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Bioaccumulation of Heavy Metals in Cetaceans and Fish from the Bulgarian Black Sea Coast

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Abstract

The accumulation of heavy metals in animal tissues is an important factor in monitoring the health of any marine ecosystem, and the Black Sea is no exception. The use of aquatic organisms as bioindicators of heavy metal pollution is common in studies, and molluscs and fish are some of the most used indicators, unlike cetaceans, which are not common. The objective of the present study is to determine the accumulation of five heavy metals (Pb, Cd, Zn, Cu, Ni) in the living tissues of three cetacean species, six fish species part of the cetacean diet, and seawater of the Bulgarian Black Sea coast. Heavy metal concentrations were analyzed using an Atomic Absorption Spectrophotometry and the results were used to calculate the bioaccumulation factor (BCF) and the biomagnification factor (BMF). The results are analyzed in a comparative plan between the northern and southern part of the Bulgarian coast. In all biota samples, the concentration of Zn was the highest. No statistical differences were found between fish and cetacean samples from the two study areas. Cetaceans have a higher BCF for Cd, while fish for Pb, and the BMF for cetaceans was confirmed for the elements Cd and Zn.

Introduction

Pollution of seas and oceans with heavy metals is a global problem and the Black Sea is no exception. The hydrobiological characteristics of the semi-enclosed sea make it extremely sensitive to threats, as many pollutants, including heavy metals, come from large rivers, coastal activities such as agriculture, tourism and factories that discharge wastewater with varying degrees of treatment directly into the sea (Jitar et al., 2013). In addition, the ongoing military conflict between Russia and Ukraine appears to be another source of pollution in the Black Sea in the past few years (Radulescu, 2023; Shukla et al., 2023).

Heavy metal pollution can have devastating effects on the ecological balance of the marine environment, as they can be easily assimilated and accumulated in the food webs (Copat et al., 2013; Jitar et al., 2013, Jakimska et al., 2011; Yoshino et al., 2020), and the greatest concern being in the upper levels of the food chain for the top predators, such as cetaceans. Accumulation of heavy metals is a phenomenon that has been documented for many cetacean species (Law, 1994; Delgado-Suarez et al., 2023) and it has been shown heavy metals can seriously affect the physiological activities and biochemical parameters of the organism (Boon et al., 1994; Gerpe et al., 2002; Jaishankar et al., 2014 et al., Kehringet et al., 2016; Xiong et al., 2019;

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Garcia-Cegarra et al., 2020). However, it should be considered that the bioaccumulation of heavy metals often affected by numerous factors, as a environment condition, species, sex, age, breeding cycle, tissues, diet and geographical location (Bossart, 2011; Kehring et al., 2016). Their trophodynamics also differ across food webs, such as concentrations of some heavy metals such as Cd and Pb can be biologically amplified, but others not (Wei et al., 2016). So, in these cases, the use of biomagnification factor (BMF) can be a good criterion to assess the ecological risk of pollutants (Tao et al., 2012; Ali & Khan, 2019).

Over the past few decades, the Black Sea ecosystem has registered major changes caused by anthropogenic factors and pollutants, so many fish species have declined dramatically. It is assumed that the reduction of fish resources leads to a decrease in the availability of prey and has a strong impact on cetacean populations (Bushuyev, 2000). Fish is a major food resource from the sea and often is subject of research for the accumulation of various heavy metals (Stancheva et al., 2010; Elnabris et al., 2013; Bat & Arici, 2016; Makedonski et al., 2020). Bioaccumulation of metals in the Black Sea food chain has been also reported for other organisms, such as algae and molluscs (Jitar et al., 2015), but there are no such reports for cetaceans and their role as bioindicators. This type of research is a few for the entire Black Sea (Das et al., 2004; Evtimova et al., 2019), and toxicological data on cetaceans from the area are scarce or even non-existent.

For object of the present study, the three cetaceans living in the Black Sea were selected - Black Sea porpoise (*Phocoena phocoena* ssp. *relicta* Abel, 1905), Black Sea common dolphin (*Delphinus delphis* ssp. *ponticus* Barabasch-Nikiforov, 1935) and Black Sea bottlenose dolphin (*Tursiops truncatus* ssp. *ponticus* Barabash-Nikiforov, 1940), due to their place in the food chain and lack of toxicological data for these species. The aim of this research was to study the presence and accumulation of five heavy metals (lead – Pb, cadmium – Cd, – zinc – Zn, copper – Cu and nickel – Ni) in the cetaceans along the Bulgarian Black Sea coast. Also, the accumulation and biomagnification of heavy metals from water into the tissues of the cetaceans and six fish species, included in their diet.

