D2.3: Bioaccumulation and potential for biomagnification of CoEC in Mediterranean Sea waters

Task 2.4: Evaluate bioaccumulation and biomagnification potential of CoEC in Mediterranean food webs

WP2: Screening, mapping and monitoring of chemical pollution

Author: Christina Zeri, Hellenic Centre for Marine Research

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CONTRIBUTORS	Ioanna Kalantzi, Soultana Zervoudaki, Elli Pitta, Evangelia Strogyloudi, Catherine Tsangaris, Manolis Tsapakis, Panagiotis Michalopoulos (HCMR); Simone Barletta (IRIS); Leo Mensel, Romane Blassel (ECSA).
ABSTRACT	Deliverable 2.3 presents a comprehensive study on the bioaccumulation and biomagnification of Contaminants of Emerging Concern (CoECs) within the marine food web of the Saronikos Gulf, eastern Mediterranean. Conducted in September 2023, the study involved the collection and analysis of 17 marine species, including fish, crustaceans, cephalopods, and zooplankton. Chemical and stable isotope analyses ($\delta^{15}N$ and $\delta^{13}C$) established a food web covering 2.5 trophic levels (2.0–4.9). The research focused on four per- and polyfluoroalkyl substances (PFAS), total mercury (THg), and selected trace metals. While ΣPFAS concentrations were below regulatory seafood safety limits (EU Regulation 2023/915), 75% exceeded the proposed EFSA Environmental Quality Standard. No consistent PFAS bioaccumulation pattern was observed with age, and biodilution trends were apparent in demersal species, likely due to dominant bioconcentration pathways. In contrast, THg showed clear age-related bioaccumulation and trophic biomagnification, with

	concentrations increasing fivefold per trophic level. Arsenic and rubidium also demonstrated magnification trends across the food web. These findings underscore the complex contaminant dynamics in benthic ecosystems and emphasize the need to integrate bioconcentration and trophic processes in environmental risk assessments.

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Executive Summary

Deliverable 2.3 presents the findings on the bioaccumulation and biomagnification of Contaminants of Emerging Concern (CoECs) in the marine food web of the Saronikos Gulf, located in the eastern Mediterranean. Dedicated sampling was carried out in September 2023 (Milestone 3 of the project ‘Sampling cruise for the bioaccumulation-biomagnification study’, achieved in M6), including direct collection of biota and supplementary purchases from local fishers. A total of seventeen species—comprising fish, crustaceans, cephalopods, and zooplankton—were investigated. Organisms were dissected, and subsamples of fish muscle and cephalopod mantle tissue were subjected to chemical analysis. Stable isotope analysis ($\delta^{15}N$ and $\delta^{13}C$) enabled the construction of a food web spanning 2.5 trophic levels, ranging from level 2.0 to 4.9. Of the sixteen species examined, twelve were demersal, meaning they reside near the seafloor and forage in benthic habitats. The study analysed four per- and polyfluoroalkyl substances (PFAS)—PFOA, PFNA, PFOS, and PFHxS—alongside total mercury (THg) and selected trace metals (Li, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Cd, Cs, Ba, Pb, U).

Sum of individual PFAS (ΣPFAS) concentrations were below the current Safety Standards for seafood as set by the Regulation (EU) 2023/915 yet exceeded the newly proposed EFSA Environmental Quality Standard (EQS) threshold (0.077 ng g^{-1}) in 75% of the samples. No consistent pattern of PFAS bioaccumulation with age was observed in the three size classes of *Pagellus erythrinus* (common pandora) and Merluccius merluccius (European hake). Despite the highest PFAS concentrations being found in *Auxis rocheii* (bullet tuna), the overall pattern within the demersal trophic group suggests "biodilution," where PFAS concentrations decrease with increasing trophic level. This is likely due to the demersal nature of the food web and the significance of bioconcentration—direct uptake from the sedimentary environment—as opposed to dietary intake. In contrast, THg concentrations, while within acceptable limits, displayed age-related bioaccumulation in both *Pagellus erythrinus* and particularly in *Merluccius merluccius*. Furthermore, a clear biomagnification trend was observed, with THg levels increasing approximately fivefold per trophic level. The highest mercury concentrations were found in *Mullus barbatus* (red mullet), *Serranus hepatus* (brown comber), *Lophius piscatorius* (anglerfish), *Pagellus erythrinus (common pandora)*, and *Auxis rocheii (bullet tuna)*. Among the other metals studied, arsenic (As) and rubidium (Rb) showed increasing concentrations across the food web. Arsenic levels doubled per trophic level, while rubidium showed a magnification factor of 1.17.

These results highlight the complexity of contaminant dynamics in demersal marine ecosystems and underscore the importance of considering both bioconcentration and trophic interactions in ecological risk assessments and monitoring practices

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Acronyms

ANOSIM	Analysis of Similarities
APM	Airborne Particulate Matter
BAF	Bioaccumulation factor
BASF	Sediment bioaccumulation factor
CoEC	Contaminants of Emerging Concern
CRM	Certified Reference Materials
EC	European Commission
ECHA	European Chemical Agency
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EQS	Environmental Quality Standards
EU	European Union
ICS-MS	Inductively Coupled Plasma-Mass Spectrometer
LOD	Limit of Detection
LOQ	Limit of Quantification
M	Month
MeHg	Methyl mercury
MS	Milestone
MSFD	Marine Strategy Framework Directive
nMDS	Non-metric Multidimensional Scaling
OECD	Organization for Economic Co-operation and Development
PFAA	Perfluoroalkyl acids
PFAS	Per- and poly-fluoroalkyl substances
PFHxS	Perfluorohexane sulfonic acid.
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluoroocatnesulfonic acid
POPs	Persistent organic pollutants
RT	Retention Time
THg	Total mercury
TL	Trophic Level
TMF	Trophic Magnification Factor
TWI	Tolerable Weekly Intake
UNEP	United Nations Environment Programme
US EPA	US Environmental Protection Agency
WFD	Water framework Directive
WHO	World Health Organization
WP	Work Package
WWTP	Waste Water Treatment Plant

1.0 Introduction

This Deliverable presents the outcomes of Task 2.4 under Work Package 2 (WP2) of the iMERMAID project. It outlines the methodology, results, and final conclusions regarding the presence of Contaminants of Emerging Concern (CoECs)—such as PFAS and legacy contaminants like mercury (Hg)—in marine biota, as well as their potential for trophic transfer. The study also includes an analysis of various other metals (Li, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Cd, Cs, Ba, Pb, U), some of which are classified as CoECs due to their emerging use in modern applications. The purpose of this work is (i) to propose a multiple species indicator of CoEC impacts for the Mediterranean Sea relating to biomagnification (ii) to provide bioaccumulation and biomagnification data for CoECs in Mediterranean food webs and compare with established Environmental Quality Standards (EQS) and Safety limits for human consumption.

1.1 Bioaccumulation and Biomagnification of contaminants in marine trophic webs

Marine biota can take up contaminants from the marine environment, often leading to their accumulation within their body. Contaminant uptake may occur through various routes including seawater, suspended particles, sediments, and food. The dominant exposure route depends largely on the organism's feeding habits. For instance, filter feeders primarily take up dissolved and particle-bound contaminants, while deposit feeders are more exposed to sediment-associated contaminants. Predatory species, in contrast, primarily take up contaminants through trophic transfer from their prey. Following uptake, contaminants can bioaccumulate within the organism's body and may magnify in the food chain. Bioaccumulation occurs when an organism absorbs a contaminant from its food or the environment faster than it can excrete it, resulting in the accumulation of the contaminant in its tissues over time (U.S. EPA, Toxics in the Food Web) [1]. Bioconcentration refers specifically to the absorption and retention of contaminants directly from the surrounding environment, such as water or sediment, resulting in concentrations within the organism that exceed those in the external environment. Biomagnification refers to the accumulation of contaminants through the trophic web, whereby their concentrations increase as they are transferred from prey to predator, often accumulating in fatty tissues (U.S. EPA, Toxics in the Food Web)[1].

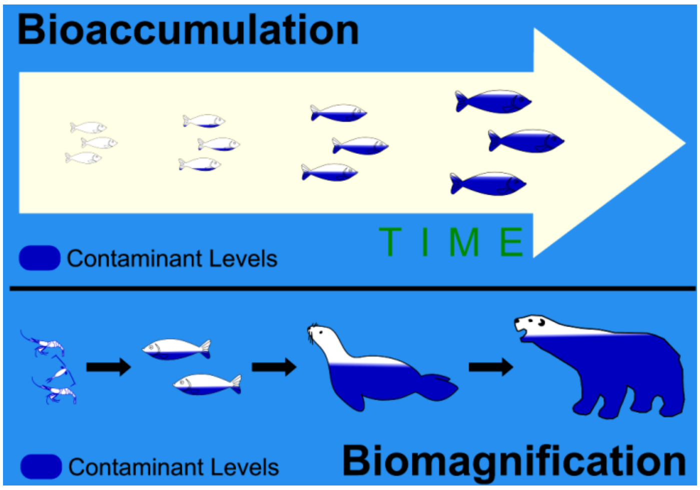


Figure 1: Schematic diagram explaining bioaccumulation of contaminants in the same species through time (age) and biomagnification within a trophic web of various species due to prey-predator relationship [Source: https://mercurypolicy.scripts.mit.edu/blog/?tag=seafood].

Smaller marine organisms, such as plankton and small fish, can ingest contaminants (e.g., PFAS or Hg) from the surrounding water and their food. When these organisms are eaten by larger predators, the contaminants they contain are transferred to the predator. Their concentration in these higher consumers increases over time either due to low degradation, low excretion rate (e.g., PFAS) or to binding to proteins (e.g., Hg to protein-SH groups). As a result, larger and longer-lived predatory fish such as tuna and sharks can accumulate higher concentrations of contaminants compared to smaller organisms further down the food chain.

Seafood consumption represents an important route of human exposure to biomagnifying contaminants, especially for coastal dwellers with a high consumption of fish, molluscs and crustaceans. Consequently, monitoring their concentrations in edible marine biota is critical for assessing ecological risks, evaluating dietary exposure and establishing regulatory measures. Recognizing the potential health risks, the European Food Safety Authority (EFSA) has established a Tolerable Weekly Intake (TWI) for the sum of certain important PFAS, including PFOA, PFOS, PFNA and PFHxS (TWI 4.4 ng Kg^{-1} of body weight).

Understanding the dynamics of trophic webs is challenging as there is a need for knowing individual consumers diet through stomach content analysis as well as their foraging habitats. It is common for species occupying the same trophic level to consume different prey types from different environments. The use of stable isotopes of nitrogen ($\delta^{15}N$‰) and carbon ($\delta^{13}C$‰) in understanding the flow of energy and matter has been proven useful over the last decades [2,3] . These isotopic signatures are defined as:

$$\delta 13C = \left(\frac{[^{13C}/_{12C}]sample}{[^{13C}/_{12C}]standard} - 1 \right) x\ 1000, \qquad \delta 15N = \left(\frac{[^{15N}/_{14N}]sample}{[^{15N}/_{14N}]standard} - 1 \right) x\ 1000,$$

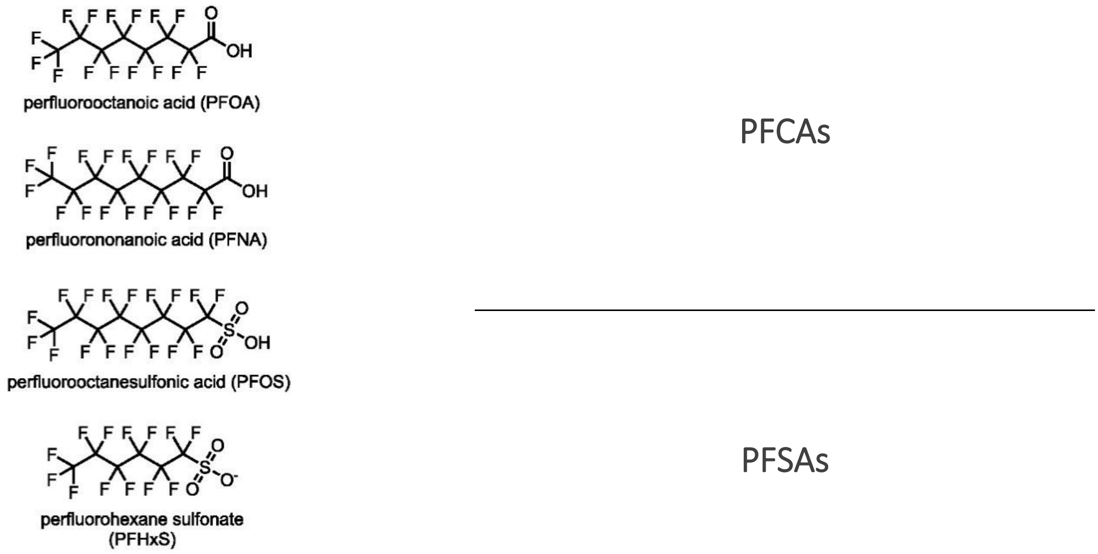
Where the standard is an established reference material. For $\delta^{15}N$ is the atmospheric N_2 which contains 99.63% ^{14}N and 0.36% ^{15}N; and for $\delta^{13}C$ used to be a standard limestone (called Pee Dee Belemnite—or PDB) from South Carolina, no longer available. Other natural organic materials are used and their isotopic ratio is expressed as of PDB.

The heavier isotopes ^{15}N and ^{13}C are much less abundant in nature and less reactive than the lighter ones ^{14}N and ^{12}C. ^{14}N is easily assimilated, hence it is relatively more abundant in primary producers, while at the same time is preferentially excreted, so that ^{15}N builds up in trophic webs. Similarly, the isotopic signature of CO_2 carbon during respiration is ^{12}C, leaving the heavier ^{13}C in the biomass. In marine systems, primary producers such as phytoplankton typically exhibit $\delta^{13}C$ values between −18‰ and −22‰, reflecting both their photosynthetic pathway and the isotopic composition of dissolved inorganic carbon. As carbon moves up the food chain—from zooplankton to higher consumers such as fish, seabirds, and marine mammals—$\delta^{13}C$ values shift minimally, usually by less than 1‰, thereby serving as an effective indicator of food sources (e.g., pelagic vs. benthic, coastal vs. offshore). In contrast, $\delta^{15}N$ values increase significantly, by approximately 3–4‰ with each trophic transfer, due to preferential excretion of ^{14}N.

1.2 Per- and polyfluoroalkyl substances (PFAS) in the marine environment

Per- and polyfluoroalkyl substances (PFAS) are a large and diverse class of anthropogenic compounds characterized by strong carbon-fluorine bonds that give them exceptional thermal stability and degradability[4]. A defining characteristic of PFAS is the presence of strong carbon-fluorine bonds, which are among the most robust chemical bonds known. Numerous terms such as ‘perfluorinated and polyfluorinated chemicals’, ‘perfluorinated organics’, ‘perfluorochemical surfactants’ and ‘highly fluorinated compounds’ have been used. Recently, the definition of PFAS has evolved, with the European Chemical Agency (ECHA) providing a more comprehensive characterization. In 2023, PFAS were defined as any chemical that contains at least one fully fluorinated carbon atom (CFx) and is persistent in the environment, meaning that it does not degrade naturally. This definition encompasses thousands of individual compounds and illustrates the scope of PFAS and the challenges associated with their regulation and management due to their environmental persistence and potential health risks. Advances in non-targeted analytical techniques have enabled the identification of many unknown PFAS in environmental and product samples. In 2021, the Organization for Economic Co-operation and Development (OECD) put forward a revised, broad definition: “PFAS are defined as fluorinated substances containing at least one fully fluorinated methyl or methylene carbon atom (without an H/Cl/Br/I atom attached), i.e., with a few exceptions, any chemical with at least one -CF3 or one perfluorinated methylene group (-CF2-) is a PFAS.” This is the latest definition for PFAS, which is easy to implement to distinguish between PFAS and non-PFAS and is understandable to experts and non-experts alike[5].

The two main classes are perfluoroalkyl substances, in which all hydrogen atoms in the carbon chain are replaced by fluorine (e.g. perfluorooctanoic acid, PFOA), and polyfluoroalkyl substances, which contain at least one perfluorinated unit but are not fully fluorinated (e.g., fluorotelomer alcohols). PFAS can also be divided into ionic PFAS and neutral PFAS, each of which has a different environmental behavior (Lindstrom et al., 2011)[6]. Among the ionic PFAS, the most widely used groups are perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). Due to their hydrophilic functional groups — such as carboxylate and sulfonate - ionic PFAS typically display high air–water (Kaw) and octanol–water partition coefficients (Kow), so they are more likely to disperse in aquatic environments than in the atmosphere [5]. The log Kow values of ionic PFAS, which range from 3 to 7, tend to increase with the length of the carbon chain[7] . Consequently, long-chain ionic PFAS (C > 7) are more likely to sorb to solids in the environment, including suspended solids, airborne particulate matter (APM), soil and sediments[8]. In contrast to ionic PFAS, neutral PFAS are generally more volatile and less stable in the environment than their ionic counterparts. Therefore, most studies on PFAS in the marine environment have focused on ionic PFAS, as shown below.



This exceptional stability makes PFAS very resistant to natural degradation processes in the environment. Consequently, they persist over long periods of time, earning them the nickname ‘eternal chemicals’. These properties have led to their use in industrial applications and consumer products such as firefighting foams, non-stick cookware, food packaging and textiles since their development in the 1940s and widespread use from the 1950s onwards[9,10]. PFAS have attracted much attention since the early 2000s when the ubiquity and potential risk of the two best known PFAS (i.e., legacy PFAS), perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), were reported [11,12]. However, their persistence in the environment and their bioaccumulative potential have led to significant environmental and health concerns [11,13–15].

The presence of PFAS in the marine environment is primarily attributed to various human activities that release these chemicals into the environment. Industrial discharges from manufacturing facilities that produce or utilize PFAS represent a significant source of contamination. These facilities can release PFAS directly into surface waters or indirectly through wastewater streams. Wastewater treatment plants (WWTPs) also play a role in the dissemination of PFAS into the marine environment. While WWTPs are designed to remove various pollutants from wastewater, they are not entirely effective at removing PFAS due to their unique chemical properties. Consequently, treated wastewater discharged from these plants can still contain detectable levels of PFAS, which eventually find their way into rivers and coastal waters. Major flood events, intensified by climate change, can also contribute substantial loads of PFAS to coastal environments [16].

Owing to their considerable solubility and polarity, PFAS are efficiently mobilized within aquatic environments, rendering the ocean a major terminal sink on a global scale[10]. Understanding their transport pathways and fate is therefore essential for assessing environmental risk and informing regulatory strategies [15]. PFAS are now detected in all environments worldwide, including marine ecosystems [15–19]. Once they enter the aquatic environment, certain PFAS, in particular perfluoroalkyl acids (PFAA) such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), can accumulate in marine organisms via water, sediment and food. In contrast to other persistent organic pollutants (POPs), which tend to accumulate in fatty tissue, PFAS have an affinity for binding to proteins in organisms [5,20]. Therefore, they are mainly found in well-perfused tissues such as the liver, kidneys and spleen of marine animals. Their accumulation varies by species and trophic level and is influenced by physiological factors (e.g., lipid content, protein affinity) and environmental conditions.

PFAS contamination is not limited to specific geographical areas but represents a global environmental challenge, as these persistent chemicals have been detected in the marine environment throughout the world [15,21]. Studies have even found PFAS in remote regions such as the Arctic and the deep sea, highlighting their widespread distribution [15]. However, the levels and types of PFAS found in seafood can vary significantly by region and species[15]. In Europe, studies have revealed the presence of PFAS in marine organisms from the North Sea, the Baltic Sea and the Mediterranean Sea [22].

PFOS is listed as a priority substance in the Water Framework Directive (WFD) and Member States have been required to report on compliance with environmental quality standards (EQS) since 2021. According to Directive 2013/39/EU, the EQS for PFOS are currently 0.65 ngL^{-1} for inland surface waters, 0.13 ng L^{-1} for transitional, coastal and territorial waters and 9.1 ng g^{-1} wet weight for biota (fish). In October 2022, the European Commission proposed quality standards for the sum of 24 PFAS, including PFOS, in surface waters and groundwater. These are based on an opinion of the European Food Safety Authority (EFSA), which is supported by opinions of the Scientific Committee on Health, Environment and Emerging Risks. The proposed standard for surface water and groundwater is 4.4 ng L^{-1} (as PFOA equivalent). For surface waters only, the new (2024) proposed EQS for biota is 0.077ng g^{-1} wet weight (as PFOA equivalents). In addition, Commission Regulation (EU) 2023/915[23] on maximum levels of contaminants in food, sets limits for PFOA, PFNA, PFOS, PFHxS for seafood for human consumption.

1.3 Mercury (Hg) in the marine environment

Mercury (Hg) is a globally distributed pollutant of significant ecological and public health concern, classified by the United Nations Environment Programme (UNEP, 2018) as a chemical element that is toxic to living organisms including humans. Its introduction into marine systems occurs through a combination of natural processes such as volcanic activity and the weathering of rocks, as well as anthropogenic activities including coal combustion, mining, waste incineration, and industrial discharges (UNEP, 2013) [24]. Once released, mercury can be transported atmospherically and deposited into oceanic waters, where it undergoes complex biogeochemical transformations.

In the marine environment, mercury is present in several forms: elemental mercury (Hg^0), inorganic divalent mercury (Hg^{2+}), and organic mercury compounds, most notably methylmercury (MeHg). Methylmercury is of particular concern due to its high toxicity and ability to bioaccumulate in organisms and biomagnify through the food web [25]. This transformation predominantly occurs via microbial methylation processes facilitated by sulfate-reducing and iron-reducing bacteria in anoxic sediments and water columns [26]. Thus, methylmercury is the predominant form of organic Hg present in seafood (84-97%)[27].

