International Baccalaureate

ENVIRONMENTAL SYSTEMS AND SOCIETIES

EXTENDED ESSAY

Research Question:

How does the tissue bioaccumulation of Cd, Pb and Hg (tested with ICP-MS) change in fish species *Sparus aurata, Merluccius merluccius* and *Merlangius merlangus* in accordance to their origins of catching (the Aegean, Mediterranean and Black Sea)?

Candidate Number: 001129 0080

Word count: 4056

TABLE OF CONTENTS

Chapter I: Introduction

1.1 Background information

The request for fish consumption has increased quickly in recent years due to its benefits in terms of nutrition and proteins.[1] As sea water is subject to increasing pollutant discharges from extensive commercial fishing, trace metal poisoning of seafood is progressively becoming a global health concern.[2] When choosing a source of nutrition, toxic heavy metals such as Cd, Pb and Hg should be taken into account among the levels of beneficial oils, minerals and proteins of fish.[3] These elements can be toxic at even very low concentrations, which is why heavy metal content in consumed goods is strictly regulated by the EU.[3] Fish can easily absorb heavy metals from surroundings as well as consuming various other sea life that benefit from sediments. Even though the accumulation of heavy metals are low in single organisms, they pose a serious threat when consumed by wild sea life either chronically or in large portions.[3,4]

This essay seeks to investigate the heavy metal bioaccumulation caused by anthropogenic and natural processes near the coasts of Turkey. It includes an analysis of the specified heavy metals and their ratios that occur within one sea, while relating to the human activities most done in the area with cause and effect. The essay will also simultaneously consider restrictions enforced regarding both commercial and supplementary fish farming and the analysis of the demographics of the fishing community based on their fishing habits. The research question, which is “How does the tissue bioaccumulation of Cd, Pb and Hg (tested with ICP-MS) change in fish species *Sparus aurata, Merluccius merluccius* and *Merlangius merlangus* in accordance to their origins of catching (the Aegean, Mediterranean and Black Sea)?”, is worthy of investigation since it is a debated topic, whether industrial processes by humans and natural events happening in coasts affect the marine composition, and most specifically aquatic life for human consumption. Light ought to be shed onto the choice of fish for weekly intake, to ensure that a two times a week consumption does not lead to bodily dysfunctions from exceeding the limit of harmful metals.

1.2 Heavy Metals and Their Posed Health Risks

Heavy metal consumption even in low concentrations can have detrimental effects on the human body and physiology. Among many dangerous heavy metals; cadmium, lead and mercury are the highest in food groups including fish types. The effects of these elements are as follows:

---

[1] Huang, H., Li, Y., Zheng, X., Wang, Z., Wang, Z., & Cheng, X. (2022). Nutritional value and bioaccumulation of heavy metals in nine commercial fish species from Dachen Fishing Ground, East China Sea. Scientific reports, 12(1), 6927. https://doi.org/10.1038/s41598-022-10975-6

[2] Baki, A. M. et al. Concentration of heavy metals in seafood (fishes, shrimp, lobster and crabs) and human health assessment in Saint Martin Island, Bangladesh. Ecotoxicol. Environ. Saf. 159, 153–163 (2018).

[3] Koch, W., Czop, M., Iłowiecka, K., Nawrocka, A., & Wiącek, D. (2022). Dietary Intake of Toxic Heavy Metals with Major Groups of Food Products-Results of Analytical Determinations. Nutrients, 14(8), 1626. https://doi.org/10.3390/nu14081626

[4] Latif, M., Zahoor, M., Muhammad, A., Naz, S., Kamran, A. W., Ullah, R., Shah, A. B., Almeer, R., & Sayed, A. (2022). Bioaccumulation of lead in different organs of Ctenopharyngodon Idella (grass fish) and Tor putitora (Mahseer) fish. Brazilian journal of biology = Revista brasleira de biologia, 84, e260355. https://doi.org/10.1590/1519-6984.260355

Figure 1: Cadmium metal (Cd)

Cadmium is a non-essential metal that produces severe toxicity at concentrations as low as ∼1 μg/g. Its buildup in the human body can cause renal, pulmonary, hepatic, skeletal, reproductive, and even cancer consequences.[5] Cadmium is detected in both terrestrial environments and is found to be released either from natural sources such as leaching of Cd-rich soils or volcanic activities, as well as anthropogenic activities such as the production of plastics stabilizers and nickel-cadmium batteries, mining, electroplating, smelting and production of pigments.

Figure 2: Lead metal (Pb)

Lead typically enters the body by inhalation and ingestion of lead-containing dust. Through the bloodstream, it accumulates in soft tissues such as the liver, kidneys, lungs, brain, spleen, muscles and heart. Lead is found to be high in concentrations in the surface due to atmospheric input, and enters the atmosphere during burning of coal, oil and waste. From the air, lead is removed by precipitation or by particles falling onto land and water bodies.[6]

Figure 3: Mercury metal (Hg)

Mercury is a neurotoxin that affects both the central and peripheral nervous systems. Inhaling mercury vapour can affect the neurological, digestive, and immunological systems, as

[5] Saha, N., Mollah, M., Alam, M. F. & Rahman, M. S. Seasonal investigation of heavy metals in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment. Food Control 70, 110–118 (2016).

[6] N.A., (2017), Mercury and Health, https://www.who.int/news-room/fact-sheets/detail/mercury-and-health#:~:text=Neurological%20and%20behavioural%20disorders%20may,and%20cognitive%20and%20motor%20dysfunction.

well as the lungs and kidneys, and can be deadly. Mercury inorganic salts are caustic to the skin, eyes, and gastrointestinal system and can cause kidney damage if consumed.

