on BOD5 according to the scheme of Naddeo et al. (2007)

	High	Good	Moderate	Poor	Bad
BOD_5 (mg/l)	< 2.5	< 4.0	< 8.0	< 15.0	> 15.0

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8 D.3.1.4 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) was determined according to the APHA 5220B standard method. In this method the oxidation of the organic matter is achieved with a boiling mixture of potassium dichromate and sulfuric acid. After oxidation the remaining potassium dichromate that has not been consumed is titrated with ammonium iron sulfate to determine the amount of dichromate consumed during oxidation. The amount of organic matter is calculated as the oxygen equivalent consumed in the oxidation.

8 D.3.1.5 Total Suspended Solids (TSS)

The determination of suspended solids in surface water samples was performed as described in the USA specification analysis of water and wastewater (APHA, 1992). The suspended solids are all the particles retained on a standard glass fiber filter and remain when the filter has dried at 105 oC. The procedure is as follows: filter with pore diameter 0,45 μm is placed in a tray of silver foil, dried for about 1.5 hours and weighed on an analytical scale of 0.1 mg precision. Then a 1L volume sample was filtered and after filtration it was transferred again to the aluminum foil and dried again for about 12 hours. Then it was allowed to cool in a desiccator and weighed. The drying cycle is repeated until the difference is not greater than 0.5 mg.

8 D.3.1.6 *Total Organic Carbon (TOC) in surface waters*

For the sampling, 40 ml glass bottles with teflon caps were used which had previously been thoroughly cleaned with acid (stay in 10% HCl for at least 12 hours) and Milli-Q deionized water. Immediately after sampling, 100 μL of HCl 2N acid was added to each bottle and the samples were refrigerated until counted. The analysis of the samples for the determination of the concentration of soluble organic carbon is carried out using the Organic Carbon Analyzer, Shimadzu TOC-L. The method used is high temperature catalytic oxidation (HTCO) (Sugimura and Suzuki, 1988) and the accuracy and precision of the analyzes are checked using standard reference samples (DOC-CRM program, University of Miami, D.A. Hansell).

8 D.3.1.7 *Total hydrocarbons in surface waters*

Water samples (2.5 L) were extracted with n-hexane and the extracts were fractionated on an activated silica. The final determination was performed by gas chromatography - flame ionization detector (Agilent 7890A GC). The quantification was based on a standard mixture of n-alkanes.

8 D.3.1.8 *Total Coliform*

For the determination of Total coliforms, the membrane filtration ISO 9308-1 method has been chosen. Through this method, 100 ml of sample were filtered through a 0.45 μm membrane then placed on lactose agar plate supplemented with Tergitol-7 and 0.5% TTC (2,3,5 triphenyltetrazolium chloride), and cultured at 36°C and 44°C. Only colonies producing yellow colour on medium were counted as coliform.

8 D.3.2 Sediment analyses

8 D.3.2.1 *Total Phosphorus (TP) and Total Nitrogen (TN) in sediments*

Total nitrogen and total phosphorus were determined using a variant of the Valderama method. Valderama (1981) method is based on the oxidation of all forms of nitrogen and phosphorus to nitrates and phosphates respectively with the use of potassium persulphate, as a strong oxidant, under intense conditions of temperature and pressure. The required pH change conditions for the completion of the reactions (start at pH 9.7 and completion at pH 5-6) are achieved using a boric acid-sodium hydroxide system.

120 ml glass bottles with a Teflon-lined screw cap were used for the determination. Because oxidation requires a temperature of 110 - 115 ° C and a pressure of 1.55 atm, an autoclave was used. The samples were weighed into glass flasks followed by double digestion in the autoclave, first with oxidant A (NaOH, H_3BO_3, K_2SO_8) for half an hour and then another half with oxidant B (K_2SO_8, H_2O). autoclave, filtrate or centrifuge and collect the filtrate or supernatant in a vial pre-diluted with HCl.

Quantities of the solution are then used to measure total nitrogen as nitrates and total phosphorus as phosphates by the following photometric techniques.

Nitrates: The nitrates in the samples are reacted with a mixed reagent containing sulfanilamide, naphthyl-ethylenediamine and VCl_3. The color development process is as follows: in a 15 mL centrifuge tube transfer 3 mL of standard or sample and 2 mL of mixed reagent, mix and mix in a water bath at 60 ° C for one hour, and photometry at 543 nm. The sediment samples require dilution 10 times (ie 0.3 mL sample and dilution up to 3 mL in the centrifuge tube).

Quantification limit 3 mg / kg.

Phosphates: The quantification was done by the photometric method of determination of phosphate ions based on their reaction with molybdenum ions (eg MoO_4^{2-}, $Mo_7O_{24}^{6-}$, $Mo_4O_{13}^{2-}$) in an acidic environment to form a phosphoromolybdate complex such as e.g. $(NH_4)_3[PMo_{12}O_{40}]$, which has a bright yellow color. This is followed by the reduction of phosphoromolybdate ions from ascorbic acid, in the presence of Sb^{3+} ions, to form a brightly colored, unknown form of product known as "molybdenum blue". The absorbance of the solution containing the molybdenum blue is measured on a UV-Vis spectrophotometer at 880 nm. The content of PO_4^{3-} phosphate ion of the sample is calculated from the absorbance value at 880 nm by means of a reference curve made with standard PO_4^{3-} solutions.

Quantification limit 3 mg / kg.

The VARIAN CARY 1E spectrophotometer was used for the determination of total nitrogen and phosphorus (Junco et al., 1983; Ladakis et al., 2003; Valderama 1981; Schnetger et al., 2014; Doane and Horwath, 2003).

There are no quality standards for total phosphorus and total nitrogen in sediments.

8 D.3.2.2 Total Organic Carbon (TOC) in sediments

Organic carbon in the sediments (in the sludge fraction) was determined by the Walkey-Black method as modified by Gaudette et al. (1974) for the determination of organic carbon in marine and lake sediments. The method is based on the oxidation of organic matter with $K_2Cr_2O_7$ in the presence of

H_2SO_4 and the back-counting of excess dichromate ions with a standard ammonium divalent iron solution, using a ferroin index to determine the equivalent point (Gaudette et al., 1974).

The limit of quantification of the method is 0.84g / kg TOC.

8 D.3.2.3 Total Hydrocarbons in sediments

8 D.3.2.3.1 Introduction

Aliphatic and polycyclic aromatic hydrocarbons constitute important classes of organic contaminants that may cause degradation and a risk of serious damage in the marine environment. Aliphatic hydrocarbons are major components of petroleum products (Wang et al., 1999), but they may also derive from natural sources such as terrestrial plants and marine algae. Polycyclic aromatic hydrocarbons (PAHs) have been classified as priority pollutants by international environmental agencies (EEA-EU, EPA-US), since certain homologues are highly carcinogenic, mutagenic and bioaccumulative for aquatic organisms. PAH can originate from three different sources (Latimer and Zheng, 2003): from the incomplete combustion of organic matter (pyrolytic origin), from the release of petroleum and its products (petrogenic origin) and the transformation of biogenic precursors (biogenic origin) (Yunker and Macdonald, 2003). Hydrocarbons are hydrophobic compounds with very low solubility in seawater and therefore, their biogeochemical cycling is controlled by their high affinity to marine particles, resulting in their downward transport through the water column and final accumulation in sediments (Prahl and Carpenter, 1979; Dachs et al., 2002).

8 D.3.2.3.2 Methodology

For analysis of aliphatic hydrocarbons C12-C40 and PAHs, 3 g of dried sediment were Soxhlet extracted with a mixture of methanol and dichloromethane. The extract was saponified with methanolic KOH, the unsaponified components were extracted with n-hexane and cleaned-up and fractionated by passing through a silica column. The final determination of both aliphatic hydrocarbons and PAH were performed by gas chromatography - mass spectrometry (Agilent 7890GC-5975MS)

For analysis of volatile hydrocarbons (<C12) the samples were collected in special headspace vials, and the determination was performed by gas chromatography - mass spectrometry (Agilent 7890GC-5975MS)

8 D.3.2.4 Polychlorinated Biphenyls (PCBs) in sediment samples

8 D.3.2.4.1 Introduction

Synthetic organochlorines such as polychlorinated biphenyls (PCBs) are widespread organic contaminants characterized by high resistance to degradation and high bioaccumulative capability and considered as hazardous to human and/or environmental health. The manufacture and use of these compounds have been banned since the mid-1970s, but as a consequence of their high persistence, they continue to be detected in the environment.

8 D.3.2.4.2 Methodology

5 g of dried sediment were Soxhlet extracted with dichloromethane. The extract was desulphurized using activated copper and cleaned-up by passing through an aluminum oxide column. The final determination was carried out by gas chromatography with an electron capture detector (Agilent 7890A GC). The following individual compounds were quantified: CB28, CB52, CB101, CB105, CB118, CB128, CB138, CB153, CB156, CB170, CB180, CB183, CB194.

8 D.3.2.5 Heavy metals in sediments

The samples were mixed for the best possible homogenization and then samples were taken. From the liquid samples a sample was collected which was kept in the refrigerator in a liquid state for the determination of hexavalent chromium [Cr (VI)].

A sample was also collected and placed in the freezer for further pre-treatment and determination of all other parameters.

8 D.3.2.5.1 Pre-treatment of samples

The liquid samples for the determination of hexavalent chromium were not further treated prior to analysis.

The frozen samples were then lyophilized to remove moisture.

Initial drying - Lyophilization

Depending on the texture and the time from sampling the coexisting water in the storage container can range from 10 -50%. In order not to disturb the concentrations of volatile chemicals, it is preferable to freeze them and then subject them to lyophilization, ie sublimation of ice water at a temperature lower than -40^{o}C and in a vacuum of 133 x 10-3 mbar).

The sediment samples for the present study were subjected to the lyophilization method described above in a LabConco lyophilizer for approximately 48 hours to remove sediment moisture and to perform further analyzes.

Sieving

The samples were passed through a sieve with holes 1mm in diameter in order to remove pebbles, shells of organisms and other solids such as e.g. roots.

Determination of humidity to express the results on dry weight

An amount of both the liquid samples (for hexavalent chromium) and the lyophilized samples (for the remaining parameters) is subjected to oven drying to quantify the moisture content to express the results of all chemical assays on dry weight.

The humidity determination procedure is as follows:

Humidity determination was based on ISO 11465: 1993.

- The moisture-proof glass capsules were placed in an oven at 105 ± 5 ° C for 1 hour.
- The capsules were removed from the oven and placed in a dryer to bring to room temperature for 45 minutes.

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Figure D-2 Moisture caps in the oven

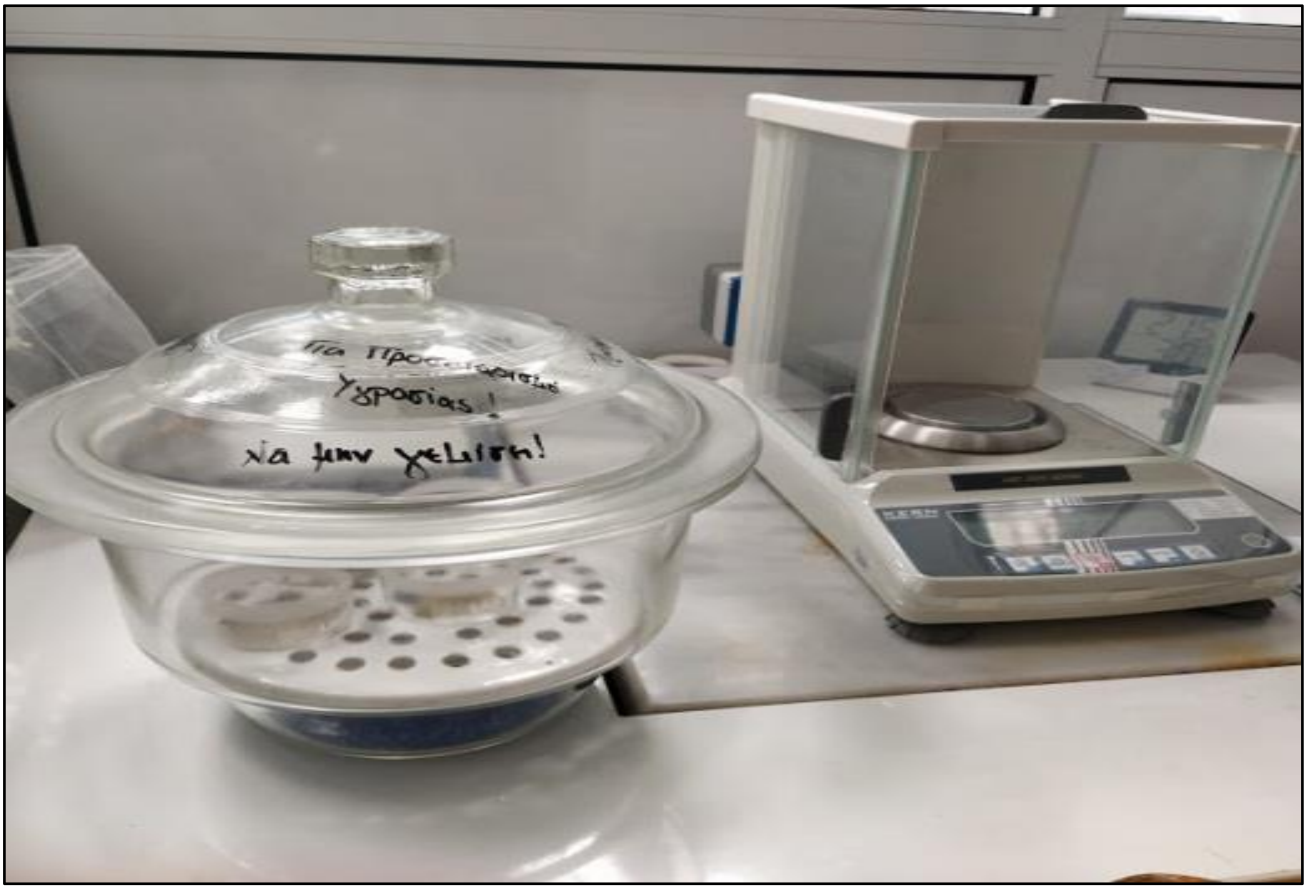

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Figure D-3 Moisture caps in the dryer

- Then blanks were weighed on a scale of 4 decimal places and their mass (m_0) was recorded.
- Approximately 1 gram of sample was added and the exact mass (m_1) recorded.
- The capsules were repositioned in the oven at 105 ± 5 ° C until the next morning.
- The sample capsules were removed from the oven and placed in a desiccator to bring to room temperature for 45 minutes.
- The exact mass was weighed and recorded after overnight drying (m2).
- The samples were repositioned in the oven at 105 ± 5 ° C for 2 hours.
- The sample capsules were removed from the oven and placed in a desiccator to bring to room temperature for 45 minutes.
- Finally, they were weighed and the exact mass was recorded after two hours of drying (m_3).
- If the last two weightings do not differ more than 0.02g the drying is completed.

Humidity is calculated based on the formula:

%Humidity (% Y) =

$$\frac{m_1 - m_3}{m_1 - m_0} \times 100$$

In the lyophilized samples the percentages of % moisture ranged from 0.2-1.8% while in the liquid samples for hexavalent chromium from 1-73%.

8 D.3.2.5.2 *Quality standards for sediment metals*

There is no Community legislation on maximum permissible metal content in sediments.

The ERL-ERM (Effects Range Low - Effects Range Median) criteria are used as useful toxicity prediction techniques to evaluate the results. ERL (Effects Range Low) and ERM (Effects Range Median) concentrations have been adopted as a tool for assessing chemicals and impacts on marine habitat. These guidelines indicate tolerable concentrations of specific pollutants in sediments to protect benthic communities.

Low-scale concentrations (ie lower than ERLs) indicate a minimal likelihood of toxic effects on marine populations, while higher-scale values (ie greater than ERM) indicate concentrations that cause certain damage to benthic populations. Concentrations higher than the ERL and lower than the ERM represent a range in which they occasionally cause biological effects (Long et al., 1998; Long et al., 1995; Smith et al., 1996; Buchman 2008).

Table D-5 ERL and ERM sediment quality criteria (in mg / kg)

Element	ERL	ERM
Cd	1,2	9,6
Cr	81	370
Cu	34	270
Hg	0,150	0,710
Pb	46,7	218
Ni	20,9	51,6
Zn	150	410

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8 D.3.2.5.3 *Determination of Hg and Cd metals by false digestion*

The sediment samples for the extraction of Cd and Hg metals were treated with concentrated nitric acid in closed Teflon containers (in house method).

The samples are weighed into dry, clean Teflon containers weighing 4 decimal places. 8 mL of concentrated nitric acid is added slowly with a dropper (per 2 mL) to prevent foaming of the samples and overflow. The lid of each container is closed tightly and placed on a heating plate for at least 6 to 8 hours at a temperature of about 70-80 °C. After cooling the containers to ambient temperature, the acid containing and extracting the metals from the sediment is transferred quantitatively to 50mL volumetric flasks by successive rinsing with bis-deionized water.

The obtained digests are filtered through a nitrocellulose filter with a pore diameter of 0.45μm and stored in pre-cleaned (diluted nitric acid) 50mL vials.

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Figure D-4 Teflon containers and heating plate

Cd was measured by atomic absorption spectrometry with VARIAN Spectra AA-460 Z GTA toner oven based on the ISO 15586: 2003 standard and Hg was measured based on the EN 16175 method by atomic cold vapor absorption spectrometry [at Cold-absorption spectromet (CV-AAS)].

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Figure D-5 Atomic absorption spectrophotometer with toner oven (VARIAN Spectra AA-460 Z GTA).

Calculation formulas of dry concentrations in the sediment

The weighed sediment mass for digestion into a Teflon container is moisture-corrected with the formula

$M_{dry} = m_{weighting} - (\%Y/100) \times m_{weighting}$

Formulas of metal concentration calculation

$$Cd\left(\frac{\mu g}{g}\right)_{dw} = Cd\left(\frac{mg}{kg}\right)_{dw} = \frac{Cd\left(\frac{\mu g}{L}\right)_{AAS} \times 0{,}05L}{m_{dry}}$$

$$Hg\left(\frac{\mu g}{g}\right)_{dw.} = \mathrm{Cd}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right)_{dw.} = \frac{\mathrm{Cd}\left(\frac{\mu g}{L}\right)_{AAS} \times 0{,}05L}{dry}$$

Where 〚Cd (μg / L)$_{AAS}$ and 〚Hg (μg / L)$_{AAS}$ are the measurement concentrations in the digested sample at atomic absorption and 0,05L are the 50mL final take-up volume of the digested sample.

8 D.3.2.5.4 Determination of total metal content (Cr, Cu, Ni, Pb, Zn) by total digestion

In order to determine the concentrations of the main constituents and trace elements in the marine sediments by liquid chemical methods, it is first necessary to solubilize the sample. This dissolution is achieved by treating (digesting) the sample with active acids or mixtures thereof (HF - HClO4 - HNO3) under high temperature conditions. The process is carried out in Teflon (poly-tetrafluoro-ethylene) containers, which is the most suitable material for the strong conditions required, as it is not affected by strong acids and does not contaminate the sample with metals. The only disadvantage is their high cost.

Complete dissolution of the sample necessarily involves the addition of HF to the acid mixture because it is ideal for the complete destruction of the silicate lattice (releasing bound metals, such as Fe, Al and Li). The use of HNO_3 - $HClO_4$ is recommended for environmental studies, as HNO_3 destroys easily oxidized organic matter, while $HClO_4$ decomposes the most durable organic matter. The addition of HNO_3 for preliminary oxidation of the organic matter is required before the use of $HClO_4$ to prevent explosion. The use of H2SO4 is avoided due to the precipitation of insoluble sulfates.

The following procedure was followed in the experimental process:

- sieve with sieve diameter 1mm and 64μm
- weighing 0,3-0,5 g of sediment and placing in Teflon (dry mass is calculated considering the moisture of the sediment)
- addition of 6 ml HNO_3 and evaporation to a heating plate at a temperature higher than 80 ° C, until about 1 ml is left
- add 5 ml HF + 1 ml HCLO4 and evaporate almost to dryness
- add 2 ml HNO3 + 5 ml HF + 1 ml $HCLO_4$ and evaporate almost to dryness
- add 2 ml HNO3 + 5 ml HF + 0.5 ml $HCLO_4$ and evaporate almost to dryness.

After the last evaporation, the residue was diluted with 2M HNO_3 solution and left on the plate at a temperature above 80 ° C overnight. The next day the digestion liquid was received with 2N HNO_3 solution, diluted in 50ml volumetric flasks, transferred to 50ml plastic vials, pre-diluted with dilute nitrate and stored in the refrigerator (ISO 14869-1: 2000).

Flame atomic absorption spectrometry (VARIAN Spectra AA-200A) was used to measure Zn, Cu and Cr metals, while graphite furnace atomic absorption spectrometry (VARIAN Spectra AA-460 Z GTA) was used for Pb and Ni metals.

Table D-6 Quantification limits.

Element	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Quantification limit	10	2	0,4	0,4	6

8 D.3.2.5.5 Determination of hexavalent chromium

Method 3060A EPA-ALKAL DIGESTION hexavalent chromium was applied.

Method summary

Method 3060A uses an alkaline extraction to solubilize non-water-soluble and water-soluble Cr (VI) compounds in solid samples. The sample is digested with a solution of 0.28 M Na_2CO_3 / 0.5 M NaOH and heated at 90-95 ˚C for 90 minutes in order to solubilize Cr (VI) and at the same time remain stable against reduction to Cr (III). The Cr (VI) in the extract is then reacts with diphenylcarbazide and quantified by the UV-Vis photometric procedure.

Sample handling

Samples should be stored in liquid form, as taken from the field at 42 ºC until analysis. Hexavalent chromium has been proved to remain quantitatively stable in field humidity samples for 30 days from the sampling day. Cr (VI) has also been proved to be stable in alkaline extract for up to 168 hours after extraction.

8 D.3.2.6 Granulometry (Micromeritics Sedigraph III Plus)

<u>Sample processing:</u> The sediment sample is dried at 65 ° C for 24 hours before being introduced into the Sedigraph to remove moisture. Then, each sample is weighted with a precision balance and 20 ml of Sodium Hexametaphosphate (C = 5.5 g/l) are added and left for 24 h at room temperature. The main aim is to break down the agglomerates so that sieving will be easier. The next day each sample is sieved through a 63 μm diameter sieve to separate the sand from the clay and sludge. Sand fractions (> 63 μm) are placed with Milly Q in the oven until are completely dry, so that we can measure the dry weight, while <63 μm diameter fractions are placed with Sodium Hexametaphosphate in SediGraph (SediGraph III Plus micromeritics) for further granulometric analysis. The results of SediGraph and the weights of the sand fractions result in the final percentage analysis (granulometric analysis) of the samples.

Principle of operation of the instrument: The Sedigraph device is a modern method for the analysis of fine sediments, for a grain size of less than 63μm. The analysis method is based on the absorption of soft X-ray radiation from the suspended particles of the sample in deionized water. The device creates a mixture of precipitate with deionized water which is homogenized with ultrasound and enters a cell where a beam of soft X-rays falls. The device uses the method of calculating the precipitation rate of the pellets, by continuously measuring the absorption of the incident beam. The values of the measurements are entered into a computer, where the particle size distribution of the sediment sample is constructed.

8 D.3.3 Benthic macroinvertebrates

8 D.3.3.1 Sampling methodology

The sampling methodology for collecting benthic macroinvertebrate samples is the semi-quantitative three-minute kick/sweep plus a 1- min effort when bank vegetation exists. Sampling is conducted from all available microhabitats of the river site proportionally according to the matrix of possible river habitats (Lazaridou et al. 2018a). Sampling is performed using a hand-net with surface area of 575 cm2 (250mm×230mm), with a mesh size of 0.9 mm and a net depth of 40 cm. Benthic samples are collected by placing the hand-net in the opposite direction of the flow and by kicking/shaking the riverbed for three minutes. Within three minutes, all identified microhabitats are covered proportionally to the area they occupy. During benthic macroinvertebrate collection, the sampling protocol is completed and the following information is recorded:

- - Sampling data:
  - name or code of the position,
  - name of researcher / sampling officer,
  - name of the person who completed the protocol,
  - sampling date and time
  - site coordinates
  - site altitude
- Photo and video of the place,
- hydromorphological parameters:
  - substrate composition,
  - hydrological measurements (width and depth of water, flow).
- Vegetation parameters (shading of the site, riparian vegetation cover, aquatic vegetation cover).
- Conditions during sampling (air temperature, meteorological conditions).
- In situ physicochemical measurements (dissolved oxygen, temperature, conductivity, pH, turbidity).
- Water samples for ex situ analysis:
  - 5-day biochemical oxygen demand (BOD_5),
  - nutrients ($N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, $P\text{-}PO_4$, TDP)

- chloride

8 D.3.3.2 Biological quality based on benthic macroinvertebrates

Classification of the biological quality into five (5) classes based on macroinvertebrates is performed with the Hellenic Evaluation System 2 (Hellenic Evaluation System 2, HESY2; Lazaridou et al., 2018a) for the river types R-M1, R-M2, R -M3, R-M4 and R-M5 and STAR ICMi for very large rivers (Lazaridou et al., 2018b).