Marine biota absorb MeHg primarily through dietary intake. It bioaccumulates in primary consumers such as zooplankton and small pelagic fish and becomes increasingly concentrated at higher trophic levels, including in long-lived predatory species such as tuna, swordfish, sharks, and marine mammals [4,28]. High levels of MeHg in marine organisms can impair adverse biological effects[29].

The Mediterranean Sea have long been considered a hotspot for Hg pollution based on Hg data reported for marine species two times higher than those for the same species and size range living in the Atlantic

Ocean. This so called 'Mediterranean anomaly' was attributed to the enclosed character of the basin, the presence of Hg pollution hot spots in small gulfs (i.e., chlor-alkali plants) and to the presence of cinnabar (HgS) deposits and volcanoes. Yet, based on mercury speciation data, it is now well established that the observed elevated concentrations of Hg in Mediterranean marine organisms is a biological issue, related to mercury methylation processes that are different between various environments[30].

Human exposure to methylmercury occurs almost exclusively through the consumption of contaminated seafood, with the Mediterranean population being vulnerable to increased exposure, due to fish base diet. Results from epidemiological studies linking exposure to Hg to neurotoxicological effects, have prompted international agencies to establish consumption guidelines and advisories to mitigate risk[31,32]. Given its persistence and global distribution, mercury is a priority for environmental monitoring and regulatory efforts of the European Union. It is included in the EU list of Priority substances with established Environmental Quality standards (EQS) in waters (0.07 μg L^{-1}) (Water Framework Directive, 2000/60/EC, 2008/105/EC) and marine biota (0.02 μg g^{-1}) and has established threshold values for seafood of human consumption (Regulation EU 2023/915). Likewise, the tolerance for human consumption Tolerable Weekly Intakes (TWI) proposed by the EFSA is equal to 1.3μg kg^{-1} of body weight.

1.4 Other metals (Li, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Cd, Cs, Ba, Pb, U) in the marine environment

Contaminants of Emerging Concern (CoECs) are chemicals or substances that have been detected in the environment but are not yet regulated or fully understood in terms of their impact on human health and ecosystems. These contaminants often come from pharmaceuticals, personal care products, industrial processes, and agricultural runoff. Several metals and metalloids are classified as CoECs due to their potential toxicity, persistence in the environment, and bioaccumulation. These metals are often introduced into ecosystems through industrial activities, mining, wastewater discharge, and electronic waste. Marine organisms can uptake metals through respiration, adsorption and ingestion, and can regulate them depending on organism biology and physiology[33,34]. Metals that cannot be excreted continue to remain in the body and are perpetually added over the life span of the organism[35]. Metal accumulation is unique among species and varies based on age, size, habitat, feeding nature, swimming behaviour and environmental factors[36,37]. A special case of bioaccumulation can be the biomagnification in which the metal concentration in the organism exceeds that of its prey, and whereby dietary absorption occurs faster than elimination[38].

The classification of metals as CoECs is a developing field, influenced by ongoing research and monitoring efforts. While some metals like cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) have well-established regulatory limits, others such as lithium (Li), vanadium (V), selenium (Se), rubidium (Rb), caesium (Cs), uranium (U) and barium (Ba) are gaining attention due to emerging evidence of their environmental presence and potential risks. Other metals presence, such as cobalt (Co), copper (Cu), chromium (Cr), nickel (Ni), and zinc (Zn), at elevated levels has raised concerns about ecological and human health impacts.

In this study, the concentrations of Li, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Cd, Cs, Ba, Pb, U were investigated for their potential for bioaccumulation and biomagnification. As, Cd, and Pb are widely acknowledged for their high toxicity and are often listed among priority pollutants by environmental agencies[39]. The European Commission (European Union, 2023) has established maximum levels for these metals in certain foods, acknowledging their toxicity and potential health risks. Ba, Cs, U are monitored due to their presence in industrial processes and potential health risks. More specifically, U, particularly in areas near mining activities, has been identified as a contaminant of emerging concern due to its radiological and chemical toxicity. The increasing occurrence of Ba and Cs in environmental samples, suggests the need for further investigation into their potential as emerging contaminants. Ba and U are already included in regulatory lists, such as reference doses by the United States Environmental Protection Agency[40], due to their toxicity and environmental persistence. Co, Cu, Cr, Ni, Se and Zn are essential nutrients that are required for various biochemical and physiological functions. However, at elevated concentrations can disrupt biological processes and pose risks to aquatic life and human health (WHO, 1996). Cu and Zn are not traditionally classified as major CoECs like PFAS or pharmaceuticals, but they are increasingly monitored due to their impact on aquatic ecosystems and potential human health risks. Li, V, and Rb are not traditionally classified as major contaminants but are gaining attention due to increased industrial use, especially in electronics and energy storage.

2.0 Methodology

2.1 Sampling

Zooplankton samples were collected at a single station (S11: 37º 52,36N, 23º 38,30E) in the Saronikos Gulf in October 2023 aboard the research vessel Aegaeo, operated by the Hellenic Centre for Marine Research. Mesozooplankton samples were collected with a 200-μm mesh, 0.57-m open-mouth net containing a mechanical flowmeter for estimation of the filtered volume. Four oblique tows were performed. Samples were then filtered through a 200-μm Nytex mesh and stored at -4°C.

Three of the samples were designated for pollutant analysis (OFAS, Hg, other metals), while the fourth was used to assess zooplankton community composition. Qualitative and quantitative analyses of mesozooplankton were conducted under an Olympus SZX12 stereomicroscope. Zooplankton were identified to the group and species level. Abundance was reported as individuals per cubic meter (ind m^{-3}), and relative abundance (%) was used to describe species evenness within the community.

The mesozooplankton community was dominated by copepods, which were the most abundant group, with a density of 351.15 individuals per cubic meter (ind m^{-3}), followed by cladocerans (127.40 ind m^{-3}), meroplankton (34.26 ind m^{-3}), chaetognaths (4.28 ind m^{-3}), and other groups (5.35 ind m^{-3}), totalling 522.45 ind m^{-3}. The meroplankton primarily consisted of echinoderm larvae. Among the mesozooplankton species, the copepod *Temora stylifera* was dominant, accounting for 56.15% of the total relative abundance. Other notable species included the cladoceran *Penilia avirostris* (23.57%), and the copepods *Corycaeus clausi* (4.71%), *Paracalanus denudatus* (1.02%), and *Oithona plumifera* (0.82%), with the remaining species collectively comprising 5.74%.

Table 1: Zooplanktonic community structure in the samples collected in the Saronikos Gulf.

Mesozooplankton Taxa	***Abundance ind m^{-3}***
Copepods	351.15
Cladocerans	127.40
Meroplankton	34.26
Chaetognaths	4.28
Others	5.35
Total Abundance	522.45
Mesozooplankton Dominant Species	**% Relative Abundance**
Temora stylifera	56.15
Penilia avirostris	23.57
Corycaeus clausi	4.71
Paracalanus denundantus	1.02
Oithona plumifera	0.82
Others	5.74

Aquatic organisms were sampled during a dedicated sampling campaign with the R/V FILIA of the HCMR, at the Saronikos Gulf, Greece. Experimental trawling was conducted on 19/9/2023 at depths ranging from 72 m to 85 m, in total 4 trawls (Figure 2). Eight fish species were collected, along with 3 species of molluscs and one crustacean. To complement the trophic food web, three more species that were not able to be sampled by trawling, were obtained from local fishermen assuring that they were collected from the same area.

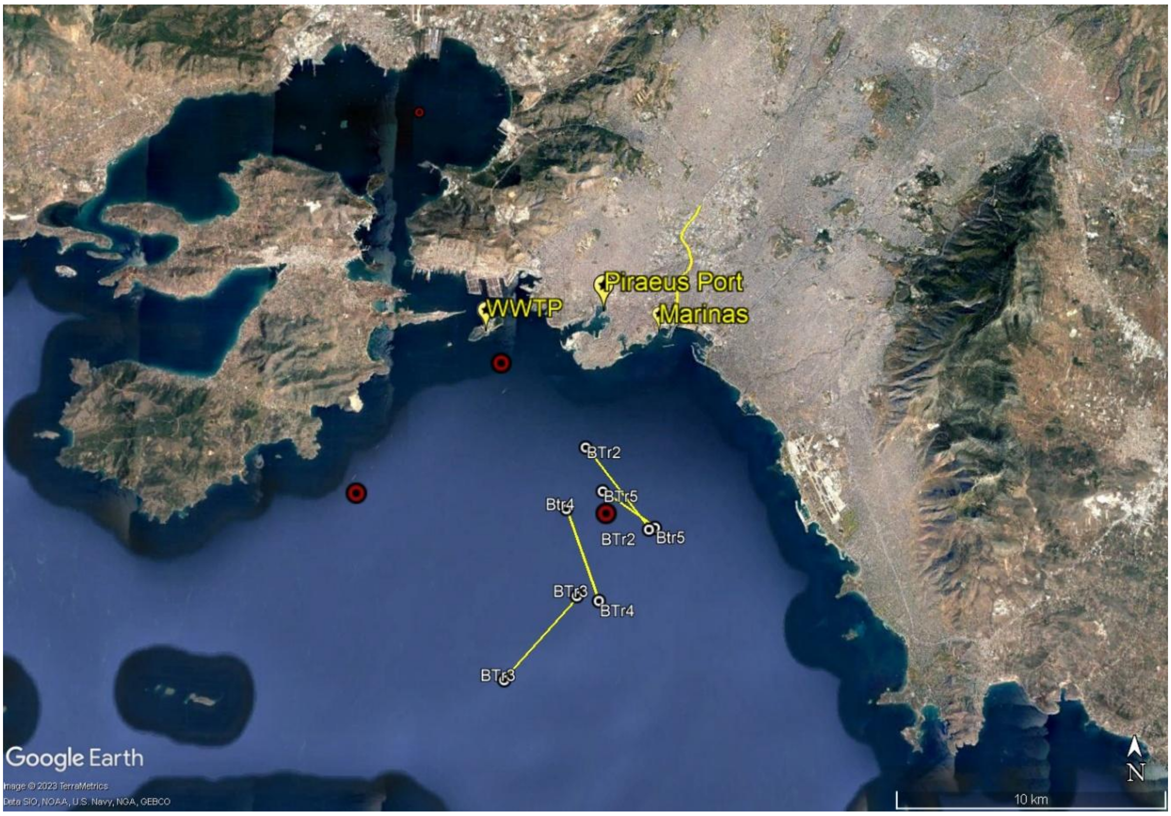


Figure 2: Trawling positions (yellow lines) in the Saronikos Gulf, Greece for the collection of marine organisms.

The species used for the present study are presented in Table 2 and Table 3 along with their morphometric parameters (body length, body weight). For assessing the biomagnification potential of specific contaminants, the dorsal muscle of fish and soft tissue of invertebrates were collected for analysis. Assessment of bioaccumulation potential of contaminants was conducted for two species *Pagellus Erythrinous* and *Merlucious merlucious*. For this purpose, muscle, liver and gills were collected for analysis from specimens of three length sizes (A, B, C). (Table 2).

Table 2: Morphometric data of sampled fish, number of individuals used as replicates and/or for pooling of replicates.

Species name	Common name	Total Length (cm)	Total weight (g)	No of individuals/replicates	
Fish					
Spicara sp	Picarels	16.3 ± 0.8	46.8 ± 7.4	8	
Mullus barbatus	Red mullet	18.7 ± 0.9	77.2 ± 9.9	8	
Pagellous erytrhinous (sizes A, B, C)	Common pandora	25.6 ± 2.1	220.7 ± 52.7	10	pooled in 2
		18.8 ± 1.1	80.0 ± 15.2	24	pooled in 2
		14.4 ± 0.8	38.9 ± 7.3	20	pooled in 3
Boops boops	Bogue	17.0 ± 1.3	46.9 ± 11.7	8	
Mullus surmuletus	Striped red mullet	17.4 ± 0.9	63.1 ± 12.4	8	
Serranus hepatous	Brown comber	9.3 ± 0.4	13.1 ± 2.1	40	pooled in 8
Lophius piscatorius	Anglerfish	37.7 ± 11.6	1285 ± 1116	3	
Merluccius merluccius (sizes A, B, C)	Hake	38.1 ± 2.3	365.0 ± 70.7	7	
		29.3 ± 1.6	221.5 ± 24.0	8	
		21.9 ± 1.5	76.1 ± 13.7	8	
Sardina pilchardus	Sardine	13.7 ± 0.6	20.3 ± 3.2	42	pooled in 8
Siganus rivulatus	Marbled spinefoot	24.9 ± 1.7	204. 2 ± 49.0	8	
Auxis rochei	Bullet tuna	40.6 ± 1.2	1030.4 ± 139.0	7	

Table 3: Morphometric data of sampled crustacean & cephalopods, number of individuals used as replicates and/or for pooling of replicates.

Species name	Common name	Mantle Length (cm)	Total weight (g)	No of individuals/replicates	
Crustacean & Cephalopods					
Parapenaeus longirostris	Deep-water rose shrimp	8.7 ± 1.0	6.0 ± 2.0	80	Pooled in 8
Loligo Vulgaris	European squid	15.0 ± 3.0	114.0 ± 53.5	6	
Illex coindetii	European flying squid	13.2 ± 1.5	79.3 ± 19.0	8	
Eledone moschata	Musky octopus	8.8 ± 1.2	161.3 ± 55.5	6	
Octopus vulgaris	Common octopus	85.9 ± 17.0	179.0 ± 98.5	7	

For each species three to four individuals were considered as replicates for each specific determination except small size species for which pooled samples were formed. Dissection was done on a clean glass table strictly avoiding contact with plastics or metals depending on the analyses to be followed. All the samples were then frozen at −20 °C, freeze-dried and ground into a homogenized fine powder prior to analyses.

2.2 Complementary parameters (isotopes, lipids)

2.2.1. Analyses for lipid content

The lipid content of freeze-dried samples was determined using an in-house extraction method and non-chlorinated solvents following Smedes (1999)[41]. Lipids extraction was performed in a Soxhlet apparatus with a 1:1 (v/v) mixture of cyclohexane – isopropanol. The extracted lipids were weighed upon solvent removal using a rotary evaporator and further drying for at least 6 hours at 105°C.

2.2.2. Stable isotopes analyses and trophic level (TL) calculation

Carbon and nitrogen isotope analysis was undertaken by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) at Sercon Analytical. The reference material used for δ13C and δ15N analysis of was IA-R068 (soy protein, δ13CV-PDB = -25.22 ‰, δ15NAIR = 0.99 ‰). IA-R068, IA-R069 (tuna protein, δ13CV-PDB = -18.88 ‰, δ15NAIR = 11.60 ‰) and a mixture of IAEA-C7 (oxalic acid, δ13CV-PDB = -14.48 ‰) and IA-R045 (ammonium sulphate, δ15NAIR = -4.71 ‰) were run as quality control check samples during analysis.

For samples with C/N ratio >3.5 $\delta^{13}C$ values were corrected for the effects of lipid content, following the equation:

$\delta^{13}C_{\text{lipid-corrected}}=\delta^{13}C_{\text{bulk}}-(\text{Correction Factor}\times\%\text{lipid})$,

where correction factor =3.32 for aquatic organisms[42] and % lipid content of dry mass.

Trophic level determination was conducted following the equation:

$TL_{\text{consumer}} = 2.0 + ({}^{15}N_{\text{consumer}} - {}^{15}N_{\text{primary consumer}})/3.8$

where $\delta^{15}N$primary consumer is the stable nitrogen isotope value of zooplankton and 3.8 is the isotopic trophic enrichment factor.

2.3 Analysis of PFAS

PFAS analysis was undertaken at the Mass Spectrometry and Dioxin Analysis Lab of NCSR Demokritos, Greece, accredited by EN ISO/IEC 17025:2017 for PFAS analysis in food products.

Materials: Standards of PFOS, PFOA, PFNA, PFHxS and stable isotope labelled standards 13C8-PFOA, 13C9-PFNA, 13C8-PFOS, 13C3-PFHxS were purchased from Wellington. Ammonium acetate, acetonitrile, methanol (LC grade), NaCl, MgSO4 and C18 (RP-18) were purchased from Merck (Darmstadt, Germany). Envi-carb was purchased from Supelco. Ultrapure water was provided by a Nanopure apparatus, Barnsted/Thermolyne, USA.

Sample clean-up: 3 gr of the tissue sample was added to a 15 mL Falcon tube. After the addition of 10 ng of isotope labelled internal standards, 10 mL of acetonitrile were added, and the sample was vortexed for 1 min and sonicated for 15 min. After centrifugation at 5000 rpm for 15 min, the supernatant was transferred to a clean Falcon tube containing 2 g of MgSO4, 0.5 g of NaCl, 0.1 g C18 and 0.1 g Envi-carb. The mixture was immediately shaken for 2 min and centrifuged at 5000 rpm for 15 min. After centrifugation the supernatant was collected and concentrated to 1 mL in a rotary evaporator. Finally, the extract was dried under a gentle stream of nitrogen and 200 µL of LC mobile phase were added.

Instrumental analysis method: A UHPLC-ESI-MS/MS system (WATERS) which consisted of an Acquity UPLC and a Xevo TQ-XS detector was used for PFAS analysis. 10 µL of the sample were injected and separated on a Waters Acquity BEH C18 column (1.7 mm, 2.1 mmx100 mm). 2 mM ammonium acetate in water: MeOH 90:10 (A) and 2 mM ammonium acetate in MeOH (B) were used as mobile phases. The elution gradient started at 100% A, changed to 100% B at 17 min linearly, and remained constant until 19 min. The detection was performed in ESI negative mode. MS/MS parameters (parent ions, product ions and collision energy) were optimized. The method has been validated for specificity, repeatability, reproducibility, recovery and sensitivity according to EURACHEM guide. For analyte identity (specificity) confirmation, retention time (RT) of the analyte should correspond to that of the labelled standard ±0.2s. Repeatability and reproducibility of the method developed were tested by multiple analyses of spiked samples. Recovery was estimated by the use of internal isotopically labelled standards and found to vary between 60-90%. The limit of detection (LOD) was calculated from the lowest concentration with acceptable signal-to-noise ratio and the limit of quantitation (LOQ) from the lowest concentration with ion abundance ratio within ±15% of the theoretical value and deviation of the relative response factor from the mean value ≤20%. The calculated LOD 0.03 ng mL^{-1} and the LOQ was 0.1 ng mL^{-1}.

2.4 Analysis of THg

In the present work total Hg (THg) including inorganic and methylated forms of mercury in biota (i.e., Hg^{2+} + MeHg) was determined. THg determination was conducted in the freeze-dried samples without any further treatment using a direct mercury analyser (DMA 80 evo, Milestone Srl, Italy) according to the EPA 7473 method (US EPA, 1998). The accuracy and precision of mercury determination will be verified by the parallel analysis of certified reference materials NIST 2976 (muscle tissue from NRC/Canada and IAEA) and DORM-4 (fish protein from NRC/Canada). Acceptable agreement was found between assigned and measured values (Table 4). The limit of detection (LOD) of the method was estimated at the 0.006 ng g-1 dw level (as the triplicated standard deviation of the blank).

Table 4: Assigned and measured THg concentrations (in µg g- 1 dw) of the certified reference materials: NIST 2976 (muscle tissue from NRC/Canada and IAEA) and DORM-4 (fish protein from NRC/Canada), presented as average (± standard deviation).

Reference material	Assigned value (*µg g⁻¹ dw*)	Measured value (*µg g⁻¹ dw*)
NIST 2976 *n*=23	0.412 ± 0.036	0.416 ± 0.034
DORM-4 *n*=22	0.061 ± 0.0036	0.059 ± 0.0037

2.5 Analysis of other metals and elements (Li, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Cd, Cs, Ba, Pb, U)

The concentrations of 15 metal and other elements (Li, V, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Cd, Cs, Ba, Pb, U) were determined according to a modified version of the USEPA method 3052 (1996) for microwave-assisted acid digestion of siliceous and organically based matrices. An amount of around 0.05 - 0.25 g of freeze-dried muscle, liver, gills, stomach content and zooplankton were transferred in acid-cleaned Teflon vessels and pre-digested with 5 mL of conc. HNO3 and 2 mL H2O2 for one hour in a sand bath. After pre-digestion, samples were digested under pressure using a closed, high pressure, microwave system (Multiwave 3000, Anton Paar, Austria). Metal concentrations in the sample digests were measured according to the USEPA 6020A (2007), using an Inductively Coupled Plasma – Mass Spectrometer (ICP–MS NexION300, PerkinElmer, Shelton, CT, U.S.). An optimal sample dilution factor of x600 was chosen for samples. The instrument was calibrated using standard solutions. An internal standard containing Indium and Bismuth (10 µg L-1) was added to each sample and calibration standard. A calibration standard was run for every 10 samples analysed. Elements concentrations were expressed in wet weight. The above-mentioned protocols are described in detail in Kalantzi et al. (2013, 2016)[34,43].