1.3 Properties of the Chosen Aquatic Environments

1.3.1 Black Sea

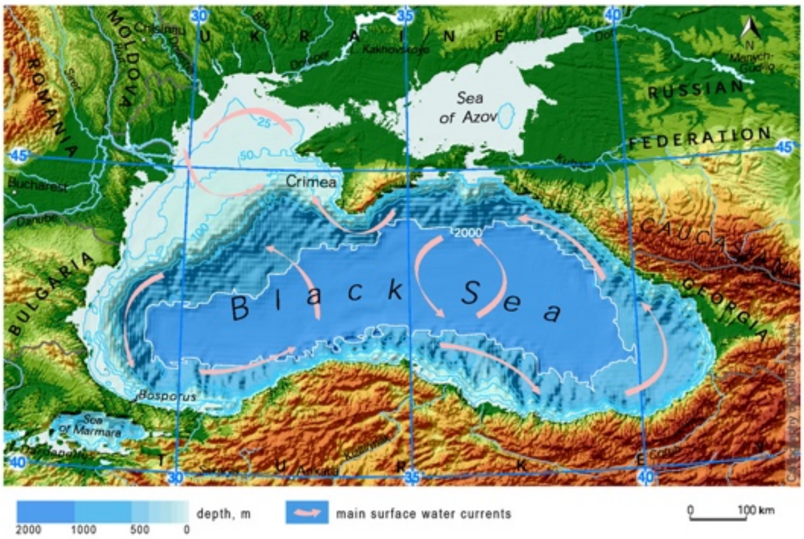


Figure 4: Map of the Black Sea, Lavrova, O., 2017. Satellite Survey of Internal Waves in the Black and Caspian Seas

The Black Sea region is positioned between Europe and Asia and is a marginal Mediterranean sea of the Atlantic Ocean. It covers 436400 km^2 and has a maximum depth of 2212 m. Under the less dense, fresher water that emerges from the Black Sea, denser, more saline water from the Aegean enters the Black Sea. As a result, a sizable and persistent layer of deep water is produced, which is anoxic because it does not mix or drain. Since the Black Sea's water level is currently high, water from the Mediterranean is being swapped with it. The first of its sort to be found, the Black Sea Undersea River is a current of particularly salty water moving through the Bosporus Strait and down the Black Sea's seabed. [7]

The Black Sea was closely subjected to the radioactive spill from the Chernobyl accident in 1986. In a research conducted in the deep western Black Sea on sediments containing plutonium and americium, it is suggested that the upper 3 cm layer of sediments was formed with the spill material during the 27-year period after this accident.[8] It can be said that the Black Sea is highly in danger in terms of quality of sediment as well as degree of tissue bioaccumulation of radioactive elements and heavy metals.

---

[7] Murray, JW; Jannasch, HW; Honjo, S.; Anderson, RF; Reeburgh, WS; Top, Z., et al. (1989). Unexpected changes in the oxic/anoxic interface in the Black Sea. *Nature*, 338(6214), 411-413. http://dx.doi.org/10.1038/338411a0 Retrieved from https://escholarship.org/uc/item/7xg3p017

[8] Proskurnin, V. Y., Tereshchenko, N. N., Paraskiv, A. A., & Chuzhikova-Proskurnina, O. D. (2021). Plutonium and americium in the deep Black Sea bottom sediments. Journal of environmental radioactivity, 229-230, 106540. https://doi.org/10.1016/j.jenvrad.2021.106540

1.3.2 Aegean Sea

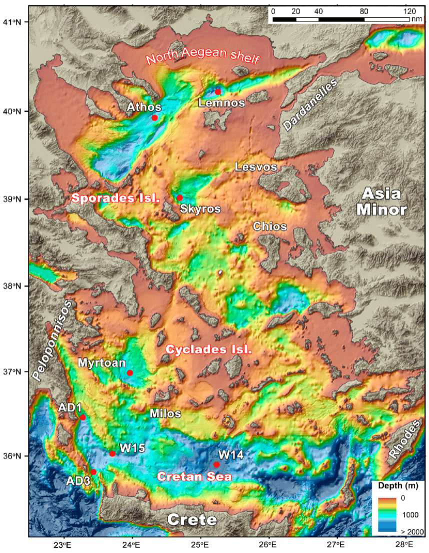


Figure 5: Bathymetric map of the Aegean Sea (data from Marine information service, 2016).

The Aegean sea is an elongated bay of the Mediterranean between Europe and Asia. It is located between the Balkans and Anatolia and covers an area of 215,000 square kilometers. The sea includes the 12 Aegean islands, and its maximum depth is 3,639m. The Aegean allows for the transfer of Mediterranean water into the Black Sea, with hypersaline water from here moving north along the west coast of Anatolia, and before being displaced by lighter Black Sea outflow.