HESY2 is based on the EQR (Ecological Quality Ratio) rationale and is the deviation of the observed HESY value (Artemiadou & Lazaridou 2005) from the reference sites of each river type. It is based on the multi-habitat sampling scheme and takes into consideration the tolerance, the abundance and the diversity/richness of benthic macroinvertebrates, which are required by the WFD. HESY2 responds effectively to multiple pressures (land use, organic pollution and hydromorphological modifications). It consists of:

(A) The Hellenic Evaluation Score (HES) of benthic macroinvertebrate families which results from the sum of the scores of all taxonomic groups of the sample according to their abundance (Table D-7).

(B) Average HES (AHES) which is similar to ASPT.

(C) The SemiHES value, which is the final result of the Hellenic Evaluation System, is the semi-total of the HES and AHES values [Semi- HES = (standardized HES+standardized AHES)/2] standardized against the habitat diversity richness (WFD requirement of habitat) (Table D-8) based on the Habitat Richness Matrix (GHRM) (Table D-9).

SemiHES values are interpreted at a five-class scale (High, Good, Moderate, Incomplete, Poor) as required by the WFD (Table D-10).

The RM typology is then used to calibrate the HESY2. Quality boundaries were set for each river type, using, as mentioned above, the EQR_Semi_HES (HESY2) values of the reference samples (Table D-11).

Table D-7 Scores of benthic macroinvertebrate taxonomic groups for the calculation of HESY2 (Lazaridou et al. 2018a, modified by Artemiadou and Lazaridou, 2005).

Sensitivity	Taxa	Present (0-1%)	Common (1.01-10%)	Abundant (>10%)
Sensitive taxa	a) Capniidae, Chloroperlidae b) Siphlonuridae c) Aphelocheiridae	100	110	120

Sensitivity	Taxa	Present (0-1%)	Common (1.01-10%)	Abundant (>10%)
	d) Blephariceridae e) Phryganeidae, Molannidae, Odontoceridae, Beraeidae, Lepidostomatidae, Uenoidae (=Thremmatidae), Brachycentridae, Helicopsychidae			
	a) Leuctridae, Perlodidae, Perlidae b) Sericostomatidae, Goeridae	90	97	100
	a) Nemouridae, Taeniopterygidae b) Ephemeridae, Heptageniidae, Leptophlebiidae c) Leptoceridae, Polycentropodidae, Psychomyiidae, Philopotamidae, Limnephilidae, Rhyacophilidae, Glossosomatidae, Ecnomidae d) Aeshnidae, Lestidae, Corduliidae, Libellulidae e) Athericidae, Dixidae f) Scirtidae (=Helodidae), Gyrinidae, Hydraenidae g) Sialidae h) Potamonidae i) Astacidae	80	86	90
Medium tolerant taxa	a) Potamanthidae b) Calopterygidae, Cordulegastridae c) Stratiomyidae d) Hydrobiidae	70	75	78
	a) Platycnemididae, Gomphidae b) Tabanidae, Ceratopogonidae, Empididae c) Elmidae (=Elminthidae) d) Viviparidae, Neritidae e) Unionidae	60	64	67
	a) Caenidae, Oligoneuriidae, Polymitarcyidae, Isonychiidae b) Hydropsychidae c) *Ancylus*[1] , Acroloxidae d) Gammaridae, Corophiidae e) Atyidae f) Planariidae, Dendrocoelidae, Dugesiidae g) Dryopidae, Helophoridae, Hydrochidae h) Psychodidae, Simuliidae	50	53	56
Tolerant taxa	a) Ephemerellidae, Baetidae b) Hydroptilidae, Ptilocolepidae c) Tipulidae, Dolichopodidae, Anthomyiidae, Limoniidae d) Haliplidae, Curculionidae, Chrysomelidae, Hydroscaphidae	40	38	35

Sensitivity	Taxa	Present (0-1%)	Common (1.01-10%)	Abundant (>10%)
	e) Hydrachnidae f)Piscicolidae, Glossiphoniidae			
	a) Coenagrionidae b) Chironomidae c) Dytiscidae, Hydrophilidae, Hygrobiidae d) Corixidae, Hebridae, Veliidae, Mesoveliidae, Hydrometridae, Gerridae, Nepidae, Pleidae, Naucoridae, Notonectidae, Belostomatidae e) Asellidae, Ostracoda f) Physidae, Bithyniidae, Thiaridae (=Melaniidae) g) Hirudinidae, h) Sphaeriidae i) Oligochaeta (except for Tubificidae)	30	25	20
	a) Rhagionidae, Culicidae, Muscidae, Thaumaleidae, Ephydridae, Chaoboridae b) Lymnaeidae, Planorbidae c) Erpobdellidae	20	12	3
	a) Tubificidae, b) Valvatidae, c) Syrphidae	10	2	1

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Table D-8 HES and AHES scores for the calculation of Semi-HES (Artemiadou & Lazaridou, 2005). Habitat diversity is defined according to the Greek Habitat Richness Matrix (Table D-9).

	Grade 5	Grade 4	Grade 3	Grade 2	Grade 1
			Rich Habitat Diversity sites		
HES form					
HESJ	>1532	1326–1532	830–1325	341–829	0–340
			Poor habitat diversity sites		
HESJ	>1052	756–1052	389–755	167–388	0–166
			Rich habitat diversity sites		
AHES form					
AHESJ	>64.72	54.57–64.72	45.82–54.56	31.73–45.81	0–31.72
			Poor habitat diversity sites		
AHESJ	>55.69	45.18–55.69	35.33–45.17	27.50–35.32	0–27.49

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Table D-9 Greek Habitat Richness Matrix. A site can be classified as rich, if at least one checked type of habitat belongs to the diagonal striped cells; otherwise it is classified as poor.

If present	Macrophyte bed	Natural substrate			Artificilal sub-strate	Slough	Woody snag
		Coarse *	Mixture **	Fine ***			
Riffle							
Channel margin							
Island margin							
Main channel							
Run							
Channel margin							
Island margin							
Main channel							
Pool							
Channel margin							
Island margin							
Main channel							

* Coarse: Substrate composition >70% of boulders and/or cobbles and/or pebbles
** Mixture: Variant substrate composition that cannot be classified as coarse or fine
*** Fine: Substrate composition >70% of gravel and/or sand and/or silt

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Table D-10 Final classification in quality classes according to the Semi-HES of benthic macroinvertebrates (Artemiadou & Lazaridou, 2005).

Nine-grade scale of semi-HES	Interpretation A
5	High
4.5	High
4	Good
3.5	Good
3	Moderate
2.5	Moderate
2	Poor
1.5	Poor
1	Bad

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Table D-11 Class boundaries of HESY2 per intercalibration type (Lazaridou et al. 2018a).

Ecological Status	R-M1	R-M2	R-M4	R-M5
High	≥0.943	≥0.944	≥0.85	≥0.963
Good	(0.943-0.75]	(0.944-0.708]	(0.85-0.637]	(0.963-0.673]
Moderate	(0.750-0.500]	(0.708-0.472]	(0.637-0.425]	(0.673-0.444]
Poor	(0.500-0.250]	(0.472-0.236]	(0.425-0.213]	(0.444-0.222]
Bad	(0.250-0]	(0.236-0]	(0.213-0]	(0.222-0]

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8 D.3.4 Benthic diatoms

Diatom sampling and preparation was based on European standards (European Committee for Standardization 2003, 2004). Samples were collected from a lit area in the center of the river, whenever possible, and were preserved with 70% ethanol. In the lab, samples were treated with hot hydrogen peroxide to remove organic matter and obtain clean frustules used for diatom species identification (Battarbee 1986). Clean frustules were mounted with Naphrax for observation in the microscope. 400 frustules per sample were identified to species level with a light microscope, at 1000X magnitude. For the taxonomy, the work of Cantonati et al. (2017) was mainly used. Diatom quality indices were calculated with the OMNIDIA software, version 5.3 (Lecointe et al. 1993, 1999 http://clci.club.fr/index.htm).

IPS - Specific Pollution Sensitivity Index

For the ecological quality based on benthic diatoms, IPS - Specific Pollution Sensitivity Index (Coste in Cemagref 1982) was used. IPS is able to detect different types of pollution (organic pollution, salinity, eutrophication) in rivers (Prygiel & Coste, 1993) and it is widely used for ecological studies (Descy & Coste, 1991), whereas it has been proved to be the most efficient index in Mediterranean rivers (Gomà et al., 2004). IPS is based on the Zelinka & Marvan (1961) equation:

$IPS = \sum_{j=1}^{n} A_j.I_j.V_j / \sum_{j=1}^{n} A_j.V_j$

Where:Aj: the relative abundance of a species in the sample

Vj: indicator value or stenoecy degree of the species (1=low value – wide distribution range, 2=medium value – intermediate distribution range, 3=great value – narrow distribution range, characteristic of specific conditions)

Ij: pollution sensitivity of the species (1 to 5): 1 = very tolerant, 2 = tolerant, 3 = indifferent, 4 = sensitive, 5 = very sensitive.

IPS ranges from 1 to 20 with increasing ecological quality, whereas after the establishment of reference values, the Ecological Quality Ratio (EQR) ranges from 0 to 1, and are divided in five quality classes (Table D-12). EQR is calculated by dividing the IPS value with the Reference IPS value of each river type, as indicated in Table D-12. In the cases that the IPS value of the site is higher than the Reference IPS value, the upper limit of EQR could exceed 1. Since there are different river types, reference sites and intercalibration was done per Mediterranean river type (R-M1, R-M2, R-M3, R-M4, R-M5, Very large) according to the Mediterranean Geographical Intercalibration Group. Intercalibration for Greece, so far, has been done for river types R-M1, R-M2 and R-M4 (catchment area <1000 km^2) but not for types R-M3, Very large (catchment area >1000 km^2) and R-M5 (temporary streams) due to lack of reference sites in the latter types (Smeti & Karaouzas 2016).

Table D-12 Quality boundaries based on EQR IPS (R-M1, R-M2, R-M4) and IPS (R-M3, R-M5, Very large)

	R-M1	R-M2	R-M4	R-M3, R-M5, Very large
Reference IPS values	16.00	16.30	16.85	
High/Good Boundary	0.956	0.953	0.932	17
Good/Moderate Boundary	0.717	0.732	0.716	13
Moderate/Poor Boundary	0.478	0.477	0.466	9
Poor/Bad Boundary	0.239	0.238	0.233	5

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8 D.3.5 Fish as BQE for ecological assessment – Implementation of the Hellenic Fish Index (HeFI)

According to the WFD 2000/60/EC standardized assessment methods are required in order to quantitatively classify the ecological status of river water bodies. Several ecological status indexes have been implemented usually using one or multiple biological elements to reveal the levels of community structure integrity and demographic completeness. Four biological quality elements (BQEs) are used in the WFD for the ecological assessment of river water bodies (fish, benthic diatoms, aquatic macrophytes and benthic macroinvertabrates).

The selected biological element of the used index for the purposes of this specific study is fish since it is considered to be the most appropriate to identify any diversions from the reference conditions caused by the dominant stresses impacting the Greek rivers.

In this work the fish community composition and fish traits are used for the determination of the ecological state (the ecological quality and integrity of the sampled ecosystems). Since 2017 the

Hellenic Fish Index- HeFI (Zogaris et al. 2018) is now the official index used in Greece for the application of the WFD.The index has been intercallibrated with other EU indices. The HeFI approach is based on the philosophy of the Index of Biotic Integrity – IBI that predicts and quantifies the response of fish communities to multiple anthropogenic pressures that occur simultaneously at aquatic systems. The index uses the collected fish sampling data in conjunction with the environmental and the habitat characteristics to compute a four metrics (which are based on a multitude of sub-indices). These sub-indices are compared with the reference conditions which are the expected values under conditions undisturbed from human presence. The fish metrics that we found to be the most responsive were: "Density of insectivores", "density of omnivores", "density of benthic species" and the "density of potamodromus species". Reference conditions for these guild-based metrics are calculated using a reference model based on the following basic environmental variables: site altitude, slope, winter temperature (January), source altitude, and catchment area.

The reference condition boundaries for each fish metric are identified through multiannual data which have recorded both the temporal and spatial natural variability for the specific ecosystems. The identified diversion from the reference conditions is standardized and aggregated to a single value of ecological quality. In our case study the water quality classification boundaries were set as:

High (1) = 0.8, Good (2) = 0.6, Moderate (3) = 0.4, Poor (4) = 0.2, Bad (5) <0.2

8 D.3.5.1 Fish sampling

Obviously one of the most important problems and challenges in monitoring and evaluating ecological quality is sampling. Fish are not static and unconcerned to annoyance, they know how to avoid predators. Fish also move and migrate. Therefore, in order to make a complete sampling, many conditions are required.

Key assumptions are the following:

- Utilization of electric fishing (technique and special tools for catching fish using electricity) with the guidelines set in the intercalibration projects of ichthyological indicators for WFD at European level.
- Involvement of experienced ichthyologists who know the identification, behavior, ecology and natural history of fish and ecosystems.
- Adequacy of the area of electro-fishing as defined by the established practices.
- Reference to the uncertainty and effectiveness of sampling in each assessment/ evaluation of ecological quality. When the preconditions for scientific evaluation are not sufficient the publication of an assessment should be avoided.

A detailed presentation of the methods applied in Greece is mentioned in the HCMR manual for sampling fish in rivers (IMBRIW-HCMR, 2012).

8 D.3.5.2 Assessment of assurance and confidence in implementation of the index

Under conditions of successful electrofishing the results of the index can be considered quite representative of the current riverine ecosystem status concerning the complexity and the broad variability which is an intrinsic characteristic in most of the sampled water bodies.

The current field campaign was conducted in spring. This season is inappropriate for fish sampling at sites located in mountainous or large low plain rivers (best period for fish-based assessment is summer or early autumn). In small and mid-sized rivers in Mediterranean region, ecological assessment using fish is possible during spring.

Due to the special morphological characteristics of the sites, the criteria of the minimum sampling effort required under the WFD guidelines were not met.

8 D.3.5.3 Aggregated results

In this project, 21 species of fish were collected and a total of 1294 fish individuals in 6 river basins.

The number of fish species at the various sampling points varies from one (1) to five species (5) and are indigenous and / or alien species belonging to typical large families of fish fauna (Cyprinidae predominates but there are also alien species of the family Poeciliidae). Only two (2) alien species were identified and they are non-native species of the basins under study (*Carassius gibelio, Gambusia holbrooki*). During the sampling, mainly small fish were collected, and in general the fishing conditions were characterized by some difficulties due to the season and peculiarities of the specific sites. Also, fish numbers are low in many cases due to severe ecosystem degradation.

In the Mediterranean rivers, the assessment of the ecological status is a challenging task, especially due to the great variety of conditions and biogeographical characteristics of many river basins. Some serious pressures are "overshadowed" by the natural diversity of the Mediterranean rivers. A major problem concerning the degradation of Greek river systems at low altitudes is the excessive water intake / abstraction of water that usually serves agricultural or other uses. These obviously provoke serious hydromorphological changes that also affect the life (biocommunities) of the river. Table D-13 shows various summary characteristics of the sampling results of May 2021, such as e.g. the relative abundance of fish at the sampling sites. Detailed results of fish sampling are presented at each respective chapter of each sampling site (Results chapter).

Table D-13. Summary results of the relative abundance of species collected at the 11 sampling sites. The sampling points are located in the South - North direction. (1-Evrotas, 2-Alfios, 3-Ladon, 4-Pinios, 5-Evinos, 6-Enotiki Tafros, 7-Acheloos, 8-Dipotamos, 9-Arachthos, 10-Louros, 11-Acheron-Mavropotamos)

Fish species	1	2	3	4	5	6	7	8	9	10	11
Anguilla anguilla										1	1
Atherina boyeri								1			
Barbus_peloponnesius		1	1		1						
*Carassius gibelio**				3							
Economidichthys trichonis						1					
Economidichthys_pygmaeus										3	
*Gambusia holbrooki**						3				3	
Gobiidae sp.						2					
Liza ramada											
Luciobarbus_albanicus					2	3	1		1		
Mugil cephalus								1			
Mugilidae sp.					1			2	1		
Pelasgus_laconicus	1	3									
Pelasgus_thesproticus										1	1
Salaria_fluviatilis			3	3	1						
Scardinius acarnanicus						3					
Squalius_keadicus	1										
Squalius_peloponensis		2	2	1							
Telestes_pleurobipunctatus			1								1
Tropidophoxinellus_hellenicus			1								
Tropidophoxinellus_spartiaticus	3										

* Note Abundance (semi-quantitative scale):

1 = Rare; individuals (less than 10), one class sizer per 100 m. along the river.

2 = Common / Large number (more than 10 individuals), more than one class size per 100 m. along the river.

3 = Abundant (more than 20 individuals) and more than two class sizes per 100 m along the river.

Fish species that have been introduced or transported into the systems are marked with an asterisk (*).

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At the same time, the ichthyological characteristics related to the structure of the fish communities are presented with a semi-quantitative classification. The so-called non-resistant species are usually specialized and sensitive to anthropogenic interventions (eg benthic fish with special needs, large predatory species, etc.). The classification of fish species in terms of resilience is mainly based on sensitivity to water pollution, changes in the aquatic habitat and includes species that show a recent decrease in distribution and abundance (such categorizations have been published, see Zogaris et al.

2018). The absence of non-resistant species is a first reaction of the ecosystem to a series of anthropogenic interventions, as highly degraded river systems are dominated mainly by resistant species. Similarly, the lack of expected migratory species indicates the fragmentation of river habitats (obstacles to movement, such as dams, overflows, etc.). It is very important to consider these characteristics in order to determine the certainty of the application effectiveness concerning the index in each sampling position. The evaluation is applied by the experts on a three-point scale of certainty as to the accuracy of the assessment: high, medium, low. The moderate rating is practically "good enough" for the rating and differs slightly from the high certainty. Obviously low certainty should not be presented as a satisfactory evaluation of the ecological assessment. In our case, five sampling sites present "low" certainty.

8 D.3.6 Hydromorphology

The 'QBR' index (Munné et al. 2003) has been used for evaluating the quality of riparian habitats which is calculated according to the following criteria: (a) the total riparian vegetation cover and its cohesion with the adjoining uncultivated habitat, (b) the cover structure, which is based on the total cover of trees as well as shrubs and other understory plants, (c) the quality of the cover, which depends on the geomorphology of the riparian habitat and the number of native tree species, and (d) the possible anthropogenic modifications to the stream or river bed.

Each of these four parameters contributes 0-25 points to the total index, which has a maximum value of 100 corresponding to excellent ecological condition. In accordance with this method (Munné et al. 2003), the quality of riparian habitats is considered to be:

- x "High" "or In a natural state" when the index reaches a value of 95 or above.
- x "Good, though with some anthropogenic intervention" with values of 75-90.
- x "Moderate" (Satisfactory with significant intervention) with values of 55-70.
- x "Poor" (marked anthropogenic modification) with values of 30-50.
- x "Bad, marked degradation" with values lower than 20.

8 D.4 OVERALL ASSESSMENT OF THE WATER BODIES ECOLOGICAL QUALITY ACCORDING TO THE WATER FRAMEWORK DIRECTIVE

8 D.4.1 Introduction

The European Water Framework Directive (2000/60/EC) requires from the Member States to monitor and assess their water bodies' quality status. For this assessment, a series of biological (phytoplankton, fishes, macroinvertebrates, angiosperms, etc.), physicochemical and hydromorphological indicators should be used. Thus, relevant indices and metrics should be developed to describe the deviation of the water bodies' status from the reference conditions (relatively undisturbed status) and classify them accordingly to ecological quality categories (Puente et al. 2008).

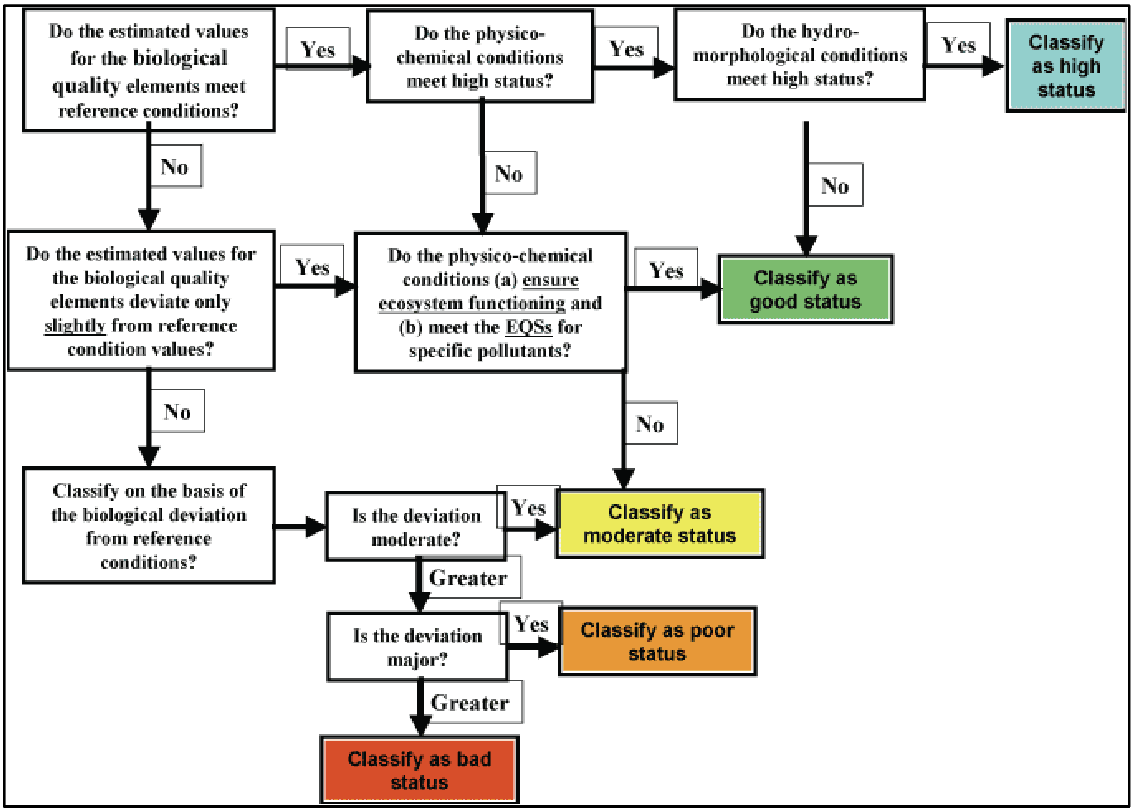
Most ecological water quality assessment techniques use biological indicators, which are based on species known to exist in a specific ecosystem and therefore reflect its water quality, or use species diversity to estimate changes in the environmental integrity of an ecosystem. In most cases biological indicators combine both properties (biotic indexes). Sampling and identification of species - indicators that belong to different biotic groups (algae, macrophytes, invertebrates in inland water and fish) provide the means to evaluate the long-term quality changes of the water and the ecosystem (Guinda et al.2008).

The combination of different groups of environmental parameters such as physicochemical, hydromorphological and biological for the assessment of the ecological water quality contributes in overcoming the aforementioned and other shortcomings that can be found when a single group of environmental parameters is used. Therefore, this multi-parametric approach eliminates stochastic errors and facilitates the holistic water quality assessment based on the ecosystem functionality principle (Everard and Powell, 2002).

In Greece, ecological assessment of rivers is mostly based on water quality and biological classification systems adopted from other Mediterranean countries. With the exception for ichthyofauna and macroinvertebrates, physicochemical quality, hydromorphology, benthic diatoms and aquatic macrophytes are asssessed using methods developed in other Member States. Nevertheless, following the intercalibration exercises, all the national assessment methods and the respective quality classes boundaries are comparable across the Member States.