The accuracy and precision of the analytical methodology were tested with certified reference materials (CRMs) and blanks with every six samples digested. The following CRMs were used: DORM-4 (fish protein), LUTS-1 (non-defatted lobster hepatopancreas) certified by the National Research Council of Canada and BCR-668 (muscle tissue) certified by the Joint Research Centre of European Commission. Average recoveries of DORM-4, LUTS-1 and BCR-668 were 101 ± 7 % (n=11); 102 ± 8 % (n=8); and 100 ± 10 % (n=9) respectively for all elements. The elements concentrations in the digestion blanks were typically very low and were subtracted from the sample values. The limits of detection (LOD) of the procedure were calculated by multiplying the standard deviation of the blanks (n=28) by three and were: 0.003 (Li); 0.017

(V); 0.064 (Cr); 0.001 (Co); 0.062 (Ni); 0.108 (Cu); 0.975 (Zn); 0.007 (As); 0.049 (Se); 0.001 (Rb); 0.002 (Cd); 0.001 (Cs); 0.041 (Ba); 0.015 (Pb) 0.002 (U) mg/kg dry weight.

For statistical analysis, if more than 50% of the samples concentration of residue exceeded the LOD, values of metals below the limit of detection (LOD) were set equal to 0.5*LOD. If the previous assumption was not met, these metals were excluded from the analysis (USEPA, 1991). To explore metal accumulation between the different tissues and between different species, non-metric multidimensional scaling (nMDS) ordinations based on Euclidean distance of log(x+1) transformed data were used. The Analysis of Similarities (ANOSIM) of the Euclidean distance matrices was used to test the significance of differences in overall metal concentrations between tissues for each fish species and between fish species for each tissue. These analyses were performed with PRIMER v7 software (Plymouth Marine Laboratory, Natural Environmental Research council, UK). Statistically significant differences of metal concentrations between tissues for each fish species, between fish species for each tissue and between the different sizes of fish were determined using the non- parametric Kruskal-Wallis test for multiple independent variables followed by multiple comparisons of mean ranks for all groups (Dunn's test). A p-value of 0.05 or less was considered to be statistically significant. Correlation between metal concentrations and trophic level was tested by Spearman correlation matrix for each tissue. The STATISTICA v.12.0 (StatSoft Inc.) software was used for the univariate analysis.

To assess the human health risks of dietary metal exposure, mean metal concentrations were compared with permitted limits for muscle tissues established by different national and international authorities. These Safety Standards are 0.30 mg/kg ww for Pb, (0.5 mg/kg for crustaceans), 0.05 mg/kg ww for Cd (0.15 mg/kg for Auxis rochei, 0.25 mg/kg for Sardina pilchardus, 0.5 mg/kg for crustaceans, 1.0 mg/kg for cephalopods), 30 mg/kg ww for Cu, 30 mg/kg ww for Zn, 1.0 mg/kg ww for Ni, 6.8 mg/kg ww for Se, 95 mg/kg ww for Ba, 0.50 mg/kg ww for Cr and 1.3 mg/kg ww for As (Kalantzi et al., 2013; European Union, 2023). Furthermore, the Estimated Daily Intake (EDI; μg/kg bw/day) of metals was calculated according to the following equation[44]:

$$EDI = [\, C \times AvC \,] / bw$$

where C (μg/g ww of organism) is the mean metal concentration in the muscle tissue of each organism, AvC is the average consumption of g fish per day and bw is the adult body weight of the general population. An AvC of 68.68 g/day of fish in Greece and an average bw of the general population of 70 kg were assumed (FAO, 2005–2012)[45]. These EDI values were compared with Reference Doses (RfD) as established by the USEPA (2017)[40] as follows:

Reference doses, USEPA (2017) μg/kg bw/d			
Li	2.0	Zn	300
V	5.0	As (inorganic)	0.3
Cr	3	Se	5.0
Co	0.3	Cd	1.0
Ni	20	Ba	200
Cu	40	U	0.2
Pb	3.57*		

*USEPA (2014)

Regarding Pb, since an RfD value was not set by USEPA in 2017, a previously established value by USEPA (2014)[46] was used, that is 3.57 μg/kg bw/d.

2.6 Bioaccumulation factor (BAF), and Trophic magnification factor (TMF)

BAF (L/kg ww) was calculated according to the equation:

$BAF = C_{biota}/C_{water}$

where C_{biota} is the concentration of contaminant in the biota sample, and C_{water} is the mean concentration (ng/L) of the respective contaminant in the ambient seawater.

According to the European Regulation EC/1907/2006, BAF>2000 indicate bioaccumulative substances and >5000 very bioaccumulative substances. In studies comparing bioaccumulation of contaminants across species the $Log_{10}BAF$ is used and a value >3 denotes bioaccumulation.

TMF is calculated as the anti -log of the slope of the regression of the contaminant concentration in the biota vs the respective trophic level.

$Log\ C_{biota} = a \times TL + b$

$TMF = 10^{a}$

TMF values >1 denote that contaminants magnify along the food web.

3.0 Results and Discussion

3.1 *Trophic level of examined species*

Results on species isotopic composition of $\delta^{15}N‰$ $\delta^{13}C‰$ along with % lipid content (dw), and trophic level (TL) are shown in Table 5. Data from zooplankton used as the basis (primary consumer) for the trophic level calculation.

Table 5: Lipid content (%),isotopic signatures δ15N‰, δ13 C‰, and trophic level (TL) of the studied species.

	% Lipids (dw)	$\delta^{15}N‰$	$\delta^{13}C‰$	$\delta^{13}C‰$ lipid corrected	Trophic Level (TL)	*Feeding habits
zooplankton	19.96	6.34±0.00	-20.55±0.00	-20.60	2.0	
Fish						
Siganus rivulatus	29.05	7.32±0.11	-18.21±1.18	-18.28	2.3	demersal
Boops boops	23.92	10.48±0.41	-20.29±0.54	-20.35	3.2	demersal
Sardina pilchardus	13.82	11.19±0.21	-19.79±0.21	-19.82	3.4	pelagic
Mullus surmuletus	18.86	11.44±0.17	-19.37±0.57	-19.41	3.5	demersal
Spicara sp	15.17	12.59 ± 0.97	-19.01±0.13	-18.68	3.8	pelagic
Merluccius merluccius	14.97	12.34±0.58	-18.65±0.17	-19.05	3.8	demersal
Mullus barbatus	13.27	12.75 ±0.53	-17.92±0.53	-17.95	3.9	demersal
Serranus hepatous	22.96	13.03±0.21	-18.01±0.10	-18.07	4.0	demersal
Lophius piscatorius	11.88	13.74±0.25	-17.79±0.12	-17.82	4.2	demersal
Pagellus erytrhinus	1.49	14.07± 0.92	-18.30±0.35	-18.33	4.3	demersal
*Auxis rochei**	22.59	-	-	-	4.9*	pelagic
Crustacean & Cephalopods						
Parapenaeus longirostris	19.80	10.97±0.14	-18.44±0.04	-18.96	3.36	
Illex coindetii	19.96	12.23±0.36	-18.96±0.21	-17.78	3.73	
Octopus vulgaris	20.15	12.41±0.21	-18.19±0.07	-17.63	3.79	
Loligo Vulgaris	17.53	12.72±0.35	-17.74±0.38	-18.49	3.88	
Eledone moschata	18.12	12.89±0.30	-17.58±0.17	-19.01	3.93	

**Isotopic data for Auxiis Rocheii were inconsistent., and its TL is calculated based on the regression line of Hg vs TL, see section 2.2, Figure 11.*

**According to [Search FishBase](https://www.fishbase.se)*

In this study, $\delta^{15}N$ signatures ranged from 6.34 ‰ in zooplankton to 14.07‰ to *Pagellus erythrinous*. TLs for most fish species were found between 3 and 4. Only two fish species, *Siganus rivulatus* and *Auxis rocheii* had low TLs, 2. 3 and 2.5 respectively. Highest TLs were found for *Lophius piscatorius* (4.2) and *Pagellus erythrinous* (4.3). *Auxis rocheii* is a type of bullet tuna fish and was selected as a representative of a higher TL. All species of cephalopods investigated in the Saronikos Gulf, belong to more or less the same TL (3.7-3.9) indicative of carnivorous feeding habits with preference to decapods and fish, while the shrimp *Parapenaeus longirostris* occupies a somewhat lower TL (3.4) which could be related to increased consumption of sediment organic detritus, especially foraminifera, during its digging phase. (FAO MEDSUDMED- Parapenaeus longirostris).

C/N ratio for all species analysed were found >3.5, thus lipid corrected $\delta^{13}C$ were calculated and ranged from -17.6‰ to -20.6‰ (Figure 3). Zooplankton had the lowest value (-20.6‰) indicating its pelagic carbon source. For *Boops boops*, *Sardina pilchardus* and *Spicara sp*. $\delta^{13}C$ values are also very negative (~-20.4‰ to -19.05‰), suggesting a planktonic/pelagic feeding source, typical for offshore or open-water plankton-based systems. $\delta^{13}C$ signatures for the rest of the fish species reflect a mixed pelagic coastal-benthic feeding source with *Lophius piscatorius* (-17.8‰) and *Mullus barbatus* (-18.0‰) being more enriched, indicating feeding near or on the seafloor where the carbon base is benthic. Cephalopods like *Octopus vulgaris* (-17.6‰) and *Loligo vulgaris* (-17.8‰) have equally high $\delta^{13}C$, consistent of coastal benthic feeding. Finally, $\delta^{13}C$ of *Parapenaeus longirostris* (-19.0‰) lies in between pelagic – benthic feeding sources — likely influenced by both. *P. longirostris* is recognized as an active predator targeting bathypelagic, benthic, and endobenthic prey, while also exhibiting scavenging behavior [47,48]. Differences in TL and in the $\delta^{13}C$ % signature between the two species *Mullus surmuletus* and *Mullus barbatus*, indicate different diet and feeding habits in the two species which is in accordance with literature findings [49,50]. Lower δ13C % values recorded in M. barbatus than in *M. surmuletus* could be related to higher consumption of sub-surface deposit-feeding polychaetes by *M. barbatus* [50].

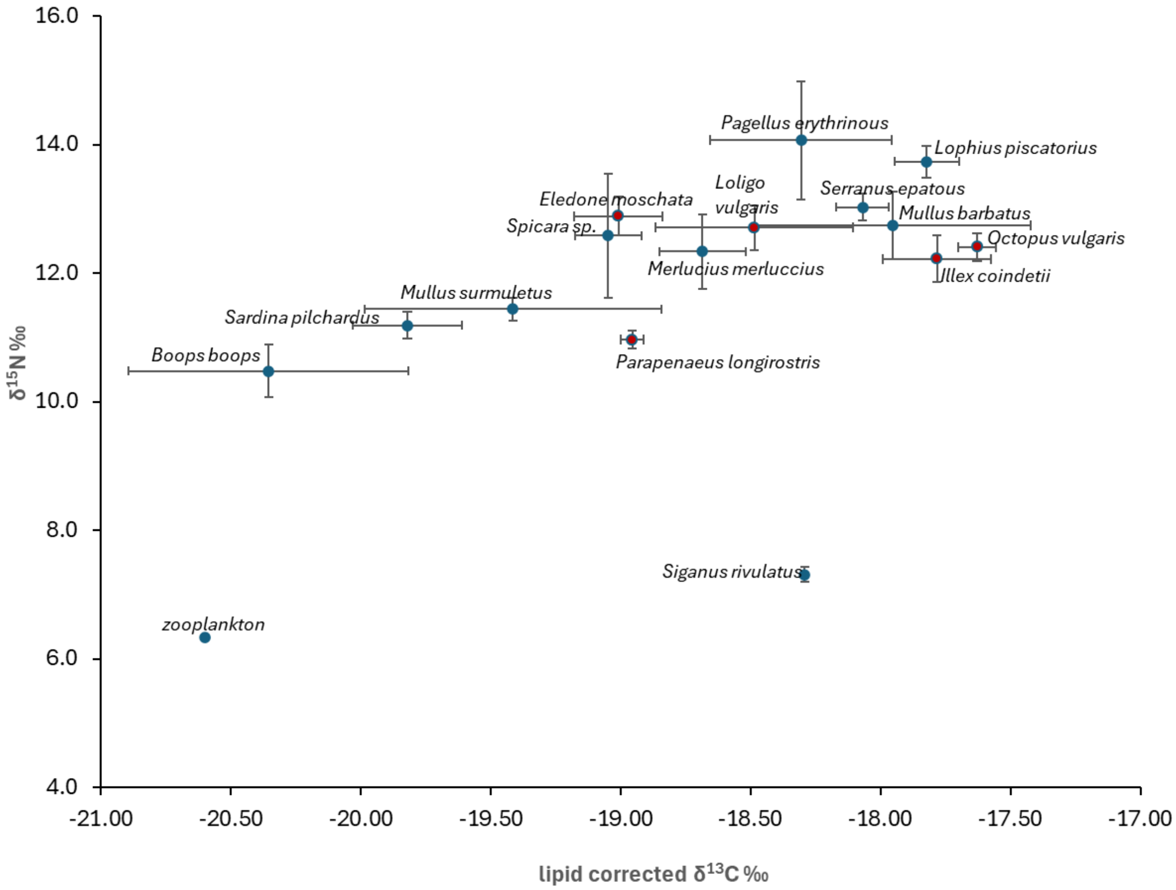


Figure 3: Plot of δ15 N and lipid corrected δ13 C values in marine organisms from the Saronikos Gulf.

Overall, the isotopic signatures of N and C indicate that the food web at the Saronikos Gulf study site is primarily dominated by species with coastal-benthic feeding habits, rather than exclusively pelagic ones.

3.2 PFAS in marine food webs of the Saronikos Gulf

3.2.1. PFAS concentrations in marine organisms

PFAS concentrations in marine organisms from the Saronikos Gulf, are shown in Table 6. All 4 PFAS compounds were detected in the 54 samples/replicates, with the highest detection frequency found for PFOA (96.3 %) and PFNA (96.3%), followed by PFOS (87.1 %) and PFHxS (40.8 %). The sum of PFAS (ΣPFAS) concentrations for all organisms ranged from 0.01 ng g^{-1} (ww) to 1.08 ng g^{-1} (ww) (mean 0.31 ng g^{-1} (ww)), with a slightly higher range in cephalopods and crustaceans (0.13 – 1.08 ng g^{-1} (ww); mean 0.45 ng g^{-1} (ww)) than in fish (0.01 -1.08 ng g^{-1} (ww) (mean 0.25 ng g^{-1} (ww)).

Table 6: PFAS concentrations (ng g^{-1} (ww)) in all samples /replicates of biota collected in the Saronikos Gulf.

Species name	PFOA	PFNA	PFOS	PFHxS	ΣPFAS
	ng g-1 (ww)				
zooplankton	0.04	0.002	0.03	0.002	0.08
Fish					
Siganus rivulatus	n.d.	0.01	n.d.	n.d.	0.01
	0.01	0.02	0.05	n.d.	0.07
	0.01	n.d.	0.03	n.d.	0.04
Auxis rochei	0.02	0.02	0.66	n.d.	0.70
	0.02	0.04	1.03	n.d.	1.08
	n.d.	0.04	0.04	n.d.	0.09
Boops boops	0.01	0.06	0.37	0.04	0.48
	0.01	0.03	0.23	0.04	0.31
	0.01	0.04	0.28	0.04	0.37
Sardine	0.06	0.08	0.64	n.d.	0.77
	0.04	0.07	0.67	0.02	0.80
	0.03	0.07	0.56	n.d.	0.65
Mullus surmuletus	0.02	0.15	0.07	n.d.	0.24
	0.03	0.13	0.05	n.d.	0.21
	0.04	0.14	0.05	n.d.	0.22
Spicara sp	0.01	0.02	0.07	n.d.	0.10
	0.01	0.01	0.06	n.d.	0.08

Species name	PFOA	PFNA	PFOS	PFHxS	ΣPFAS
	ng g-1 (ww)				
	0.01	0.01	0.04	0.01	0.07
Merluccius merluccius Size A	0.02	0.01	0.12	n.d.	0.15
	0.01	0.01	0.06	n.d.	0.08
	0.01	0.03	0.09	n.d.	0.12
Merluccius merluccius Size B	0.01	0.04	0.26	0.01	0.31
	0.01	0.04	0.19	n.d.	0.25
	0.02	0.05	0.29	n.d.	0.37
Merluccius merluccius Size C	0.01	0.05	0.17	n.d.	0.23
	0.02	0.05	0.15	n.d.	0.22
	0.01	0.05	0.12	n.d.	0.19
Mullus barbatus	0.04	0.06	0.13	0.12	0.34
	0.03	0.05	0.12	0.08	0.28
	0.06	0.08	0.19	0.09	0.42
Serranus hepatus	0.02	0.05	0.02	n.d.	0.09
	0.02	0.04	0.02	n.d.	0.08
	0.02	0.06	0.01	n.d.	0.08
Lophius piscatorius	0.02	0.03	0.21	n.d.	0.26
	0.02	0.01	0.20	n.d.	0.24
	0.01	0.02	0.24	0.01	0.28
Pagellous erytrhinus	0.01	0.01	n.d.	0.01	0.03

Species name	PFOA	PFNA	PFOS	PFHxS	ΣPFAS
	ng g-1 (ww)				
Size A	0.01	0.02	n.d.	n.d.	0.02
Pagellous erytrhinus Size B	0.01	n.d.	n.d.	n.d.	0.01
	0.01	0.01	n.d.	n.d.	0.02
Pagellous erytrhinus Size C	0.01	0.01	n.d.	0.02	0.05
mean	**0.02**	**0.04**	**0.18**	**0.01**	**0.25**
SD	**0.01**	**0.04**	**0.23**	**0.03**	**0.24**
min	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**0.01**
max	**0.06**	**0.15**	**1.03**	**0.12**	**1.08**
Crustacean & Cephalopods					
Parapenaeus longirostris	0.09	0.35	0.64	n.d.	1.08
	0.07	0.30	0.60	0.01	0.98
	0.08	0.27	0.55	n.d.	0.91
Illex coindetii	0.02	0.01	0.34	n.d.	0.37
	0.04	0.02	0.17	n.d.	0.23
	0.03	0.02	0.16	n.d.	0.20
Octopus vulgaris	0.03	0.09	0.46	0.01	0.59
	0.03	0.08	0.25	0.02	0.38
	0.04	0.08	0.55	0.02	0.68
Loligo Vulgaris	0.02	0.01	0.19	n.d.	0.23
	0.02	0.03	0.08	n.d.	0.13

Species name	PFOA	PFNA	PFOS	PFHxS	ΣPFAS
	ng g-1 (ww)				
	0.04	0.04	0.17	n.d.	0.26
Eledone moschata	0.02	0.08	0.09	n.d.	0.19
	0.05	0.08	n.d.	n.d.	0.13
	0.02	0.07	0.27	0.01	0.36
mean	**0.04**	**0.10**	**0.30**	**n.d.**	**0.45**
SD	**0.02**	**0.11**	**0.21**	**0.01**	**0.32**
min	**0.02**	**0.01**	**n.d.**	**n.d.**	**0.13**
max	**0.09**	**0.35**	**0.64**	**0.02**	**1.08**

Among the four PFAS compounds analyzed, PFOS contributed the most (>50%) to ΣPFAS in all species except for *Pagellus erythrinus*, where it was not detected, and *Serranus hepatus* and *Mullus surmuletus*, where its contribution dropped to less than 20% (Figure 4). PFOA and PFNA generally contributed equally to ΣPFAS, except in *Pagellus erythrinus*, *Serranus hepatus*, and *Mullus surmuletus*, where they exhibited the highest contributions. PFHxS showed the lowest contribution (on average 5.9%) to ΣPFAS, with the exception of *Pagellus erythrinus* and *Mullus barbatus* where it peaked to levels >19%.

According to the European Regulation (EU) 2023/915[23] on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006, the permitted maximum level of PFOA, PFNA, PFOS, PFHxS, and ΣPFAS in muscle meat of fish are 0.2, 0.5, 2.0, 0.2, and 2.0 ng g^{-1} (ww), respectively, except in sardine where these levels are 1.0, 2.5, 7.0, 0.2, and 8.0 ng g^{-1} (ww), respectively, and in crustaceans where these values are 0.7, 1.0, 3.0, 1.5, 5.0 ng g^{-1} (ww), respectively. The concentrations of PFAS in fish muscle, crustaceans and cephalopods from Saronikos Gulf were found to be below these permitted levels, but exceed the proposed stricter EQS of 0.077 ng g^{-1} ww for marine biota at 75% of species investigated. In particular ΣPFAS (mostly PFOS) were found above the proposed EQS in Boops boops, Sardina pilchardus, Mullus surmuletus, Merluccius merluccius, Mullus barbatus, Lophius piscatorius, Auxis rocheii,in the shrimp Parapernaeus longirostris and all the cephalopods examined. It should be noted here that this EQS is still not part of any legislative document.