The majority of the Aegean's floor rocks are limestone, though volcanic activity that has wracked the area in relatively recent geologic ages has frequently dramatically altered them. Vibrant sediments in the southern Aegean islands of Santorini and Milos are of great importance.[9]

1.3.3 Mediterranean Sea – Turkish Coasts

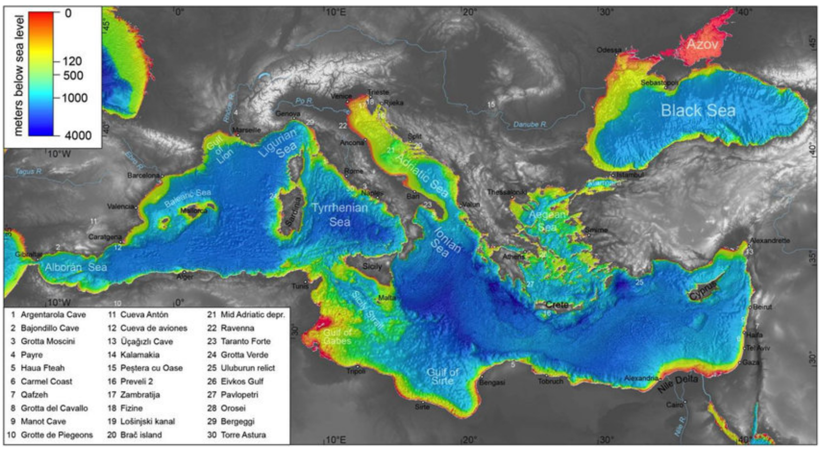


Figure 6: Map of the Mediterranean Sea, Benjamin J., 2017. Late Quaternary sea-level changes and early human societies in the central and eastern Mediterranean Basin: An interdisciplinary review

---

[9] "Aegean Sea | Mediterranean Sea". *Encyclopedia Britannica*. Retrieved 17 January 2023

The Mediterranean Sea has a surface area of approximately 2 500 000 km^2 or 0.7% of the world's oceans, with an average depth of 1500 m.

With a long history of mercury mining and a large concentration of sub-marine volcanoes, the Mediterranean region has a significantly high mercury budget. A growing number of research initiatives are being conducted to determine the effects of mercury contamination on human health and the environment as the result of the increases in mercury concentrations in biota that have been seen over the past forty years.[10]

1.4 Fish species

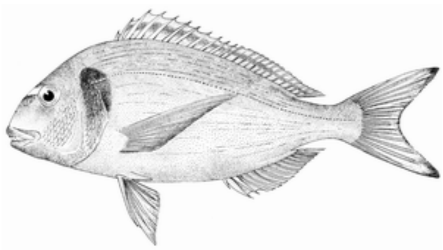


Figure 7: FAO 2023. Sparus aurata. Cultured Aquatic Species Information Programme. Text by Colloca, F.; Cerasi, S.. Fisheries and Aquaculture Division [online]. Rome. Updated 2005-05-17 [Cited January 19th 2023].
https://www.fao.org/fishery/en/culturedspecies/sparus_aurata?lang=en

Aegean Sea selected species: *Sparus aurata,* commonly known as gilt-head sea bream, is found in the Mediterranean sea and the Aegean Sea. It generally reaches about 35 cm in length and weighs up to 7.4 kg. It is typically found at depths of less than 30 metres but this depth can reach up to 150 m. The desired sea flooring is near sea grass and over sandy bottoms, but at some times in estuaries, which pose as great ecotone areas with many species. The species mainly feeds on shellfish along with some plant material. Their main diet is made up of zoobenthos with 68 percent, and they have the trophic level of 4.1.

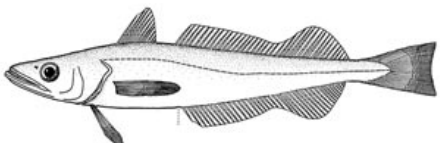


Figure 8: FAO 2023. Merluccius merluccius Linneaus,1758. Fisheries and Aquaculture Division [online]. Rome. [Cited January 19th 2023].
https://www.fao.org/fishery/en/aqspecies/2238/en

Mediterranean sea selected species: *Merluccius merluccius,* commonly known as the European hake, is found in the eastern Atlantic from Norway and in the Mediterranean sea. It is a crucial species in European fisheries and is believed to be exploited by some fishing communities. This species is normally found at depths between 70 m and 370 m, yet spawning occurs in 100m to 300m in the Mediterranean. The species organisms are up to 16cm long, and they feed mostly on crustaceans such as krill. Their main diet consists of nekton with 98 percent, and they have a trophic level of 4.5.

[10] "Marmara, Sea of ." The Columbia Encyclopedia, 6th ed.. . Retrieved September 23, 2022 from Encyclopedia.com: https://www.encyclopedia.com/reference/encyclopedias-almanacs-transcripts-and-maps/marmara-sea

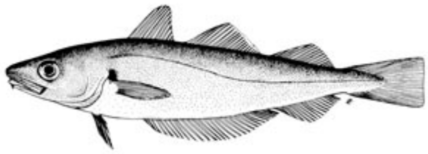


Figure 9: FAO 2023. Merlangius merlangus Linnaeus,1758. Fisheries and Aquaculture Division [online]. Rome. [Cited January 19th 2023].
https://www.fao.org/fishery/en/aqspecies/3022/en

Black Sea selected species: *Merlangius merlangus,* commonly known as whiting, is found in the eastern North Atlantic ocean and the Black sea. It is more commonly habited from depths of 30m to 100m and mainly on mud and gravel bottoms, but also preferring sandy and rocky areas. The species feeds on shrimps, crabs, mollusks and small fish. Their main diet includes zooplankton with 99 percent, and they have a trophic level of 4.0.