8 D.4.2 Surface waters

Physicochemical quality is assessed based on concentrations of dissolved oxygen and nutrients according to respective weight coefficients. The weight coefficients range from 1 to 5 reflecting ecological classes (1=Bad, 2=Poor, 3=Moderate, 4=Good, 5=High). The average weight (Total weight per number of physicochemical parameters) indicates the final physicochemical quality class. The biological quality class is determined by the worst biological element. In our case, since biological quality was assessed with the use of fish, benthic macroinvertebrates and/or benthic diatoms, whichever BQE has the worst classification will determine the final biological quality. After applying these classification schemes in the various parameters, we get the physicochemical and biological status at each study site (worst case principle applied). Then the methodology illustrated in Figure D-6 has been used by combining initially the physicochemical with the biological and then the hydromorphological quality classes at each study area, in order to get the final ecological status classification.



Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-6 Flow-diagram for assigning final ecological status classification according to the 2000/60/EC Directive.

8 D.5 RESULTS

8 D.5.1 Mariorema

8 D.5.1.1 Physicochemical parameters of surface waters

During the visit of the HCMR personnel to the Mariorema sampling station, the site was completely dry and therefore the sampling campaign included only sediment sampling.

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Figure D-7 Sampling station in Mariorema river.

8 D.5.1.2 *Physicochemical analyses in sediment*

The sediment in Mariorema had a low concentration of TOC (organic carbon) and a moderate charge of total nitrogen and total phosphorus although there are no relative quality limits.

Table D-14 Concentrations of total phosphorus, nitrogen and organic carbon.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.037	247	0.29

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.1.3 *Heavy metals in sediment*

In Mariorema, low Cd and Hg concentrations were measured, lower than the ERL limits of the two metals, so no negative effects on benthic organisms are possible. The metals Cr, Cu, Pb, Zn were also measured at low concentrations, lower than the ERL limits so no adverse effects on benthic organisms are possible. Ni metal was measured slightly higher (36.9mg / kg) than the ERL limit (20mg / kg). Cr (VI) also showed a low concentration corresponding to 1% of total Cr.

Table D-15. Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.154	78.7	0.79	0.034	36.9	18.6	30.8	<5	67.0	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.1.4 *Hydrocarbons and PCBs in sediment samples*

Since sediment sample of Mariorema river was very coarse and could not pass through the sieve of 500 m hole-diameter, it is assumed that all compounds were undetectable, because the organic substances are not absorbed by coarse materials.

8 D.5.1.5 *Granulometry*

The predominant class of sediments in Mariorema is sand.

Table D-16. Sediment granulometry analysis.

	Sand	Silt	Clay
	%	%	%
Mariorema	87.52	0.78	11.70

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.1.6 *Hydromorphological assessment*

The QBR score for Mariorema was 85 which indicated a good status of the riparian vegetation and channel conditions. However, the site was completely dry which indicates that hydrologically behaves mostly as torrent with an ephemeral flow regime. The drone derived orthophoto map of this site is accessible here:

https://cloud.pix4d.com/dataset/944959/map?shareToken=204ada68-7b51-4ca4-987f-9983106560a4

8 D.5.1.7 Ecological status

Ecological status of Mariorema river cannot be assessed since the sampling site was water-dry and there is no information about its biological quality and the physicochemical status. Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/1C2PXZKJqxiCenzP9.

8 D.5.2 Evrotas river

8 D.5.2.1 Physicochemical parameters of surface waters

Table D-17 below presents the basic physicochemical parameters measured in the context of water and sediment sampling in May 2021. The values range among normal levels for the season while the concentration of dissolved oxygen indicates high quality.

Table D-17 Physicochemical parameters.

Date	Temperature (°C)	EL Conductivity (µS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	Dissolved Oxygen (mg/L)
17/05/2021	20.5	575	369	0.24	6.7	4.68	9.2

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Figure D-8 Sampling station located in Evrotas river.

8 D.5.2.2 Chemical parameters of surface waters

The concentrations of nutrients in combination with the dissolved oxygen characterize the physicochemical condition of both stations of Evrotas river as high. TOC concentrations at both stations are normal, do not indicate unusual enrichment or pollution of water with organic carbon and are much lower than the values found in rivers where water quality is characterized as poor.

The extremely low measured values of total coliforms, which are <100 cfu / 100 ml, characterize the water of the stations as unpolluted (EPA, 2003b). BOD_5 concentrations indicate good quality. In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-18 Chemical parameters (a).

	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Evrotas upstream	687	3	14	1	4	HIGH
Evrotas downstream	667	3	19	1	4	HIGH

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-19 Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD5 (mg/l)	COD (mg/l)
Upstream	799	2.2	31	22.46	3.60	<10
Downstream	429	1.1	45	9.54	3.10	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.2.3 Physicochemical analyses in sediments

The sediment in Evrotas showed a low load in TOC, and a moderate load in total nitrogen and total phosphorus although there are no relative quality limits. These may be due to diffuse agricultural sources of pollution.

Table D-20 Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.015	246	0.21

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.2.4 Heavy metals in sediments

In Evrotas, low Cd concentration was measured, lower than the ERL quality limit, so no negative effects on benthic organisms are possible. For Hg in Evrotas the concentration measured was 0.178mg / kg, slightly higher than the ERL limit for this metal which is 0.150mg / kg. Possible sources of mercury are the burning of fossil fuels and wastewater from settlements (mercury amalgams are still used in dentistry; UN Environment, 2019). All other metals (Cr, Ni, Pb, Cu, Zn) presented very low concentrations, less than the ERL limit for each metal and therefore no adverse effects on benthic organisms are possible. Cr (VI) also showed a very low value corresponding to 0.6% of total Cr.

Table D-21 Concentrations of heavy metals in sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.054	27.5	0.16	0.178	18.2	4.4	8.6	<5	29.0	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.2.5 Hydrocarbons and PCBs in sediments

Since sediment sample of Evrotas river was very coarse and could not pass through the sieve of 500 m hole-diameter, it is assumed that all compounds were undetectable, because the organic substances are not absorbed by coarse materials.

8 D.5.2.6 Granulometry

In the sediment of Evrotas the predominant class is sand.

Table D-22 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Evrotas	99.10	0.03	0.87

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.2.7 Benthic macroinvertebrates

The biological status at the Evrotas River sampling site was classified as high based on benthic macroinvertebrate fauna. A total of 591 individuals belonging to 30 families were collected, with the Diptera families Chironomidae and Simuliidae being the most dominant (219 and 32 individuals, respectively) accounting for 45% of the total abundance (Figure D-9). The Ephemeroptera followed with the families Baetidae (102 individuals), Ephemerellidae (52 individuals) and Caenidae (28 individuals) with 34% of the total abundance (Figure D-9). The remaining groups of invertebrates were represented with relatively small abundances as shown in Figure D-9.

Table D-23 Biological status based on benthic macroinvertebrate fauna.

River Name	Evrotas
Date	17/05/2021
Typology	R-M2
Total abundance	591
Number of Taxa	30
Shannon-Wiener Diversity Index	2,29
Number of sensitive families	7
Number of tolerant families	11
HESY2 Score	1,00
HESY2 Quality	High
% EPT	41,46
% EPTC	46,02

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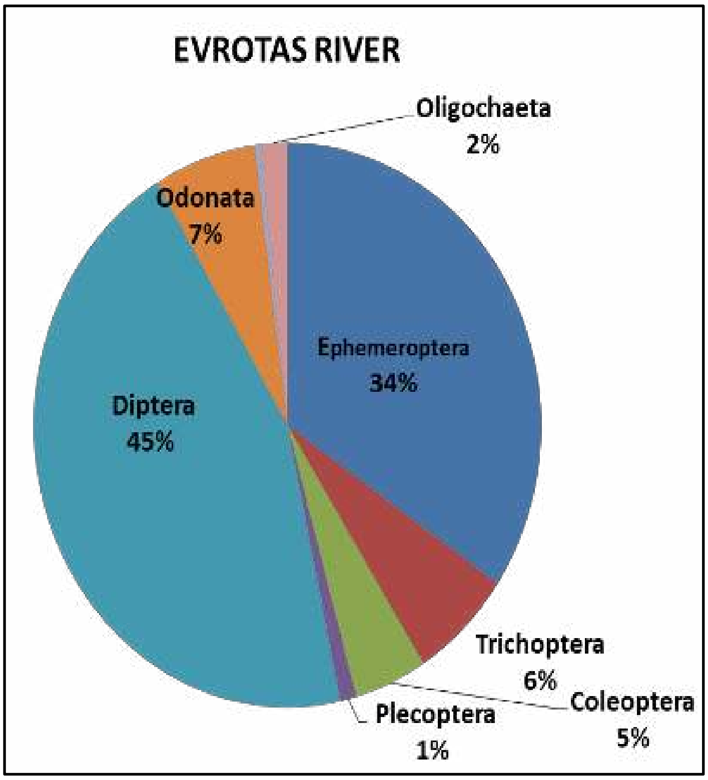

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-9 Abundance percentages of macroinvertebrate groups at the sampling site of Evrotas river.

8 D.5.2.8 Benthic diatoms assessment

In this site, 24 species of diatoms were identified, and the assemblage presented relatively high evenness (E=0.65) and relatively high diversity based on Shannon diversity index (H=2.97). Dominant species were Achnanthidium minutissimum (47.6%) and Cyclotella distinguenda (11.4%). A. minutissimum is a cosmopolitan species, usually found in well oxygenated, clean, fresh waters, whereas C. distinguenda is usually found in alkaline waters with low nutrient concentration (Taylor et al 2007, Bey & Ector 2013). Biological quality of the site based on benthic diatoms is high (EQR IPS=1.043), with no organic pollution or anthropogenic eutrophication (Table D-24).

Table D-24 EQR IPS and IPS values and quality based on a color code.

River name	River Type	EQR IPS/IPS	High Good Moderate Poor Bad
EVROTAS	R-M2	1.043	

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.2.9 Ichthyological assessment

A total of three species were detected, generally low numbers of fish. This site is initially evaluated as of "bad" ecological quality based on the sampling of the fish fauna.

In general, this area’s degradation (due to water pollution) is also reflected through the low density of fish populations and the very low populations of certain fish species that were expected to be found in this type of river and the specific geographical location. At the sampled river section dominates the species Tropidophoxinellus spartiaticus which is characterized by high tolerance to eutrophic conditions. Only a few individuals Squalius keadicus were found in this section, species that compared to the previous one has a more "rheophile" behavior and is absent or maintains lower population densities in stagnant waters and / or waters encumbered by high organic loads and eutrophication. The eel (Anguilla anguilla) is also absent from the area although there are ideal habitats for this species and has been recorded in this part of the river in the past.

The composition of species in the area as a whole is very different from what is expected, with a strong predominance of the Tropidophoxinellus spartiaticus.

Table D-25 Summary results of the relative abundance of species collected at Evrotas river.

Species	Evrotas
Anguilla anguilla	
Atherina boyeri	
Barbus_peloponnesius	
Carassius gibelio*	
Economidichthys trichonis	
Economidichthys_pygmaeus	
Gambusia holbrooki*	
Gobiidae sp.	
Liza ramada	
Luciobarbus_albanicus	
Mugil cephalus	
Mugilidae sp.	
Pelasgus_laconicus	1
Pelasgus_thesproticus	
Salaria_fluviatilis	
Scardinius acarnanicus	
Squalius_keadicus	1
Squalius_peloponensis	
Telestes_pleurobipunctatus	
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	3
**Abundance (semi-quantitive):* 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

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Table D-26 Fish characteristics and confidence assessment and bioassessment at Evrotas river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Evrotas
Total fish species	3
Diversity of expected typespecific species	Moderate
Reproduction data	Moderate
Presence of intolerant species	Moderate
Presence of expected migratory species	Bad

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Evrotas
Sampling effort assessment	Bad
Uncertainty estimation for bioassessment	High

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-27 HeFI index implementation at Evrotas river.

Sampling parameters	Evrotas
Density of insectivores	2
Density of omnivores	2
Density of benthic species	1
Density of potamodromus species	0
Degree of Certainty	4
Preliminary assessment	1.25
Preliminary ecological quality (three-level scale)	Bad

Prepared by HCMR on behalf of ASPROFOS,2022

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-10 *Tropidophoxinelus spartiaticus* (Golden Menida) in sampling in Evrotas (left - during electric fishing, right - in a portable aquarium during sampling).

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-11 Performing habitat parameter measurements at Evrotas river (left), Fish sampling (electrofishing; right).

8 D.5.2.10 Hydromorphological assessment

The QBR score for Evrotas was 40 which indicated a poor status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 7 of this document. The drone derived orthophoto map of this site is accessible here:

https://cloud.pix4d.com/dataset/944952/map?shareToken=38f5dfb0-ddd8-4fb8-9c9f-04e162700325

8 D.5.2.11 Ecological status

For the complete assessment of the ecological quality of Evrotas river the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality. After the implementation of all classification schemes in the various parameters, we get the biological, physicochemical and hydromorphological quality class at each study site (worst case principle applied). Then the methodology illustrated in Figure D-6 has been used by combining initial the biological and the physicochemical and then the hydromorphological quality classes at each study area, in order to get the final ecological status classification. Evrotas river is characterized by BAD ecological status due to BAD biological quality. Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/fqRrySyMAzRfqkoi6.

Table D-28 Biological quality.

Water body	HESY2	EQR IPS/IPS	Ichthyofauna	Biological quality
EVROTAS	HIGH	HIGH	BAD	BAD

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-29. Final ecological quality.

Water body	Biological quality	Physicochemical quality	Hydromorphological quality	Ecological Status
EVROTAS	BAD	HIGH	POOR	BAD

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.3 Alfios river

8 D.5.3.1 Physicochemical parameters of surface waters

Below are the basic physicochemical parameters (Table D-30) measured in the context of water and sediment sampling in May 2021. The values are at normal levels for the season and the concentration of dissolved oxygen indicates good quality for Alfios river.

Table D-30 Physicochemical parameters

Date	Temperature (ºC)	El. Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	D. Oxygen (mg/L)
18/05/2021	16	490	312	0.16	7.5	0.88	8.2

Prepared by HCMR on behalf of ASPROFOS,2022

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-12 Sampling station located in Alfios river.

8 D.5.3.2 Chemical parameters of surface waters

The physicochemical condition based on nutrients and oxygen is characterized as high in both stations. In addition, the TOC concentrations at both stations are normal, show no unusual enrichment or pollution of water with organic carbon and are much lower than the values found in rivers whose water quality is characterized as poor. The measured values of total coliforms are also very low, characterizing the waters as unpolluted (EPA, 2003b). BOD_5 concentrations classify water as good quality. In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-31 Chemicals parameters (a).

	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Alfios Upsteram	6	<LOQ	5	1	4	HIGH
Alfios Downstream	6	1	<LOQ	1	3	HIGH

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-32. Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD_5 (mg/l)	COD (mg/l)
Upstream	582	0.8	52	2.56	3.90	<10
Downstream	843	2.0	36	3.49	3.10	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.3.3 *Physicochemical analyses in sediment*

The sediment in Alfios demonstrated a moderate load in TOC, low content of total phosphorus and high load in total nitrogen, although there are no relative quality limits.

Table D-33. Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.09	56	1.02

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.3.4 *Heavy metals in sediment*

Low levels of Cd and Hg were measured at Alfios sediment, below the ERL limits of the two metals, thus no adverse effects on benthic organisms are possible. The metals Cu, Pb, Zn had relatively low concentrations, lower than the respective ERL quality limits and therefore are also not likely to have adverse effects on benthic organisms.

Cr metal was measured to have a higher value concentration than ERL and therefore adverse effects on benthic organisms are potential. Ni metal was measured above the ERM limit so there are definite negative effects on benthic organisms. This is probably due to the natural origin of these two elements that are common in many watersheds of Greece from the disintegration of basic and ultrabasic rocks which show natural enrichment in Cr and Ni (Karageorgis et al., 2005). Cr (VI) was measured at a very low content of just 0.5% of total Cr. Low Cu, Pb and Zn levels were measured, below the ERL limit, so no adverse effects on benthic organisms are possible.

Table D-34. Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.172	155	0.72	0.040	101	14.1	28.8	<5	54.6	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.3.5 Hydrocarbons in sediments

Aliphatic hydrocarbon concentrations were very low and very close to expected background levels. Similar values have been measured in unpolluted sediments in different parts of Greece (Bouloubassi et al., 2012; Lipiatou and Saliot, 1991; Gogou et al., 2000; Hatzianestis and Sklivagou, 2001,). The levels of natural hydrocarbons in the sediments are about ~ 10 μg / g, while in areas with high productivity natural hydrocarbons of 100 μg/g have been measured (Bouloubassi and Saliot, 1993). Concentrations higher than this value indicate petroleum pollution. Therefore, it seems that there is no petroleum related pollution in the study areas.

Regarding PAHs, depending on their total concentrations, the sediments can be classified into four categories: (Baumard et al., 1998): (a) unpolluted, 0–100 ng/g; (b) moderately polluted, 100–1000 ng/g, (c) highly polluted, 1000–5000 ng/g and (d) extremely polluted> 5000 ng/g. In Alfios river the total PAH concentrations were <100 ng/g and indicate the absence of pollution (Botsou and Hatzianestis, 2012; Parinos et al., 2013; Hatzianestis et al., 2020).

Table D-35. Hydrocarbon concentrations in sediment (n.d.: not detected, detection limit: 0.1 μg/kg).

	Alfios-R
Total Hydrocarbons C_{12}-C_{40} (mg/kg)	7.2
Total Hydrocarbons < C_{12} (mg/kg)	0.8
Polycyclic aromatic hydrocarbons (PAH) (μg/kg)	
Naphthalene	2.4
Methyl - naphthalenes	2.0
Acenaphthylene	0.1
Acenaphthene	0.2

	Alfios-R
Dimethyl - naphthalenes	1.7
Triethyl - naphthalenes	n.d.
Fluorene	0.2
Dibenzothiophene	0.1
Methyl - dibenzothiophenes	0.1
Dimethyl - dibenzothiophenes	n.d.
Phenanthrene	1.4
Anthracene	0.1
Methyl- phenanthrenes	2.7
Dimethyl- phenanthrenes	1.6
Trimethyl- phenanthrenes	0.3
Fluoranthene	0.5
Pyrene	0.5
Methyl-pyrenes	0.9
Dimethyl-pyrenes	0.4
Retene	0.1
Benzo(a)anthracene	0.5
Chrysene	0.5
Methyl chrysenes	0.9
Dimethyl chrysenes	0.4
Benzo(b)fluoranthene	0.6
Benzo(k)fluoranthene	0.1
Benzo(e)pyrene	0.5
Benzo(a)pyrene	0.2
Perylene	1.5
Indeno(1,2,3-cd)pyrene	n.d.
Bibenzo(ghi_perylene	0.4
DEbenzo(ah)anthracene	0.1
ΣPAH	20.9

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.3.6 *PCBs in sediment*

The concentrations of PCBs are given in the following table (Table D-36). The quantified compounds include also the seven PCBs (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) selected by ICES (International Council for the Exploration of the Sea) and recommended for monitoring by the European Union. These PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The PCBs concentrations in Alfios river's sample were very low and clearly lower than those measured in sediments collected from both the coastal zone and the open sea in the Mediterranean area (De Lazzari et al, 2004, Hatzianestis et al, 2000, Tolosa et al, 1995). Therefore, is seems that no pollution from PCBs exists. Most congeners were not detectable, whereas in all cases, in higher quantities the hexachloro- CB153 and CB138 were detected, followed by the heptachloro- CB180, in accordance with the commercial formulations such as Arochlor 1260.

Table D-36. Concentrations of polychlorinated biphenyls (PCBs) (ng/g) in sediment samples. As total PCBs the sum of the individual congeners is calculated. (n.d.: not detected, detection limit: 0.01 ng/g).

Stations	CB 28	CB 52	CB 101	CB 118	CB 153	CB 105	CB 138	CB 183	CB 128	CB 156
ALFIOS -R	0.01	0.01	n.d.	n.d.	0.01	0.01	0.03	n.d.	n.d.	n.d.

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.3.7 *Granulometry*

The predominant class in the sediment of Alfios is sand, followed by clay.

Table D-37 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Alfios	59.61	3.32	37.07

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.3.8 *Benthic macroinvertebrates*

The biological status at Alfeios River sampling site was classified as good based on benthic macroinvertebrates. A total of 725 individuals belonging to 23 families were collected, with the predominant families of Baetidae (98 individuals), Leptophlebiidae (92 individuals), Ephemerellidae (69 individuals), Heptageniidae (62 individuals) and Caenidae (26 individuals) accounting for 48% of the total abundance (Figure D-13). Diptera of the family Chironomidae followed (263 individuals) with 37% of the total abundance (Figure D-13). The remaining groups of invertebrates were present with relatively small number of taxa and abundances as shown in Figure D-13.

Table D-38. Biological status based on benthic macroinvertebrate fauna.

River Name	Alfios
Date	**18/05/2021**
Typology	**R-M1**
Total abundance	725
Number of Taxa	23
Shannon-Wiener Diversity Index	2,08
Number of sensitive families	9
Number of tolerant families	6
HESY2 Score	0,89
HESY2 Quality	Good
% EPT	53,93
% EPTC	57,10

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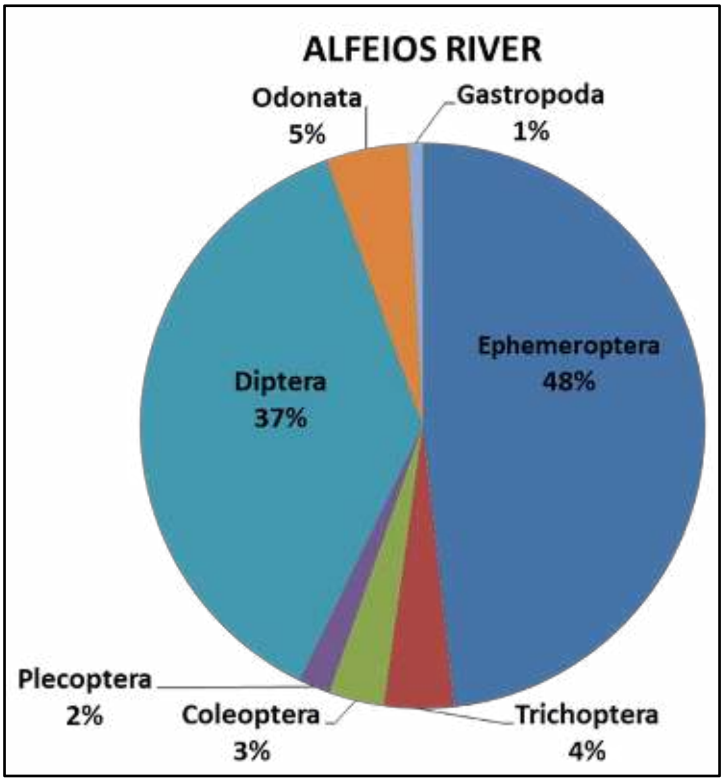


Prepared by HCMR on behalf of ASPROFOS,2022.

Figure D-13 Abundance percentages of macroinvertebrate groups at the sampling site of Alfeios river.

8 D.5.3.9 Benthic diatom assessment

In this site, 21 species of diatoms were identified, and the assemblage presented very low evenness (E=0.47) and thus low diversity based on Shannon diversity index (H=2.06). The most dominant species was *Achnanthidium minutissimum* (65.5%). A. *minutissimum* is usually found in well oxygenated, clean, fresh waters (Taylor et al 2007). Biological quality of the site based on benthic diatoms is high (EQR IPS=1.15), with no organic pollution or anthropogenic eutrophication (Table D-39).

Table D-39 EQR IPS and IPS values and quality based on color code.

River name	River Type	EQR IPS/IPS	High Good Moderate Poor Bad
ALFEIOS_R	R-M1	1.15	

8 D.5.3.10 Ichthyological assessment

A total of three species were detected, relatively low numbers of fish. This site is initially evaluated with "moderate" ecological quality based on the sampling of the fish fauna.