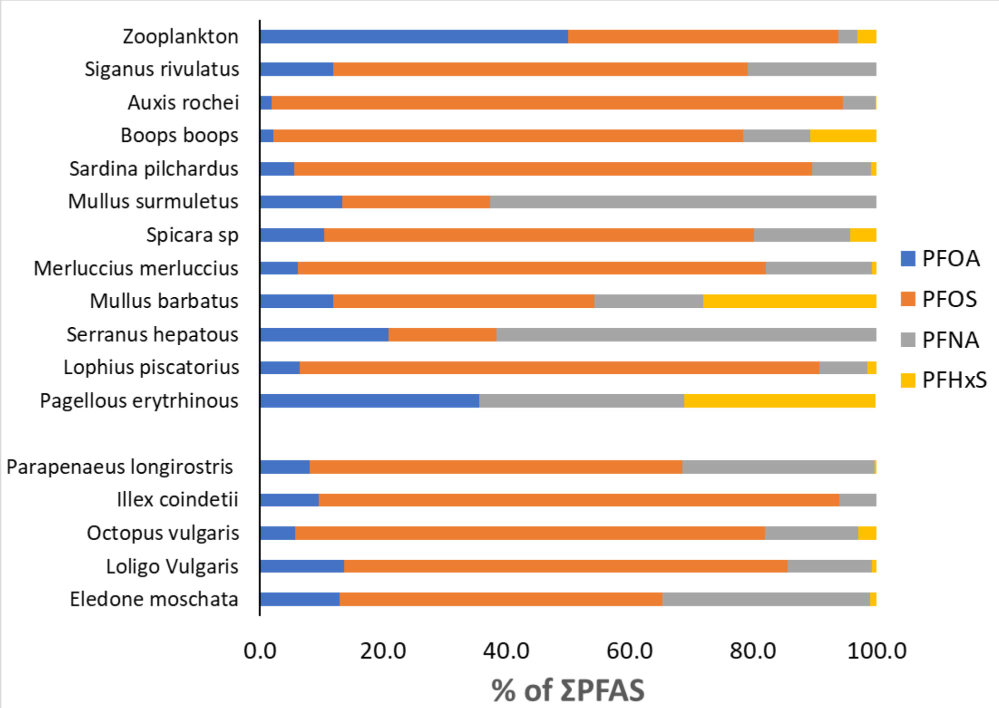


Figure 4: Percentage contribution of PFAS compounds in ΣPFAS in the various marine organisms investigated.

PFOS concentrations fluctuated within a relatively elevated range, from not detected (n.d.) to 1.02 ng g^{-1}, (ww), followed by PFNA, which ranged from n.d. to 0.35 ng g^{-1} (ww). PFOA and PFHxS were detected at considerably lower levels (n.d.–0.09 ng g^{-1} (ww) and n.d.–0.04 ng g^{-1} (ww), respectively), with some outlier PFHxS concentrations (0.09–0.12 ng g^{-1} (ww)) recorded in *Mullus barbatus*.

Spearman correlation analysis revealed a strong correlation between PFOA and PFNA ($r = 0.788$; $p = 0.000$). No significant correlation was observed between any other pairs of compounds.

Both PFOA and PFNA are long-chain PFAS (C8 and C9, respectively) characterized by hydrophilicity due to the presence of a carboxylic group. PFOS is also a long-chain compound (C8) but exhibits both hydrophobic and lipophobic properties due to its sulfonic group. In contrast, PFHxS is a short-chain PFAS (C6) containing a sulfonic group. Short-chain PFAS, such as PFHxS, typically exhibit lower bioaccumulation potential [20,51], which likely explains the low concentrations of PFHxS detected in the muscle tissues of organisms from the Saronikos Gulf and the absence of correlation with other PFAS compounds. On the other hand, the strong correlation observed between PFOA and PFNA is likely attributed to their similar chemical structures, resulting in common pathways in marine organisms.

Studies on PFAS compounds in marine biota, waters, and sediments reveal considerable variability, as shown in Table 6. Our results for marine biota fall within the lower range of reported values, consistent with the low PFAS concentrations observed in the waters and sediments of the Saronikos Gulf, as reported by Lougkovois et al. (2025)[16]. The PFAS levels in marine species from this study are comparable to those found in other Mediterranean regions, such as the Ebro Estuary [52] and the Bizerte Lagoon [53], as well as in the Beibu Gulf of the South China Sea [54].

Table 6: PFAS concentrations in biota (ng g-1), seawater (ng L-1) and sediments (ng g-1) from selected marine areas. Average value sin parentheses.

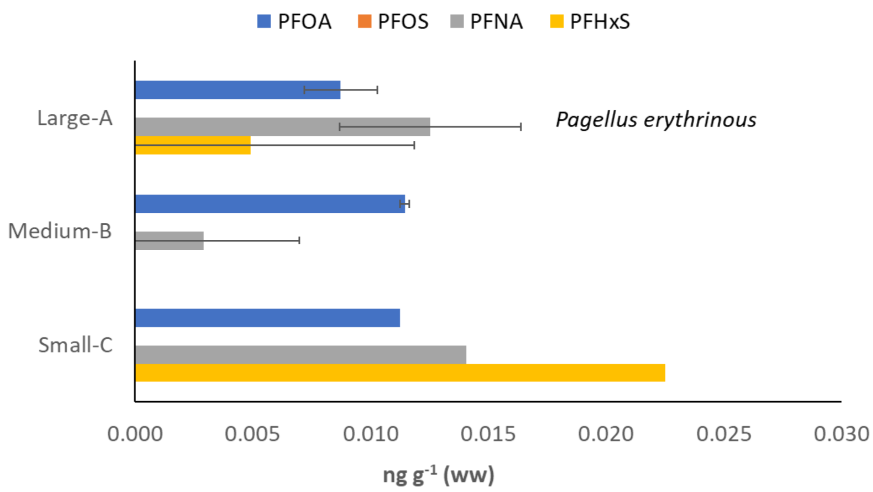
Area	*Matrix	PFOA	PFNA	PFOS	PFHxS	*Source*
Beibou Gulf, South China Sea	**Biota (ng g^{-1})** *Fish, Crus., Ceph.*	<LOD – 0.61 (0.15)	<LOD – 0.26 (0.10)	<LOD – 1.53 (0.56)	<LOD	*[54]*
	Waters (ng L^{-1})	0.36 ± 0.03	0.12 ± 0.02	0.18 ± 0.12	0.14 ± 0.02	
	Sediments (ng g^{-1})	0.035	0.035	0.066	<LOD	
Xiaoqing River Estuary Bohai Sea	**Biota (ng g^{-1})** *Fish, Crus., Moll.*	23.5 – 682 (154) (dw)	-	2.52 – 23.7 (6.07) (dw)	-	*[59]*
	Waters (ng L^{-1})	27599 ± 11367	11.99 ± 5.16	22.07 ± 6.6	5.92 ± 1.5	
	Sediments (ng g^{-1})	10.17 ± 5.8	0.01 ± 0.0	0.07 ± 0.02	<LOD	
South China Sea	**Biota (ng g^{-1})** *Fish, Crus., Moll.,*	<LOD – 11.64 (2.77)	<LOD – 4.89 (1.73)	<LOD – 16.24 (5.22)	<LOD – 6.00 (0.37)	*[55]*
	Waters (ng L^{-1})	1.55 – 9.47 (2.8)	<LOD – 0.75 (0.13)	<LOD – 0.23 (0.15)	0.03 – 0.37 (0.20)	
	Sediments (ng g^{-1})	<LOD – 0.93 (0.60)	<LOD – 0.26 (0.14)	0.01	<LOD- 0.06	
South China Sea	**Biota (ng g^{-1})** *Fish, Crus.*	0.03 – 4.07 (0.41)	<LOQ – 0.61 (0.10)	<LOQ – 23.1 (1.62)	-	*[56]*
	Waters (ng L^{-1})	0.28 – 25.2 (4.47)	0.06 – 0.43 (0.17)	<LOD – 2.39 (0.38)	0.02 – 0.19 (0.07)	
European marine waters	**Biota (ng g^{-1})** *Fish*	0.38 – 1.37	0.22 – 1.15	1.35 – 62.7	0.14 – 3.48	*[57]*
Aegean Sea, Mediterranean	**Biota (ng g^{-1})** *Fish, Crus., Moll.*	<LOD	0.60 ± 0.03	5.29	<LOD	*[58]*
Bizerte Lagoon, Mediterranean Sea	**Biota (ng g^{-1})** *Fish, Crus., Moll.,*	<LOD – 0.07 (0.018) (dw)	<LOD – 0.26 (0.069) (dw)	0.03 – 1.19 (307) (dw)	-	*[53]*
Ebro River estuary, Mediterranean Sea	**Biota (ng g^{-1})** Fish	<LOD – 0.46 (0.05)	<LOD – 0.95 (0.18)	<LOD – 6.2 (1.92)	-	*[52]*
	Waters (ng L^{-1})	<LOD – 8.7 (1.14)	<LOD – 3.3 (0.34)	<LOD – 4.3 (0.42)	<LOD – 0.67	
	Sediments	<LOD – 12.0	<LOD – 4.2	<LOD – 22.6	<LOD – 1.5	

Area	*Matrix	PFOA	PFNA	PFOS	PFHxS	*Source*
	(ng g^{-1})	(2.28)	(0.36)	(1.10)	(0.14)	
Saronikos Gulf, Mediterranean Sea	**Biota (ng g^{-1})** *Fish, Crus., Ceph*	<LOD – 0.09 (0.02)	<LOD – 0.35 (0.06)	<LOD – 1.03 (0.21)	<LOD – 0.12 (0.01)	*Present work*
		<LOD – 0.39 (0.11) (dw)	<LOQ – 1.46 (0.27) (dw)	<LOQ – 3.36 (0.97) (dw)	<LOD – 0.64 (0.05) (dw)	
	Waters (ng L^{-1})	<LOD	<LOD	0.735 – 4.35	<LOD	*[16]*
	Sediments (ng g^{-1})	1.19	<LOD	1.22 – 1.69	<LOD	

**All data on biota tissues based on freeze-dried samples*

3.2.2. Bioaccumulation potential of PFAS

The bioaccumulation potential of PFAS was investigated in two species, *Pagellus erythrinus* and *Merluccius merluccius*, by analysing PFAS concentrations across three size groups of individuals, as presented in Table 1. As shown in Figure 5, no increase in PFAS concentrations was observed with increasing fish size. In *Merluccius merluccius*, a reverse trend was noted for PFOA and PFNA, with higher concentrations detected in smaller individuals. Similarly, in *Pagellus erythrinus*, the highest concentrations were recorded in the smallest size group.



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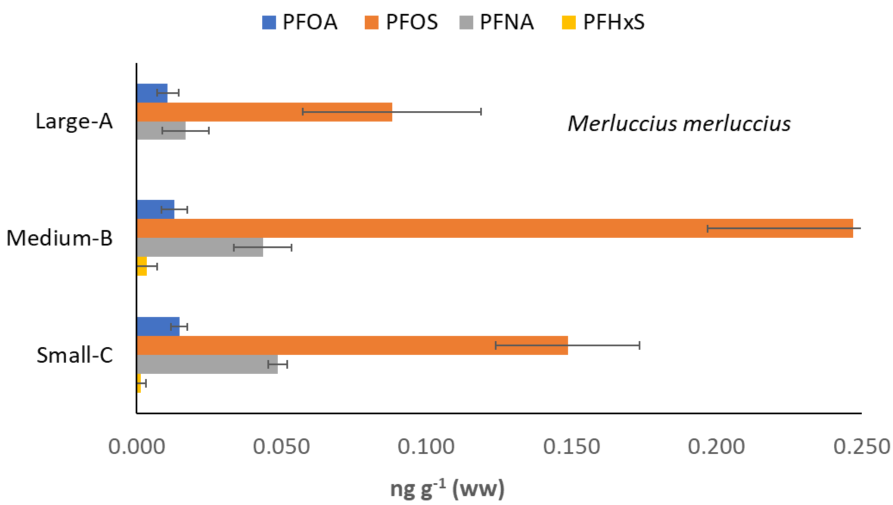


Figure 5: Distribution of PFAS compounds in the three sizes of (a) Pagellus erythrinous and (b) Merluccius merluccius.

(b)

To further assess the bioaccumulation potential of individual PFAS compounds and organisms, bioaccumulation factors (BAFs) were calculated. PFAS concentrations in the marine waters and sediments of the Saronikos Gulf and specifically at our study area, were measured by Lougkovois et al. (2025)[16], in June 2023, 4 months before our biota sampling. In line with our results, only PFOS was detected in seawater at concentrations ranging from 0.735 to 4.35 ngL^{-1} mean 1.52 ngL^{-1}. PFOA, PFNA and PFHxS were below the detection limit i.e. <0.164 ngL^{-1}; <0.319 ngL^{-1}; <0.0461 ngL^{-1} respectively. In sediments PFOS fluctuated between 1.22 to 1.69 μg/kg dw, PFOA between <LOD. to 1.19 μg kg-1 dw, while PFNA and PFHxS were not detected.

Figure 6 illustrates the distribution of BAF values, calculated based on the average value of PFOS detected in seawater (1.52 ng L^{-1}). The highest BAF -PFOS values ($2.5 < \log BAF < 3$) were observed in *Sardina pilchardus*, *Auxis rocheii* and *Parapenaeus Longirostris*. For the rest of PFAS compounds, for which concentrations were found <LOD, the BAFs were calculated using the LOD/2 value.

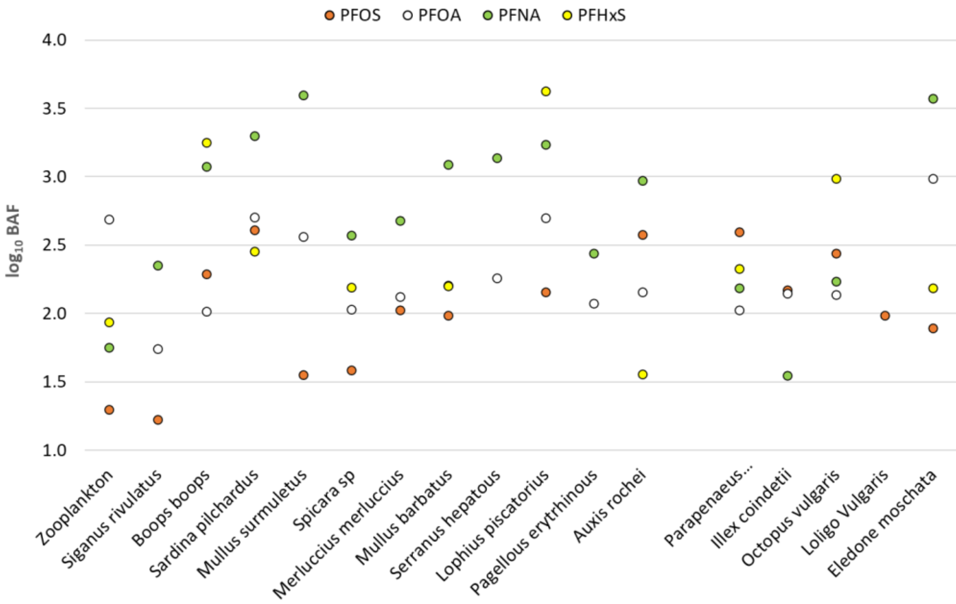
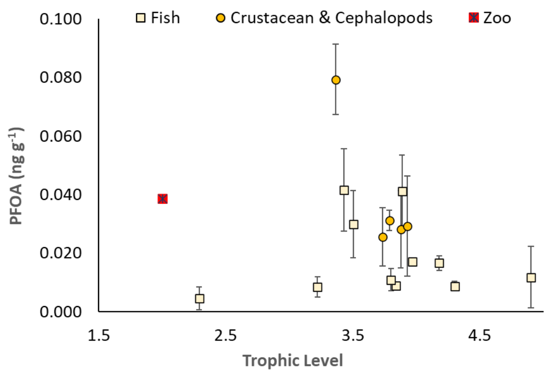


Figure 6: Bioaccumulation factors (BAF) of PFAS compounds in the investigated marine organisms. For PFOA, PFNA and PFHxS, the respective seawater concentrations were found <LOD and BAFs are calculated based on the LOD/2 value.

Despite its low occurrence in both seawater and biota PFNA seems to have a high tendency to bioaccumulate showing BAFs> 3, in fish species *Boops, boops, Sardina pilchardus, Mullus surmuletus, Mullus barbatus, Serranus hepatous, Lophius piscatorius, Auxis Rocheii* and in cephalopod *Eledone moschata*.

3.2.3. *Trophic transfer of PFAS in the Saronikos Gulf*

In Figure 7**¡Error! No se encuentra el origen de la referencia.**, PFAS concentrations in fish, crustacean & cephalopods muscle/mantle tissues are plotted against the trophic level of each species. Despite that *Siganus rivulatus* at TL 2.3 retains the lowest concentrations, it becomes apparent that for all species with TL>3 concentrations of all PFAS compounds follow a diminishing trend with increasing trophic level.



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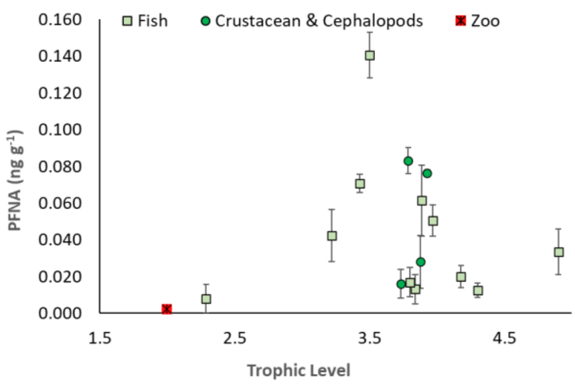
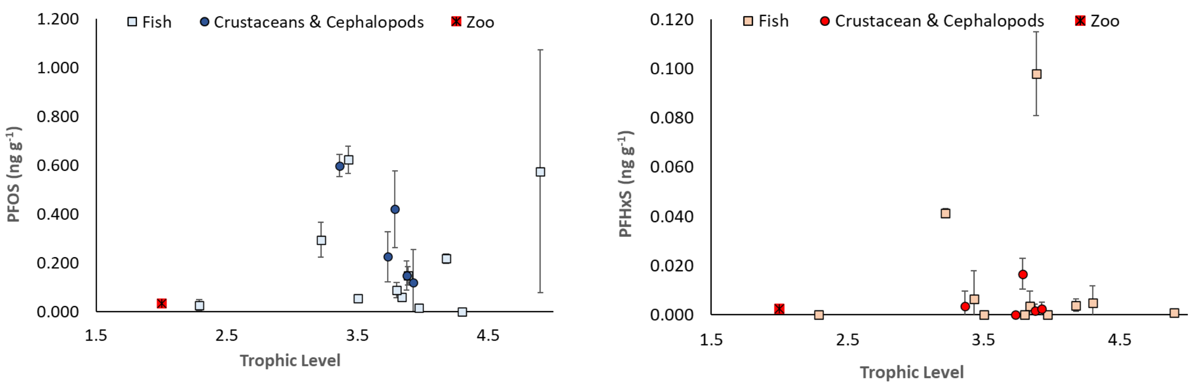


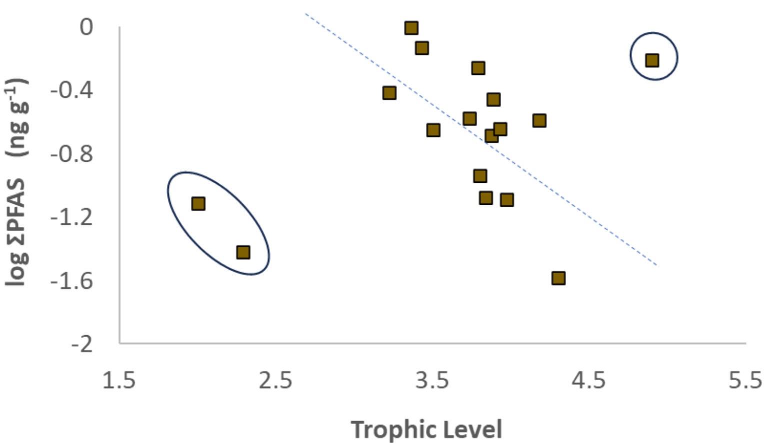
(b)



(c) (d)

Figure 7: Concentrations (ng g-1) (ww) of (a) PFOA, (b) PFNA, (c) PFOS and (d) PFHxS against the trophic level of the studied organisms.

Similarly, TMF values of ΣPFAS, calculated as the antilog of the slope of the log (ΣPFAS) versus trophic level (TL) regression line (Figure 8), were found to be less than 1 for both fish (TMF = 0.7) and cephalopods (TMF = 0.07), supporting the occurrence of PFAS biodilution within the Saronikos Gulf marine food web.



y=-0.9509x + 2.9377

R^2 = 0.48, p =0.000

TMF = 0.1

With the exception of zooplankton Siganus rivulatus and Auxis Rocheii data

Figure 8: The regression of logΣPFAS vs TL shows a clear bio-dilution pattern with TMF <1.

Biodilution of contaminants in marine food webs is often associated with systems dominated by planktivores or organisms characterized by fast metabolism and low lipid content. In such cases, species feeding directly on plankton or detritus may exhibit higher contaminant levels than their predators due to greater direct exposure from the surrounding water, resulting in apparent biodilution along the food web. For instance, biodilution has been observed in Mediterranean food webs for various metals [35]. For PFAS compounds Li et al. (2024)[59] report biodilution of PFMOAA in an estuarine food web (Xiaoqing River estuary) and they attribute this pattern to the sedimentary abundance of this compound, directly affecting benthic feeding organisms. In the present study, most of the investigated species are demersal exhibiting mixed feeding strategies typical of coastal benthic ecosystems. Consequently, direct ingestion of sedimentary detritus with relatively elevated PFAS concentrations may explain higher PFAS accumulation in these organisms, potentially exceeding levels found in their predators. Accordingly, Cara et al. (2022)[60]found no clear signs of PFAS biomagnification or biodilution in fish and crustaceans from the Belgian North Sea, suggesting that bioconcentration from the environment was most likely dominating over biomagnification in the studied species. However, they noted that the trophic range studied was too restricted to draw solid conclusions on the trophic transfer of PFAS, which also true for our case.

3.3 THg in the marine food web of theSaronikos Gulf

3.3.1. THg concentration in marine organisms

Total Hg (THg) concentrations (μg g^{-1}) determined in the muscle/mantle tissues of the organisms investigated are shown in Table 8. THg ranged from as low as 0.005 μg g^{-1} (ww) in *Siganus rivulatus* to 0.567 μg g^{-1} (ww) in *Mullus barbatus*. Elevated THg concentrations (> 0.200 μg g^{-1} (ww)) were recorded in the long-lived predatory fish species *A. rochei, S. hepatus*, *L. piscatorius* and *P. erythrinus* followed by *M. barbatus*. The lowest THg concentrations were observed in the fish *S. rivulatus*. Detailed data are given in Table A1 in Annex.