Chapter II: Methodology

2.1 Variables

Independent variable:

- Aquatic regions in which species were obtained from – the Black, Mediterranean and Aegean seas

Dependent variable:

- Concentrations of Cd, Hg and Pb in tissue– the bioaccumulation in the muscles of the selected fish, determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Controlled variables

Controlled variable	**Effect on the result**	**Method for control**
Incubation and processing of the fish	The processed fish (frozen, packaged etc) affect the amount of heavy metal by either contaminating them from the package or deforming them because of low temperatures[11]	all the fish samples are bought from the fish store and ensured that they are brought in fresh, that they were collected in max 5 days of time prior to selling
sample place	the place where the sample is taken, the part of the body of the fish, can affect the results as different parts of the body can have different accumulation[12]	the samples are all taken from the back myomeres (body muscles) near the caudal fin (tail)
mass of samples	the mass of the samples may affect the result by increasing the chance for error in the estimation of the results of ICP-MS, as the method converts the concentration in mg/kg	the samples are collected in exact masses of 5.00 g
size of selected fish	the size of the fish affects how much bioaccumulation occurs, with a strong	the fish are each be selected to match the average length of its

[11] (n.d.). *Freezing and refrigerated storage in fisheries - 2.Influence of temperature*. www.fao.org. https://www.fao.org/3/v3630e/v3630e03.htm

[12] Varol, M., Kaçar, E., & Akın, H. K. (2020). Accumulation of trace elements in muscle, gill and liver of fish species (Capoeta umbla and Luciobarbus mystaceus) in the Tigris River (Turkey), and health risk assessment, Environmental Research. https://doi.org/10.1016/j.envres.2020.109570

	positive correlation between the accumulation amount and the length of the fish[13]	species, to make an applicable conclusion
season/period of catching	the season, such as winter and summer, affects which heavy metal(s) would be more present. This variation is primarily due to the chemical properties of the metals.[14]	all fish is collected in mid-November, winter season
trophic levels of fish species	the trophic level of a species affects biomagnification, higher levels show more significant take-up of toxic materials with more complex food sources[15]	selected species are in the 4th trophic level, as researched beforehand
fishing method	wild fish and farm-raised fish differ in their diets, with wild fish having a more diverse one. This gives rise to the varying of sources for heavy metal consumption of organisms.	the samples are all wild-caught

2.2 Experimental method

There are various methods used to detect metals and their levels in marine animals, such as Flame Atomic Absorption Spectrometric (FAAS) Electro-Thermal Evaporation Inductively Coupled Plasma Mass Spectrometry (ETV-ID-ICP-MS) or Inductively Coupled Plasma Optical Spectrometry (ICP-OES). Yet, Inductively Coupled Plasma Mass Spectrometry method (ICP-MS) has become much more common in food chemical analysis. This method has some advantages, compared to the others, like simultaneous multielement measurement capability. Additionally, this technique offers a larger linear dynamic range, simplifies spectral interpretation, and provides isotopic data; allowing for interpretations and detection of errors.[16]

2.3 Justification and Risk Assessment

The most prominent risk involved in this research is the interaction with hazardous heavy metals. However, the investigations are conducted in a proper lab environment (in the GOP branch of Düzen Laboratory Group in Ankara) and with appropriate precautions. It should also be taken into account that the experiment is not conducted by myself but rather by an experienced senior chemist. Another risk is the handling of organism based investigations, however it is eliminated by the fact that the organisms are not living and rather decapitated

---

[13] Sackett, Dana. "The Influence of Fish Length on Tissue Mercury Dynamics: Implications for Natural Resource Management and Human Health Risk." *Int J Environ Res Public Health*, 2013, https://doi.org/10.3390/ijerph10020638.

[14] Fallah, A. A. (2011). Seasonal bioaccumulation of toxic trace elements in economically important fish species from the Caspian Sea using GFAAS. *Journal für Verbraucherschutz und Lebensmittelsicherheit*. https://doi.org/10.1007/s00003-011-0666-7

[15] Drouillard, K G. Biomagnification. 2nd ed., vol. 1, Encyclopedia of Ecology, 2008, https://doi.org/10.1016/B978-0-444-63768-0.00377-2. pp. 353-358.

[16] KUPLULU, O., IPLIKCIOGLU CIL, G., KORKMAZ, S., AYKUT, O., & OZANSOY, G. (2018). Determination of Metal Contamination in Seafood from the Black, Marmara, Aegean and Mediterranean Sea Metal Contamination in Seafood. Journal of the Hellenic Veterinary Medical Society, 69(1), 749-758. doi:http://dx.doi.org/10.12681/jhvms.16400

beforehand by a professional. The dealing with spectrometry processes is a risk that can be ignored, since the machine operating is designed to not harm any individual in terms of radiation.

The fish species chosen for sampling are based on the accessibility of them, while also relying on their similarities so that there also is not different variables not considered. The three species are chosen from higher trophic levels within their ecosystem, live in around the same depths and bottoms, while having somewhat similar of diets. All the species are ensured to be wild-caught commonly by fishers, so that the effect of heavy metals reach the consumers and affect the general health of a population.

The three heavy metals chosen for investigation, Cd, Hg and Pb, are based on the research [2,3,5] which show that they are the most prominent metals in fish and the biggest toxicants.

The handling of the discards of experimented fish is an environmental issue, while around 5 grams of one fish will be used for experimentation, it is unavoidable to discard the rest of the fish, as it might pose a health risk to consume food that has been used in a lab. Of course, as these discards are organic, they will be added to a compost to make use for agricultural processes of small extent.

Chapter III: Experimentation

3.1 Sample collection

For this investigation, different predator fish species were obtained from the Black, Mediterranean and Aegean Sea seacoasts. The selected species are as follows: *Sparus aurata* from the Aegean Sea, *Merluccius merluccius* from the east Mediterranean sea, and *Merlangius merlangus* from the southern Black Sea. One kilogram from each fish species were purchased from three different fish markets, all selling species caught from only one region. All the samples were purchased during the same fishing season, the winter season. Samples were then washed in clean water and 5g of the muscles of each sample were homogenized. The samples were stored in -18°C.