This area is a peculiar part of the upper reaches of the southern branch of the Alfios River. The river is located in an open valley area on a plateau and has some special fish characteristics that make it stand out as a river area with a distinctly different fish community from other parts of the main river of Alfios. Some species of fish are absent and a species that is not usually found in many parts of the main river (Pelasgus laconicus) predominates. Pelasgus laconicus is limited only at this site and in Evrotas river as a narrow endemic species of the Southern Peloponnese. However, fish populations were not particularly dense or high in absolute numbers during sampling campaign. It is also important to note how low was the population density of Briana (Barbus peloponnesius). The location and area as a whole are ichthyologically peculiar and cannot be easily assessed in terms of its fish-based ecological quality. Uncertainty regarding the assessment of ecological quality based on fish ("Moderate" on the five-point scale) is high. However, due to the very rare endemic species and the crystal-clear cold waters of the area, special attention should be paid in order to maintain the conditions of this site.

Table D-40 Summary results of the relative abundance of species collected at Alfios river.

Species	Alfios
Anguilla anguilla	
Atherina boyeri	
Barbus_peloponnesius	1
Carassius gibelio*	
Economidichthys trichonis	
Economidichthys_pygmaeus	
Gambusia holbrooki*	
Gobiidae sp.	
Liza ramada	
Luciobarbus_albanicus	
Mugil cephalus	
Mugilidae sp.	
Pelasgus_laconicus	3

Species	Alfios
Pelasgus_thesproticus	
Salaria_fluviatilis	
Scardinius acarnanicus	
Squalius_keadicus	
Squalius_peloponensis	2
Telestes_pleurobipunctatus	
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-41 Fish characteristics and confidence assessment and bioassessment at Alfios river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Alfios
Total fish species	3
Diversity of expected typespecific species	Moderate
Reproduction data	Good
Presence of intolerant species	Moderate
Presence of expected migratory species	Bad
Sampling effort assessment	Good
Uncertainty estimation for bioassessment	Low

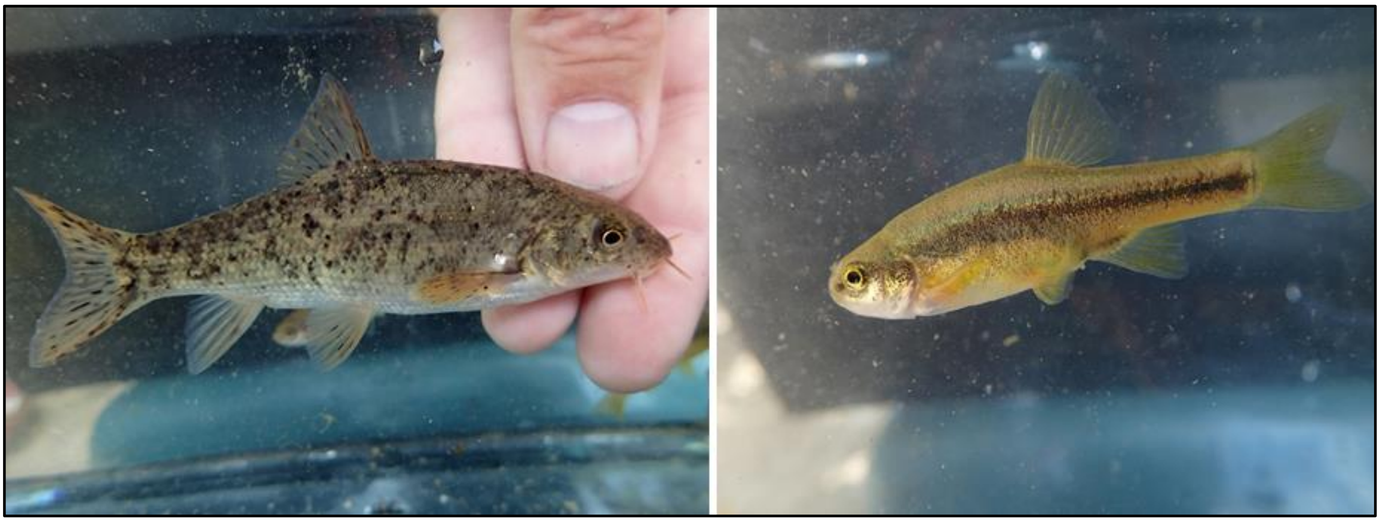
Prepared by HCMR on behalf of ASPROFOS,2022

Table D-42 HeFI index implementation at Alfios river.

Sampling parameters	Alfios
Density of insectivores	4
Density of omnivores	2
Density of benthic species	3
Density of potamodromus species	2

Sampling parameters	Alfios
Degree of Certainty	1
Preliminary assessment	2.75
Preliminary ecological quality (three-level scale)	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022



Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-14 Important species of fish fauna in the upper reaches of the Alfios. Left: Barbus peloponnesius (Peloponnesian briana). Right: Pelasgus laconicus (Laconic Pelasgian). The last species has only two populations (Evrotas, upstream Alfios) and this individual bears the bright colors of the breeding season.

8 D.5.3.11 Hydromorphological assessment

The QBR score for Alfios was 65 which indicated a moderate status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 8 D.6 of this document. The drone derived orthophoto map of this site is accessible here:

https://cloud.pix4d.com/dataset/944948/map?shareToken=b3a0229a-2600-489b-8624-2c9dd2836ffe

8 D.5.3.12 Ecological status

For the complete assessment of the ecological quality of Alfios river the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality. Since the uncertainty regarding the assessment of ecological quality based on fish ("Moderate") is high in Alfios river, the biological quality is assessed based only on the indices HESY2 and EQR IPS/IPS. After the implementation of all classification schemes in the various parameters, we get the biological, physicochemical and hydromorphological quality class at each study site (worst case principle applied). Then the methodology illustrated in Figure D-6has been used by combining initial the biological and the physicochemical and then the hydromorphological quality classes at each study area, in order to get the final ecological status classification. Ecological status of the site located in Alfios river is characterized as **GOOD** based on its biological quality (worst case principle applied). Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/F8ztKxeF7X4X2hu5A.

Table D-43 Biological quality.

Water body	HESY2	EQR IPS/IPS	Ichthyofauna	Biological quality
ALFIOS	Good	High	Moderate	Good

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Table D-44 Final ecological quality.

Water body	Biological quality	Physicochemical quality	Hydromorphological quality	Ecological Status
ALFIOS	Good	High	Moderate	Good

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.4 Ladonas Pinios

8 D.5.4.1 Physicochemical parameters of surface waters

The following table (Table D-45) below presents the basic physicochemical parameters measured in the context of water and sediment sampling in May 2021. The values are at normal levels for the season and the concentration of dissolved oxygen indicates good quality.

Table D-45 Physicochemical parameters

Date	Temperature (°C)	E. Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	D. oxygen (mg/L)
18/05/2021	22.9	357	228	0.11	7.43	1.02	7.5

Prepared by HCMR on behalf of ASPROFOS,2022

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-15 Sampling station located in Ladon river.

8 D.5.4.2 Chemical parameters of surface waters

Despite the presence of increased nitrate values, the final physicochemical state of both stations is characterized as high. TOC concentrations at both stations are normal, do not indicate unusual enrichment or pollution of water with organic carbon and are much lower than the values found in rivers whose water quality is characterized as poor. The measured values of total coliforms are also very low. High quality is also indicated by the concentration of Biochemically Required Oxygen (Table D-47). In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-46 Chemical parameters (a).

	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Ladon Upstream	718	2	<LOQ	1	4	HIGH
Ladon Downstream	658	2	<LOQ	1	3	HIGH

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-47 Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD_5 (mg/l)	COD (mg/l)
Upstream	420	1.1	19	3.35	0.00	<10
Downstream	413	1.1	27	3.60	1.10	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.4.3 Physicochemical analyses in sediments

The sediment in Ladonas presented a low load on TOC and total phosphorus and a moderate concentration regarding the total nitrogen values (Table D-48), although there are no relative quality limits.

Table D-48 Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.016	43.2	0.17

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.4.4 Heavy metals in sediments

In sediment of Ladon river, low Cd and Hg concentrations were measured, lower than the ERL limits of the two metals, thus no negative effects on benthic organisms are possible.

The metals Cr, Cu, Pb, Zn were also measured at low concentrations, lower than the ERL limits thus no adverse effects on benthic organisms are possible. Ni metal was measured slightly higher (24.7mg/kg) than the ERL limit (20mg/kg). Cr (VI) also showed a very low concentration corresponding to 0.6% of total Cr.

Table D-49 Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.177	35.4	0.21	0.039	24.7	4.8	14.6	<5	17.9	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.4.5 Hydrocarbons in sediments

Aliphatic hydrocarbon concentrations were very low and very close to expected background levels. Similar values have been measured in unpolluted sediments in different parts of Greece (Bouloubassi et al., 2012; Lipiatou and Saliot, 1991; Gogou et al., 2000; Hatzianestis and Sklivagou, 2001,). The levels of natural hydrocarbons in the sediments are about ~ 10 μg / g, while in areas with high productivity natural hydrocarbons of 100 μg/g have been measured (Bouloubassi and Saliot, 1993). Concentrations higher than this value indicate petroleum pollution. Therefore, it seems that there is no petroleum related pollution in the study areas.

Regarding PAHs, depending on their total concentrations, the sediments can be classified into four categories: (Baumard et al., 1998): (a) unpolluted, 0–100 ng/g; (b) moderately polluted, 100–1000 ng/g, (c) highly polluted, 1000–5000 ng/g and (d) extremely polluted> 5000 ng/g. In Ladon river the total PAH concentrations were <100 ng/g and indicate the absence of pollution (Botsou and Hatzianestis, 2012; Parinos et al., 2013; Hatzianestis et al., 2020).

Table D-50 Hydrocarbon concentrations in sediment (n.d.: not detected, detection limit: 0.1 μg/kg).

	Ladon-R
Total Hydrocarbons C_{12}-C_{40} (mg/kg)	4.3
Total Hydrocarbons < C_{12} (mg/kg)	0.6
Polycyclic aromatic hydrocarbons (PAH) (μg/kg)	
Naphthalene	2.6
Methyl - naphthalenes	2.5
Acenaphthylene	n.d.
Acenaphthene	0.2
Dimethyl - naphthalenes	1.6
Triethyl - naphthalenes	0.8
Fluorene	0.2

	Ladon-R
Dibenzothiophene	0.1
Methyl - dibenzothiophenes	n.d.
Dimethyl - dibenzothiophenes	n.d.
Phenanthrene	0.9
Anthracene	0.1
Methyl- phenanthrenes	1.5
Dimethyl- phenanthrenes	0.8
Trimethyl- phenanthrenes	0.5
Fluoranthene	0.4
Pyrene	0.5
Methyl-pyrenes	0.6
Dimethyl-pyrenes	0.1
Retene	0.4
Benzo(a)anthracene	0.6
Chrysene	0.3
Methyl chrysenes	0.4
Dimethyl chrysenes	0.1
Benzo(b)fluoranthene	0.5
Benzo(k)fluoranthene	0.2
Benzo(e)pyrene	0.3
Benzo(a)pyrene	0.3
Perylene	2.1
Indeno(1,2,3-cd)pyrene	0.1
Bibenzo(ghi_perylene	0.2
DEbenzo(ah)anthracene	n.d.
ΣPAH	**18.7**

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.4.6 PCBs in sediments

The concentrations of PCBs are given in the following table (Table D-51). The quantified compounds include also the seven PCBs (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) selected by ICES (International Council for the Exploration of the Sea) and recommended for monitoring by the European Union. These PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The PCBs concentrations in Ladon river's sample were very low and clearly lower than those measured in sediments collected from both the coastal zone and the open sea in the Mediterranean area (De Lazzari et al, 2004, Hatzianestis et al, 2000, Tolosa et al, 1995). Therefore, is seems that no pollution from PCBs exists. Most congeners were not detectable, whereas in all cases, in higher quantities the hexachloro- CB153 and CB138 were detected, followed by the heptachloro- CB180, in accordance with the commercial formulations such as Arochlor 1260.

Table D-51 Concentrations of polychlorinated biphenyls (PCBs) (ng/g) in sediment samples. As total PCBs the sum of the individual congeners is calculated. (n.d..: not detected, detection limit: 0.01 ng/g).

Stations	CB 28	CB 52	CB 101	CB 118	CB 153	CB 105	CB 138	CB 183	CB 128	CB 156	CB 180	CB 170	CB 194	Sum of PCBs
LADON-R	0.01	0.01	ND	ND	0.01	ND	0.04	ND	ND	0.01	0.01	0.02	ND	**0.111**

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.4.7 Granulometry

The predominant granulometric class of Ladon river's sediment is sand.

Table D-52 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Ladon	89.98	0.28	9.73

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.4.8 Benthic macroinvertebrates

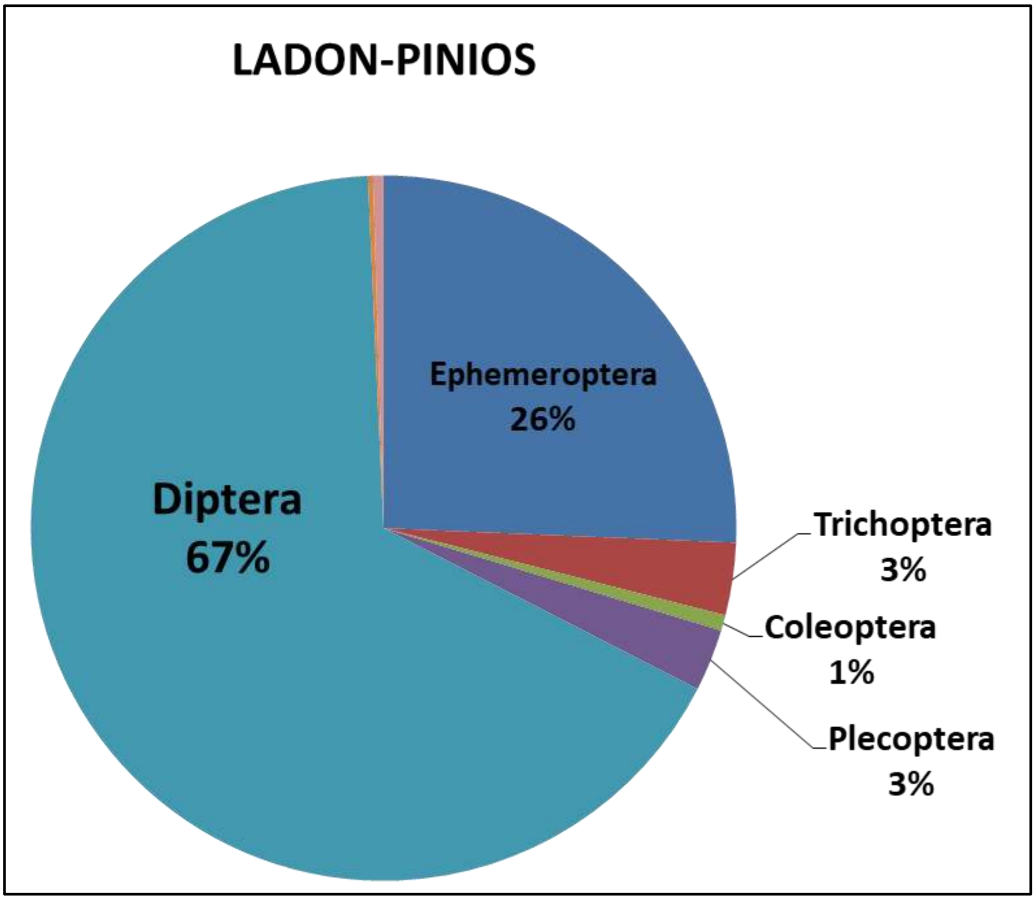
The biological status of Ladon-Pinios sampling site was classified as moderate based on benthic invertebrate fauna. A total of 421 individuals belonging to 18 families, with Diptera and

Ephemeroptera being the predominant groups were collected with 67% and 26% of the total abundance, respectively (Figure D-16). Simuliidae (152 individuals) and Chironomidae (110 individuals) were the most dominant Diptera families, whereas Baetidae (71 individuals) and Ephemerellidae (29 individuals) were the most dominant Ephemeroptera families. The rest of the invertebrate groups were present with very low abundances as shown in Figure D-16. It should be noted that there is a significant hydromorphological degradation in the river.

Table D-53 Biological status based on benthic macroinvertebrate fauna.

River Name	Ladon-Pinios
Date	18/05/2021
Typology	R-M2
Total abundance	421
Number of Taxa	18
Shannon-Wiener Diversity Index	1,79
Number of sensitive families	3
Number of tolerant families	7
HESY2 Score	0,67
HESY2 Quality	Moderate
% EPT	31,83
% EPTC	32,54

Prepared by HCMR on behalf of ASPROFOS,2022



Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-16 Abundance percentages of macroinvertebrate groups at the sampling site of Ladon-Pinios river.

8 D.5.4.9 Bethic diatoms assessment

In this site, only 13 species of diatoms were identified, and the assemblage presented relatively low evenness (E=0.6) and thus low diversity based on Shannon diversity index (H=2.23). Dominant species were Cymbella affinis (19.4%) and Encyonema minutum (11.4%), followed by Encyonopsis microcephala (9.5%). These species are cosmopolitan and common in oligo to mesotrophic systems (Cantonati et al 2017). Biological quality of the site based on benthic diatoms is high (EQR IPS=1.049), with no organic pollution and low anthropogenic eutrophication (Table D-54).

Table D-54 EQR IPS and IPS values and quality based on a color code.

River name	River Type	EQR IPS/IPS	High / Good / Moderate / Poor / Bad
LADON_PINEIOS	R-M2	1.049	

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.4.10 Ichthyological assessment

A total of four species were found, moderate numbers of fish. This site is initially evaluated with "moderate" ecological quality based on the sampling of the fish fauna.

The area has undergone hydromorphological degradation (mainly gravel extraction) over an extensive length of the river section and there are many points with surface water extractions (irrigation pumps). However, the river area maintains a high interest in fish fauna (high number of species). Based on the sampling, the river area at this point is classified as moderate. The fish are very small in size and the distribution of fish is concentrated in deeper parts of the river. The degradation of fish fauna is obviously mainly due to the summer extractions and diversions of water for the irrigation of adjacent crops. The reduction of the natural flow and the low water levels affect negatively the fish (especially the large ones). For these reasons, this site is classified as of "moderate" ecological quality based on the fish fauna sampling with high certainty concerning the evaluation. The area is probably affected by the downstream dam which, strangely, can "benefit" some species of fish (eg *Salaria fluviatlis, Luciobarbus albanicus*). On the other hand, the dam lake causes various structural conditions in the fish fauna (probably from year to year). Changes in composition (structure) of the fish fauna are obviously always in relation to the reproductive success and the composition of the populations in the adjacent lake. At least one species (Carassius sp.) is favored by the presence of the artificial lake. These characteristics indicate a degradation compared to the expected natural reference conditions in this part of the river.

Table D-55 Summary results of the relative abundance of species collected at Ladon river.

Species	Ladon
Anguilla anguilla	
Atherina boyeri	
Barbus_peloponnesius	1
Carassius gibelio*	

Species	Ladon
Economidichthys trichonis	
Economidichthys_pygmaeus	
Gambusia holbrooki*	
Gobiidae sp.	
Liza ramada	
Luciobarbus_albanicus	
Mugil cephalus	
Mugilidae sp.	
Pelasgus_laconicus	
Pelasgus_thesproticus	
Salaria_fluviatilis	3
Scardinius acarnanicus	
Squalius_keadicus	
Squalius_peloponensis	2
Telestes_pleurobipunctatus	1
Tropidophoxinellus_hellenicus	1
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-56 Fish characteristics and confidence assessment and bioassessment at Ladon river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Ladon
Total fish species	5
Diversity of expected typespecific species	High
Reproduction data	Good
Presence of intolerant species	Moderate
Presence of expected migratory species	Moderate
Sampling effort assessment	Good

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Ladon
Uncertainty estimation for bioassessment	High

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-57 HeFI index implementation at Ladon river.

Sampling parameters	Ladon
Density of insectivores	4
Density of omnivores	3
Density of benthic species	4
Density of potamodromus species	2
Degree of Certainty	4
Preliminary assessment	3.25
Preliminary ecological quality (three-level scale)	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.4.11 Hydromorphological assessment

The QBR score for Ladon was 90 which indicated a good status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 7 of this document. The drone derived orthophoto map of this site is accessible here:

https://cloud.pix4d.com/dataset/944955/map?shareToken=0f0a1bc8-bca4-43b3-b10f-718121ff0e1d

8 D.5.4.12 Ecological status

For the complete assessment of the ecological quality of Ladon river the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality (worst case principle applied). After the implementation of all classification schemes in the various parameters, we get the biological, physicochemical and hydromorphological quality class at each study site. Then the methodology illustrated in Figure D-6 has been used by combining initial the biological and the physicochemical and then the hydromorphological quality classes at each study area, in order to get the final ecological status classification. Ladon river is characterized by **MODERATE** ecological status due to moderate biological quality. Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/F7NnbuyKN1v3nqnF6.

Table D-58 Biological quality.

Water body	HESY2	EQR IPS/IPS	Ichthyofauna	Biological quality
LADON	Moderate	High	Moderate	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-59 Final ecological quality.

Water body	Biological quality	Physicochemical quality	Hydromorphological quality	Ecological Status
LADON	Moderate	High	Good	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.5 Pinios River

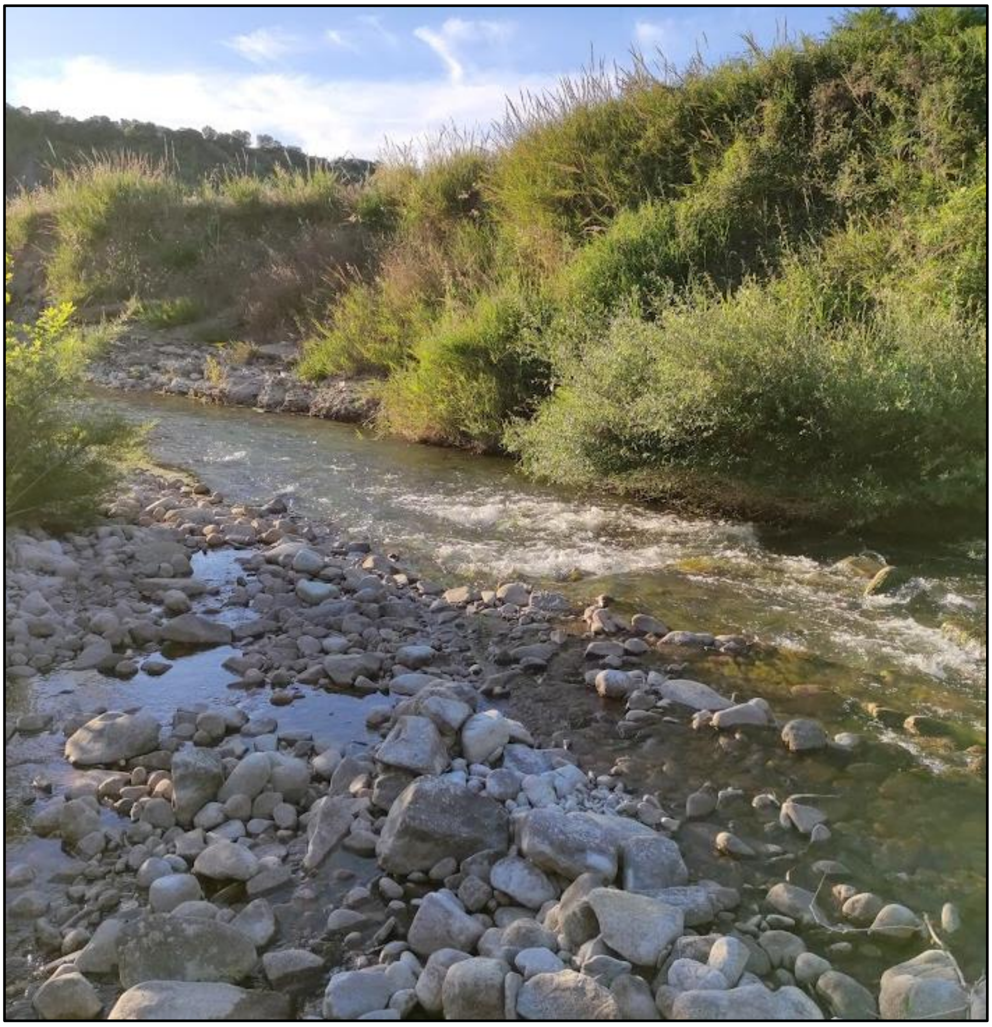
8 D.5.5.1 Physicochemical parameters of surface waters

The following table (Table D-60) below presents the basic physicochemical parameters measured during water and sediment sampling in May 2021. The values are at normal levels for the season and the concentration of dissolved oxygen indicates good quality.