The muscle is both the final target tissue of the metabolism and the main edible part of the fish, and the estimation of metal concentrations in this tissue is mandatory not only for understanding bioaccumulation/biomagnification patterns but also for the protection of public health. The liver, on the other hand, is the main organ for nutrient metabolism while the gills are the organ in direct contact with seawater and the primary site of metal uptake into the body from the soluble phase.

Especially for two fish species (*P. erythrinus* and *M. merluccius*) in addition to the muscle, the gills and liver were dissected and analyzed for their THg content (Table 8). In both species, the gills are the organ with the lower THg concentrations.

Table 7: THg (µg g-1) concentrations in muscle/mantle tissues of marine organisms.

Species name		THg µg g-1 ww	THg µg g-1 dw
*Zooplankton** *n=7*	mean ± SD range	0.005 ± 0.001 0.004 - 0.006	0.033 ± 0.005
Fish			
Siganus rivulatus *n = 4*	mean ± SD range	0.011 ± 0.008 0.005 - 0.021	0.040 ± 0.020
Boops boops *n = 4*	mean ± SD range	0.076 ± 0.018 0.059 - 0.101	0.320 ± 0.099
Sardina Pilchardus *n = 4*	mean ± SD range	0.022 ± 0.006 0.015 - 0.029	0.101± 0.020
Mullus surmuletus *n = 4*	mean ± SD range	0.126 ± 0.015 0.112 - 0.143	0.529 ± 0.088
Spicara sp *n = 4*	mean ± SD range	0.189 ± 0.066 0.130 - 0.261	0.672 ± 0.183

Species name		THg µg g-1 ww	THg µg g-1 dw
Merluccius merluccius *Size A* *n = 4*	mean ± SD range	0.145 ± 0.032 0.113 - 0.179	0.398 ± 0.158
Merluccius merluccius *Size B* *n = 3*	mean ± SD range	0.088 ± 0.038 0.055 - 0.129	0.346 ± 0.190
Merluccius merluccius *Size C* *n = 4*	mean ± SD range	0.063 ± 0.035 *0.035 - 0.112*	0.781 ± 0.273
Mullus barbatus *n= 4*	mean ± SD range	0.292 ± 0.193 0.145 - 0.567	1.456 ± 1.024
Serranus hepatous *n = 4*	mean ± SD range	0.335 ± 0.083 0.248 - 0.441	1.538 ± 0.384
Lophius piscatorius *n = 3*	mean ± SD range	0.320 ± 0.035 0.282 - 0.349	1.726 ± 0.232
Pagellous erytrhinous *Size A* *n =2*	mean ± SD range	0.379 - 0.476	1.836 ± 0.169
Pagellous erytrhinous *Size B* *n = 2*	mean ± SD range	0.312 - 0.313	1.327 ± 0.014
Pagellous erytrhinous *Size C* *n= 1*	mean ± SD range	0.128	0.522
Auxis rochei *n = 4*	mean ± SD range	0.325 ± 0.090 0.219-0.427	1.110 ± 0.282
Crustacean & Cephalopods			
Parapenaeus longirostris *n = 4*	mean ± SD range	0.057 ± 0.008 0.050 - 0.065	0.393 ± 0.043
Illex coindetii *n = 4*	mean ± SD range	0.123 ± 0.040 0.085 - 0.168	0.563 ± 0.202
Octopus vulgaris *n = 4*	mean ± SD range	0.123 ± 0.040 0.085 - 0.168	0.513 ± 0.379
Loligo Vulgaris *n = 4*	mean ± SD range	0.088 ± 0.030 0.045 - 0.111	0.340 ± 0.111
Eledone moschata *n = 4*	mean ± SD range	0.113 ± 0.090 0.032 - 0.192	0.579 ±0.420

Table 8: THg concentrations (μg g-1 ww) in liver and gills of P. erythrinus and M. merluccius.

Species name		**THg** $\mu g\, g^{-1}$ ww	**THg** $\mu g\, g^{-1}$ ww
		liver	*gills*
P. erythrinus			
size A	range	0.616 - 1.069 *n* = 2	0.166 - 0.180 *n* = 2
size B	range	0.398 - 0.484 *n* = 2	0.093 - 0.093 *n*=2
size C	range	0.232 *n* = 1	0.055 *n* = 1
M. merluccius			
size A	Mean ± SD range	0.051 ± 0.012 0.040 -0 .067 *n* = 4	0.024 - 0.070 n = 2
size B	Mean ± SD range	0.032 ± 0.007 0.028 - 0.042 *n* = 4	0.029 - 0.038 n = 2

As shown in Table 9, for several species the data presented in this work are comparable to those from fish collected from the Italian fishing areas (FAO 37) including the Adriatic and Tyrrhenian Sea during 2009 – 2011[70] . For *Boops boops*, *Merluccius, merluccius* and *Sardina pilchardus* one order of magnitude lower THg concentrations were recorded in Saronikos Gulf than in the other areas.

According to the European Regulation (EU) 2023/915 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006, the permitted maximum level of THg in crustaceans and muscle meat of fish should not exceed 0.5 μg g^{-1} ww, except in *Pagellus erythrinus, Mullus barbatus* and *Mullus surmuletus* where the level is 1.0 μg g^{-1} ww, and except cephalopods and *Sardina pilchardus* where the limit is 0.3 μg g^{-1}. The concentrations of THg in fish muscle, crustaceans and cephalopods from Saronikos Gulf were found to be below these permitted levels.

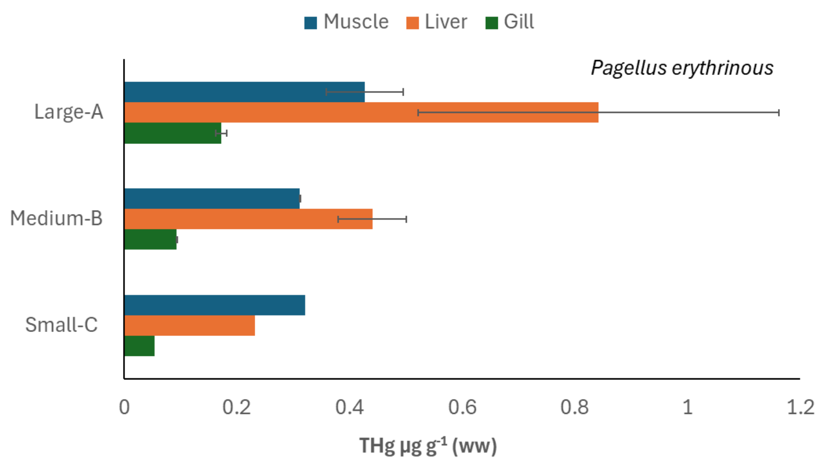
Table 9: THg (μg g-1) (ww) concentrations in fish collected from fishing grounds of the Adriatic, Tyrrhenian Seas and the Saronikos Gulf.

Fish species	**Italian fishing grounds [70]**	**Saronikos Gulf this study**
	THg (μg g^{-1}) (ww)	THg (μg g^{-1}) (ww)
Auxis Rocheii	0.230 ± 0.066	0.325 ± 0.090
Boops sp.	0.125 ± 0.027	0.076 ± 0.018
Lophius piscatorius	0.100 ± 0.000	0.320 ± 0.035
Merluccius merluccius	0.132 ± 0.057	0.092 ± 0.052

Fish species	Italian fishing grounds [70]	Saronikos Gulf this study
Mullus barbatus	0.235 ± 0.055	0.292 ± 0.193
Mullus surmuletus	0.129 ± 0.079	0.126 ± 0.015
Pagellus erythrinus	0.990 ± 0.470	0.321 ± 0.127
Sardina pilchardus	0.190 ± 0.028	0.022 ± 0.006
Spicara sp.	0.154 ± 0.060	0.189 ± 0.066

3.3.2. *Bioaccumulation potential of THg*

The bioaccumulation potential of THg was investigated in two species, *Pagellus erythrinus* and *Merluccius merluccius*, by analysing THg concentrations across three size groups of individuals, as presented in Table 1. As shown in Figure 9, there is a gradual increase in THg concentrations with increasing size of *Pagellus erythrinous* for all 3 types of tissues analysed (muscle, liver, gills), with liver showing the highest accumulation in the large and medium sizes. For *Merluccius merluccius,* there was a clear trend of increasing THg concentrations in muscle tissue with increasing fish size. Similarly, THg increased from the medium to the large size in the liver and gills. The small size of *M. merluccius* however, appears relatively enriched in THg in both the muscle and liver (gills sample was not analysed). Statistical differences among tissues are given in Figure A1 in Annex.



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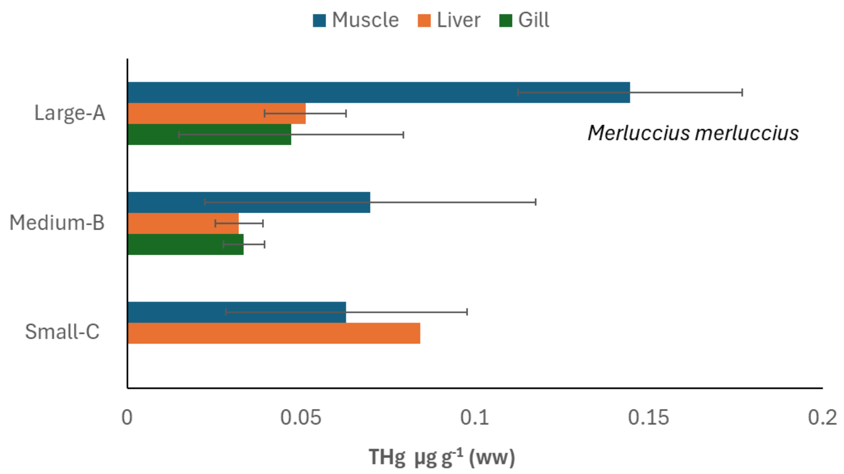


(b)

Figure 9: Distribution of THg (μg g-1) (ww) in the three sizes and three tissue types of (a) Pagellus erythrinous and (b) Merluccius merluccius.

Elevated concentrations of metals in fish liver compared to other fish tissues is a well -documented pattern related to the liver's role in metal storage and detoxification [71]. Surprisingly this pattern is obvious only for large and medium sizes of *Pagellus erythrinous* and not for *Merluccius merluccius* in our study area. For the latter, only in the smallest size the liver showed elevated THg content. It is possible that age differences affect metal metabolism differently.

To further assess the bioaccumulation potential of THg in organisms, the bioaccumulation factor (BAF) was calculated. THg concentrations in the marine waters of the Saronikos Gulf and specifically at our study area, are monitored within the framework of the National monitoring programme for the implementation of the WFD (2000/60/EC). Based on data from the period 2018-2023, average THg concentration for seawater was found 0.04 ± 0.01 ngL^{-1}, (n=33). THg BAF was calculated accordingly, and the resulting values for all species investigated, are shown in Figure 9.

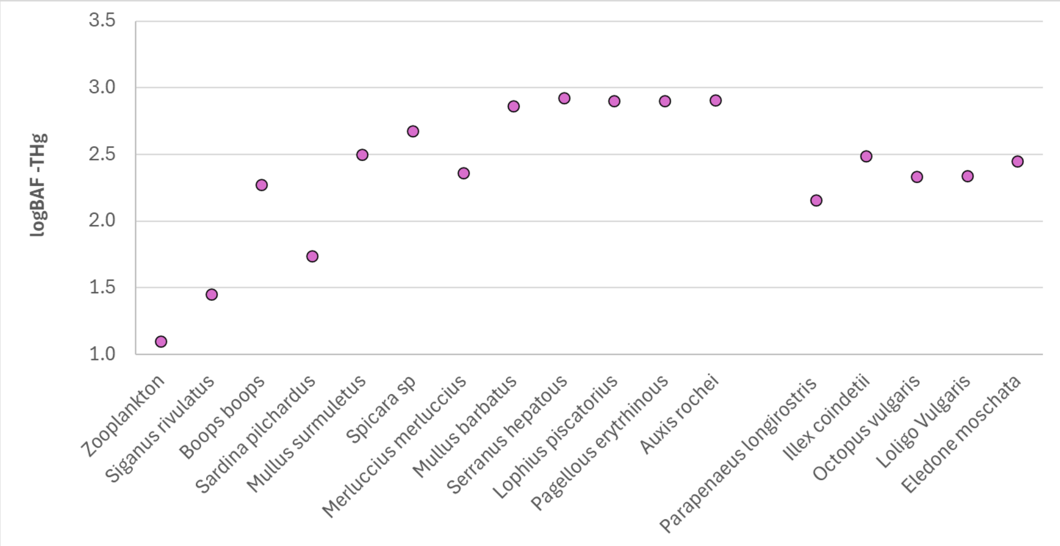


Figure 10: Bioaccumulation factors (BAF) of THg in the investigated marine organisms.

For most species logBAFs were found >2.0, except for zooplankton, *S. rivulatus* and *S. Pilchardus*. The highest BAF -THg values (log BAF ~ 3) were observed in *Mullus barbatus, Serranus hepatous, Lophius piscatorius, Pagellous erythrinous and Auxis rocheii*, implying that THg tends to accumulate in the muscle tissue of these species.

BAF calculations are highly dependent on exposure concentrations, often resulting in lower values under conditions of high seawater contamination. Additionally, BAFs are sensitive to the analytical accuracy of contaminant measurements in seawater. Some studies report contaminant concentrations on a wet weight (ww) basis, while others use a dry weight (dw) basis—the latter typically yielding higher BAFs. Due to these variations, comparisons among studies are often challenging.

3.3.3. Trophic transfer of THg in the Saronikos Gulf

To investigate trophic transfer of THg within the food web studied, the regression of LogTHg (μg g^{-1}) against species TL was drawn (Figure 11). The strong positive relationship implies that THg is effectively transferred from zooplanktonic organisms to higher trophic level in the Saronikos Gulf, with a TMF value equal to 5.

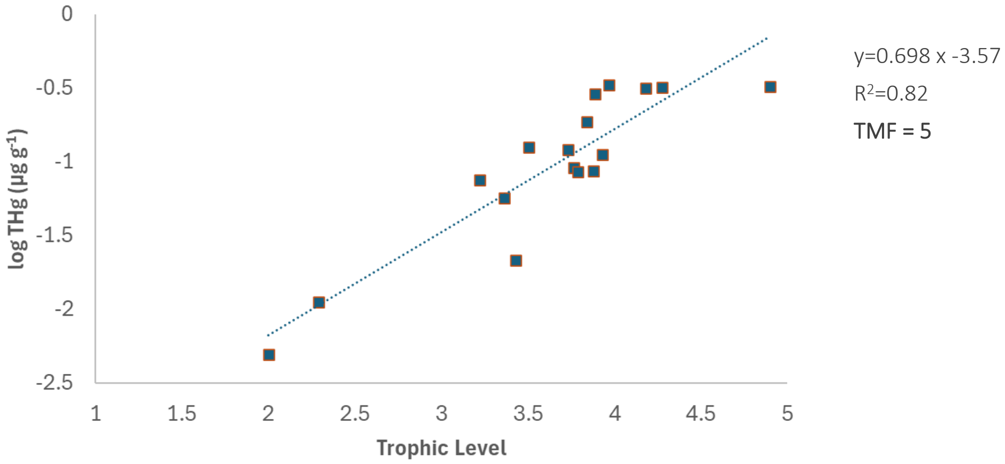


Figure 11: Log-transformed THg (μg g-1) (ww) concentrations in the Saronikos Gulf studied species vs corresponding trophic level.

Bioaccumulation and biomagnification of Hg in marine organisms is related to its methylated form, which is produced by bacterial action during organic matter mineralization. In the marine environment, Hg methylation occurs both in coastal and shelf sediments, and in sub-thermocline low-oxygen oceanic waters, at depths where organic matter is intensively remineralized. Increased mercury bioaccumulation in the Mediterranean trophic webs as opposed to other marine areas with similar Hg concentrations in seawater and sediments has been attributed to a more effective bacterial methylation of inorganic forms of Hg. Chouvelon et al. (2018)[71] have compared Hg bioaccumulation in a NW Mediterranean and a NE Atlantic trophic web and have shown a significantly higher Hg bioaccumulation by Mediterranean organisms, which held significantly lower δ^{13}C‰ and δ^{15}N‰ signatures, and lower individual sizes for most of them. Similarly, Tesan-Onrubia et al. (2023) [72] have focused on Hg biomagnification in Mediterranean planktonic trophic webs and have documented effective biomagnification of Methylmercury (MeHg) from phyto- to zooplanktonic microorganisms. Several factors are considered responsible for the high bioaccumulation and biomagnification of Hg in the oligotrophic Mediterranean Sea. Bacterial methylation of mercury (MeHg) appears to be more active due to the higher proportion of slow-sinking pico- and nanoplankton in the water column, which increases exposure time to bacteria, as well as the lower growth rates of organisms, which result to lower excretion rates of MeHg. Moreover, in benthic dominated trophic webs such as the one studied in the Saronikos Gulf, Hg methylation is more prevalent, due to local anoxic conditions and sediment resuspension which may release methylated forms of Hg.

Published TMFs for Hg ([73] and references therein) from various areas in the world, show high variability ranging from 1.1 to 13.9. Several factors such as the structure of the trophic web studied, geographical differences as well as the amount of Hg entering the base of the food web[61] are responsible for the variation observed in THg TMFs. In the present study TMF value indicates that THg increases 5 times per trophic level which is consistent with the global average for marine sites 6.2 ± 4.1 as reported by [74].

3.4 Other metals in the marine food web of the Saronikos Gulf

3.4.1. Concentration of metals in marine organisms

The concentrations of the 15 metals and other elements (μg kg^{-1}) (ww) in marine organisms from the Saronikos Gulf, are shown in Table 10. The higher concentrations of metals in fish muscle, liver, and gills were found for Zn and As, followed by Cu, Se, and the lower for U, Cd (except liver), Cs (except muscle), and intermediate values for Ni, Cs, Ba, Pb, Li. In fish stomach content, the higher concentrations were found for Ba, Zn, Cu, As and the lower for U, Cs. For crustaceans and cephalopods, the higher concentrations were found for Se and Pb and the lower for Ba, As, V.

Mean concentrations of Cr, Ni, Cu, Zn, As (1%), Se, Cd, Ba, and Pb in muscle were well below Safety Standards established for all organisms from Saronikos Gulf. Similarly, the Estimated Daily Intake (EDI) of Li, V, Cr, Co, Ni, Cu, Zn, Se, Cd, Ba, Pb, and U was found in all cases lower than the established Reference Doses (RfD) (USEPA, 2017) (Table 11). Though, the EDI of As (1%) in Eledone moschata was 0.51 μg/kg bw/d above the established limit of 0.30 μg/kg bw/d. It should be noted that for risk assessment estimations only the potentially toxic, inorganic form of As was considered and assumed to be a content of 1% of the total As. However, the real inorganic As form may be even less than 1% as has been previously found for other fish species [44] thus the EDI could be lower for As. Speciation analysis of As is recommended to conclude for its toxicity.