3.2 Standards and reagents

Every reagent was of the analytical reagent standard. All dilutions were carried out using ultra-pure water. Nitric acid had a concentration of at least 64% and a density of about 1,5 g/mL. For the calibrations, Merck Millipore's element standard solutions were utilized, and they were made by diluting mg/L stock solutions.

3.3 Standard preparation

The stock solutions contained 10 mg/L of Cd, and 10 mg/L of Pb. In a 100 ml volumetric flask, 1 mL of mercury and 1 mL of nitric acid were diluted to create a stock solution.

3.4 Extraction and clean up

The Nordic Committee on Food Analyses No. 186 2007 technique for heavy metal analysis was used (Anon, 2007). All of the plastics and glassware were left in 10% (v/v) nitric acid overnight. It was rinsed with distilled water and deionized water before usage, then it was dried. Using a stainless steel knife, boneless muscle tissues were removed, together with the skin in the case of fish samples, and were then subjected to a vigorous acid digestion. 2 aliquots of 1 g of the homogenized specimen from each individual sample were taken for extraction.

The samples were extracted with a microwave technique. 5 ml of 64% nitric acid and 5 ml of ultra-pure water were combined to create a digestion solution, and samples were digested in this solution for 10 minutes at 600 W of electricity. Ramp time was 10 minutes at 180 °C and 450 psi of pressure. Then, for the ICP-MS quantification, the contents were decanted into falcon tubes and 50 ml of ultra-pure water was added. Three different metal solution concentrations were employed for the calibration. Cd, Hg, and Pb were measured at 0.5 g/L, 2.5 g/L, and 10 g/L. During the test of mercury, gold was added in order to stabilize the Hg. Analytical blanks were run in the same way.

3.5 ICP-MS operation

Parameter	Setting
RF power	1500W
Carrier gas flow	1.2 min^{-1}
Plasma gas flow	15 min^{-1}
Auxiliary gas flow	1.0 min^{-1}
Lens voltage	4.5 V
Mass resolution	0.8
Integration time	3 points/ms
Points per peak	3
Replicates	3
Nebuliser	Babbington
Spray chamber	Water cooled double pass
Spray chamber temperature	2°C

Table 1: Settings for ICP-MS instrument

The mass range of the inductively coupled argon plasma mass spectrometer was 5 to 240 AMU. Table 1 provides an illustration of the ICP-configurations. The calibration's recovery was within 10% of the initial value. Table 2 is a list of the limits of detection (LOD) and limits of quantification (LOQ) for instruments. The detection limit is calculated using the three times standard deviation of the blank solution. The test solutions were examined once the device had been calibrated. To prevent interference from highly concentrated matrix constituents, the samples obtained by pressure digestion were diluted before measurement. The blank solution and one calibration solution were routinely examined at suitable short intervals. The concentration was calculated automatically by the ICP-MS instrument itself.[16]

Metal	LOD (µg/g)	LOQ (mg/kg)
Pb	0.3	0.0013
Hg	1	0.047
Cd	0.5	0.033

Table 2: Instrumental detection limits and limits of quantification

Chapter IV: Results and analysis

4.1 Results

The species samples were each tested three times to ensure the results' accuracy and prepare data from the mean of these results. The raw data is stored in Table 3. Alongside the

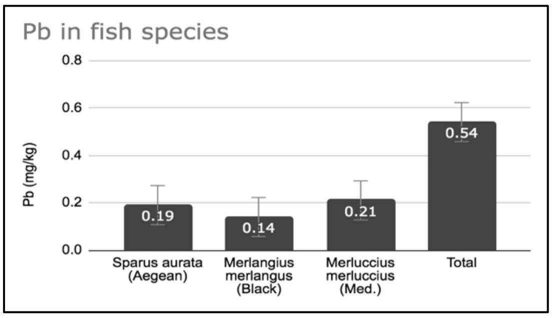
	Cd [No Gas]	Hg [No Gas]	Pb [No Gas]	Cd [No Gas]	Hg [No Gas]	Pb [No Gas]
	Conc. [ug/l]	Conc. [ug/l]	Conc. [ug/l]	mg/kg	mg/kg	mg/kg
Sparus aurata-1	0,03	7,58	3,40	0,002	0,379	0,170
Sparus aurata-2	0,02	3,52	4,41	0,001	0,176	0,220
Sparus aurata-3	0,04	4,00	3,90	0,002	0,200	0,195
Merlangius merlangus-1	0,01	6,68	2,82	0,001	0,334	0,141
Merlangius merlangus-2	0,01	5,90	6,75	0,000	0,295	0,338
Merlangius merlangus-3	0,01	4,70	2,83	0,000	0,235	0,141
Merluccius merluccius-1	0,03	4,10	4,36	0,001	0,205	0,218
Merluccius merluccius-2	0,03	3,41	3,18	0,001	0,170	0,159
Merluccius merluccius-3	0,03	3,55	3,96	0,001	0,178	0,198

Table 3: Concentrations of every heavy metal (in micrograms per liter and milligrams in kilogram) in each fish species, three trials.

raw data table with all trials, Tables 4, 5 and 6 show the mean data of the presence of each heavy metal according to species and graphs 1, 2 and 3 will be useful for the comparison of one heavy metal abundance in each species.