Table D-60 Physicochemical parameters.

Date	Temperature (°C)	El. Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	Dissolved Oxygen (mg/L)
18/05/2021	22.1	402	257	0.13	7.41	2.32	8.44

Prepared by HCMR on behalf of ASPROFOS,2022



Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-17 Sampling station located in Pinios river.

8 D.5.5.2 Chemical parameters of surface waters

The physicochemical quality of both stations is high as well as the BOD_5 concentrations indicated high water quality. The TOC values are slightly higher than 1000 μg/l, but no unusual enrichment or pollution of water with organic carbon is indicated and those values are much lower than those found in rivers whose water quality is characterized as poor. Furthermore, measured values of total coliforms are also low (<100 cfu / 100 ml, unpolluted water) (EPA, 2003b). In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-61 Chemical parameters (a).

	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Pinios Upstream	100	3	11	<LOQ	5	HIGH
Pinios Downstream	95	3	5	<LOQ	5	HIGH

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-62 Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD_5 (mg/l)	COD (mg/l)
Upstream	1170	1.3	7	12.20	0.80	<10
Downstream	1123	1	7	3.66	1.70	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.5.3 Physicochemical analyses in sediments

The sediment in Pinios presented a low concentration of organic carbon (TOC) and a moderate load concerning total nitrogen and total phosphorus, although there are no relative quality limits.

Table D-63 Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.042	320	0.20

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.5.4 Heavy metals in sediments

In Pinios, low Cd and Hg concentrations were measured, less than the ERL limits of the two metals, therefore they are not likely to have negative effects on benthic organisms. The metals Cr, Cu, Pb, Zn were also measured at low concentrations, lower than the ERL limits thus no adverse effects on benthic organisms are possible. Ni metal was measured above the ERM limit, therefore there are definite negative effects on benthic organisms. The presence of Ni is of geological origin from ophiolite rocks in the catchment area of the river Pinios (Karageorgis et al., 2005). Cr (VI) also showed a low concentration, corresponding to 0.7% of total Cr.

Table D-64 Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.104	78.6	0.599	0.041	76.1	14.8	26.6	<5	54.2	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.5.5 Hydrocarbons in sediments

Aliphatic hydrocarbon concentrations were very low and very close to expected background levels. Similar values have been measured in unpolluted sediments in different parts of Greece (Bouloubassi et al., 2012; Lipiatou and Saliot, 1991; Gogou et al., 2000; Hatzianestis and Sklivagou, 2001,). The levels of natural hydrocarbons in the sediments are about ~ 10 μg / g, while in areas with high productivity natural hydrocarbons of 100 μg/g have been measured (Bouloubassi and Saliot, 1993). Concentrations higher than this value indicate petroleum pollution. Therefore, it seems that there is no petroleum related pollution in the study areas.

Regarding PAHs, depending on their total concentrations, the sediments can be classified into four categories: (Baumard et al., 1998): (a) unpolluted, 0–100 ng/g; (b) moderately polluted, 100–1000 ng/g, (c) highly polluted, 1000–5000 ng/g and (d) extremely polluted> 5000 ng/g. According to this criterion, moderate pollution was detected in Pinios where the methylated derivatives of PAHs predominate, indicating petrogenic-petroleum origin.

Table D-65 Hydrocarbon concentrations in sediment (n.d. : not detected, detection limit: 0.1 μg/kg).

	Pinios
Total Hydrocarbons C12-C40 (mg/kg)	14.6
Total Hydrocarbons < C12 (mg/kg))	0.3
Polycyclic aromatic hydrocarbons (PAH) (μg/kg)	
Naphthalene	29.7
Methyl - naphthalenes	72.3
Acenaphthylene	0.2
Acenaphthene	0.8
Dimethyl - naphthalenes	41.3

	Pinios
Triethyl - naphthalenes	37.8
Fluorene	0.9
Dibenzothiophene	0.7
Methyl - dibenzothiophenes	3.2
Dimethyl - dibenzothiophenes	1.7
Phenanthrene	16.6
Anthracene	1.4
Methyl- phenanthrenes	33.7
Dimethyl- phenanthrenes	28.2
Trimethyl- phenanthrenes	17.5
Fluoranthene	4.6
Pyrene	4.8
Methyl-pyrenes	12.9
Dimethyl-pyrenes	14.2
Retene	6.6
Benzo(a)anthracene	3.6
Chrysene	4.1
Methyl chrysenes	7.9
Dimethyl chrysenes	8.5
Benzo(b)fluoranthene	4.9
Benzo(k)fluoranthene	1.0
Benzo(e)pyrene	3.4
Benzo(a)pyrene	2.0
Perylene	11.7
Indeno(1,2,3-cd)pyrene	1.5
Bibenzo(ghi_perylene	4.3
DEbenzo(ah)anthracene	0.8
ΣPAH	382.7

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.5.6 PCBs in sediments

The concentrations of PCBs are given in the following table (Table D-66). The quantified compounds include also the seven PCBs (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) selected by ICES (International Council for the Exploration of the Sea) and recommended for monitoring by the European Union. These PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The PCBs concentrations in Pinios river's sample were very low and clearly lower than those measured in sediments collected from both the coastal zone and the open sea in the Mediterranean area (De Lazzari et al, 2004, Hatzianestis et al, 2000, Tolosa et al, 1995). Therefore, is seems that no pollution from PCBs exists. Most congeners were not detectable, whereas in all cases, in higher quantities the hexachloro- CB153 and CB138 were detected, followed by the heptachloro- CB180, in accordance with the commercial formulations such as Arochlor 1260.

Table D-66 Concentrations of polychlorinated biphenyls (PCBs) (ng/g) in sediment samples. As total PCBs the sum of the individual congeners is calculated. (n.d..: not detected, detection limit: 0.01 ng/g).

Stations	CB 28	CB 52	CB 101	CB 118	CB 153	CB 105	CB 138	CB 183	CB 128	CB 156	CB 180	CB 170	CB 194	Sum of PCBs
PINEIOS	0.02	0.01	n.d.	n.d.	0.02	0.01	0.01	0.01	0.03	0.03	0.03	0.02	n.d.	0.203

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.5.7 Granulometry

The predominant classes in the sediment of Pinios are the sand and clay.

Table D-67 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Pinios	47.61	5.23	47.16

Prepared by HCMR on behalf of ASPROFOS,2022

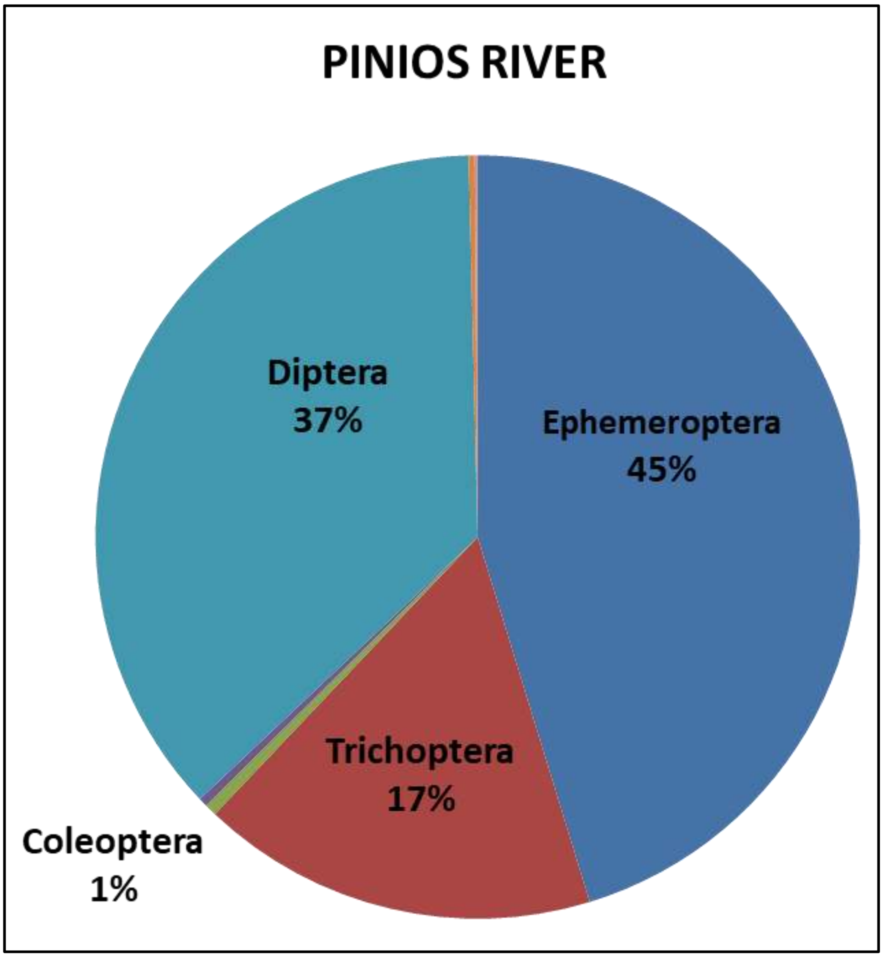
8 D.5.5.8 Benthic macroinvertebrates

The biological status at the sampling site of Pinios river was characterized as good based on the benthic invertebrate fauna. A total of 791 individuals belonging to 20 families were collected. The predominant groups were Ephemeroptera, Diptera and Trichoptera with 45%, 37% and 17% of the total abundance, respectively (Figure D-18). The most dominant families were Chironomidae (260 individuals) and Oligoneuriidae (217 individuals) followed by families Hydropsychidae (126 individuals), Baetidae (75 individuals), Leptophlebiidae (26 individuals) and Simuliidae (20 individuals) (Figure D-18). These families are relatively tolerant to moderate levels of pollution and their abundances increase considerably under these conditions. The remaining groups of invertebrates were represented with relatively small abundances as shown in Figure D-18.

Table D-68 Biological status based on benthic macroinvertebrate fauna.

River Name	Pinios
Date	18/05/2021
Typology	R-M2
Total abundance	791
Number of Taxa	20
Shannon-Wiener Diversity Index	1,83
Number of sensitive families	4
Number of tolerant families	6
HESY2 Score	0,78
HESY2 Quality	Good
% EPT	62,45
% EPTC	62,96

Prepared by HCMR on behalf of ASPROFOS,2022



Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-18 Abundance percentages of macroinvertebrate groups at the sampling site of Pinios river.

8 D.5.5.9 Benthic diatoms assessment

In this site, 28 species of diatoms were identified, and the assemblage presented high evenness (E=0.74) and thus high diversity based on Shannon diversity index (H=3.57). Dominant species were *Achnanthidium minutissimum* (21.5%), *Cocconeis pediculus* (16.8%) and *Navicula cryptotenella* (12.9%). *A. minutissimum* is usually found in well oxygenated, clean, fresh waters. *C. pediculus* and *N. cryptotenella* are usually found in alkaline waters, in moderate trophic levels (Taylor et al 2007, Cantonati et al 2017). A high relative abundance of *Achnanthidium saprophilum* (8.8%) suggests an impact of organic waste water. Biological quality of the site based on benthic diatoms is good (EQR IPS=0.945), with moderate anthropogenic eutrophication (Table D-69).

Table D-69 EQR IPS and IPS values and ecological quality classes with color code.

River name	River Type	EQR IPS/IPS	High / Good / Moderate / Poor / Bad
PINIOS	R-M2	0.945	

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.5.10 *Ichthyological assessment*

A total of three species were detected, low numbers of fish. This site is initially evaluated with "moderate" ecological quality based on the sampling of the fish fauna.

The sampling site is located at the main branch of the Pinios river and very close to its confluence with the homonymous artificial lake. As a result of the proximity of the river section with the high artificial level of the dam are influences on the fish fauna that affect and degrade their natural character. In the past, sampling campaigns conducted by HCMR have revealed the presence of foreign species that are favored by the lake. Due to aforementioned criteria, the overall assessment of ecological quality is "poor". The HCMR sampling time series also confirm that this site has been altered by the lake and possibly by increased organic load favoring some species of fish over others (eg *Barbus peloponnesius* and *Telestes pleurobipunctatus* have obviously been negatively affected). Those that are vulnerable to eutrophic conditions/stagnant waters present low populations and a low percentage of species composition in this part of the river.

Table D-70 Summary results of the relative abundance of species collected at Pinios river.

Species	Pinios
Anguilla anguilla	
Atherina boyeri	
Barbus_peloponnesius	
Carassius gibelio*	3
Economidichthys trichonis	
Economidichthys_pygmaeus	
Gambusia holbrooki*	
Gobiidae sp.	
Liza ramada	

Species	Pinios
Luciobarbus_albanicus	
Mugil cephalus	
Mugilidae sp.	
Pelasgus_laconicus	
Pelasgus_thesproticus	
Salaria_fluviatilis	3
Scardinius acarnanicus	
Squalius_keadicus	
Squalius_peloponensis	1
Telestes_pleurobipunctatus	
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-71 Fish characteristics and confidence assessment and bioassessment at Pinios river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Pinios
Total fish species	3
Diversity of expected typespecific species	Low
Reproduction data	Bad
Presence of intolerant species	Bad
Presence of expected migratory species	Bad
Sampling effort assessment	Good
Uncertainty estimation for bioassessment	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-72 HeFI index implementation at Pinios river.

Sampling parameters	Pinios
Density of insectivores	2
Density of omnivores	2
Density of benthic species	2
Density of potamodromus species	2
Degree of Certainty	3
Preliminary assessment	2
Preliminary ecological quality (three-level scale)	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.5.11 Hydromorphological assessment

The QBR score for Pinios was 75 which indicated a good status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 7 of this document. The drone derived orthophoto map of this site is accessible here:

https://cloud.pix4d.com/dataset/944961/map?shareToken=01b8c410-a82d-42ec-a6e0-a75d787a34d4

8 D.5.5.12 Ecological status

For the complete assessment of the ecological quality of Pinios river the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality (worst case principle applied). After the implementation of all classification schemes in the various parameters, we get the biological, physicochemical and hydromorphological quality class at each study site. Then the methodology illustrated in Figure D-6 has been used by combining initial the biological and the physicochemical and then the hydromorphological quality classes at each study area, in order to get the final ecological status classification. Pinios river is characterized by MODERATE ecological status due to moderate biological quality. Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/Gy77UEuXDCX7799o9.

Table D-73 Biological quality.

Water body	HESY2	EQR IPS/IPS	Ichthyofauna	Biological quality
PINIOS	Good	Good	Moderate	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-74 Final ecological quality.

Water body	Biological quality	Physicochemical quality	Hydromorphological quality	Ecological Status
PINIOS	Moderate	High	Good	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.6 Evinos River

8 D.5.6.1 Physicochemical parameters of surface waters

The following table (Table D-75) below presents the basic physicochemical parameters measured in the context of water and sediment sampling in May 2021. The values range in normal levels according to the sampling season and the concentration of dissolved oxygen indicates high quality.

Table D-75 Physicochemical parameters.

Date	Temperature (ºC)	El. Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	D. Oxygen (mg/L)
19/05/2021	19.7	319	204	0.1	7.51	1.13	9.27

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Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-19 Sampling station located in Evinos river.

8 D.5.6.2 *Chemical parameters of surface waters*

The final physicochemical quality of both stations of Evinos river is characterized as high, based on concentration of both BOD_5 and nutrients. The measured concentrations of TOC are less than 1000 μg/l, indicating no unusual enrichment or pollution of water with organic carbon and the values are lower than those found in rivers whose water quality is characterized as poor. Furthermore, measured values of total coliforms are equally low (EPA, 2003b). In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-76 Chemical parameters (a).

	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Evinos Upstream	98	1	12	1	4	HIGH
Evinos Downstream	95	1	6	<LOQ	3	HIGH

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-77 Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD_5 (mg/l)	COD (mg/l)
Upstream	308	1.9	40	4.16	1.70	<10
Downstream	424	1.2	33	3.60	1.40	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.6.3 Physicochemical analyses in sediments

The sediment of Evinos indicated a low concentration of TOC, and a moderate load in total nitrogen and total phosphorus, although there are no relative quality limits.

Table D-78 Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.023	280	0.32

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.6.4 Heavy metals in sediments

In Evinos, low Cd and Hg contents were measured, lower than the ERL limits of the two metals, thus no negative effects on benthic organisms are possible.

Cr metal was measured lower than the respective ERL value and therefore no adverse effects on benthic organisms are possible. Ni metal was measured above the ERM limit so there are definite negative effects on benthic organisms. Cr (VI) was measured at a relatively low concentration corresponding to 1% of total Cr. Low Cu, Pb and Zn values, below the ERL limit for each metal were measured, thus no adverse effects on benthic organisms are possible.

Table D-79 Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.092	138	0.88	0.040	64.5	9.6	27.0	<5	37.0	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.6.5 Hydrocarbons in sediments

Aliphatic hydrocarbon concentrations were very low and very close to expected background levels. Similar values have been measured in unpolluted sediments in different parts of Greece (Bouloubassi et al., 2012; Lipiatou and Saliot, 1991; Gogou et al., 2000; Hatzianestis and Sklivagou, 2001,). The levels of natural hydrocarbons in the sediments are about ~ 10 μg / g, while in areas with high productivity natural hydrocarbons of 100 μg/g have been measured (Bouloubassi and Saliot, 1993). Concentrations higher than this value indicate petroleum pollution. Therefore, it seems that there is no petroleum related pollution in the study areas.

Regarding PAHs, depending on their total concentrations, the sediments can be classified into four categories: (Baumard et al., 1998): (a) unpolluted, 0–100 ng/g; (b) moderately polluted, 100–1000 ng/g, (c) highly polluted, 1000–5000 ng/g and (d) extremely polluted> 5000 ng/g. According to this criterion, moderate pollution was detected in Evinos where the methylated derivatives of PAHs predominate, indicating petrogenic-petroleum origin.

Table D-80 Hydrocarbon concentrations in sediment (n.d.: not detected, detection limit: 0.1 μg/kg).

	Evinos
Total Hydrocarbons C12-C40 (mg/kg)	6.2
Total Hydrocarbons < C12 (mg/kg))	0.5
Polycyclic aromatic hydrocarbons (PAH) (μg/kg)	
Naphthalene	7.8
Methyl - naphthalenes	15.1
Acenaphthylene	0.3
Acenaphthene	0.5
Dimethyl - naphthalenes	16.4
Triethyl - naphthalenes	11.3
Fluorene	10.0

	Evinos
Dibenzothiophene	0.7
Methyl - dibenzothiophenes	0.4
Dimethyl - dibenzothiophenes	0.4
Phenanthrene	62.1
Anthracene	6.3
Methyl- phenanthrenes	17.9
Dimethyl- phenanthrenes	10.1
Trimethyl- phenanthrenes	5.8
Fluoranthene	14.6
Pyrene	4.4
Methyl-pyrenes	5.9
Dimethyl-pyrenes	6.1
Retene	1.2
Benzo(a)anthracene	1.5
Chrysene	2.2
Methyl chrysenes	3.0
Dimethyl chrysenes	2.8
Benzo(b)fluoranthene	2.5
Benzo(k)fluoranthene	0.4
Benzo(e)pyrene	2.0
Benzo(a)pyrene	0.9
Perylene	2.1
Indeno(1,2,3-cd)pyrene	0.5
Bibenzo(ghi_perylene	1.6
DEbenzo(ah)anthracene	0.3
ΣPAH	216.7

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.6.6 PCBs in sediments

The concentrations of PCBs are given in the following table (Table D-81). The quantified compounds include also the seven PCBs (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) selected by ICES (International Council for the Exploration of the Sea) and recommended for monitoring by the European Union. These PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The PCBs concentrations in Evinos river's sample were very low and clearly lower than those measured in sediments collected from both the coastal zone and the open sea in the Mediterranean area (De Lazzari et al, 2004, Hatzianestis et al, 2000, Tolosa et al, 1995). Therefore, is seems that no pollution from PCBs exists. Most congeners were not detectable, whereas in all cases, in higher quantities the hexachloro- CB153 and CB138 were detected, followed by the heptachloro- CB180, in accordance with the commercial formulations such as Arochlor 1260.

Table D-81 Concentrations of polychlorinated biphenyls (PCBs) (ng/g) in sediment samples. As total PCBs the sum of the individual congeners is calculated. (n.d..: not detected, detection limit: 0.01 ng/g).

Stations	CB 28	CB 52	CB 101	CB 118	CB 153	CB 105	CB 138	CB 183	CB 128	CB 156	CB 180	CB 170	CB 194	Sum of PCBs
EVINOS	0.03	0.02	n.d.	0.02	0.01	0.01	0.05	n.d.	n.d.	0.02	0.02	0.02	n.d.	0.194

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.6.7 Granulometry

Granulometry analysis in the sediment of the Evinos river highlighted sand and clay as the predominant classes.

Table D-82 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Evinos	66.46	3.36	30.18

Prepared by HCMR on behalf of ASPROFOS,2022

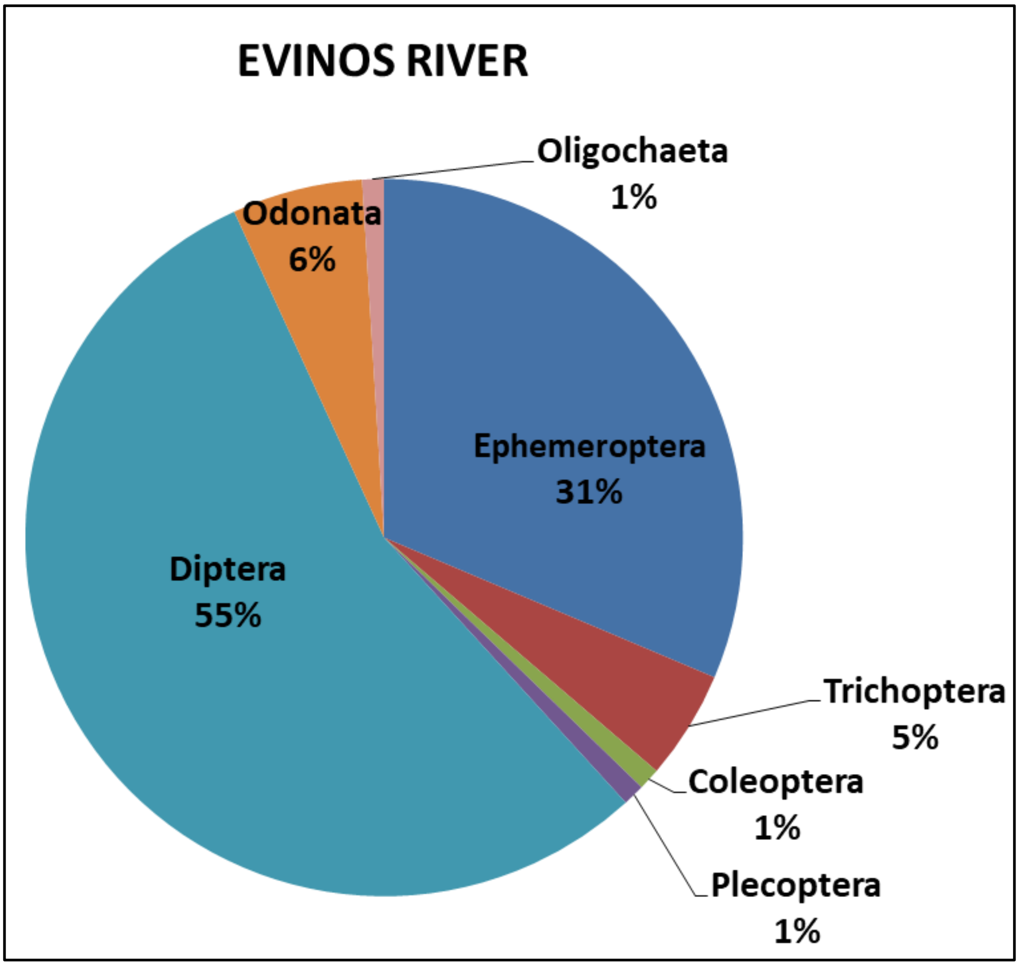
8 D.5.6.8 Benthic macroinvertebrates

The biological status at the sampling site of the Evinos River was classified as moderate based on benthic invertebrate fauna. A total of 104 specimens belonging to 13 families were collected. The most predominant groups were Diptera and Ephemeroptera with 55% and 31% of the total abundance, respectively (Figure D-20). Diptera were mainly represented by Chironomidae (56 individuals) and Ephemeroptera by Caenidae and Baetidae (15 and 14 individuals, respectively). The remaining invertebrate groups were present with very small numbers as shown in Figure D-20. Due to the poor habitat type consisting mainly of sand, the diversity of benthic fauna is not supported. Thus, the benthic fauna in the specific site is limited with a small number of species and abundances.