Table 10: Mean concentrations of metals and other elements (μg/kg ww) in the marine food web of the Saronikos Gulf. MU: muscle; LI: liver; GI: gills; ST: stomach content; SA: size A; SB: size B; SC: size C; WW: wet weight; DW: dry weight; BDL: below detection limit

Species name	Tissue	Li	V	Cr	Co	Ni	Cu	Zn	As	Se	Rb	Cd	Cs	Ba	Pb	U
								μg/kg ww								
zooplankton		325.7	1054.0	1287.3	226.5	838.1	4347.8	24178.3	4719.1	1073.4	696.6	166.5	4.8	2199.7	292.2	48.9
							Fish									
Siganus rivulatus	MU	9.5	8.2	96.3	1.6	**BDL**	120.9	1194.8	2555.3	135.3	465.2	**BDL**	9.7	**BDL**	**BDL**	**BDL**
Auxis rochei	MU	5.2	13.7	134.8	3.5	**BDL**	633.8	4870.0	1398.3	385.4	695.2	2.6	31.6	19.4	**BDL**	**BDL**
Boops boops	MU	41.4	38.9	132.7	4.8	12.1	348.0	4885.3	6601.8	506.8	704.8	**BDL**	13.4	48.2	**BDL**	**BDL**
	LI	221.7	682.8	384.6	280.0	164.0	4598.5	32241.6	9979.4	4159.7	532.3	611.7	21.0	141.3	184.2	8.0
	GI	678.6	1032.1	180.9	73.8	982.9	631.2	19693.2	4803.0	2786.9	451.9	9.8	7.2	1957.5	541.1	46.4
	ST	998.4	8722.2	3971.1	565.1	1454.4	8515.2	11114.0	11213.9	3072.8	1328.9	260.7	280.7	103515.0	715.9	158.0
Sardina pilchardus	MU	43.1	93.5	102.0	7.5	36.9	698.1	10024.5	7887.5	718.7	595.8	0.9	15.8	**BDL**	**BDL**	**BDL**
Mullus Surmuletus	MU	18.8	35.8	142.9	4.7	35.7	574.8	2881.7	8188.7	318.0	866.7	0.2	36.1	**BDL**	7.0	**BDL**
	LI	37.4	177.6	247.9	185.8	95.9	3003.1	29607.2	10419.9	2879.3	1137.6	101.9	49.0	**BDL**	158.4	**BDL**
	GI	190.6	611.0	343.2	74.9	819.2	1075.0	15608.4	7958.8	1962.2	803.5	3.9	34.7	1205.0	509.2	21.7
	ST	609.7	2277.0	1901.2	429.2	3116.1	2322.7	23397.8	4778.6	1408.1	1333.5	56.9	87.6	3966.2	2084.7	183.1
Spicara sp	MU	53.0	35.8	111.8	4.5	29.7	258.1	4093.4	8438.2	569.4	705.7	0.3	12.0	34.5	1.8	**BDL**
	LI	56.8	249.3	169.2	101.9	86.0	3758.0	31154.9	9532.1	2608.1	486.6	234.1	3.7	49.9	173.1	3.6
	GI	484.6	805.8	167.6	78.3	966.3	615.7	24425.2	10792.6	2277.3	433.1	7.8	5.5	4083.9	472.0	28.9
	ST	755.4	2609.4	3556.8	287.5	3417.0	4659.9	53980.6	11045.1	1530.7	1756.1	150.5	27.0	18846.2	1441.1	123.2
Merluccius merluccius-SA	MU	12.1	18.7	79.6	**BDL**	7.1	94.9	1836.0	4148.0	272.0	689.1	**BDL**	21.8	**BDL**	4.9	**BDL**
	LI	8.7	29.1	167.7	11.3	14.6	1797.9	13246.9	1342.8	316.8	186.8	7.4	3.6	52.5	25.4	**BDL**
	GI	13.9	21.3	39.7	2.3	21.5	206.7	4710.1	610.6	334.0	128.9	0.4	3.1	20.0	8.7	0.4

Species name	Tissue	Li	V	Cr	Co	Ni	Cu	Zn	As	Se	Rb	Cd	Cs	Ba	Pb	U
								μg/kg ww								
Merluccius merluccius-SB	MU	9.8	15.9	77.6	**BDL**	2.8	107.5	1812.0	4040.5	252.4	689.0	**BDL**	20.7	**BDL**	**BDL**	**BDL**
	LI	8.1	22.4	208.1	10.5	5.9	1531.2	12355.2	1292.4	399.8	166.4	10.2	3.1	**BDL**	5.8	**BDL**
	GI	16.8	19.8	41.1	2.4	20.5	173.1	4176.4	603.0	339.3	144.8	0.6	3.8	20.7	6.2	0.4
Merluccius merluccius-SC	MU	9.4	12.1	45.0	0.2	6.4	52.9	1238.3	3375.9	129.0	411.9	**BDL**	13.6	**BDL**	**BDL**	**BDL**
	LI	7.1	20.0	51.8	8.9	8.9	711.9	4417.3	846.8	193.3	99.7	10.8	2.2	1.8	4.6	**BDL**
	GI	7.4	14.9	29.1	1.0	14.6	80.5	1758.9	393.7	122.2	63.5	0.3	1.5	8.7	1.7	0.1
Mullus Barbatus	MU	12.1	23.9	100.4	5.4	42.2	175.7	2006.4	14923.4	293.5	767.3	**BDL**	10.6	**BDL**	18.7	**BDL**
Serranus hepatous	MU	37.9	50.8	86.9	4.0	30.2	166.3	2348.6	10218.9	399.9	620.6	**BDL**	19.9	**BDL**	**BDL**	**BDL**
	LI	38.0	143.1	134.9	56.8	111.8	1670.2	17649.4	11788.1	1316.4	448.7	152.9	10.4	31.4	67.6	0.9
	GI	276.7	409.7	136.0	88.0	1071.3	635.3	18288.5	11806.9	899.3	464.7	8.5	12.0	805.0	209.2	18.1
	ST	177.4	454.8	796.0	119.5	1590.7	14769.3	21760.7	8203.0	493.7	552.1	73.0	30.0	3571.6	589.8	24.5
Lophius piscatorius	MU	21.7	28.4	81.0	1.3	4.2	142.9	2736.1	14152.6	651.9	974.9	**BDL**	14.7	**BDL**	**BDL**	**BDL**
	LI	24.3	101.5	328.6	114.3	73.4	14845.2	27853.9	3639.3	1382.3	619.2	96.6	5.9	**BDL**	110.7	0.7
	GI	154.3	118.3	56.9	30.3	368.1	369.2	15475.6	2531.5	492.3	359.0	3.7	4.2	179.7	39.7	7.4
Pagellous erytrhinous-SA	MU	15.5	29.9	129.1	2.1	15.6	232.5	3291.0	5206.8	441.6	889.9	0.2	18.4	**BDL**	5.8	**BDL**
	LI	31.5	785.7	145.8	149.5	236.1	4300.9	33641.0	7481.2	2725.6	830.5	782.8	13.6	23.3	390.1	10.1
	GI	282.1	878.2	180.9	87.4	1173.0	555.9	17243.8	3777.3	1149.6	522.3	9.1	9.6	2777.5	958.1	47.2
	ST	738.6	1715.6	4037.5	395.3	3061.0	3074.7	39421.0	6850.1	1073.2	1801.1	302.4	116.0	3838.6	2134.3	114.7
Pagellous erytrhinous-SB	MU	20.8	34.7	134.1	2.6	**BDL**	226.2	2557.8	4780.0	398.9	796.5	0.3	17.3	13.8	6.6	**BDL**
	LI	32.1	340.0	125.3	153.1	144.2	4145.9	31019.0	8419.5	2347.1	731.8	274.0	11.6	13.2	624.1	4.3
	GI	253.7	744.5	182.0	84.8	1141.0	621.0	18459.9	4524.5	1326.8	558.7	7.7	10.2	2110.8	1128.7	34.5
	ST	595.5	1527.7	2303.9	280.3	2315.4	3467.3	32356.7	7554.3	1143.0	1278.8	139.2	71.7	2752.7	1515.4	105.6
Pagellous erytrhinous-SC	MU	26.1	35.7	97.1	2.9	**BDL**	170.4	2683.6	5416.0	339.4	761.3	**BDL**	15.3	**BDL**	1.7	**BDL**
	LI	34.8	110.0	114.8	75.2	90.3	2966.4	40322.8	7445.1	1738.8	742.1	103.1	10.9	**BDL**	316.0	1.2
	GI	234.8	419.8	177.3	78.4	972.7	683.6	19380.7	4334.3	1147.5	645.0	8.2	10.9	1715.0	769.7	25.3
	ST	281.7	604.9	1022.7	175.8	1155.5	4468.6	52073.5	9597.4	946.2	1135.0	427.9	39.6	1079.4	973.8	36.6
								Crustacean & Cephalopods								
Prapenaeus longirostris	MU	36.3	50.4	59.9	3.0	28.5	2495.0	6205.3	18096.4	324.9	538.9	1.4	9.2	22.8	**BDL**	**BDL**
Illex coindetii	MU	65.8	106.1	125.4	9.7	45.7	6246.5	13802.2	22544.0	659.5	1601.9	29.2	5.7	46.6	12.9	2.0
Octopus vulgaris	MU	80.4	126.7	101.0	17.3	199.2	4615.5	11396.8	27741.7	491.5	916.7	5.7	2.6	27.4	52.2	2.5
Loligo Vulgaris	MU	56.1	80.5	104.0	3.8	**BDL**	1821.9	11103.7	15915.6	698.8	1374.0	8.8	4.7	24.2	8.3	0.7
Eledone moschata	MU	102.8	128.0	109.4	16.5	161.0	11317.8	17626.5	52466.6	534.3	990.9	4.0	2.8	469.5	33.0	**BDL**

Table 11: Estimated Daily Intake (EDI) of metal concentrations in the muscle of marine organisms from Saronikos Gulf. For risk estimations of As, only for the potentially toxic, inorganic form was used As which was assumed to be 1% of total As.

Species	Li	V	Cr	Co	Ni	Cu	Zn	As (1%)	Se	Cd	Ba	Pb	U
Spicara sp	0.05	0.04	0.11	0.00	0.03	0.25	4.02	0.08	0.56	0.00	0.03	0.00	0.00
Mullus Barbatus	0.01	0.02	0.10	0.01	0.04	0.17	1.97	0.15	0.29	0.00	0.01	0.02	0.00
Pagellous erytrhinous-A	0.02	0.03	0.13	0.00	0.02	0.23	3.23	0.05	0.43	0.00	0.00	0.01	0.00
Pagellous erytrhinous-B	0.02	0.03	0.13	0.00	0.01	0.22	2.51	0.05	0.39	0.00	0.01	0.01	0.00
Pagellous erytrhinous-C	0.03	0.04	0.10	0.00	0.01	0.17	2.63	0.05	0.33	0.00	0.00	0.00	0.00
Boops boops	0.04	0.04	0.13	0.00	0.01	0.34	4.79	0.06	0.50	0.00	0.05	0.00	0.00
Mullus Surmuletus	0.02	0.04	0.14	0.00	0.04	0.56	2.83	0.08	0.31	0.00	0.02	0.01	0.00
Serranus hepatus	0.04	0.05	0.09	0.00	0.03	0.16	2.30	0.10	0.39	0.00	0.01	0.00	0.00
Lophius piscatorius	0.02	0.03	0.08	0.00	0.00	0.14	2.68	0.14	0.64	0.00	0.00	0.00	0.00
Merluccius merluccius-A	0.01	0.02	0.08	0.00	0.01	0.09	1.80	0.04	0.27	0.00	0.00	0.00	0.00
Merluccius merluccius-B	0.01	0.02	0.08	0.00	0.00	0.11	1.78	0.04	0.25	0.00	0.00	0.00	0.00
Merluccius merluccius-C	0.01	0.01	0.04	0.00	0.01	0.05	1.21	0.03	0.13	0.00	0.00	0.00	0.00
Sardina pilchardus	0.04	0.09	0.10	0.01	0.04	0.68	9.84	0.08	0.71	0.00	0.02	0.00	0.00
Siganus rivulatus	0.01	0.01	0.09	0.00	0.01	0.12	1.17	0.03	0.13	0.00	0.00	0.00	0.00
Prapenaeus longirostris	0.04	0.05	0.06	0.00	0.03	2.45	6.09	0.18	0.32	0.00	0.02	0.00	0.00
Loligo Vulgaris	0.06	0.08	0.10	0.00	0.01	1.79	10.89	0.16	0.69	0.01	0.02	0.01	0.00
Illex coindetii	0.06	0.10	0.12	0.01	0.04	6.13	13.54	0.22	0.65	0.03	0.05	0.01	0.00
Eledone moschata	0.10	0.13	0.11	0.02	0.16	11.10	17.29	0.51	0.52	0.00	0.46	0.03	0.00
Octopus vulgaris	0.08	0.12	0.10	0.02	0.20	4.53	11.18	0.27	0.48	0.01	0.03	0.05	0.00
Auxis rochei	0.01	0.01	0.13	0.00	0.01	0.62	4.78	0.01	0.38	0.00	0.02	0.00	0.00
Rfd (μg/kg/d)	***2***	***5***	***3***	***0.3***	***20***	***40***	***300***	***0.3***	***5***	***1***	***200***	***3.57***	***3***

Metals' distribution in the tissues of the examined fish species is depicted in nMDS ordination plot (Figure 12 (a)). In this plot, the closest points to each other have similar values in metal content whereas points further apart correspond to very different values. As indicated by nMDS ordination (Figure 12 (a)), the samples when analysed in terms of metal composition form clusters according to tissues rather than according to species. The liver was grouped to the bottom and right of the ordination plot whereas the muscle was grouped to the top and right. The gills were grouped on the top and middle of the ordination plot. The stomach content was grouped on the left of the ordination plot close to zooplankton. These observations are further supported by the analysis of similarity (ANOSIM) (Table A2 in Annex), whereby strong tissue differentiation was indicated through statistically significant differences ($p < 0.005$) between samples of all fish tissues studied with R values ranging from 0.322 to 1.000.

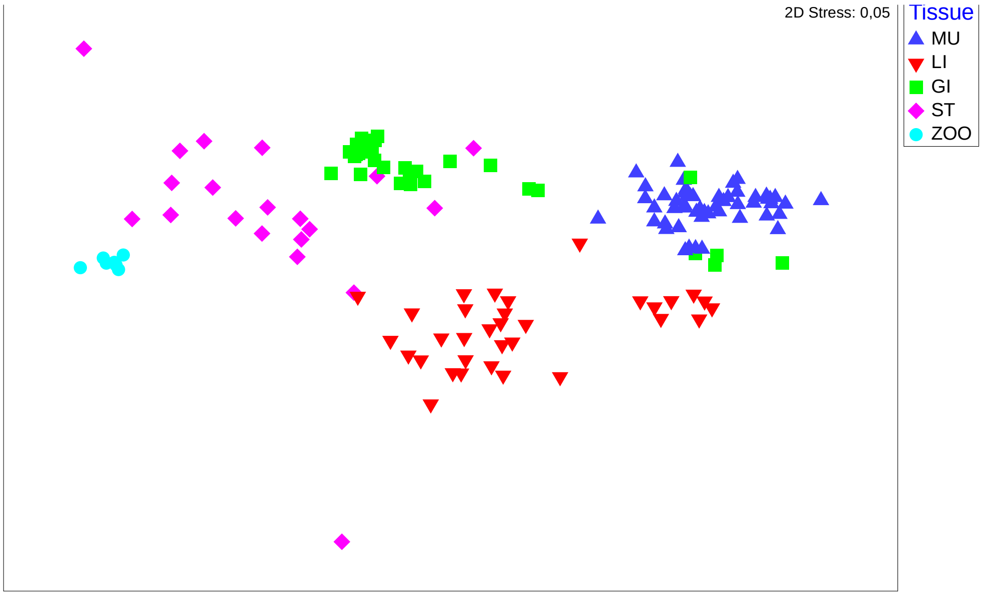
Based on Kruskal-Wallis analysis, the lowest concentrations were found in muscle and the highest in liver and gills. However, in muscle, Cs, Rb and As had their highest content compared to liver and gills. Muscle has a relatively low metabolic activity and does not accumulate high concentrations of contaminants [62] and/or has a large fraction in comparison with the total organism weight, which may contribute to the dilution of metals when compared to other organs such as liver, and gills [63]. However, muscle is a long-term accumulation tissue and some metals show a higher affinity to the muscle tissue compared to other tissues[64]. The highest concentrations of Cd, Cu, Zn, Se, Cr, and Co were found in liver and the highest concentrations of Pb, U, Li, V, Ni, and Ba were found in gills. Metabolically active organs such as liver play a key role in metal metabolism, uptake, storage and detoxification/elimination processes[65] through the induction of metal-binding proteins to protect against toxic effects[63]. The liver reflects the past exposure of the organism to metals[64]. Gills reflect the metal bioavailability in the water column with which they come into close and permanent contact[65], especially at the beginning of the exposure before the transition of metals to other fish tissues[64]. Similar accumulation trend was observed in Kalantzi et al. (2019)[63] except V concentration which was highest in liver, and Cr concentrations which was non significantly different between muscle, liver, gills. It should be noted that stomach content had the highest concentrations of most metals compared to muscle, liver and gills.

Similarly, the metal distribution in fish species per each tissue was investigated and illustrated in nMDS ordination plots (Figure 12 (b, c, d, f)). In fish muscle plot (Figure 12 (b)), most of fish species are clearly different from each of the other e.g. *Auxis rochei - Merluccius merluccius*, whereas other fish species do not differentiate e.g. *Spicara sp. - Boops boops*. This was verified by ANOSIM results (Table A3) where the R values ranged from 0.306 to 1.000 for the significantly different fish species. In fish liver (Figure 12 (c)) and gills plots (Figure 12 (d)), all possible species combinations clearly differentiate as verified by ANOSIM results (Table A4). However, this differentiation is not the case for stomach content (Figure 12 (e)) where only *Spicara sp, Pagellous erytrhinous* and *Pagellous erytrhinous, Boops boops* significantly differentiate according to their metal concentrations. This was verified by ANOSIM results (Table A4). The distribution of metals in crustaceans and cephalopods is shown in nMDS ordination plot (Figure 12(f)) and validated by ANOSIM results (Table A4). Most of the combinations of species differentiate according to their metal content except for *Illex coindetii* from the other species (except *Parapenaeus longirostris*) and *Eledone moschata*, *Octopus vulgaris*.

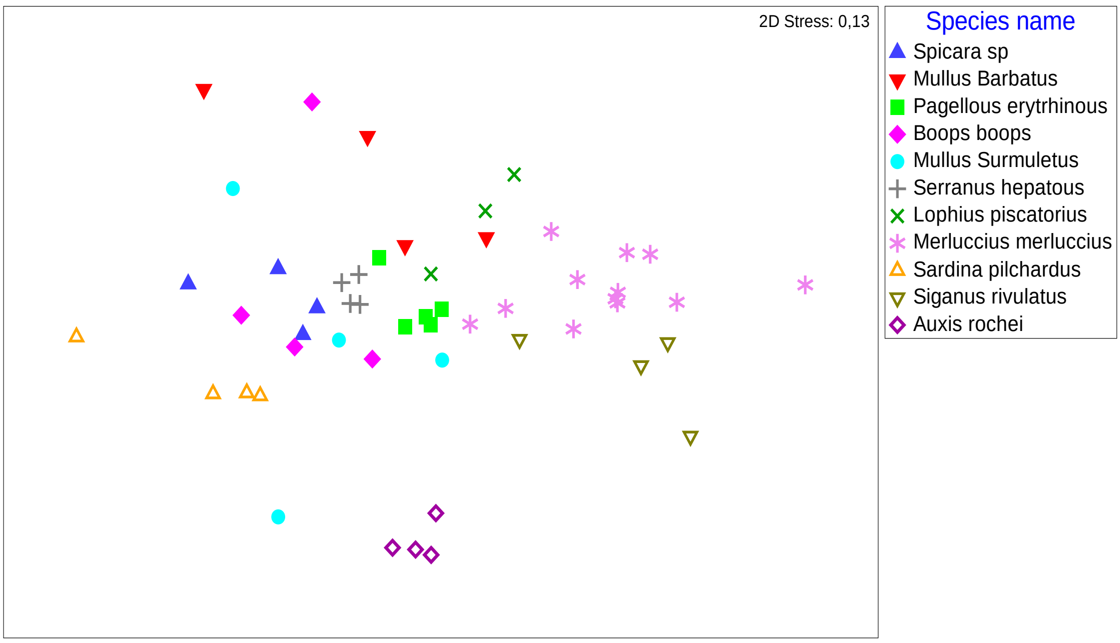
These results indicate that species separate statistically significantly between each other based on their metal content. More specifically, according to Kruskal-Wallis results, in the muscle, *Merluccius merluccius* and Siganus rivulatus had the lowest concentrations of most metals, and cephalopods (*Illex coindetii, Eledone moschata, Octopus vulgaris*) had the highest concentrations of metals. Cephalopods increased metal content may be linked to their habitat which is the sediment and is more enriched with metals[66]. Disturbance events of the sediment may cause resuspension of sediment into the water column, re-mobilisation of metals and increased bioavailability of metals[67]. Conversely, Cs had its highest concentrations in *Merluccius merluccius, Mullus surmuletus*, and *Serranus hepatus* and its lowest concentrations in cephalopods (*Loligo vulgaris, Illex coindetii, Eledone moschata, Octopus vulgaris*).

In liver and gills, the lowest concentrations of metals were found in *Merluccius merluccius* and the highest in *Spicara sp, Pagellous erytrhinous*, and *Boops boops*. Levels of elements in fish tissues can be influenced by seasonal and biological differences, for example, species biology and proximate composition, size, dark/white muscle, age, sex and sexual maturity, and environmental factors, such as water chemistry, salinity, temperature and contaminants[68].

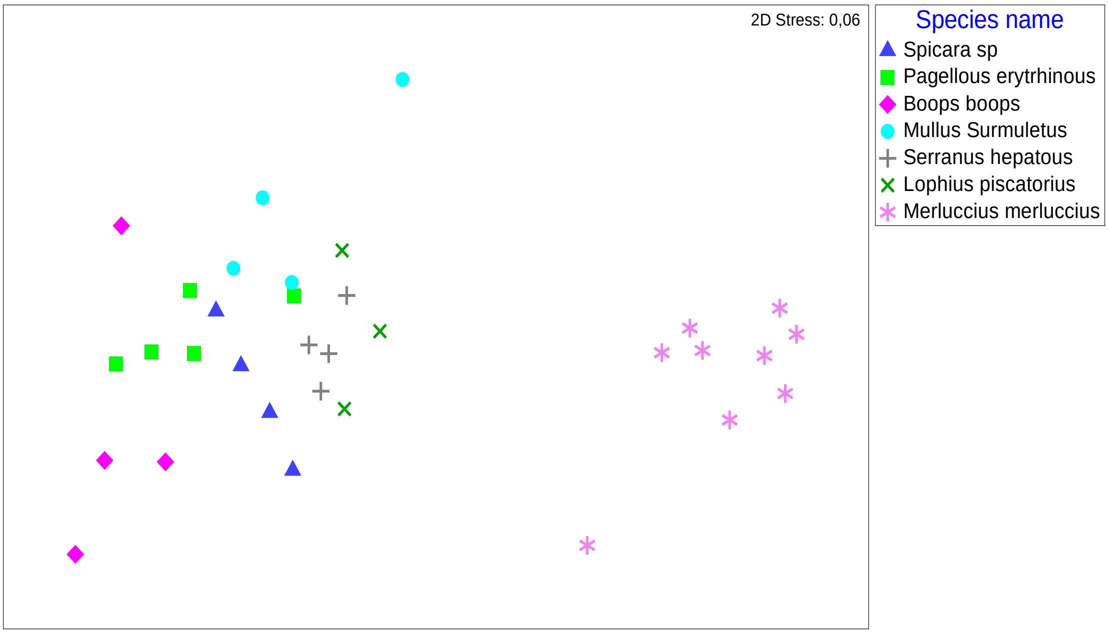
In the stomach content, no statistically significant differences were found between fish species indicating similar content of metals in their diet. Thus, metal transfer to fish cannot be solely attributed to fish dietary exposure but also to aqueous exposure, habitat type and environmental pollution, as well as, to excretion rates through feces, urine, and respiratory membranes[33].