	Pb (mg/kg)
Sparus aurata (Aegean)	0.19
Merlangius merlangus (Black)	0.14
Merluccius merluccius (Med.)	0.21
Standard deviation	0.0823
Total	0.6223

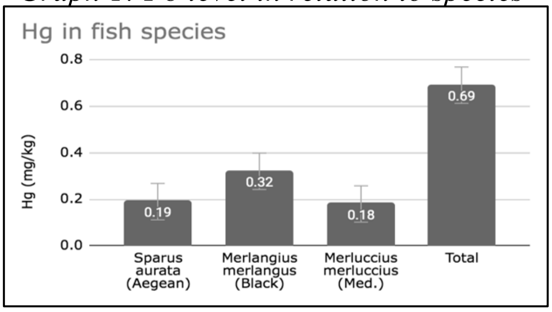
Table 4: Mean concentrations of Pb in species



Graph 1: Pb level in relation to species

	Hg (mg/kg)
Sparus aurata (Aegean)	0.19
Merlangius merlangus (Black)	0.32
Merluccius merluccius (Med.)	0.18
Standard deviation	0.078
Total	0.768

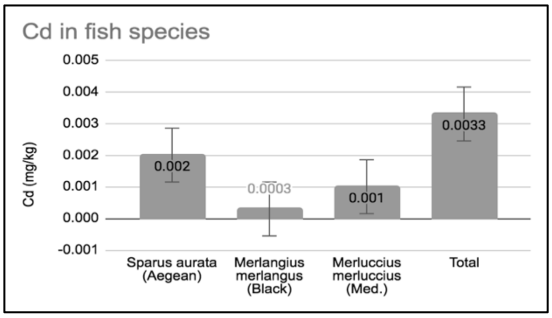
Table 5: Mean concentrations of Hg in species



Graph 2: Hg level in relation to species

	Cd (mg/kg)
Sparus aurata (Aegean)	0.002
Merlangius merlangus (Black)	0.0003
Merluccius merluccius (Med.)	0.001
Standard deviation	0.00085
Total	0.00415

Table 6: Mean concentrations of Cd in species



Graph 3: Cd level in relation to species

The error bars in the graphs are drawn according to the individual standard deviations of each species.

Of these results, it was found that the total concentrations of heavy metals were Hg>Pb>Cd and Cd showed very little presence, below the method detection limit (<0.002), for all species. Hg has been detected to be 0.69 mg/kg and Pb to be 0.54 mg/kg in total. Statistics of Hg showed the highest standard deviation of 0.082 and Pb came second with 0.078. Means of heavy metal levels are 0.18 for Pb, 0.23 for Hg and 0.0011 for Cd.

The highest level of Pb was seen in *Merluccius merluccius* with a fluctuation of 0.3 from the mean level, of Hg in *Merlangius merlangus* but with a 0.9 difference from the mean, and of Cd in *Sparus aurata* with 0.0008 difference.

Sparus aurata was found to have near-average Pb and Hg levels detected (with 0.1 and 0.4 difference from mean), while for Cd it was the highest among species. *Merlangius merlangus* was the species with the most fluctuating levels of each metal. It showed the highest level of Hg with 0.32 mg/kg, lowest level of Cd and Pb with 0.0003 and 0.14 mg/kg respectively.

Merluccius merluccius was the species with the second least fluctuation in heavy metal levels, yet it had the least Hg and the highest Pb level.

Chapter V: Discussion and Conclusion

5.1 Discussion

Investigating the relative concentrations of heavy metals in marine life allows for the observation of how bioaccumulation works, how coastal natural and anthropogenic activities affects the marine composition and to assess the health concerns caused by the consumption of marine life.

In this study, the muscles of the fish were studied, which normally are not the main area of bioaccumulation, yet were chosen since they are the edible parts. Pb, Hg and Cd were all detected in all species, yet Cd was further below the limits of detection, meaning it does not show a major bioaccumulation. Many studies report Cd as the lowest contaminant, which is favorable since it is the most dangerous for human health. Cd mostly enters the aquatic environment through atmospheric deposition and industrial discharges. Compared to other heavy metals, cadmium and its derivatives are relatively water soluble, which increases their bioavailability and bioaccumulative potential.[17] However, relating to the findings of this study, it can be said that no or very little cadmium is released to the marine environment of any sea. This means, that the wastages from industries near coasts are taken care of and processed properly without a deposition to the nearby sea.[18]

When it comes to Pb, the species *Merluccius merluccius* had the most detected Pb, with 0.21 mg/kg and 0.3 higher than the mean. This species lives in the inner shores of the Mediterranean and feeds on nektons, which means that they are predators. Since their trophic level is the highest among the studied species, with 4.5, the high Pb level can be explained with the fact that this species simply consumes biomagnified Pb from other marine life. However, the Mediterranean sea is an almost closed basin with many densely populated and industrialized countries surrounding it, and it is subjected to a constant rate of pollution with toxic compounds including 3800 tons of lead dumped into the sea each year. Yet, 70 percent of wastewater is left untreated.[19] Meaning that the amount of Pb present even in low trophic levels are significant, and the diet consisting of other fish adds up to this bioaccumulation in the species *Merluccius merluccius.*

Hg is known to be distributed to a marine life through the burning of coal and then precipitation of its particles onto the surface of the water body. Mercury is later converted into methyl-mercury with the help of bacteria in the marine ecosystem, and this compound gets into the bodies of fish living within the ecosystem.[15] Hg was found to have its highest amount in the species sample *Merlangius merlangus* and within the Black Sea. The concentration was detected to be 0.32 mg/kg, 0.9 higher than the mean of 0.23 mg/kg. This result can be linked to several reasons, including the properties of the sea and the discharging from coastal cities. The

[17] Ansari, T. M., Marr, I. L., & Tariq, N. (2004). Heavy metals in marine pollution perspective - a mini review. *Journal of Applied Sciences*, *4*(1), 5-6.

[18] Neff, Jerry. "Cadmium in the Ocean." *Bioaccumulation in Marine Organisms (pp.89-102)*, 2002, https://doi.org/10.1016/B978-008043716-3/50006-3. Accessed 19 Jan. 2023.