Table D-83 Biological status based on benthic macroinvertebrate fauna.

River Name	Evinos River
Date	19/05/2021
Typology	R-M3
Total abundance	104
Number of Taxa	13
Shannon-Wiener Diversity Index	1,56
Number of sensitive families	4
Number of tolerant families	4
HESY2 Score	0,67
HESY2 Quality	Moderate
% EPT	36,54
% EPTC	37,50

Prepared by HCMR on behalf of ASPROFOS,2022



Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-20 Abundance percentages of macroinvertebrate groups at the sampling site of Evinos river.

8 D.5.6.9 Benthic diatoms assessment

In this site, 32 species of diatoms were identified, and the assemblage presented high evenness (E=0.79) and thus high diversity based on Shannon diversity index (H=3.97). The most dominant species were Achnanthidium minutissimum (15.8%) and Eucocconeis flexella (12.2%), followed by Cymbella affinis (9.2%). A. minutissimum is usually found in well oxygenated, clean, fresh waters (Taylor et al 2007). E. flexella is found in oligotrophic, calcium-bicarbonate rich waters and is an indicator of very good ecological quality (Cantonati et al 2017). Biological quality of the site based on benthic diatoms is high (IPS=17.5), with no organic pollution or anthropogenic eutrophication (Table D-84).

Table D-84 EQR IPS and IPS values and ecological quality classes with color code.

River name	River Type	EQR IPS/IPS	High / Good / Moderate / Poor / Bad
EVINOS	R-M3	17.5	

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.6.10 Ichthyological assessment

A total of four species were detected, very small numbers of fish. This site is initially evaluated with "bad" ecological quality based on the sampling of the fish fauna.

This site is particularly degraded by extensive gravel mining and the operation of the Evinos hydroelectric dam. In general, this part of the river is degraded and presents a reduced number of species. It should also be noted that the eel (Anguilla anguilla) is absent from this site.

Table D-85 Summary results of the relative abundance of species collected at Evinos river.

Species	Evinos
Anguilla anguilla	
Atherina boyeri	
Barbus_peloponnesius	1
Carassius gibelio*	
Economidichthys trichonis	
Economidichthys_pygmaeus	
Gambusia holbrooki*	
Gobiidae sp.	
Liza ramada	
Luciobarbus_albanicus	2
Mugil cephalus	
Mugilidae sp.	1
Pelasgus_laconicus	
Pelasgus_thesproticus	
Salaria_fluviatilis	1

Species	Evinos
Scardinius acarnanicus	
Squalius_keadicus	
Squalius_peloponensis	
Telestes_pleurobipunctatus	
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-86 Fish characteristics and confidence assessment and bioassessment at Evinos river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Evinos
Total fish species	4
Diversity of expected typespecific species	Low
Reproduction data	Bad
Presence of intolerant species	Bad
Presence of expected migratory species	Bad
Sampling effort assessment	Good
Uncertainty estimation for bioassessment	High

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-87 HeFI index implementation at Evinos river.

Sampling parameters	Evinos
Density of insectivores	1
Density of omnivores	1
Density of benthic species	1
Density of potamodromus species	3
Degree of Certainty	4
Preliminary assessment	1.5

Sampling parameters	Evinos
Preliminary ecological quality (three-level scale)	Bad

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.6.11 Hydromorphological assessment

The QBR score for Evinos was 55 which indicated a moderate status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 7 of this document. The drone derived orthophoto map of this site is accessible here:

https://cloud.pix4d.com/dataset/944951/map?shareToken=56399645-a666-4c92-af58-814975632437

8 D.5.6.12 Ecological status

For the complete assessment of the ecological quality of Evinos river the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality (worst case principle applied). After the implementation of all classification schemes in the various parameters, we get the biological, physicochemical and hydromorphological quality class at each study site. Then the methodology illustrated in Figure D-6has been used by combining initial the biological and the physicochemical and then the hydromorphological quality classes at each study area, in order to get the final ecological status classification. Evinos river is characterized by **BAD** ecological status due to bad biological quality. Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/J4Gh4RHusFEo8Mkw9.

Table D-88 Biological quality.

Water body	HESY2	EQR IPS/IPS	Ichthyofauna	Biological quality
EVINOS	Moderate	High	Bad	Bad

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-89 Final ecological quality.

Water body	Biological quality	Physicochemical quality	Hydromorphological quality	Ecological Status
EVINOS	Bad	High	Moderate	Bad

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.7 Enotiki Tafros

8 D.5.7.1 *Physicochemical parameters of surface waters*

The following table (Table D-90) below presents the basic physicochemical parameters measured in the context of water and sediment sampling in May 2021. The values are normal for the season and the concentration of dissolved oxygen indicates good quality.

Table D-90 Physicochemical parameters.

Date	Temperatre (ºC)	El. Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	D. Oxygen (mg/L)
19/05/2021	23.1	320	250	0.1	7.52	2.20	7.44

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.7.2 *Chemical parameters of surface waters*

The final physicochemical quality of both stations is High. In natural waters the values of TOC are less than 1000 μg/l while in this case the values exceed this limit, because lakes generally have higher values of organic matter than rivers due to stagnant water. The colonies of total coliform bacteria were measured particularly low while BOD_5 indicated Good and High quality in the upstream and downstream stations, respectively. In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-91 Chemical parameters (a).

	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Enotiki Tafros Upstream	9	<LOQ	7	<LOQ	5	HIGH
Enotiki Tafros Downstream	8	<LOQ	7	<LOQ	5	HIGH

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-92 Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD_5 (mg/l)	COD (mg/l)
Upstream	1929	5.5	41	6.46	2.80	<10
Downstream	1579	2.2	15	8.60	1.70	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.7.3 Physicochemical analyses in sediments

The sediment analysis concerning the Enotiki Tafros indicated a high concentration of TOC, total nitrogen and total phosphorus although there are no relative quality limits. The highest TOC and total nitrogen values and the second highest total phosphorus concentration were measured in Enotiki Tafros. These values may be attributed to diffuse agricultural sources of pollution.

Table D-93 Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.318	499	2.61

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.7.4 Heavy metals in sediments

Sediment analysis of Enotiki Tafros indicated low Cd and Hg concentrations, lower than the ERL limits of the two metals, thus no negative effects on benthic organisms are possible. Cr metal was measured as higher than its respective ERL and therefore adverse effects on benthic organisms are potential. Ni metal was measured above the ERM limit thus there are definite negative effects on benthic organisms. This is probably due to the natural origin of these two elements that are common in many watersheds of Greece from the disintegration of basic and ultrabasic rocks which indicates natural enrichment in Cr and Ni (Karageorgis et al., 2005). Cr (VI) was measured at a higher value compared to all other rivers but corresponds only to 3% of total Cr. The Cu concentration was higher than the ERL threshold, so negative effects on benthic organisms are possible, but for the Pb and Zn metals the measured values were lower than the respective ERL thresholds, so no adverse effects on benthic organisms are possible. In Enotiki Tafros were measured the highest Cu and the second highest Zn concentrations, which are probably attributed to diffuse agricultural sources of pollution.

Table D-94 Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.158	63.3	4.61	0.080	204	22.8	60.8	<5	101	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.7.5 Hydrocarbons in sediments

Aliphatic hydrocarbon concentrations were very low and very close to expected background levels. Similar values have been measured in unpolluted sediments in different parts of Greece (Bouloubassi et al., 2012; Lipiatou and Saliot, 1991; Gogou et al., 2000; Hatzianestis and Sklivagou, 2001,). The levels of natural hydrocarbons in the sediments are about ~ 10 μg / g, while in areas with high productivity natural hydrocarbons of 100 μg/g have been measured (Bouloubassi and Saliot, 1993). Concentrations higher than this value indicate petroleum pollution. Therefore, it seems that there is no petroleum related pollution in the study areas.

Regarding PAHs, depending on their total concentrations, the sediments can be classified into four categories: (Baumard et al., 1998): (a) unpolluted, 0–100 ng/g; (b) moderately polluted, 100–1000 ng/g, (c) highly polluted, 1000–5000 ng/g and (d) extremely polluted> 5000 ng/g. In Enotiki Tafros sediment the total PAH concentrations were <100 ng/g and indicate the absence of pollution (Botsou and Hatzianestis, 2012; Parinos et al., 2013; Hatzianestis et al., 2020).

Table D-95 Hydrocarbon concentrations in sediment (n.d. : not detected, detection limit: 0.1 μg/kg).

	Enotiki
Total Hydrocarbons C_{12}-C_{40} (mg/kg)	21.7
Total Hydrocarbons < C_{12} (mg/kg)	0.2
Polycyclic aromatic hydrocarbons (PAH) (μg/kg)	
Naphthalene	4.9
Methyl - naphthalenes	6.4
Acenaphthylene	0.2
Acenaphthene	0.4
Dimethyl - naphthalenes	4.2
Triethyl - naphthalenes	5.7
Fluorene	0.3
Dibenzothiophene	0.2
Methyl - dibenzothiophenes	0.2
Dimethyl - dibenzothiophenes	0.5
Phenanthrene	2.4
Anthracene	0.2
Methyl- phenanthrenes	4.5

	Enotiki
Dimethyl- phenanthrenes	3.8
Trimethyl- phenanthrenes	1.7
Fluoranthene	1.2
Pyrene	1.1
Methyl-pyrenes	2.3
Dimethyl-pyrenes	2.6
Retene	1.1
Benzo(a)anthracene	0.8
Chrysene	1.3
Methyl chrysenes	2.0
Dimethyl chrysenes	1.6
Benzo(b)fluoranthene	2.1
Benzo(k)fluoranthene	0.3
Benzo(e)pyrene	1.4
Benzo(a)pyrene	0.5
Perylene	25.3
Indeno(1,2,3-cd)pyrene	0.5
Bibenzo(ghi_perylene	1.4
DEbenzo(ah)anthracene	0.3
ΣPAH	**81.0**

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.7.6 PCBs in sediments

The concentrations of PCBs are given in the following table (Table D-96). The quantified compounds include also the seven PCBs (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) selected by ICES (International Council for the Exploration of the Sea) and recommended for monitoring by the European Union. These PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The PCBs concentrations in Enotiki Tafros sediment's sample were very low and clearly lower than those measured in sediments collected from both the coastal zone and the open sea in the Mediterranean area (De Lazzari et al, 2004, Hatzianestis et al, 2000, Tolosa et al, 1995). Therefore, is seems that no pollution from PCBs exists. Most congeners were not detectable, whereas in all cases, in higher quantities the hexachloro- CB153 and CB138 were detected, followed by the heptachloro- CB180, in accordance with the commercial formulations such as Arochlor 1260.

Table D-96 Concentrations of polychlorinated biphenyls (PCBs) (ng/g) in sediment samples. As total PCBs the sum of the individual congeners is calculated. (n.d..: not detected, detection limit: 0.01 ng/g).

Stations	CB 28	CB 52	CB 101	CB 118	CB 153	CB 105	CB 138	CB 183	CB 128	CB 156	CB 180	CB 170	CB 194	Sum of PCBs
ENOTIKI	0.02	n.d.	n.d.	n.d.	0.01	0.01	0.03	0.01	n.d.	0.02	0.01	0.02	n.d.	0.169

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.7.7 Granulometry

Sediment granulometric analysis highlighted the clay as the predominant class.

Table D-97 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Enotiki Tafros	2.21	14.81	82.98

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.7.8 Benthic macroinvertebrates and diatoms

The station of Enotiki Tafros is practically located in a ditch that connects Lake Trichonida with Lake Lysimacheia. Therefore, the access was to perform appropriate sampling of macroinvertebrates and diatoms was not possible.

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-21 Sampling station of Enotiki Tafros, located in a ditch connencting Lake Trichonida with Lake Lysimacheia.

8 D.5.7.9 Ichthyological assessment

A total of five species were recorded, large numbers of fish. The site is a heavily modified water body (artificial canal in a wetland area) and the fish index cannot be applied.

It is a highly modified water body in a wetland area that connects Trichonida with Lysimachia lakes. The composition of the fish fauna originates from Trichonida Lake and the presence of some species with special interest in the protection of biodiversity cannot affect the assessment of water quality. As far as the ecological potential is concerned, the area is characterized higher than average (ie probably as of "good" ecological potential). This assumption cannot be based on an index or measurements but mainly on indications concerning species richness, the presence of species with low pollution tolerances, the non-predominance of alien species and the presence of species with particular interest regarding the conservation of local biological diversity (rare, endangered and endemic species). This site hosts more than 16 fish species and is of special ichthyological interest.

Table D-98 Summary results of the relative abundance of species collected at Enotiki Tafros.

Species	Enotiki Tafros
Anguilla anguilla	
Atherina boyeri	
Barbus_peloponnesius	
Carassius gibelio*	
Economidichthys trichonis	1
Economidichthys_pygmaeus	
Gambusia holbrooki*	3
Gobiidae sp.	2
Liza ramada	
Luciobarbus_albanicus	3
Mugil cephalus	
Mugilidae sp.	
Pelasgus_laconicus	
Pelasgus_thesproticus	
Salaria_fluviatilis	
Scardinius acarnanicus	3
Squalius_keadicus	
Squalius_peloponensis	

Species	Enotiki Tafros
Telestes_pleurobipunctatus	
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-99 Fish characteristics and confidence assessment and bioassessment at Enotiki Tafros.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Enotiki Tafros
Total fish species	5
Diversity of expected typespecific species	High
Reproduction data	Good
Presence of intolerant species	Good
Presence of expected migratory species	Moderate
Sampling effort assessment	Bad
Uncertainty estimation for bioassessment	Low

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-100 HeFI index implementation at Enotiki Tafros.

Sampling parameters	Enotiki Tafros
Density of insectivores	
Density of omnivores	
Density of benthic species	
Density of potamodromus species	
Degree of Certainty	1
Preliminary assessment	Visual
Preliminary ecological quality (three-level scale)	Good Ecological Potential

Prepared by HCMR on behalf of ASPROFOS,2022

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-22 One of the most peculiar endemic species of freshwater fish and one of the smallest fishes in Europe. Here is the Economidichthys trichonis (Nanogovios) which is limited to its worldwide distribution in Trichonida and Lysimacheia lakes. This individual is a sturdy adult male with 2.5 cm length.

8 D.5.7.10 Hydromorphological assessment

The QBR score for Enot. Tafros was 10 which indicated a bad status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 8 D.6 of this document. No drone flight could be realized in this site due to the proximity of an airport (no flight zone).

8 D.5.7.11 Ecological status

For the complete assessment of the ecological quality of Enotiki Tafros the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality. Since the uncertainty regarding the assessment of ecological quality based on fish is high in Enotiki Tafros, and there is no information about the rest of biological quality elements (macroinvertabrates, diatoms) the biological quality cannot be assessed. Therefore, ecological status cannot be estimated. Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/v2Uz8F1dMYBfP55G6.

8 D.5.8 Acheloos River

8 D.5.8.1 *Physicochemical parameters of surface waters*

The table below (Table D-101) presents the basic physicochemical parameters measured in the context of water and sediment sampling in May 2021. The values are typical for the season while the concentration of dissolved oxygen indicates good quality.

Table D-101 Physicochemical parameters.

Date	Temperature (°C)	El. Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	D. Oxygen (mg/L)
19/05/2021	20.4	296	190	0.09	7.56	0.80	8.75

8 D.5.8.2 *Chemical parameters of surface waters*

Both stations of the Acheloos river are characterized by High physicochemical condition based on the concentrations of both nutrients and of dissolved oxygen. TOC concentrations at both stations are normal, do not indicate unusual enrichment or pollution of water with organic carbon and are much lower than the values found in rivers whose water quality is characterized as poor. The BOD_5 concentration indicated Good and High quality at stations located upstream and downstream, respectively. As far as microbiology is concerned, the measured values are very low. In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-102 Chemical parameters (a).

	$N\text{-}NO_3^-$ (μg/l)	$N\text{-}NH_4^+$ (μg/l)	$N\text{-}NO_2^-$ (μg/l)	Ptotal (μg/l)	$P\text{-}PO_4^{3-}$ (μg/l)	Status
Acheloos Upstream	56	1	6	<LOQ	4	HIGH
Acheloos Downstream	56	1	6	1	4	HIGH

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-103 Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD_5 (mg/l)	COD (mg/l)
Upstream	858	1.4	12	2.40	2.80	<10
Downstream	1274	2.0	9	3.06	1.40	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.8.3 Benthic macroinvertebrates and diatoms

Sampling station located in Acheloos river is practically detected in a concrete channel, where no sediment sampling or collection of macroinvertebrates and diatoms could be feasible.

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-23 Sampling station located in Acheloos river.

8 D.5.8.4 Ichthyological assessment

Overall one species of fish was recorded, very small numbers of fish were detected. This site is evaluated as of "bad" ecological quality based on the sampling of fish fauna.

This part of the Acheloos river is highly degraded while is located downstream of a large dam. These characteristics are indicated by the sum of all the samplings that have been conducted in the recent past at this area (relatively recent HCMR samplings recorded a complete absence of fish in the area downstream of the dam).

Table D-104 Summary results of the relative abundance of species collected at Acheloos river.

Species	Acheloos
Anguilla anguilla	
Atherina boyeri	
Barbus_peloponnesius	
Carassius gibelio*	
Economidichthys trichonis	
Economidichthys_pygmaeus	
Gambusia holbrooki*	
Gobiidae sp.	
Liza ramada	
Luciobarbus_albanicus	1
Mugil cephalus	
Mugilidae sp.	
Pelasgus_laconicus	
Pelasgus_thesproticus	
Salaria_fluviatilis	
Scardinius acarnanicus	
Squalius_keadicus	
Squalius_peloponensis	
Telestes_pleurobipunctatus	
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m.. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-105 Fish characteristics and confidence assessment and bioassessment at Acheloos river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Acheloos
Total fish species	1
Diversity of expected typespecific species	Low
Reproduction data	Bad
Presence of intolerant species	Bad
Presence of expected migratory species	Bad
Sampling effort assessment	Moderate
Uncertainty estimation for bioassessment	High

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-106 HeFI index implementation at Acheloos river.

Sampling parameters	Acheloos
Density of insectivores	1
Density of omnivores	1
Density of benthic species	1
Density of potamodromus species	1
Degree of Certainty	4
Preliminary assessment	1
Preliminary ecological quality (three-level scale)	Bad

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.8.5 Hydromorphological assessment

The QBR score for Acheloos was 5 which indicated a bad status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 7 of this document. The drone flight in this site was not possible due to official flight restrictions.

8 D.5.8.6 Ecological status

For the complete assessment of the ecological quality of Acheloos river the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality. The biological quality is assessed based only on ichthyofauna index. After the implementation of all classification schemes in the various parameters, we get the biological, physicochemical and hydromorphological quality class at each study site (worst case principle applied). Then the methodology illustrated in Figure D-6has been used by combining initial the biological and the physicochemical and then the hydromorphological quality classes at each study area, in order to get the final ecological status classification. Ecological status of the site located in Acheloos river is characterized as **BAD** based on its biological quality (worst case principle applied). Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/bthAeJL7VL4DurT27.

Table D-107 Biological quality.

Water body	Ichthyofauna	Biological quality
ACHELOOS	BAD	BAD

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-108 Final ecological quality.

Water body	Biological quality	Physicochemical quality	Hydromorphological quality	Ecological Status
ACHELOOS	BAD	HIGH	BAD	BAD

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.9 Dipotamos

8 D.5.9.1 Physicochemical parameters of surface waters

The following table (Table D-109) below presents the basic physicochemical parameters measured during water and sediment sampling in May 2021. The values are typical for the season and the concentration of dissolved oxygen indicated good quality.

Table D-109 Physicochemical parameters.

Date	Temperature (ºC)	El. Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	D. Oxygen (mg/L)
20/05/2021	24.3	22680	14510	13.64	7.57	1.22	7.01

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.9.2 Chemical parameters of surface waters

The final physicochemical quality of both stations of Dipotamos river, based on the nutrients and dissolved oxygen, is Good. The TOC values measured at both stations do not indicate unusual enrichment or pollution of water with organic carbon and are much lower than the values found in rivers whose water quality is characterized as poor. Estimated colonies of total coliforms are also particularly low. BOD_5 concentrations indicated high quality at both stations of Dipotamos. In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-110 Chemical parameters (a).

	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Dipotamos Upstream	428	13	22	1	5	GOOD
Dipotamos Downstream	392	10	21	2	6	GOOD

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-111 Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD5 (mg/l)	COD (mg/l)
Upstream	1629	1.7	8	4.85	2.00	<10
Downstream	1601	1.9	11	3.53	1.40	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.9.3 Physicochemical analyses in sediments

The sediment in Dipotamos river showed a moderate load of TOC, total nitrogen and total phosphorus although there are no relative quality limits.

Table D-112 Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.041	30.1	0.74

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.9.4 Heavy metals in sediments

In sediment of Dipotamos river were measured low Cd and Hg concentrations, lower than the ERL limits of the two metals, therefore they are not likely to affect negatively benthic organisms.

Measured value of Cr metal was higher than ERL and therefore adverse effects on benthic organisms are potential. Ni metal was measured above the ERM limit thus there are definite negative effects on benthic organisms. This may be probably attributed to the natural origin of those two elements that are common in many watersheds of Greece, originating from the disintegration of basic and ultrabasic rocks which show natural enrichment in Cr and Ni (Karageorgis et al., 2005). Measured concentration of Cr (VI) was very low, about 0.1% of total Cr. Low Cu, Pb and Zn contents were measured, below their respective ERL limits, thus no adverse effects on benthic organisms are possible.

Table D-113 Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.053	254	0.26	0.052	143	12.8	20.7	<5	50.8	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.9.5 Hydrocarbons in sediments

Aliphatic hydrocarbon concentrations were very low and very close to expected background levels. Similar values have been measured in unpolluted sediments in different parts of Greece (Bouloubassi et al., 2012; Lipiatou and Saliot, 1991; Gogou et al., 2000; Hatzianestis and Sklivagou, 2001,). The levels of natural hydrocarbons in the sediments are about ~ 10 μg / g, while in areas with high productivity natural hydrocarbons of 100 μg/g have been measured (Bouloubassi and Saliot, 1993). Concentrations higher than this value indicate petroleum pollution. Therefore, it seems that there is no petroleum related pollution in the study areas.

Regarding PAHs, depending on their total concentrations, the sediments can be classified into four categories: (Baumard et al., 1998): (a) unpolluted, 0–100 ng/g; (b) moderately polluted, 100–1000 ng/g, (c) highly polluted, 1000–5000 ng/g and (d) extremely polluted> 5000 ng/g. According to this criterion, moderate pollution was detected in Dipotamos river where the methylated derivatives of PAHs predominate, indicating petrogenic-petroleum origin.

Table D-114 Hydrocarbon concentrations in sediment (n.d.: not detected, detection limit: 0.1 μg/kg).

	Dipotamo
Total Hydrocarbons C12-C40 (mg/kg)	3.5
Total Hydrocarbons < C12 (mg/kg))	0.2
Polycyclic aromatic hydrocarbons (PAH) (μg/kg)	
Naphthalene	8.6
Methyl - naphthalenes	20.5
Acenaphthylene	0.2
Acenaphthene	0.4
Dimethyl - naphthalenes	21.0
Triethyl - naphthalenes	16.6
Fluorene	0.5
Dibenzothiophene	0.4
Methyl - dibenzothiophenes	0.8
Dimethyl - dibenzothiophenes	n.d.
Phenanthrene	5.6
Anthracene	0.4
Methyl- phenanthrenes	13.9
Dimethyl- phenanthrenes	12.0
Trimethyl- phenanthrenes	11.1
Fluoranthene	1.4
Pyrene	2.0
Methyl-pyrenes	5.5
Dimethyl-pyrenes	5.8
Retene	2.5
Benzo(a)anthracene	1.5
Chrysene	2.0
Methyl chrysenes	3.6
Dimethyl chrysenes	4.4
Benzo(b)fluoranthene	2.1

	Dipotamo
Benzo(k)fluoranthene	0.4
Benzo(e)pyrene	1.9
Benzo(a)pyrene	0.8
Perylene	6.9
Indeno(1,2,3-cd)pyrene	0.6
Bibenzo(ghi_perylene	1.9
DEbenzo(ah)anthracene	0.2
ΣPAH	155.2

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.9.6 PCBs in sediments

The concentrations of PCBs are given in the following table (Table D-115). The quantified compounds include also the seven PCBs (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) selected by ICES (International Council for the Exploration of the Sea) and recommended for monitoring by the European Union. These PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The PCBs concentrations in Dipotamos sediment's sample were very low and clearly lower than those measured in sediments collected from both the coastal zone and the open sea in the Mediterranean area (De Lazzari et al, 2004, Hatzianestis et al, 2000, Tolosa et al, 1995). Therefore, is seems that no pollution from PCBs exists. Most congeners were not detectable, whereas in all cases, in higher quantities the hexachloro- CB153 and CB138 were detected, followed by the heptachloro- CB180, in accordance with the commercial formulations such as Arochlor 1260.