(A) Fish (muscle, liver gills, stomach) & zooplankton

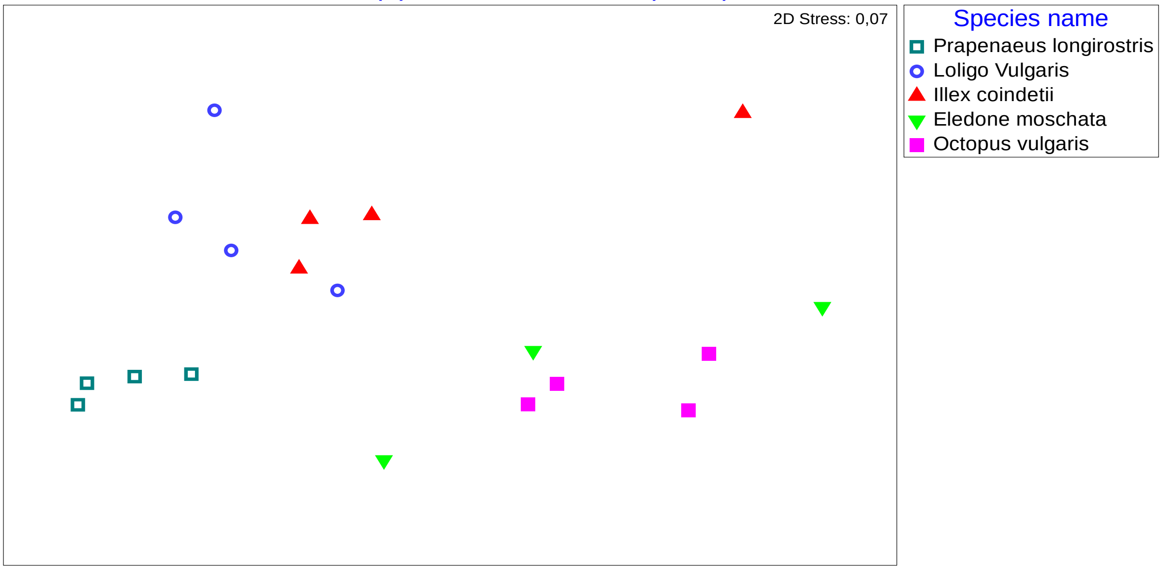


(B) Fish muscle



(C) Fish liver

(D) Fish gills



(E) muscle crustaceans & cephalopods

Figure 12: nMDS ordination plot of the distribution of metals (a) among the different tissues considering all fish species and tissues; (b) in fish muscle, (c) in fish liver, (d) in fish gills, (e) in muscle among the different crustaceans and cephalopods species.

3.4.2. Bioaccumulation potential of other metals

Concerning the bioaccumulation of metals in the different tissues, the three size groups of individuals for, *Pagellus erythrinus* and *Merluccius merluccius*, were compared. As shown in Figure 13for the muscle, liver, gills of *Pagellus erythrinus*, no statistically significant differences between the three size classes were found. In *Merluccius merluccius* (Figure 14), in muscle tissue, the concentrations of Cr, Ni, Se, Cd, Cs, Pb and U were found to be higher in the largest size group compared to the smallest whereas the concentrations of Li, V, Co, Cu, Zn, As, Rb, and Ba in muscle did not differ significantly between size classes. The positive correlation between metal concentrations and fish size may be attributed to the high dissolved content of water and the low capacity of organisms to excrete and regulate these concentrations. Consequently, the accumulation of metals will be continuous and the reduction of their content due to dilution effect from organism growth and the low metabolic rate of older organisms may not be observed[75]. No statistically significant differences were found in metal concentrations in liver and gills of *Merluccius merluccius* (Figure 14).

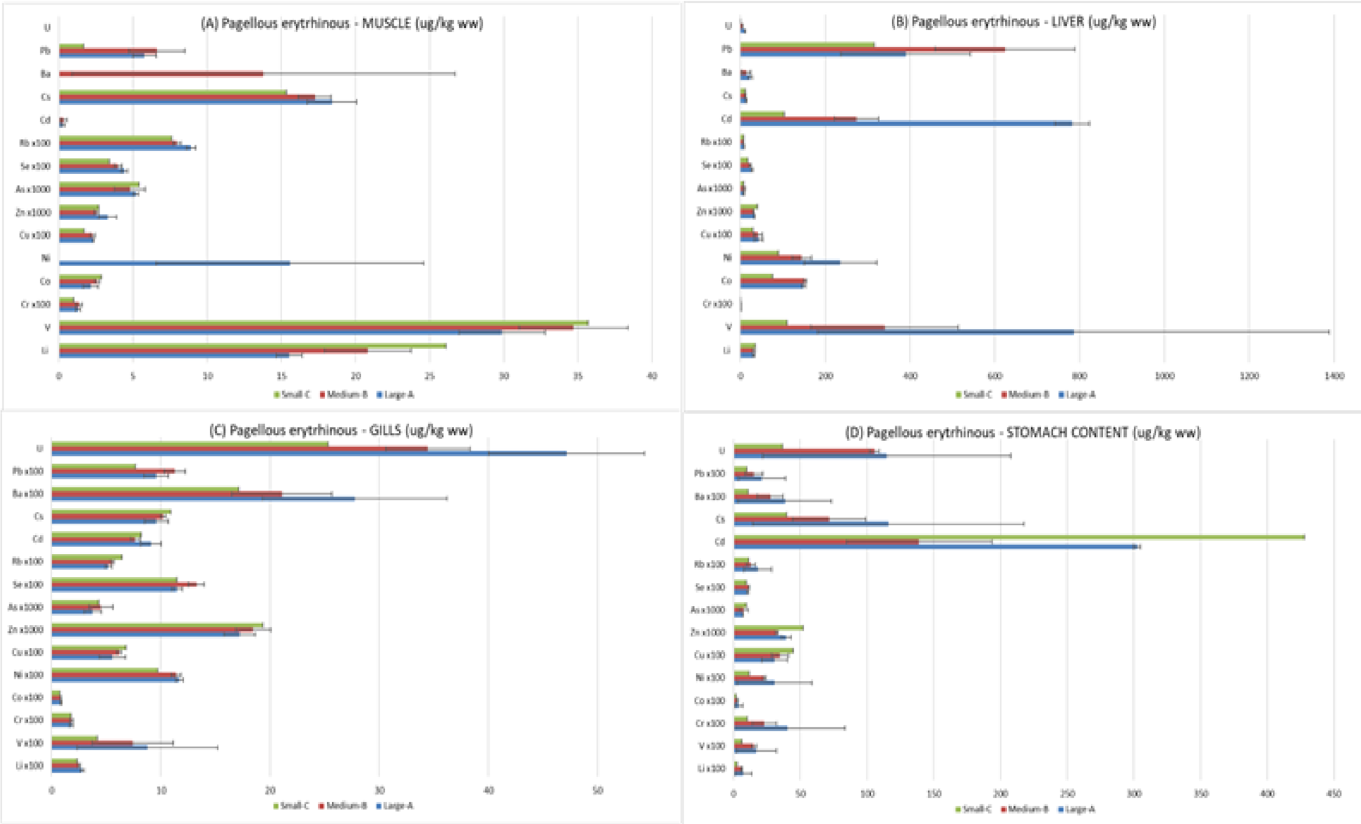


Figure 13: Metal concentrations in (a) muscle, (b) liver, (c) gills, and (d) stomach content of Pagellus erythrinus across three size groups of individuals (Small-C, Medium-B, Large-A).

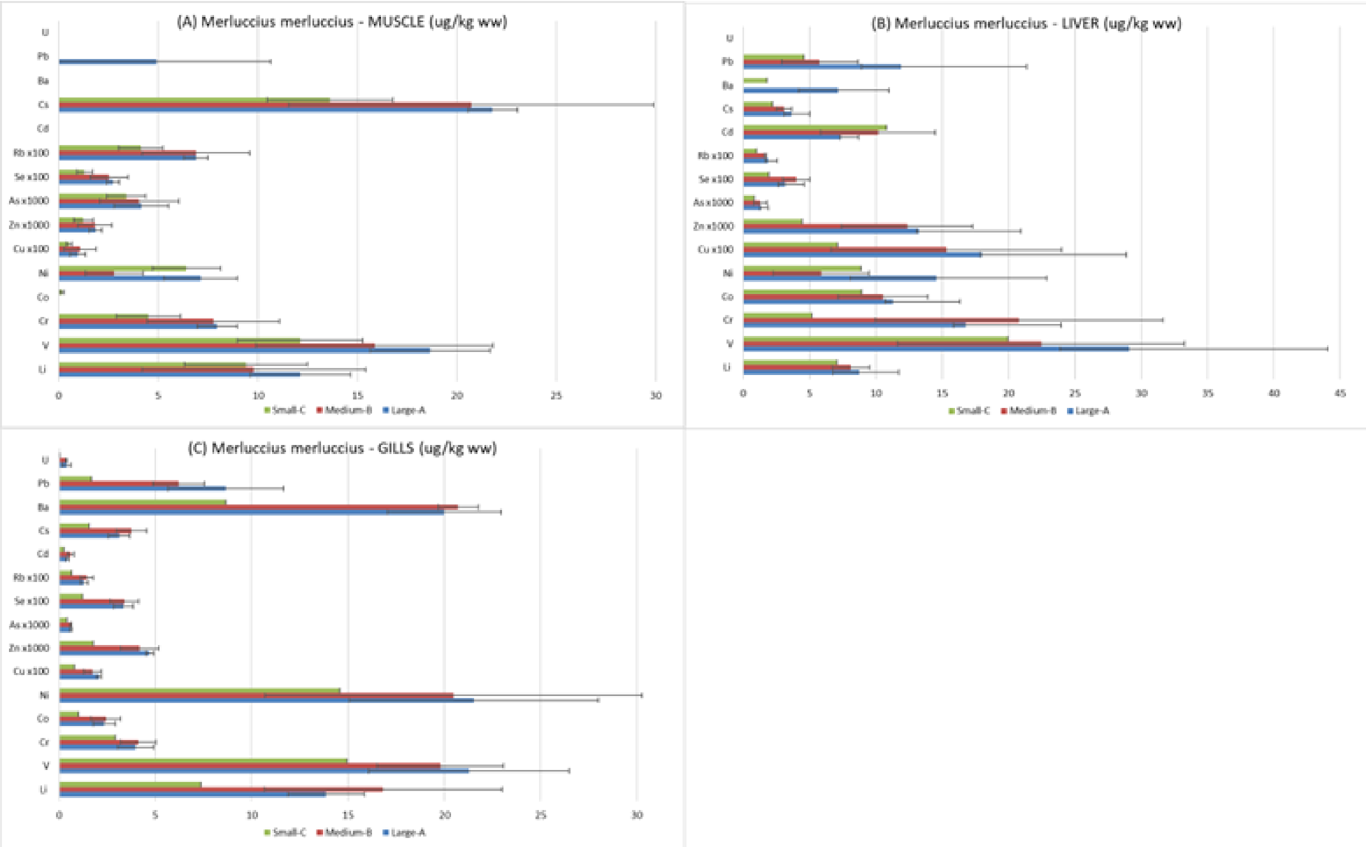


Figure 14: Metal concentrations in (a) muscle, (b) liver, and (c) gills of Merluccius merluccius across three size groups of individuals (Small-C, Medium-B, Large-A).

3.4.3. Trophic transfer of other metals in the Saroniikos Gulf

The metal concentrations in muscle of fish, crustacean & cephalopods (Figures A2), in fish gills (Figures A3), and in fish liver (Figures A4) are plotted against the trophic level of each species. Most metals follow a diminishing trend with increasing trophic level except As, Se, Rb and Cs (muscle), Li, Ni, Zn, As, Se, Cs, Ba, Pb, U (gills) and Cu, Zn, As, Se, Rb, Cd, Cs, Pb (liver). Likewise, TMF values of metals (Table 12) were found to be less than 1 for most metals (TMF = 0.1 - 0.9), supporting the biodilution of most metals within the Saronikos Gulf marine food web. Furthermore, no significant trend was found in relation to most of the metals, especially in gills and liver. Conversely, TMF values of As, and Rb in muscle were found to be higher than 1 (1.97 and 1.17, respectively) which indicate the biomagnification of these metals in the food web.

Table 12: Trophic Magnification Factors (TMF) among log of metal concentrations in the tissues and trophic levels of the species. In gray highlight are marked the values higher than 1.0.

TMF	Muscle	p	Gills	p	Liver	p
Li	0.67	*	0.69	ns	0.27	***
V	0.54	***	0.50	*	0.35	***
Cr	0.56	***	0.42	***	0.49	***
Co	0.28	***	0.45	**	0.51	**
Ni	0.36	***	0.79	ns	0.30	***
Cu	0.48	***	0.36	***	0.88	ns
Zn	0.65	**	0.79	ns	0.96	ns
As	1.97	***	0.91	ns	0.98	ns
Se	0.89	ns	0.86	ns	1.01	ns
Rb	1.17	***	0.76	*	0.82	ns
Cd	0.15	***	0.19	***	0.70	ns
Cs	1.13	ns	1.19	ns	1.11	ns
Ba	0.21	***	0.54	ns	0.10	***
Pb	0.39	***	0.76	ns	0.60	ns
U	0.20	***	0.48	ns	0.16	***

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$; ns: non significant

Even though elements are more available at lower trophic levels, fish have the capacity to reject large amounts ingested[69]. In this study, biodilution was observed for all examined metals in muscle except Se which did not have a statistically significant trend (p>0.05) and except As and Rb and Cs with TMF values indicating biomagnification (not significant for Cs).

In gills and liver, almost half of the metals show biodilution and half non-significant trend. Thus, in liver, V, Cr, Co, Cu, Rb, and Cd and, in gills, Li, V, Cr, Co, Ni, Ba, and U showed biodilution.

This pattern of biodilution with increasing trophic level may be the result of the effective efflux of metals by prey, the very low assimilation by marine fish[76] [77] [78] and the increased lipid content of organisms occupying higher trophic levels[79], which may lead to decreased element accumulation[44]. These results are in line with other studies on food webs[35] [76] [80] [81].

Conversely, in this study, As and Rb concentrations in the muscle were found to increase going up the food chain indicating biomagnification. The build-up in marine food chains could be attributed to these elements' high assimilation efficiency and low efflux rate constants which has been previously found for Cd, Co, Cs, Mn, Se, and Zn[82]. Although the TMF values of Cs in muscle, gills, and liver were higher than 1 which corresponds to biomagnification, it was not statistically significant (p>0.05). Thus, the biomagnification of Cs found in [35] was not the case in our study.

4.0 Conclusions

The work conducted under Task 2.4 represents the first comprehensive investigation of a Mediterranean marine trophic web that incorporates both legacy and emerging contaminants. This study includes sixteen marine species along with zooplankton, forming a trophic web that spans 2.5 trophic levels. The majority of species analysed are demersal—organisms that inhabit areas just above the seafloor and feed on sedimentary fauna, detritus, and organic material. Only three species (*Sardina pilchardus, Spicara spp., and Auxis rocheii*), along with zooplankton, are pelagic, feeding from the water column. Consequently, the trophic dynamics of the constructed food web are predominantly shaped by the ecological behaviour of demersal organisms.

This distinction is critical for understanding contaminant uptake, as many pollutants tend to accumulate in sediments. In such settings, bioconcentration—the direct assimilation of contaminants from the surrounding environment—can play a more significant role than dietary exposure (biomagnification).

All PFAS concentrations were found below the current EQS and safety limits for human consumption. Yet, PFNA and PFOS, fluctuated in relatively elevated concentrations across most species, in some cases exceeding the newly proposed Environmental Quality Standard (EQS) for marine biota by EFSA. No clear pattern of trophic magnification was observed. Instead, within the group of demersal species, a reverse trend—biodilution—was evident. This supports the hypothesis that bioconcentration from sedimentary detritus is more influential than biomagnification via the food chain. Notable deviations from this trend included low ΣPFAS concentrations in zooplankton and *Siganus rivulatus* (a second-level demersal species) (negative deviation) and elevated in *Auxis rocheii* (a fourth-level pelagic species) (positive deviation). Yet, no definitive conclusions on inter-group transfer can be drawn from the studied trophic range which is not very broad.

The highest ΣPFAS concentrations were recorded in the fourth-level *Auxis rocheii*, third-level *Sardina pilchardus* (small size class), and *Parapenaeus longirostris* (red shrimp).

In contrast, total mercury (THg) did not exceed EQS thresholds or safety limits but showed a clear pattern of biomagnification, with concentrations increasing approximately 5-fold per trophic level. This trend was consistent across both demersal and pelagic species, with *Auxis rocheii* again exhibiting the highest mercury accumulation.

These findings emphasize the unique contaminant dynamics in a Mediterranean demersal-dominated food web and highlight the critical role of habitat-specific exposure pathways in shaping contaminant distribution across trophic levels.

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6.0 Annex

The present Annex includes i) detailed raw data on THg concentrations; ii) the results from statistical tests on the intercomparison of THg (Kruskal-Wallis) and 'other metals' (ANOSIM) concentrations among species and among tissues; iii) regression analysis of 'other metals' concentrations vs trophic level.

Table A1: Total Hg (µg g-1) concentrations in all samples/replicates of species and tissues collected in the Saronikos gulf.

Species	Tissue	Total Hg	
		μ g g^{-1} ww	μ g g^{-1} dw
mesozooplankton		0.040	0.006
		0.030	0.005
		0.032	0.005
		0.038	0.006
		0.025	0.004
		0.035	0.006
		0.031	0.005
Siganus rivulatus	muscle	0.006	0.026
		0.014	0.051
		0.021	0.062
		0.005	0.020
Boops boops	muscle	0.069	0.250
		0.101	0.463
		0.059	0.258
		0.073	0.310
Sardina pilchardus	muscle	0.029	0.114
		0.023	0.122
		0.015	0.085
		0.021	0.082
Mullus surmuletus	muscle	0.112	0.443
		0.115	0.469
		0.143	0.632
		0.135	0.570
Spicara sp.	muscle	0.261	1.096
		0.230	0.922
		0.137	0.541
		0.130	0.564
Merluccius merluccius size A	muscle	0.165	0.630
		0.121	0.551
		0.179	0.941

Species	Tissue	Total Hg	
		$\mu g g^{-1}$ ww	$\mu g g^{-1}$ dw
		0.113	0.565
Merluccius merluccius size A	liver	0.040	0.060
		0.067	0.154
		0.052	0.080
		0.046	0.087
Merluccius merluccius size A	gills	0.070	0.502
		0.024	0.208
Merluccius merluccius size B	muscle	0.129	0.402
		0.055	0.295
		0.080	0.275
Merluccius merluccius size B	liver	0.042	0.067
		0.031	0.049
		0.028	0.041
		0.028	0.052
Merluccius merluccius size B	gills	0.029	0.192
		0.038	0.231
Merluccius merluccius size C	muscle	0.035	0.182
		0.064	0.345
		0.112	0.612
		0.042	0.245
Mullus barbatus	muscle	0.145	0.721
		0.567	2.938
		0.175	0.831
		0.281	1.334
Serranus hepatus	muscle	0.248	1.125
		0.441	2.041
		0.299	1.404
		0.354	1.582
Lophius piscatorius	muscle	0.282	1.468
		0.349	1.918
		0.329	1.792
Pagellus erythrinus Size A	muscle	0.379	1.717
		0.476	1.955
Pagellus erythrinus Size A	liver	1.069	3.514
		0.616	2.007
Pagellus erythrinus Size A	gills	0.166	0.632
		0.180	0.608

Species	Tissue	Total Hg μ g g⁻¹ ww	Total Hg μ g g⁻¹ dw
Pagellus erythrinus	muscle	0.312	1.317
Size B		0.313	1.337
Pagellus erythrinus	liver	0.484	1.758
Size B		0.398	1.380
Pagellus erythrinus	gills	0.093	0.329
Size B		0.093	0.333
Pagellus erythrinus Size C	muscle	0.128	0.522
Pagellus erythrinus Size C	liver	0.232	0.852
Pagellus erythrinus Size C	gills	0.054	0.206
Auxis rochei	muscle	0.427	1.456
		0.291	1.036
		0.362	1.168
		0.219	0.779
Crustaceans & Cephalopods			
Parapenaeus longirostris	muscle	0.062	0.426
		0.065	0.433
		0.052	0.362
		0.050	0.351
Illex coindetii	mantle	0.168	0.809
		0.093	0.396
		0.085	0.400
		0.144	0.648
Octopus vulgaris		0.049	0.424
		0.042	0.211
		0.072	0.352
Loligo vulgaris	mantle	0.111	0.425
		0.107	0.408
		0.089	0.346
		0.045	0.182
Eledone moschata	mantle	0.189	0.938
		0.037	0.240
		0.192	0.945
		0.032	0.192

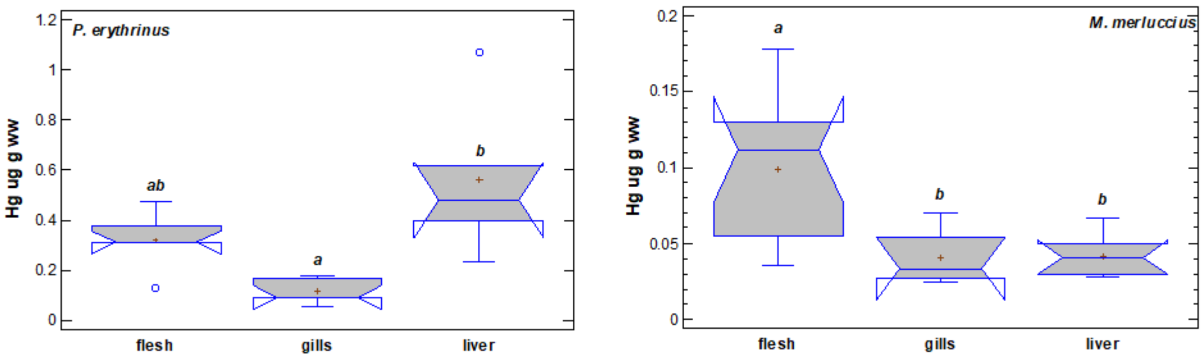

Figure A1: Differences in THg concentrations among tissues in two fish species (P. erythrinus and M. merluccius) in the Saronikos gulf (Kruskal-Wallis after the Bonferroni test for p<0.05). Different letters indicate statistically significant differences at the 95% level.