[19] *Toxics* 2014, *2*(3), 417-442; https://doi.org/10.3390/toxics2030417
Received: 3 March 2014 / Revised: 21 July 2014 / Accepted: 6 August 2014 / Published: 18 August 2014

Black Sea does not have the same ability for filtration as the open oceans because it is semi-enclosed and frequently experiences poor rates of water renewal. Up until recently, there were multiple instances where industrial and municipal discharges, including direct dumping from ships and agricultural run-off, had brought about ecological calamity. Because of this poor water renewal, the sea is especially prone to contaminants that accumulate without a degradation process. On top of this vulnerability, many rivers that carry contaminants from inlands flow into this sea, including Danube, Dnieper, Dniester, Kizilirmak, Yesilirmak and Sakarya. These rivers also cause low salinity since they constantly bring freshwater. According to studies conducted in the Black Sea, industrial discharges, human inputs, and sewer wastes directly enter rivers and the sea as a whole. Of all rivers, Danube, Dnieper and Dniester are the ones that contribute to the pollution the most. The increase in metals in the Black Sea has been attributed in large part to waste from European nations transported by the Danube and pollution transported by rivers passing through Russia and Ukraine to the Black Sea.[20]

This experiment and analysis circle around organisms in the 4th trophic level, so to reach a balanced conclusion it is only reasonable to also shed light on the heavy metal contamination and bioaccumulation in low trophic levels. Although plants can accumulate significant quantities of metals, species at lower trophic levels are generally exposed to less pollution.[21] Yet, fish at the top of the food chain are more likely to accumulate metals and contaminate humans through their diet, which can lead to chronic and acute disorders.[22] Therefore, some actions to be less exposed to heavy metals as consumers include preferring fish with the prey role in its food web, fish that has an overall diet of rock-inhabited plants that do not pick up on the sediments on the bottom and choosing fish markets that provide a proper cleaning of organs that store toxicants.

Heavy metal contamination is not only impacting fish, but also elements such as water composition, dissolved oxygen in aquatic environments. Groundwater movements leads to the further spreading of contamination to terrestrial ecosystems. The buildup of heavy metals in soil and plants negatively affects their physiological processes like photosynthesis, gas exchange and nutrient uptake, which reduces plant development and causes the accumulation of dry matter.[23]

5.2 Evaluation

This study sheds light to the topic of heavy metal bioaccumulation in predator fish species living in the different seas of Turkey, and how natural and anthropogenic elements affect it. It ensured that the Black Sea and the Mediterranean Sea is highly contaminated and prone to further contamination with heavy coastal industrial processes. It is ever more important now, to enforce the laws on filtering and waste disposal in the coastal cities, so that the seas

---

[20] Bat, L., Öztekin, A., Şahin, F., Arıcı, E., Özsandıkçı, U. (2018). An overview of the Black Sea pollution in Turkey. MedFAR., 1(2):67-86

[21] Peakall, D., & Burger, J. (2003). Methodologies for assessing exposure to metals: Speciation, bioavailability of metals, and ecological host factors. Ecotoxicology and Environmental Safety, 56, 110–121.

[22] Has-Schön, E., Bogut, I., & Strelec, I. (2006). Heavy metal profile in five fish species included in human diet, domiciled in the end flow of river Neretva (Croatia). Archives of Environmental Contamination and Toxicology, 50, 545–551.

[23] Nyiramigisha, P. "Harmful Impacts of Heavy Metal Contamination in the Soil and Crops Grown Around Dumpsites." Reviews in Agricultural Science, vol. 9, 2021, pp. 271-282, https://doi.org/10.7831/ras.9.0_271. Accessed 20 Mar. 2023.

Turkey profits the most with fishing has a sustainable income. A further study based on this investigation may be the testing of species in different trophic levels in the same food web. This study will be helpful in determining the accumulation of heavy metals in the biotic and abiotic environment and how the aquatic accumulation affects the ecosystem as a whole.

ICP-MS testing has been useful for obtaining the concentrations of each heavy metal in each species samples, and in a simple format to discuss. The method for experimentation (ICP-MS) has been reported to be the most optimal for heavy metal testing within fish, and it is approved of by many other research done within environmental and ecological studies. There are some reasons to doubt the findings however, since the scope of testing was not as wide as similar research, with only one species chosen from each marine ecosystem, and three species in total. This choice may have added a limitation concerning the actual bioaccumulation within a sea. Species living on the bottom sediments, which are widely known to have exceedingly high levels of heavy metal bioaccumulation compared to fish, were not chosen to be tested nor compared with fish. This conscious avoidance may have caused a bias as well.

The limitations include the limited budget put into the testing. The budget only allowed for the testing of three samples and for 3 elements, thus more sampling and more data was not accessed, limiting the scope and the accuracy of the investigation.

A direct testing of the abiotic elements of the aquatic environments would give reliable data of the composition of water and thus the accumulation of the tested heavy metals. This additional data would allow for more interpretation on the results, since the ecosystems would be discussed both biotically and abiotically.

5.3 Conclusion

The results and discussions of this study revealed that heavy metal bioaccumulation show considerable variations between species based on their origins, diet and roles within their food web. This variation is abiotically caused by the different activities done on the coasts of different seas, as well as natural properties such as river flows and salinity.