Table D-115 Concentrations of polychlorinated biphenyls (PCBs) (ng/g) in sediment samples. As total PCBs the sum of the individual congeners is calculated. (n.d..: not detected, detection limit: 0.01 ng/g).

Stations	CB 28	CB 52	CB 101	CB 118	CB 153	CB 105	CB 138	CB 183	CB 128	CB 156	CB 180	CB 170	CB 194	Sum of PCBs
DIPOTAMOS	0.03	0.02	n.d.	0.01	0.01	0.01	0.05	n.d.	0.01	0.01	0.01	0.02	n.d.	0.183

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.9.7 Granulometry

The granulometry of the Dipotamos river's sediment indicated that the predominant classes are the sand and clay.

Table D-116 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Dipotamos	50.77	5.20	44.02

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.9.8 Benthic macroinvertebrates and diatoms

Due to the high depth of the sampling station and the semi artificial banks (wire boxes) of Dipotamos river, benthic macroinvertebrates and diatoms could not be sampled.

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-24 Sampling station located in Dipotamos river.

8 D.5.9.9 Ichthyological assessment

Overall one fish species was recorded, small numbers of fish were detected. This site is initially assessed as a "degraded" site based on fish fauna sampling.

The low river area is extremely degraded and characterized by significant hydrological and hydro morphological alterations.

Table D-117 Summary results of the relative abundance of species collected at Dipotamos river.

Species	Dipotamos
Anguilla anguilla	
Atherina boyeri	1
Barbus_peloponnesius	
*Carassius gibelio**	
Economidichthys trichonis	
Economidichthys_pygmaeus	
*Gambusia holbrooki**	
Gobiidae sp.	
Liza ramada	
Luciobarbus_albanicus	
Mugil cephalus	1
Mugilidae sp.	2
Pelasgus_laconicus	
Pelasgus_thesproticus	
Salaria_fluviatilis	
Scardinius acarnanicus	
Squalius_keadicus	
Squalius_peloponensis	
Telestes_pleurobipunctatus	
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-118 Fish characteristics and confidence assessment and bioassessment at Dipotamos river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Dipotamos
Total fish species	1
Diversity of expected typespecific species	Low
Reproduction data	Bad
Presence of intolerant species	Bad
Presence of expected migratory species	Unknown
Sampling effort assessment	Bad
Uncertainty estimation for bioassessment	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-119 HeFI index implementation at Dipotamos river.

Sampling parameters	Dipotamos
Density of insectivores	
Density of omnivores	
Density of benthic species	
Density of potamodromus species	
Degree of Certainty	3
Preliminary assessment	Visual
Preliminary ecological quality (three-level scale)	Degraded

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.9.10 Hydromorphological assessment

The QBR score for Dipotamos was 5 which indicated a bad status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 8 D.6 of this document. No drone flight was possible at this site due to official restrictions.

8 D.5.9.11 Ecological status

For the complete assessment of the ecological quality of each sampling site the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality. As far as the Dipotamos river is concerned, the application of fish index is characterized by moderate uncertainty while no other element is available. Therefore, the biological quality of this particular site cannot be assessed. In particular, we can only present the physicochemical quality of its surface waters which is characterized as good. Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/jn6xG7kyVEvcKfPN9.

8 D.5.10 Arachthos River

8 D.5.10.1 Physicochemical parameters of surface waters

The following table (Table D-120) below presents the basic physicochemical parameters measured during water and sediment sampling in May 2021. The values are typical for the season and the concentration of dissolved oxygen indicates good quality. TDS and salinity values could not be measured accurately since the multiparameter instrument was calibrated for fresh water and the water during the sampling campaign was characterized as brackish /sea water due to sea invasion.

Table D-120 Physicochemical parameters.

Date	Temperature (ºC)	El. Conductivity (µS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	D. Oxygen (mg/L)
21/05/2021	26.8	37040			8.3	1.47	7.8

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.10.2 Chemical parameters of surface waters

The final physicochemical quality is High and Good for the upstream and downstream station, respectively of the Arachthos river. TOC concentrations at both stations are normal, do not indicate unusual enrichment or pollution of water with organic carbon and are much lower than the values found in rivers whose water quality is characterized as poor. The number of colonies of total coliforms is also very small. BOD_5 concentration indicated high quality at both stations. In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-121 Chemical parameters (a).

	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Arachthos Upstream	402	8	14	5	11	HIGH
Arachthos Downstream	427	8	26	7	14	GOOD

Prepared by HCMR on behalf of ASPROFOS,2022

Table D- 122. Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD_5 (mg/l)	COD (mg/l)
Upstream	1065	2.3	24	3.02	1.10	<10
Downstream	997	2.2	4	7.03	0.00	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.10.3 Physicochemical analyses in sediments

The sediment analysis of Arachthos river indicated a low load of TOC and total nitrogen and a moderate concentration of total phosphorus, although there are no relevant quality limits.

Table D-123 Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.014	183	0.36

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.10.4 Heavy metals in sediments

In the sediment of the Arachthos river were measured low Cd and Hg concentrations, lower than their respective ERL limits, therefore they are not likely to have negative effects on benthic organisms.

Cr metal was measured higher than its respective ERL threshold and therefore adverse effects on benthic organisms are potential. Ni metal was measured higher than the ERM limit so there are definite negative effects on benthic organisms. This may probably be attributed to the natural origin of these two elements, that are common in many watersheds of Greece, originating from the disintegration of basic and ultrabasic rocks, showing natural enrichment in Cr and Ni (Karageorgis et al., 2005). Cr (VI) was measured low, only 0.5% of total Cr. Low Cu, Pb and Zn levels were measured below the ERL limit, thus no adverse effects on benthic organisms are possible.

Table D-124 Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.145	195	0.96	0.037	82.4	4.0	10.6	<5	24.7	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.10.5 Hydrocarbons in sediments

Aliphatic hydrocarbon concentrations were very low and very close to expected background levels. Similar values have been measured in unpolluted sediments in different parts of Greece (Bouloubassi et al., 2012; Lipiatou and Saliot, 1991; Gogou et al., 2000; Hatzianestis and Sklivagou, 2001,). The levels of natural hydrocarbons in the sediments are about ~ 10 μg / g, while in areas with high productivity natural hydrocarbons of 100 μg/g have been measured (Bouloubassi and Saliot, 1993). Concentrations higher than this value indicate petroleum pollution. Therefore, it seems that there is no petroleum related pollution in the study areas.

Regarding PAHs, depending on their total concentrations, the sediments can be classified into four categories: (Baumard et al., 1998): (a) unpolluted, 0–100 ng/g; (b) moderately polluted, 100–1000 ng/g, (c) highly polluted, 1000–5000 ng/g and (d) extremely polluted> 5000 ng/g. In Arachthos river's sediment, the total PAH concentrations were <100 ng/g and indicate the absence of pollution (Botsou and Hatzianestis, 2012; Parinos et al., 2013; Hatzianestis et al., 2020).

Table D-125 Hydrocarbon concentrations in sediment (n.d. : not detected, detection limit: 0.1 μg/kg).

	Aracthos
Total Hydrocarbons C_{12}-C_{40} (mg/kg)	6.9
Total Hydrocarbons < C_{12} (mg/kg)	0.2
Polycyclic aromatic hydrocarbons (PAH) (μg/kg)	
Naphthalene	1.9
Methyl - naphthalenes	2.9
Acenaphthylene	0.1
Acenaphthene	0.2
Dimethyl - naphthalenes	3.1
Triethyl - naphthalenes	2.5

	Aracthos
Fluorene	0.3
Dibenzothiophene	0.1
Methyl - dibenzothiophenes	0.3
Dimethyl - dibenzothiophenes	0.4
Phenanthrene	1.7
Anthracene	0.1
Methyl- phenanthrenes	3.7
Dimethyl- phenanthrenes	2.8
Trimethyl- phenanthrenes	1.7
Fluoranthene	0.7
Pyrene	0.7
Methyl-pyrenes	1.4
Dimethyl-pyrenes	1.4
Retene	0.4
Benzo(a)anthracene	0.7
Chrysene	0.7
Methyl chrysenes	1.3
Dimethyl chrysenes	1.5
Benzo(b)fluoranthene	0.8
Benzo(k)fluoranthene	0.2
Benzo(e)pyrene	0.7
Benzo(a)pyrene	0.3
Perylene	4.6
Indeno(1,2,3-cd)pyrene	0.2
Bibenzo(ghi_perylene	0.6
DEbenzo(ah)anthracene	0.1
ΣPAH	**37.7**

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.10.6 PCBs in sediments

The concentrations of PCBs are given in the following table (Table D-126). The quantified compounds include also the seven PCBs (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) selected by ICES (International Council for the Exploration of the Sea) and recommended for monitoring by the European Union. These PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The PCBs concentrations in Arachthos sediment's sample were very low and clearly lower than those measured in sediments collected from both the coastal zone and the open sea in the Mediterranean area (De Lazzari et al, 2004, Hatzianestis et al, 2000, Tolosa et al, 1995). Therefore, is seems that no pollution from PCBs exists. Most congeners were not detectable, whereas in all cases, in higher quantities the hexachloro- CB153 and CB138 were detected, followed by the heptachloro- CB180, in accordance with the commercial formulations such as Arochlor 1260.

Table D-126 Concentrations of polychlorinated biphenyls (PCBs) (ng/g) in sediment samples. As total PCBs the sum of the individual congeners is calculated. (n.d..: not detected, detection limit: 0.01 ng/g).

Stations	CB 28	CB 52	CB 101	CB 118	CB 153	CB 105	CB 138	CB 183	CB 128	CB 156	CB 180	CB 170	CB 194	Sum of PCBs
ARACTHOS	0.02	0.01	n.d.	0.01	0.02	n.d.	0.06	n.d.	0.01	0.02	0.02	0.02	n.d.	0.185

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.10.7 Granulometry

The sand prevails in the sediment of the Arachthos river.

Table D-127 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Arachthos	97.97	0.06	1.97

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.10.8 Benthic macroinvertebrates and diatoms

Sampling station located in Arachthos river is a deep canal with brackish /salty water due to sea invasion. Therefore, due to the depth and the high salinity values, benthic macroinvertabrates and diatoms could not be sampled.

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-25 Sampling station located in Arachthos river.

8 D.5.10.9 Ichthyological assessment

A total of two fish species were recorded, moderate numbers of fish were detected. This site is initially assessed as a "degraded" site based on fish fauna sampling.

The sampled area is characterized by significant hydrological degradation, mainly due to the operation of dams and the existence of hydroelectric projects at the upstream part.

Table D-128 Summary results of the relative abundance of species collected at Arachthos river.

Species	Arachthos
Anguilla anguilla	
Atherina boyeri	
Barbus_peloponnesius	
Carassius gibelio*	

Species	Arachthos
Economidichthys trichonis	
Economidichthys_pygmaeus	
Gambusia holbrooki*	
Gobiidae sp.	
Liza ramada	
Luciobarbus_albanicus	1
Mugil cephalus	
Mugilidae sp.	1
Pelasgus_laconicus	
Pelasgus_thesproticus	
Salaria_fluviatilis	
Scardinius acarnanicus	
Squalius_keadicus	
Squalius_peloponensis	
Telestes_pleurobipunctatus	
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-129 Fish characteristics and confidence assessment and bioassessment at Arachthos river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Arachthos
Total fish species	2
Diversity of expected typespecific species	Low
Reproduction data	Bad
Presence of intolerant species	Bad
Presence of expected migratory species	Unknown
Sampling effort assessment	Bad

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Arachthos
Uncertainty estimation for bioassessment	Low

Prepared by: ASPROFOS, 2021.

Table D-130 HeFI index implementation at Arachthos river.

Sampling parameters	Arachthos
Density of insectivores	
Density of omnivores	
Density of benthic species	
Density of potamodromus species	
Degree of Certainty	1
Preliminary assessment	Visual
Preliminary ecological quality (three-level scale)	Degraded

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.10.10 Hydromorphological assessment

The QBR score for Aracthos was 45 which indicated a poor status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 7 of this document. The drone derived orthophoto map of this site is accessible here:

https://cloud.pix4d.com/dataset/944949/map?shareToken=880e34e4-6f3a-4cdd-b67b-d971f829e1f7

8 D.5.10.11 Ecological status

For the complete assessment of the ecological quality of each sampling site the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality. As far as the Arachthos river is concerned, the application of fish index is characterized by high uncertainty while no other element is available. Therefore, the biological quality of this particular site cannot be assessed. In particular, we can only present the physicochemical quality of its surface waters which is characterized as **good**. Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/gj2ge9TUd8K78erZ6.

8 D.5.11 Louros river

8 D.5.11.1 Physicochemical parameters of surface waters

The following table (Table D-131) below presents the basic physicochemical parameters measured in the context of water and sediment sampling in May 2021. The values are typical for the season and the concentration of dissolved oxygen indicates high quality.

Table D-131 Physicochemical parameters.

Date	Temperature (ºC)	E. Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	D. Oxygen (mg/L)
20/05/2021	20	887	567	0.37	7.69	0.75	9.99

Prepared by HCMR on behalf of ASPROFOS,2022

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-26 Sampling station located in Louros river.

8 D.5.11.2 *Chemical parameters of surface waters*

The final physicochemical quality of both stations of the Louros river is characterized as High. TOC concentrations at both stations are normal, do not indicate unusual enrichment or pollution of water with organic carbon and are much lower than the values found in rivers whose water quality is characterized as poor. The concentrations of total coliform bacteria are also particularly low (EPA, 2003b), while BOD_5 values indicated high water quality. In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-132 Chemical parameters (a).

	$N\text{-}NO_3^-$	$N\text{-}NO_2^-$	$N\text{-}NH_4^+$	$P\text{-}PO_4^{3-}$	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Louros Upstream	524	16	19	28	38	HIGH
Louros Downstream	514	16	18	39	50	HIGH

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-133 Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD_5 (mg/l)	COD (mg/l)
Upstream	650	1.7	12	2.42	0.00	<10
Downstream	628	1.4	10	2.72	0.00	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.11.3 *Physicochemical analyses in sediment*

The sediment of Louros river presented a high load of TOC and total nitrogen (the second highest compared to all other rivers) and the highest load on total phosphorus although there are no relative quality limits.

Table D-134 Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.270	610	1.96

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.11.4 Heavy metals in sediment

Low levels of Cd and Hg were measured in Louros river's sediment, lower than the respective ERL limits, thus no negative effects on benthic organisms are possible. Cr value was measured higher than the ERL threshold, therefore adverse effects on benthic organisms are potential. Ni metal was measured higher than the ERM limit thus there are definite negative effects on benthic organisms. This is probably due to the natural origin of those two elements that are common in many watersheds of Greece, originating from the disintegration of basic and ultrabasic rocks showing natural enrichment in Cr and Ni (Karageorgis et al., 2005). Cr (VI) was measured at a low content corresponding to 0.1% of the total Cr. Sediment of Louros river presented the highest Zn and the second highest Cu concentrations. Cu was measured higher than the ERL limit and therefore adverse effects on benthic organisms are possible. The relative high loads of nitrogen, phosphorus, organic carbon, copper and zinc may be due to diffuse agricultural sources of pollution.

Table D-135 Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.759	156	0.11	0.074	194	18.4	54.2	<5	144	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.11.5 Hydrocarbons in sediments

Aliphatic hydrocarbon concentrations were very low and very close to expected background levels. Similar values have been measured in unpolluted sediments in different parts of Greece (Bouloubassi et al., 2012; Lipiatou and Saliot, 1991; Gogou et al., 2000; Hatzianestis and Sklivagou, 2001,). The levels of natural hydrocarbons in the sediments are about ~ 10 μg / g, while in areas with high productivity natural hydrocarbons of 100 μg/g have been measured (Bouloubassi and Saliot, 1993). Concentrations higher than this value indicate petroleum pollution. Therefore, it seems that there is no petroleum related pollution in the study areas.

Regarding PAHs, depending on their total concentrations, the sediments can be classified into four categories: (Baumard et al., 1998): (a) unpolluted, 0–100 ng/g; (b) moderately polluted, 100–1000 ng/g, (c) highly polluted, 1000–5000 ng/g and (d) extremely polluted> 5000 ng/g. In Louros river's sediment, the total PAH concentrations were <100 ng/g and indicate the absence of pollution (Botsou and Hatzianestis, 2012; Parinos et al., 2013; Hatzianestis et al., 2020).

Table D-136 Hydrocarbon concentrations in sediment (n.d. : not detected, detection limit: 0.1 μg/kg).

	Louros
Total Hydrocarbons C_{12}-C_{40} (mg/kg)	14.7
Total Hydrocarbons < C_{12} (mg/kg)	0.3
Polycyclic aromatic hydrocarbons (PAH) (μg/kg)	
Naphthalene	2.8
Methyl - naphthalenes	2.9
Acenaphthylene	0.1
Acenaphthene	0.2
Dimethyl - naphthalenes	4.1
Triethyl - naphthalenes	3.5
Fluorene	0.3
Dibenzothiophene	0.1
Methyl - dibenzothiophenes	0.2
Dimethyl - dibenzothiophenes	0.5
Phenanthrene	1.4
Anthracene	0.1
Methyl- phenanthrenes	2.8
Dimethyl- phenanthrenes	1.9
Trimethyl- phenanthrenes	0.8
Fluoranthene	1.1
Pyrene	1.3
Methyl-pyrenes	1.5
Dimethyl-pyrenes	n.d.
Retene	0.3
Benzo(a)anthracene	0.8
Chrysene	1.2
Methyl chrysenes	2.5
Dimethyl chrysenes	1.1
Benzo(b)fluoranthene	1.8

	Louros
Benzo(k)fluoranthene	0.5
Benzo(e)pyrene	1.5
Benzo(a)pyrene	0.7
Perylene	12.3
Indeno(1,2,3-cd)pyrene	1.1
Bibenzo(ghi_perylene	1.4
DEbenzo(ah)anthracene	0.4
ΣPAH	50.9

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.11.6 PCBs in sediment

The concentrations of PCBs are given in the following table (Table D-137). The quantified compounds include also the seven PCBs (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) selected by ICES (International Council for the Exploration of the Sea) and recommended for monitoring by the European Union. These PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The PCBs concentrations in Louros sediment's sample were very low and clearly lower than those measured in sediments collected from both the coastal zone and the open sea in the Mediterranean area (De Lazzari et al, 2004, Hatzianestis et al, 2000, Tolosa et al, 1995). Therefore, is seems that no pollution from PCBs exists. Most congeners were not detectable, whereas in all cases, in higher quantities the hexachloro- CB153 and CB138 were detected, followed by the heptachloro- CB180, in accordance with the commercial formulations such as Arochlor 1260.

Table D-137 Concentrations of polychlorinated biphenyls (PCBs) (ng/g) in sediment samples. As total PCBs the sum of the individual congeners is calculated. (n.d..: not detected, detection limit: 0.01 ng/g).

Stations	CB 28	CB 52	CB 101	CB 118	CB 153	CB 105	CB 138	CB 183	CB 128	CB 156	CB 180	CB 170	CB 194	Sum of PCBs
LOUROS	0.03	0.01	0.01	0.01	0.03	0.03	0.01	n.d.	0.01	0.02	0.01	0.04	0.01	0.220

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.11.7 Granulometry

The sediments of Louros river are dominated by clay.

Table D-138 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Louros	4.84	12.83	82.33

Prepared by HCMR on behalf of ASPROFOS,2022

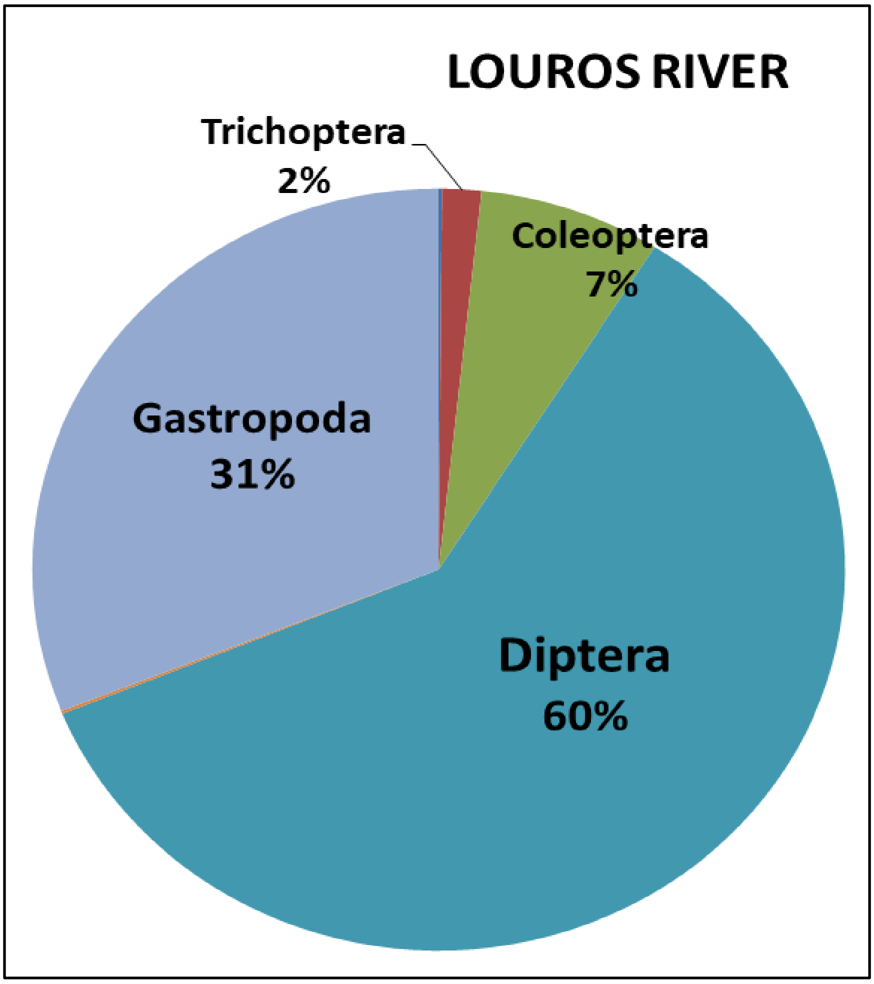
8 D.5.11.8 Benthic macroinvertebrates

The biological status at the sampling site of the Louros River was classified as moderate based on benthic macroinvertebrates (Table D-139). A total of 1106 specimens belonging to 23 families were collected. The most predominant groups were Diptera and Gastropoda with 60% and 31% of the total abundance, respectively (Figure D-27). The most dominant family from the Diptera order were Chironomidae (458 individuals) which represented most of the Diptera. Regarding Gastropoda, the most dominant families were Bithyniidae (198 individuals), Planorbidae (23 individuals) and Neritidae (11 individuals). In addition, the Crustaceans Gammaridae (115 individuals) and Atyidae (151 individuals) were present in the site with relatively large abundances.

Table D-139 Biological status based on benthic macroinvertebrate fauna.

River Name	Louros River
Date	**20/05/2021**
Typology	**R-M2**
Total abundance	1.106
Number of Taxa	23
Shannon-Wiener Diversity Index	1,85
Number of sensitive families	3
Number of tolerant families	14
HESY2 Score	0,56
HESY2 Quality	Moderate
% EPT	1,18
% EPTC	6,24

Prepared by HCMR on behalf of ASPROFOS,2022



Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-27 Abundance percentages of macroinvertebrate groups at the sampling site of Louros river.