Table A2: ANOSIM results of the differences in overall metal concentrations between fish tissues considering all fish species and between crustaceans and cephalopods species. Pairwise comparisons were made for each possible combination of tissues and species

(A) Fish tissues			(B) Crustaceans & Cephalopods		
Global R	0.831		Global R	0.722	
p	***		p	***	
Groups	Statistic R	p	Groups	Statistic R	p
MUSCLE, LIVER	0.863	***	*Prapenaeus longirostris, Loligo Vulgaris*	0.917	*
MUSCLE, GILLS	0.858	***	*Prapenaeus longirostris, Illex coindetii*	0.635	*
MUSCLE, STOMACH	0.992	***	*Prapenaeus longirostris, Eledone moschata*	0.870	*
MUSCLE, ZOO	1.000	***	*Prapenaeus longirostris, Octopus vulgaris*	1.000	*
LIVER, GILLS	0.575	***	*Loligo Vulgaris, Illex coindetii*	0.167	ns
LIVER, STOMACH	0.754	***	*Loligo Vulgaris, Eledone moschata*	0.778	*
LIVER, ZOO	0.983	***	*Loligo Vulgaris, Octopus vulgaris*	0.990	*
GILLS, STOMACH	0.423	***	*Illex coindetii, Eledone moschata*	0.389	ns
GILLS, ZOO	0.716	***	*Illex coindetii, Octopus vulgaris*	0.625	ns
STOMACH, ZOO	0.322	*	*Eledone moschata, Octopus vulgaris*	0.389	ns

*: p < 0.05; ***: p < 0.005; ns: non significant

Table A3: ANOSIM results of the differences in overall metal concentrations between fish species considering only fish muscle. Pairwise comparisons were made for each possible combination of species

Fish muscle					
		Global R	0.722		
		p	***		
Groups	Statistic R	p	Groups	Statistic R	p
Spicara sp, Mullus Barbatus	0.854	*	*Boops boops, Mullus Surmuletus*	0.135	ns
Spicara sp, Pagellous erytrhinous	0.913	**	*Boops boops, Serranus hepatous*	0.490	*
Spicara sp, Boops boops	0.094	ns	*Boops boops, Lophius piscatorius*	0.519	ns

Fish muscle					
Spicara sp, Mullus Surmuletus	0.479	ns	*Boops boops, Merluccius merluccius*	0.925	***
Spicara sp, Serranus hepatous	0.646	*	*Boops boops, Sardina pilchardus*	0.198	ns
Spicara sp, Lophius piscatorius	1.000	*	*Boops boops, Siganus rivulatus*	0.927	*
Spicara sp, Merluccius merluccius	0.951	***	*Boops boops, Auxis rochei*	0.865	*
Spicara sp, Sardina pilchardus	0.615	*	*Mullus Surmuletus, Serranus hepatous*	0.323	*
Spicara sp, Siganus rivulatus	0.990	*	*Mullus Surmuletus, Lophius piscatorius*	0.444	ns
Spicara sp, Auxis rochei	1.000	*	*Mullus Surmuletus, Merluccius merluccius*	0.834	***
Mullus Barbatus, Pagellous erytrhinous	0.556	**	*Mullus Surmuletus, Sardina pilchardus*	0.375	*
Mullus Barbatus, Boops boops	0.708	*	*Mullus Surmuletus, Siganus rivulatus*	0.833	*
Mullus Barbatus, Mullus Surmuletus	0.292	ns	*Mullus Surmuletus, Auxis rochei*	0.708	*
Mullus Barbatus, Serranus hepatous	0.615	*	*Serranus hepatous, Lophius piscatorius*	0.944	*
Mullus Barbatus, Lophius piscatorius	0.630	ns	*Serranus hepatous, Merluccius merluccius*	0.888	***
Mullus Barbatus, Merluccius merluccius	0.832	***	*Serranus hepatous, Sardina pilchardus*	0.635	*
Mullus Barbatus, Sardina pilchardus	0.917	*	*Serranus hepatous, Siganus rivulatus*	0.958	*
Mullus Barbatus, Siganus rivulatus	0.760	*	*Serranus hepatous, Auxis rochei*	1.000	*
Mullus Barbatus, Auxis rochei	0.990	*	*Lophius piscatorius, Merluccius merluccius*	0.661	***
Pagellous erytrhinous, Boops boops	0.531	*	*Lophius piscatorius, Sardina pilchardus*	0.926	*
Pagellous erytrhinous, Mullus Surmuletus	0.306	*	*Lophius piscatorius, Siganus rivulatus*	0.944	*
Pagellous erytrhinous, Serranus hepatous	0.825	**	*Lophius piscatorius, Auxis rochei*	1.000	*
Pagellous erytrhinous, Lophius piscatorius	0.805	*	*Merluccius merluccius, Sardina pilchardus*	0.983	***
Pagellous erytrhinous, Merluccius merluccius	0.645	***	*Merluccius merluccius, Siganus rivulatus*	0.444	*
Pagellous erytrhinous, Sardina pilchardus	0.85	**	*Merluccius merluccius, Auxis rochei*	0.958	***
Pagellous erytrhinous, Siganus rivulatus	0.888	**	*Sardina pilchardus, Siganus rivulatus*	1.000	*
Pagellous erytrhinous, Auxis rochei	1.000	**	*Sardina pilchardus, Auxis rochei*	1.000	*
			Siganus rivulatus, Auxis rochei	0.990	*

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$; ns: non significan

Table A4: ANOSIM results of the differences in overall metal concentrations between fish species considering liver, gills, stomach content. Pairwise comparisons were made for each possible combination of species

	LIVER		GILLS		STOMACH CONTENT	
Global R	0,774		0,852		0,183	
p	***		***		***	
Groups	Statistic R	p	Statistic R	p	Statistic R	p
Spicara sp, Pagellous erytrhinous	0.419	*	0.938	**	0.3	*
Spicara sp, Boops boops	0.552	*	0.667	*	0.042	ns
Spicara sp, Mullus Surmuletus	0.573	*	0.667	*	-0.083	ns
Spicara sp, Serranus hepatous	0.635	*	1.000	*	-0.111	ns
Spicara sp, Lophius piscatorius	0.722	*	1.000	*		
Spicara sp, Merluccius merluccius	0.995	***	1.000	**		
Pagellous erytrhinous, Boops boops	0.638	**	0.963	**	0.494	**
Pagellous erytrhinous, Mullus Surmuletus	0.513	*	0.638	**	0.206	ns
Pagellous erytrhinous, Serranus hepatous	0.819	*	1.000	**	0.426	ns
Pagellous erytrhinous, Lophius piscatorius	0.867	*	1.000	*		
Pagellous erytrhinous, Merluccius merluccius	1.000	***	1.000	**		
Boops boops, Mullus Surmuletus	0.646	*	0.656	*	0.135	ns
Boops boops, Serranus hepatous	0.792	*	1.000	*	0.148	ns
Boops boops, Lophius piscatorius	0.852	*	1.000	*		
Boops boops, Merluccius merluccius	1.000	***	1.000	**		
Mullus Surmuletus, Serranus hepatous	0.500	*	0.521	*	0.111	ns
Mullus Surmuletus, Lophius piscatorius	0.537	*	0.944	*		
Mullus Surmuletus, Merluccius merluccius	1.000	***	1.000	**		
Serranus hepatous, Lophius piscatorius	1.000	*	1.000	*		
Serranus hepatous, Merluccius merluccius	0.985	***	1.000	**		
Lophius piscatorius, Merluccius merluccius	0.975	***	1.000	*		

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$; ns: non significant

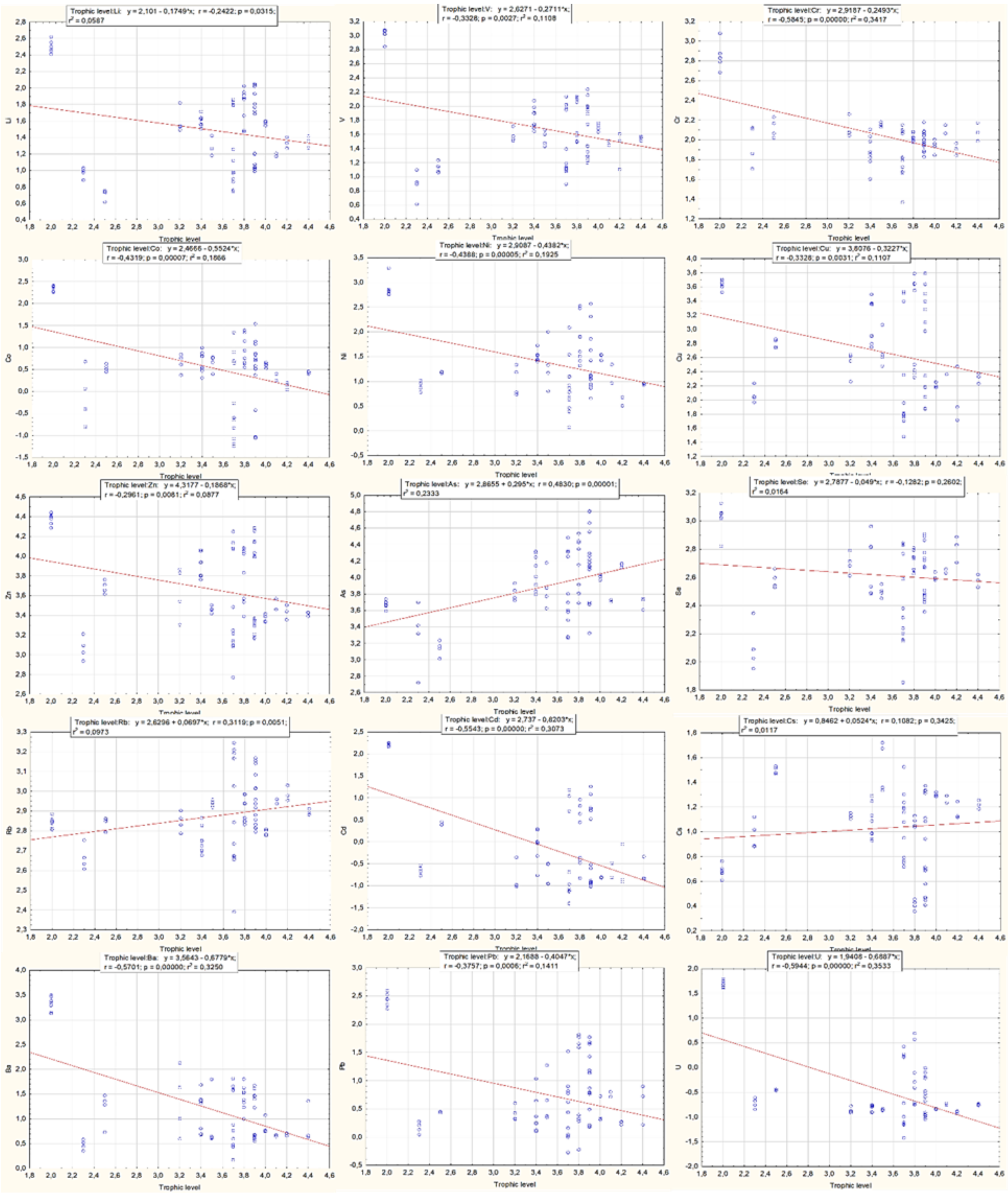

Figure A2: Log transformed metal concentrations in fish muscle plotted against the trophic level of species

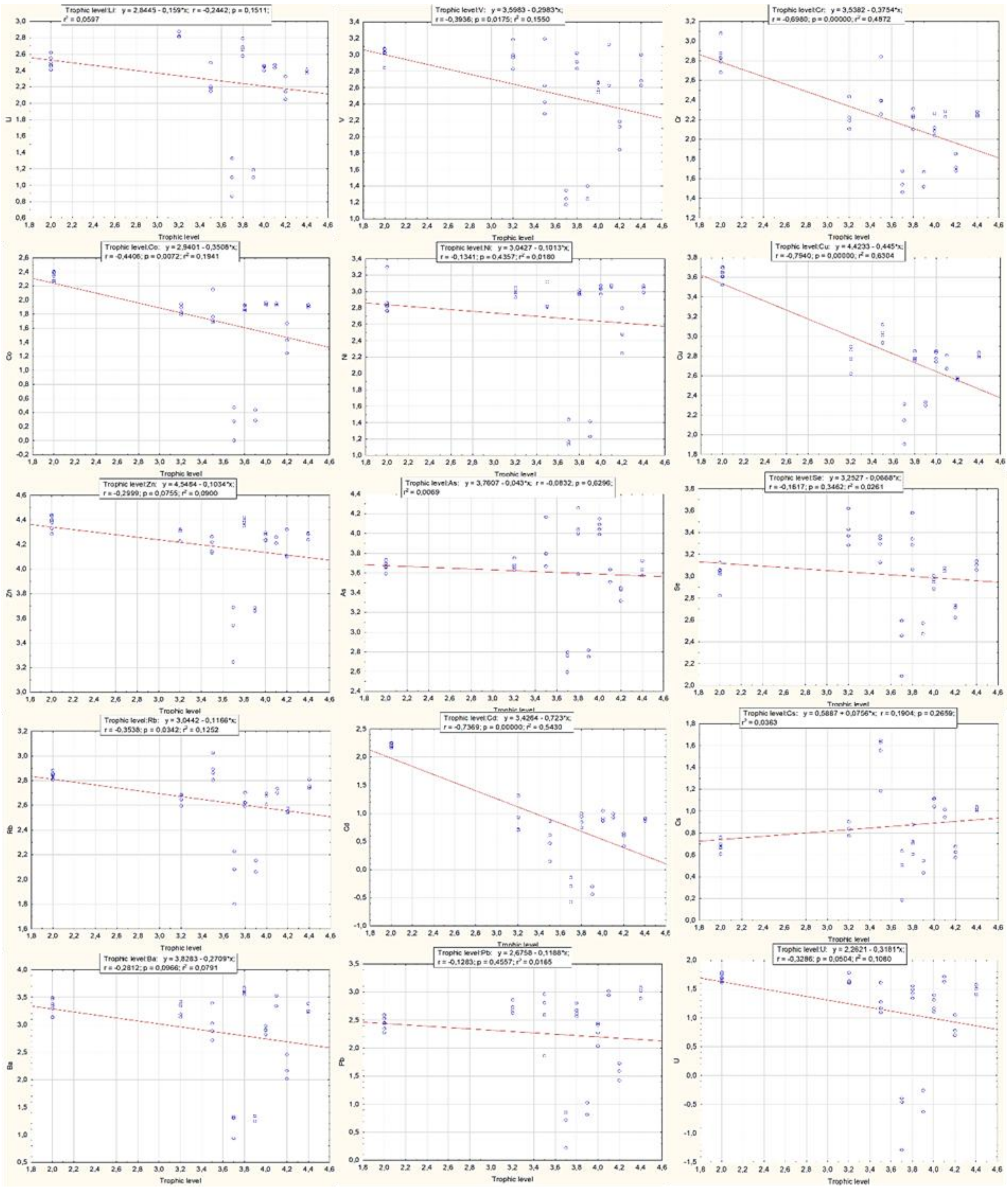

Figure A3: Log transformed metal concentrations in fish gills plotted against the trophic level of species

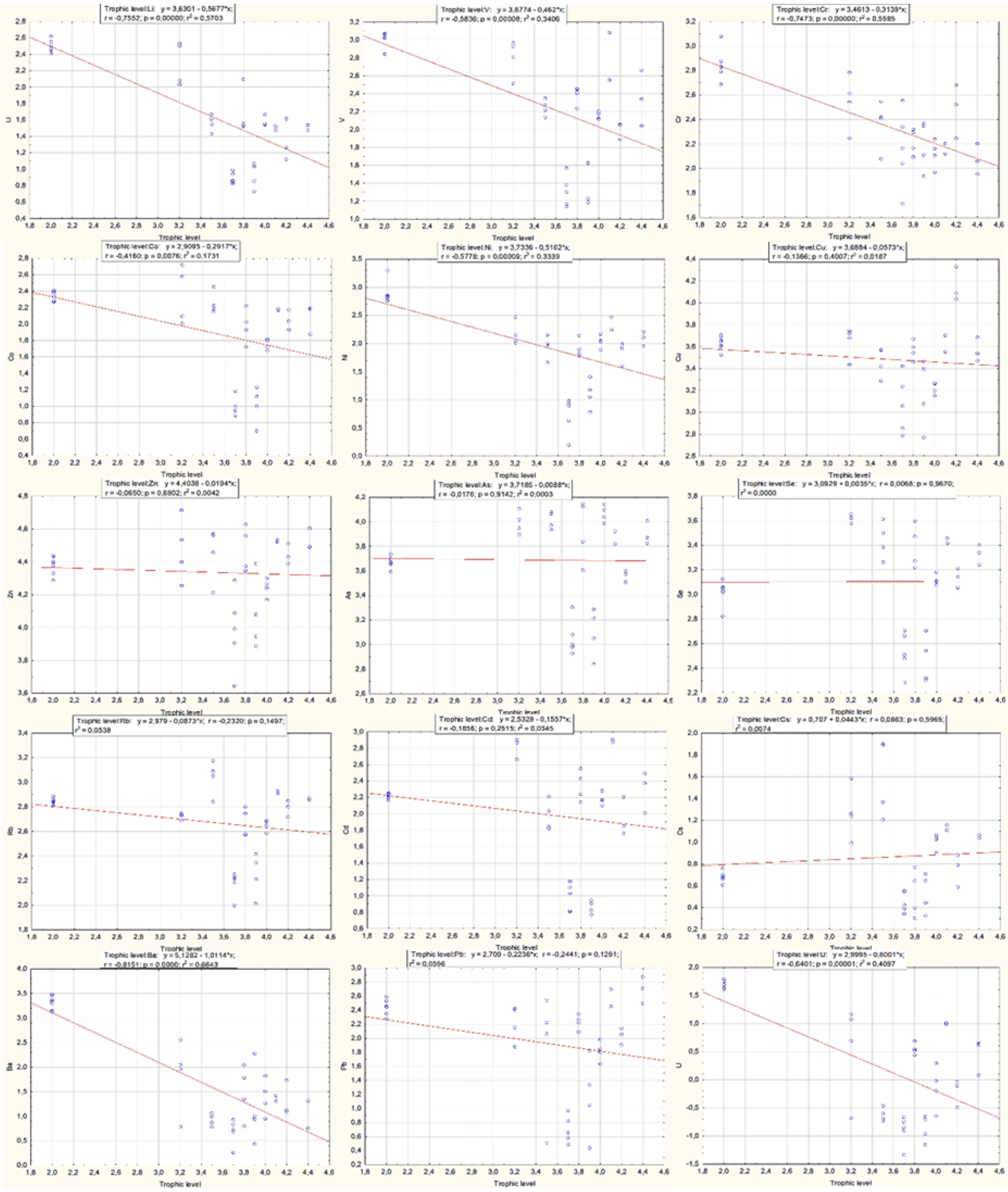

Figure A4: Log transformed metal concentrations in fish liver plotted against the trophic level of species

The Mediterranean Sea and its surrounding regions support a diverse variety of essential socioeconomic activities. It is one of the highly exploited water ways and the influence of anthropogenic activities on its marine habitats and ecosystems has grown significantly since the industrial revolution. Because of this, the Mediterranean Sea basin is very vulnerable to chemical contamination and build-up. To safeguard the Mediterranean Sea basin from contaminants for emerging concerns (CoEC), iMERMAID will integrate, coordinate, and synergize innovative preventive, monitoring, and remediation solutions. iMERMAID will build an evidence-based multidimensional framework that will guide policymaking and transform societal perceptions to reduce CoEC usage, emissions, and pollution. Furthermore, next generation sensor and remediation solutions will be developed within iMERMAID to monitor and remove prioritized chemicals from its source while reducing upstream pollution. iMERMAID builds an ideal interdisciplinary team by bringing together prominent SMEs, researchers, regulators, and innovation professionals who have been essential in improving the knowledge and awareness of CoEC. Beyond state-of-the-art techniques, iMERMAID will strive to strengthen regulations against CoEC, expand economic possibilities and competitiveness, improve the standard of living for EU residents, while preventing the accumulation of chemical pollution in the Mediterranean Sea basin. iMERMAID will empower the efforts to create a zero pollution, contaminant free waters by enabling the Chemical Strategy's goals to become a practical reality.

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