References

[1] Huang, H., Li, Y., Zheng, X., Wang, Z., Wang, Z., & Cheng, X. (2022). Nutritional value and bioaccumulation of heavy metals in nine commercial fish species from Dachen Fishing Ground, East China Sea. Scientific reports, 12(1), 6927. https://doi.org/10.1038/s41598-022-10975-6

[2] Baki, A. M. et al. Concentration of heavy metals in seafood (fishes, shrimp, lobster and crabs) and human health assessment in Saint Martin Island, Bangladesh. Ecotoxicol. Environ. Saf. 159, 153–163 (2018).

[3] Koch, W., Czop, M., Iłowiecka, K., Nawrocka, A., & Wiącek, D. (2022). Dietary Intake of Toxic Heavy Metals with Major Groups of Food Products-Results of Analytical Determinations. Nutrients, 14(8), 1626. https://doi.org/10.3390/nu14081626

[4] Latif, M., Zahoor, M., Muhammad, A., Naz, S., Kamran, A. W., Ullah, R., Shah, A. B., Almeer, R., & Sayed, A. (2022). Bioaccumulation of lead in different organs of Ctenopharyngodon Idella (grass fish) and Tor putitora (Mahseer) fish. Brazilian journal of biology = Revista brasleira de biologia, 84, e260355. https://doi.org/10.1590/1519-6984.260355

[5] Saha, N., Mollah, M., Alam, M. F. & Rahman, M. S. Seasonal investigation of heavy metals in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment. Food Control 70, 110–118 (2016)

[6] N.A., (2017), Mercury and Health, https://www.who.int/news-room/fact-sheets/detail/mercury-and-health#:~:text=Neurological%20and%20behavioural%20disorders%20may,and%20cognitive%20and%20motor%20dysfunction.

[7] Murray, JW; Jannasch, HW; Honjo, S.; Anderson, RF; Reeburgh, WS; Top, Z., et al. (1989). Unexpected changes in the oxic/anoxic interface in the Black Sea. *Nature*, 338(6214), 411-413. http://dx.doi.org/10.1038/338411a0 Retrieved from https://escholarship.org/uc/item/7xg3p017

[8] Proskurnin, V. Y., Tereshchenko, N. N., Paraskiv, A. A., & Chuzhikova-Proskurnina, O. D. (2021). Plutonium and americium in the deep Black Sea bottom sediments. Journal of environmental radioactivity, 229-230, 106540. https://doi.org/10.1016/j.jenvrad.2021.106540

[9] "Aegean Sea | Mediterranean Sea". Encyclopedia Britannica. Retrieved 17 January 2023

[10] "Marmara, Sea of ." The Columbia Encyclopedia, 6th ed.. . Retrieved September 23, 2022 from Encyclopedia.com: https://www.encyclopedia.com/reference/encyclopedias-almanacs-transcripts-and-maps/marmara-sea

[11] (n.d.). *Freezing and refrigerated storage in fisheries - 2.Influence of temperature*. www.fao.org. https://www.fao.org/3/v3630e/v3630e03.htm

[12] Varol, M., Kaçar, E., & Akın, H. K. (2020). Accumulation of trace elements in muscle, gill and liver of fish species (Capoeta umbla and Luciobarbus mystaceus) in the Tigris River (Turkey), and health risk assessment, *Environmental Research*. https://doi.org/10.1016/j.envres.2020.109570\

[13] Sackett, Dana. "The Influence of Fish Length on Tissue Mercury Dynamics: Implications for Natural Resource Management and Human Health Risk." *Int J Environ Res Public Health*, 2013, https://doi.org/10.3390/ijerph10020638.
[14] Fallah, A. A. (2011). Seasonal bioaccumulation of toxic trace elements in economically important fish species from the Caspian Sea using GFAAS. *Journal für Verbraucherschutz und Lebensmittelsicherheit*. https://doi.org/10.1007/s00003-011-0666-7
[15] Drouillard, K G. Biomagnification. 2nd ed., vol. 1, Encyclopedia of Ecology, 2008, https://doi.org/10.1016/B978-0-444-63768-0.00377-2. pp. 353-358.
[16] KUPLULU, O., IPLIKCIOGLU CIL, G., KORKMAZ, S., AYKUT, O., & OZANSOY, G. (2018). Determination of Metal Contamination in Seafood from the Black, Marmara, Aegean and Mediterranean Sea Metal Contamination in Seafood. Journal of the Hellenic Veterinary Medical Society, 69(1), 749-758. doi:http://dx.doi.org/10.12681/jhvms.16400
[17] Ansari, T. M., Marr, I. L., & Tariq, N. (2004). Heavy metals in marine pollution perspective - a mini review. Journal of Applied Sciences, 4(1), 5-6.
[18] Neff, Jerry. "Cadmium in the Ocean." *Bioaccumulation in Marine Organisms (pp.89-102)*, 2002, https://doi.org/10.1016/B978-008043716-3/50006-3. Accessed 19 Jan. 2023.
[19] Toxics 2014, 2(3), 417-442; https://doi.org/10.3390/toxics2030417 Received: 3 March 2014 / Revised: 21 July 2014 / Accepted: 6 August 2014 / Published: 18 August 2014
[20] Bat, L., Öztekin, A., Şahin, F., Arıcı, E., Özsandıkçı, U. (2018). An overview of the Black Sea pollution in Turkey. MedFAR., 1(2):67-86
[21] Peakall, D., & Burger, J. (2003). Methodologies for assessing exposure to metals: Speciation, bioavailability of metals, and ecological host factors. Ecotoxicology and Environmental Safety, 56, 110–121.
[22] Has-Schön, E., Bogut, I., & Strelec, I. (2006). Heavy metal profile in five fish species included in human diet, domiciled in the end flow of river Neretva (Croatia). Archives of Environmental Contamination and Toxicology, 50, 545–551.