8 D.5.11.9 Benthic diatom assessment

In this site, 27 species of diatoms were identified, and the assemblage presented relatively low evenness (E=0.68) and relatively high diversity based on Shannon diversity index (H=3.23). Dominant species were Achnanthidium saprophilum (43.3%) followed by lower abundances of Cocconeis euglypta (9.3%). A. saprophilum suggests the impact of organic waste water or sewage, whereas the ecology of C. euglypta is not clearly determined (Cantonati et al 2017). Biological quality of the site based on benthic diatoms is moderate (EQR IPS=0.613), with low organic pollution and moderate degradation (Table D-140).

Table D-140 EQR IPS and IPS values and ecological quality classes with color code.

River name	River Type	EQR IPS/IPS	High / Good / Moderate / Poor / Bad
LOUROS	R-M2	0.613	

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.11.10 Ichthyological assessment

A total of two fish species were recorded, moderate numbers of fish were detected. This site is initially assessed as a "degraded" site based on fish fauna sampling.

This area presents indications of degradation in hydromorphological level but there is no possibility to describe the degradation with great accuracy.

Table D-141 Summary results of the relative abundance of species collected at Louros river.

Species	Louros
Anguilla anguilla	1
Atherina boyeri	
Barbus_peloponnesius	
Carassius gibelio*	
Economidichthys trichonis	
Economidichthys_pygmaeus	3
Gambusia holbrooki*	3
Gobiidae sp.	
Liza ramada	
Luciobarbus_albanicus	
Mugil cephalus	
Mugilidae sp.	
Pelasgus_laconicus	
Pelasgus_thesproticus	1
Salaria_fluviatilis	
Scardinius acarnanicus	

Species	Louros
Squalius_keadicus	
Squalius_peloponensis	
Telestes_pleurobipunctatus	
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-142 Fish characteristics and confidence assessment and bioassessment at Louros river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Louros
Total fish species	4
Diversity of expected typespecific species	Moderate
Reproduction data	Good
Presence of intolerant species	Moderate
Presence of expected migratory species	Moderate
Sampling effort assessment	Moderate
Uncertainty estimation for bioassessment	Low

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-143 HeFI index implementation at Louros river.

Sampling parameters	Louros
Density of insectivores	
Density of omnivores	
Density of benthic species	
Density of potamodromus species	
Degree of Certainty	1
Preliminary assessment	Visual
Preliminary ecological quality (three-level scale)	Degraded

8 D.5.11.11 Hydromorphological assessment

The QBR score for Louros was 75 which indicated a good status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 7 of this document. The drone derived orthophoto map of this site is accessible here:

https://cloud.pix4d.com/dataset/944958/map?shareToken=5a3512f8-4380-4662-98ca-2a6c42124b9e

8 D.5.11.12 Ecological status

For the complete assessment of the ecological quality of Louros river the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality. Since the uncertainty regarding the assessment of ecological quality based on fish is high in Louros river, the biological quality is assessed based only on the indices HESY2 and EQR IPS/IPS (both indicated moderate quality). After the implementation of all classification schemes in the various parameters, we get the biological, physicochemical and hydromorphological quality class at each study site (worst case principle applied). Then the methodology illustrated in Figure D-6 has been used by combining initial the biological and the physicochemical and then the hydromorphological quality classes at each study area, in order to get the final ecological status classification. Ecological status of the site located in Louros river is characterized as **MODERATE** based on its biological quality (worst case principle applied). Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/r25xM6TzjKibjYvj6.

Table D-144 Biological quality.

Water body	HESY2	EQR IPS/IPS	Ichthyofauna	Biological quality
LOUROS	Moderate	Moderate	Degraded	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-145 Final ecological quality.

Water body	Biological quality	Physicochemical quality	Hydromorphological quality	Ecological Status
LOUROS	Moderate	High	Good	Moderate

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.12 Acheron River (Mavropotamos)-Koktos

8 D.5.12.1 Physicochemical parameters of surface waters

The following table (Table D-146) below presents the basic physicochemical parameters measured in the context of water and sediment sampling in May 2021. The values are typical for the season and the concentration of dissolved oxygen indicates good quality.

Table D-146 Physicochemical parameters.

Date	Temperature (°C)	El. Conductivity (μS/cm)	TDS (mg/l)	Salinity (ppt)	pH	Turbidity (NTU)	D. Oxygen (mg/L)
20/05/2021	18.4	547	351	0.23	7.67	2.44	8.74

Prepared by HCMR on behalf of ASPROFOS,2022

Prepared by HCMR on behalf of ASPROFOS,2022

Figure D-28 Sampling station located in Acheron river.

8 D.5.12.2 Chemical parameters of surface waters

The final physicochemical quality is Good. TOC concentrations at both stations are normal, do not indicate unusual enrichment or pollution of water with organic carbon and are much lower than the values found in rivers whose water quality is characterized as poor. The number of colonies of total coliforms at both stations is similarly small. BOD_5 values also indicated high water quality. In all samples, total hydrocarbon concentrations were normal and similar to those measured in non-polluted surface water samples (Parinos et al, 2019).

Table D-147 Chemical parameters (a).

	N-NO_3^-	N-NO_2^-	N-NH_4^+	P-PO_4^{3-}	Total P	
	μg/l	μg/l	μg/l	μg/l	μg/l	Status
Acheron Upstream	1235	5	17	12	16	GOOD
Acheron Downstream	976	4	18	7	9	GOOD

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-148 Chemical parameters (b).

Station	TOC (μg/l)	Total hydrocarbons comp. as n-hexane (μg/l)	SF cfu/100ml	TSS (mg/l)	BOD_5 (mg/l)	COD (mg/l)
Upstream	482	2.0	200	9.18	0.00	<10
Downstream	489	1.5	187	7.55	0.00	<10

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.12.3 Physicochemical analyses in sediment

Sediment analysis of Acheron river showed moderate load of TOC and total phosphorus and high load of total nitrogen, although there are no relevant quality limits.

Table D-149 Concentrations of total nitrogen, total phosphorus and TOC.

Nitrogen total (weight %)	Phosphorus total (mg/kg)	TOC (weight %)
0.088	202	1.17

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.12.4 Heavy metals in sediment

In Acheron, low Cd and Hg contents were measured, lower than the respective ERL limits, therefore they are not likely to have adverse effects on benthic organisms. Cr metal was measured higher than the ERL threshold and therefore adverse effects on benthic organisms are potential. Ni metal was measured higher than the ERM limit so there are definite negative effects on benthic organisms. This may be attributed to the natural origin of those two elements that are common in many watersheds of Greece, originate from the disintegration of basic and ultrabasic rocks and indicate natural enrichment in Cr and Ni (Karageorgis et al., 2005). Cr (VI) was measured at a very low content of less than 0.1% of total Cr. Low Cu, Pb and Zn levels were measured, below the ERL limit, thus no adverse effects on benthic organisms are possible.

Table D-150 Concentrations of heavy metals in the sediment.

As (mg/kg)	Cd (mg/kg)	Cr total (mg/kg)	Cr VI (mg/kg)	Hg (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cu (mg/kg)	Se (mg/kg)	Zn (mg/kg)	AOX (mg/kg)
<5	0.174	246	0.10	0.047	203	10.9	21.1	<5	47.4	<1

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.12.5 Hydrocarbons in sediment

Aliphatic hydrocarbon concentrations were very low and very close to expected background levels. Similar values have been measured in unpolluted sediments in different parts of Greece (Bouloubassi et al., 2012; Lipiatou and Saliot, 1991; Gogou et al., 2000; Hatzianestis and Sklivagou, 2001,). The levels of natural hydrocarbons in the sediments are about ~ 10 μg / g, while in areas with high productivity natural hydrocarbons of 100 μg/g have been measured (Bouloubassi and Saliot, 1993). Concentrations higher than this value indicate petroleum pollution. Therefore, it seems that there is no petroleum related pollution in the study areas.

Regarding PAHs, depending on their total concentrations, the sediments can be classified into four categories: (Baumard et al., 1998): (a) unpolluted, 0–100 ng/g; (b) moderately polluted, 100–1000 ng/g, (c) highly polluted, 1000–5000 ng/g and (d) extremely polluted> 5000 ng/g. In Acheron river's sediment, the total PAH concentrations were <100 ng/g and indicate the absence of pollution (Botsou and Hatzianestis, 2012; Parinos et al., 2013; Hatzianestis et al., 2020).

Table D-151 Hydrocarbon concentrations in sediment (n.d.: not detected, detection limit: 0.1 μg/kg).

	Acheron
Total Hydrocarbons C12-C40 (mg/kg)	12.7
Total Hydrocarbons < C12 (mg/kg))	0.3
Polycyclic aromatic hydrocarbons (PAH) (μg/kg)	
Naphthalene	3.1
Methyl - naphthalenes	3.3
Acenaphthylene	0.2
Acenaphthene	0.4
Dimethyl - naphthalenes	4.5
Triethyl - naphthalenes	4.0
Fluorene	0.4

	Acheron
Dibenzothiophene	0.2
Methyl - dibenzothiophenes	0.3
Dimethyl - dibenzothiophenes	0.4
Phenanthrene	1.6
Anthracene	0.1
Methyl- phenanthrenes	6.7
Dimethyl- phenanthrenes	6.5
Trimethyl- phenanthrenes	4.2
Fluoranthene	1.4
Pyrene	1.7
Methyl-pyrenes	3.8
Dimethyl-pyrenes	4.4
Retene	1.4
Benzo(a)anthracene	0.8
Chrysene	1.5
Methyl chrysenes	3.3
Dimethyl chrysenes	3.2
Benzo(b)fluoranthene	1.9
Benzo(k)fluoranthene	0.3
Benzo(e)pyrene	1.9
Benzo(a)pyrene	0.6
Perylene	13.5
Indeno(1,2,3-cd)pyrene	0.5
Bibenzo(ghi_perylene	1.4
DEbenzo(ah)anthracene	0.3
ΣPAH	77.6

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.12.6 PCBs in sediment

The concentrations of PCBs are given in the following table (Table D-152). The quantified compounds include also the seven PCBs (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) selected by ICES (International Council for the Exploration of the Sea) and recommended for monitoring by the European Union. These PCBs were selected as indicators due to their relatively high concentrations in technical mixtures and their wide chlorination range (3–7 chlorine atoms per molecule). The PCBs concentrations in Acheron sediment's sample were very low and clearly lower than those measured in sediments collected from both the coastal zone and the open sea in the Mediterranean area (De Lazzari et al, 2004, Hatzianestis et al, 2000, Tolosa et al, 1995). Therefore, is seems that no pollution from PCBs exists. Most congeners were not detectable, whereas in all cases, in higher quantities the hexachloro- CB153 and CB138 were detected, followed by the heptachloro- CB180, in accordance with the commercial formulations such as Arochlor 1260.

Table D-152 Concentrations of polychlorinated biphenyls (PCBs) (ng/g) in sediment samples. As total PCBs the sum of the individual congeners is calculated. (n.d..: not detected, detection limit: 0.01 ng/g).

Stations	CB 28	CB 52	CB 101	CB 118	CB 153	CB 105	CB 138	CB 183	CB 128	CB 156	CB 180	CB 170	CB 194	Sum of PCBs
ACHERON	0.02	n.d.	n.d.	n.d.	0.01	0.01	0.02	n.d.	n.d.	0.03	0.02	0.01	0.01	0.146

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.12.7 Granulometry

The sediments of the Acheron river are dominated by sand, followed by clay.

Table D-153 Sediment granulometric analysis.

	Sand	Silt	Clay
	%	%	%
Acheron	59.36	4.25	36.39

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.12.8 Benthic macroinvertebrates

Due to the depth and sandy composition of the substrate of the sampling station, benthic macroinvertebrates could not be sampled.

8 D.5.12.9 Benthic diatom assessment

In this site, 52 species of diatoms were identified, and the assemblage presented high evenness (E=0.79) and thus high diversity based on Shannon diversity index (H=4.12). Dominant species were Achnanthidium minutissimum (18.8%), Achnanthidium saprophilum (15.6%) and Cocconeis euglypta (12.3%). A. minutissimum is usually found in well oxygenated, clean, fresh waters but is generally one of the most common diatoms (Taylor et al 2007). C. euglypta is also very common, which makes difficult the determination of its ecology (Cantonati et al 2017). A. saprophilum suggests the impact of organic waste water or sewage (Cantonati et al 2017). Furthermore, the occurrence of Fistulifera saprophila (6%) in relatively low numbers indicates a moderately polluted water. F. saprophila is one of the most pollution-tolerant diatoms and it can dominate heavily degraded systems (Cantonati et al 2017). Biological quality of the site based on benthic diatoms is moderate (EQR IPS=0.607), with moderate organic pollution and degradation (Table D-154).

Table D-154 EQR IPS and IPS values and quality based on color code.

River name	River Type	EQR IPS/IPS	High Good Moderate Poor Bad
Acheron river	R-M2	0.607	

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.12.10 Ichthyologic assessment

A total of three fish species were recorded, low numbers of fish were detected.

This is an area with signs of degradation but there is no possibility to describe the degradation with great accuracy. According to sampling campaigns conducted by HCMR in the past, the presence of fish is reduced concerning species and populations.

Table D-155 Summary results of the relative abundance of species collected at Acheron river.

Species	Acheron
Anguilla anguilla	1
Atherina boyeri	
Barbus_peloponnesius	
Carassius gibelio*	

Species	Acheron
Economidichthys trichonis	
Economidichthys_pygmaeus	
Gambusia holbrooki*	
Gobiidae sp.	
Liza ramada	
Luciobarbus_albanicus	
Mugil cephalus	
Mugilidae sp.	
Pelasgus_laconicus	
Pelasgus_thesproticus	1
Salaria_fluviatilis	
Scardinius acarnanicus	
Squalius_keadicus	
Squalius_peloponensis	
Telestes_pleurobipunctatus	1
Tropidophoxinellus_hellenicus	
Tropidophoxinellus_spartiaticus	
*Abundance (semi-quantitive): 1= Rare; Few individuals (less than 10), one class size per 100 m. 2= Common/ Large number (more than 10), more than one class size per 100 m. 3= Abundant (more than 20) and more than two size classes per 100 m. Invasive and translocated species are marked with an asterisk.	

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-156 Fish characteristics and confidence assessment and bioassessment at Acheron river.

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Acheron
Total fish species	3
Diversity of expected typespecific species	Low
Reproduction data	Moderate
Presence of intolerant species	Moderate
Presence of expected migratory species	Moderate

FISH CHARACTERISTICS – CONFIDENCE ASSESSMENT & BIOASSESSMENT	Acheron
Sampling effort assessment	Bad
Uncertainty estimation for bioassessment	Low

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-157 HeFI index implementation at Acheron river.

Sampling parameters	Acheron
Density of insectivores	
Density of omnivores	
Density of benthic species	
Density of potamodromus species	
Degree of Certainty	1
Preliminary assessment	Visual
Preliminary ecological quality (three-level scale)	Degraded

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.5.12.11 Hydromorphological assessment

The QBR score for Acheron was 35 which indicated a poor status of the riparian vegetation and channel conditions. More details about the particular assessment is given at chapter 8 D.6 of this document. No drone flight was possible at this site since it was located in an inhabited area.

8 D.5.12.12 Ecological status

For the complete assessment of the ecological quality of Acheron river the biological quality element (BQE) that has the worst classification has been used to characterize the biological quality. Since the uncertainty regarding the assessment of ecological quality based on fish is high in Acheron river and sampling of macroinvertebrates was impossible, the biological quality is assessed based only on the index EQR IPS/IPS (moderate quality). After the implementation of all classification schemes in the various parameters, we get the biological, physicochemical and hydromorphological quality class at each study site (worst case principle applied). Then the methodology illustrated in Figure D-6has been used by combining initial the biological and the physicochemical and then the hydromorphological quality classes at each study area, in order to get the final ecological status classification. Ecological status of the site located in Acheron river is characterized as **MODERATE** based on its biological and physicochemical quality (worst case principle applied). Complete photographic documentation can be found at the following link address: https://photos.app.goo.gl/BaghYRpQNSBMuEPw9.

Table D-158 Biological quality.

Water body	EQR IPS/IPS	Ichthyofauna	Biological quality
ACHERON	MODERATE	Degraded	MODERATE

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-159 Final ecological quality.

Water body	Biological quality	Physicochemical quality	Hydromorphological quality	Ecological Status
ACHERON	MODERATE	GOOD	POOR	MODERATE

Prepared by HCMR on behalf of ASPROFOS,2022

8 D.6 HYDROMORPHOLOGICAL ASSESSMENT

Most sites are degraded when considering hydromorphological conditions and as they area represented within the short segments of the QBR sampling. Three sites are assessed as Poor and another three as being in poor condition. No site is in high condition, and only four are in Good condition (Table D-160).

Riparian vegetation

The rapid survey of the woody flora in the riparian zone are documented using a DAFOR scale to produce a semi-quantitative representation of the observed species on-site. Dominant and prominent species at each site are summarized in Table D-161. Notes on the conditions of the vegetation and on anthropogenic pressures at each site were also made with special reference to ongoing pressures that may affect the extent, quality and condition of woody vegetation and naturally occurring vegetation patterns.

Table D-160 QBR index and status of riparian and channel conditions at the assessed sites.

Site Name	QBR Index	Status of Riparian Vegetation and channel conditions
Mariorema R.	85	Good
Evrotas R.	40	Poor
Alfeios R.	65	Moderate
Ladon R.	90	Good

Site Name	QBR Index	Status of Riparian Vegetation and channel conditions
Pineios R.	75	Good
Evinos R.	55	Moderate
Enot. Tafros	10	Bad
Acheloos R.	5	Bad
Dipotamon R.	5	Bad
Arachthos R.	45	Poor
Louros R.	75	Good
Acheron Mavropotamos R.	35	Poor

Prepared by HCMR on behalf of ASPROFOS,2022

Table D-161 QBR channel condition score, geomorphological type and dominant woody flora at the assessed sites.

Site	Channel Alteration Metric (out of 25)	River Type (QBR)	Riparian vegetation type	Vegetation degradation (main pressures)
Mariorema R.	25	2	Quercus coccifera, Pistatcia lentiscus	Ephemeral stream
Evrotas R.	25	3	Salix alba, Platanus orientalis, Populus sp., Arundo donax	Crop expansion, tree cutting, *Arundo donax* invasion, *Ceratocystis platani* fungus
Alfeios R.	10	2	Salix alba, Platanus orientalis,	Road works, embankments, *Ceratocystis platani* fungus
Ladon R.	25	3	Salix alba, Platanus orientalis,	Gravel extraction/gravel mining, water abstraction, tree cutting
Pineios R.	25	2	Salix alba, Platanus orientalis, Ulmus sp.	Water pollution, livestock grazing, tree cutting,

Site	Channel Alteration Metric (out of 25)	River Type (QBR)	Riparian vegetation type	Vegetation degradation (main pressures)
				Ceratocystis platani fungus
Evinos R.	25	3	Vitex agnus-castus, Nerium oleander, Tamarix sp.	Gravel extraction/gravel mining, hydropeaking, Livestock grazing
Enot. Tafros	0	3	Salix alba, Phragmites australis	Artificial canal
Acheloos R.	0	3	Salix alba,Vitex agnus-castus	Hydropeaking and hydropower changes/pressures to flow regime, artificial embankments
Dipotamon R.	5	3	Salix alba, Arundo donax	*Arundo donax* invasion, crop expansion (citrus fruit), flow regime degradation
Arachthos R.	15	3	Salix alba, Platanus orientalis, Ulmus sp., Arundo donax	Hydropeaking and hydropower changes/pressures to flow regime, artificial embankments, tree cutting, crop expansion, *Ceratocystis platani* invasion
Louros R.	10	3	Salix alba, Fraxinus angustifolia, Ulmus sp., Populus alba	Hydromorphological changes (embankment), salinity changes, tree cutting, livestock grazing

Site	Channel Alteration Metric (out of 25)	River Type (QBR)	Riparian vegetation type	Vegetation degradation (main pressures)
Acheron Mavropotamos R.	10	3	Salix alba, Platanus orientalis, Arundo donax	Hydromorphological degradation (embankments etc), *Ceratocystis platani* fungus

Prepared by HCMR on behalf of ASPROFOS,2022

The survey and assessment provide the following main results:

- Only four sites are assessed as in good condition, none is in excellent or high condition and this is reflected by the overall structure of the woody vegetation in the riparian zones.
- Lowland areas are most degraded.
- Most site belong to lowland QBR type (type 3) and as expected there are multiple human-induced pressures and degradation drivers at these sites.
- Not many alien woody plant species were documented.
- Significant expansion of the weedy alien reed-cane Arundo donax is observed in many areas.
- One of the most important human induced pressures related to alien invasion concerns the fungus Ceratocystis platani which creates serious and extensive "die-back" of Oriental plane woodlands (Platanus orientalis).

8 D.7 EURASIAN OTTER (LUTRA LUTRA) SURVEYING

The Eurasian otter (*Lutra lutra*) is rather widespread in Greece although it had suffered a substantial decline in Europe from the 1970s to the 1990s. A strong recovery of the species has been recorded in Western Europe, where it is now considered "Near Threatened" (Duplaix & Savage 2018). At the European level, the otter is included in Annexes II and IV of the EU Habitats Directive 92/43/EEC, protected in the Special Areas of Conservation (SACs) of the Natura 2000 network (European Environment Agency 2015). The species is also listed in Appendix I of CITES and included in the Bern Convention. In Greece, the Eurasian otter was given protection by a Presidential Decree in 1981 (PD no. 67/1981) and is classified as "Endangered" in the latest update of the Red Data Book of Threatened Animals of Greece (Galanaki et al. 2019). Major threats for the species' survival in Greece are anthropogenic, including habitat alteration (i.e. loss, degradation, and fragmentation), wetland drainage, water abstraction, human disturbance, pollution and persecution. Otters are important indicator species for river and wetland conservation especially in the Mediterranean countries where water stress and other pressures may limit populations, their food resources (mainly fish) and their riparian and aquatic habitats.

During the on-site inspection otter signs and tracks were searched for and documented. Signs include scat (known as "spraints") that are often positioned on river-side rocks or prominent structures to mark territories (home ranges etc.). In each case, an area of at least 100 m. on at least one side of the river or waterways was carefully inspected for otter signs and evidence of otter territories. Well-known otter spraint locations were also inspected nearby the inspected site (e.g. areas and refuges under bridges). Moreover, the habitat conditions of the river segment were assessed with respect to otter presence and habitat suitability based on expert judgement and the literature (where there is knowledge of otter presence). Table D-162presents the results of the survey.

Table D-162 Summary of evidence of Eurasian otter presence, potential presence and habitat suitability at inspected sites. Colour gradient pertains to confirmation of viable population utilizing sites (Green: confirmed; warm colours: red "unlikely" presence of population, yellow: "possible" presence of population.

Site	Confirmation of otter presence during survey	Potential for presence in wider river segment	Habitat Suitability at Site
Mariorema R.	NO	NONE	Unsuitable
Evrotas R.	NO	LOW	Suitable
Alfeios R.	YES	CONFIRMED	Very Suitable
Ladon R.	YES	CONFIRMED	Very Suitable
Pineios R.	NO	HIGH	Suitable
Evinos R.	NO	LOW	Suitable-Degraded
Enot. Tafros	NO	HIGH	Suitable
Acheloos R.	NO	LOW	Suitable-Degraded
Dipotamon R.	YES	CONFIRMED	Suitable
Arachthos R.	YES	CONFIRMED	Suitable
Louros R.	YES	CONFIRMED	Very Suitable
Acheron Mavropotamos R.	NO	HIGH	Suitable

The results of the survey show that five of the sites have a confirmation of the presence of an otter population (i.e. likely a permeant population on-site). Two sites have a low likelihood of having a population nearby or within the wider area of the inspected river segment (primarily due to degradation and poor food resources available at these sites). These two sites include Evinos R. (which is severely degraded by hydropeaking and gravel extraction works immediately upstream of the inspected site) and the Acheloos R site which has very low fish populations due to severe hydropeaking due to the hydroelectric dam functioning very close to the site. Despite these local degraded conditions, it is still possible that otters may visit these two sites from the surrounding region. Finally, at one site (**Mariorema R.) it is highly unlikely that an otter would ever exist in the river segment or site due to the ephemeral flow conditions and lack of suitable habitat and food resources. It should be noted that the Eurasian otter in Greece is largely dependent on aquatic food resources (fish, aquatic amphibians and reptiles, large insects, water birds and rodents).** Maintenance of good quality habitats, the main characteristics of which are an adequate food supply, resting and breeding sites and cover are vital for long-term otter survival.

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