Materials and Methods

Study Area

The present study was conducted along the Bulgarian Black Sea coast. Fish species and sea water were sampling in two points - one located on the Northern Black Sea coast, in the area of Cape Kaliakra (43°22'47"N 28°28'21"E) and the other one on southern coast, in the area of the town of Tsarevo (42°10'23"N 27°51'46"E). Both points are located about 200 meters from the coast (Figure 1), and were chosen due to increased presence of fish and cetaceans in these coastal area.

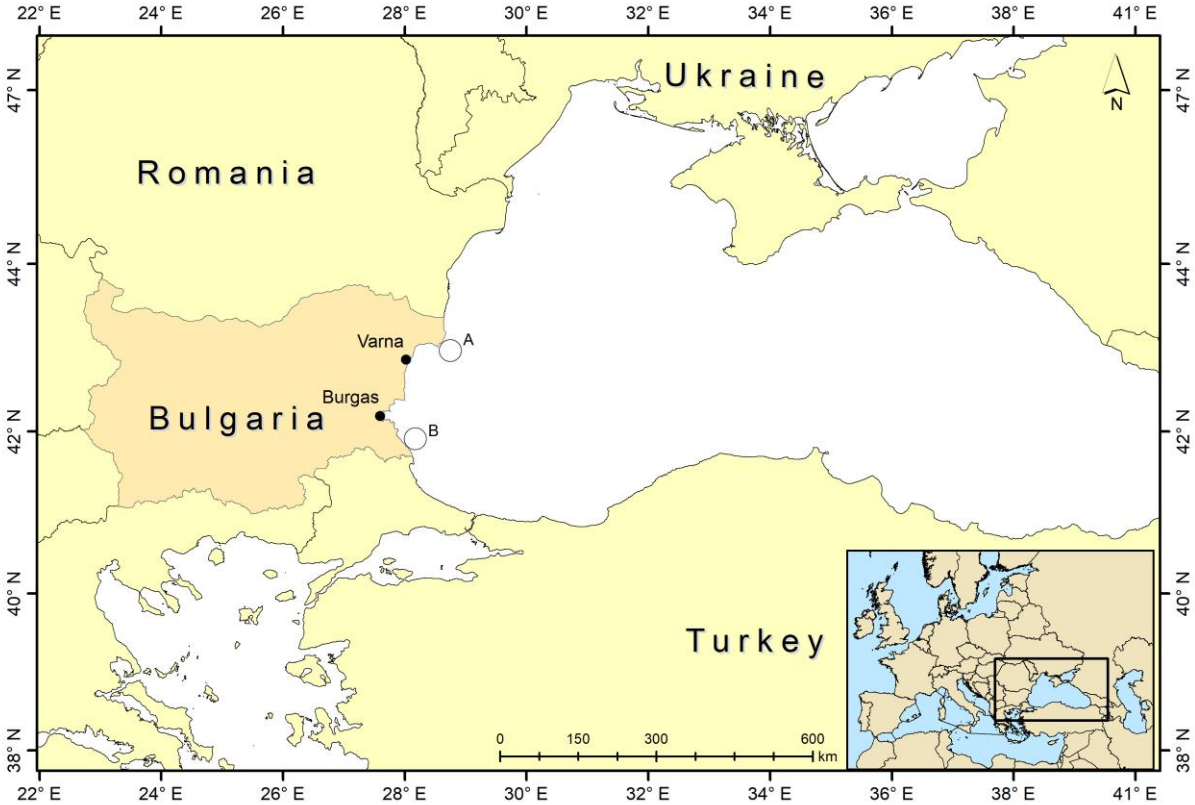


Figure 1. Study area: **A**: Northern fish and water sampling site; **B**: South fish and water sampling site on the Bulgarian Black Sea coast.

Data Collection

A total of 38 samples (n=38) were analysed in the present study - 9 (n=9) samples from cetaceans - 25 (n=25) from fish, and 4 (n=4) seawater. The data were collected from fieldwork between November 2022 and July 2023. Three approaches were used to collect samples of cetaceans, fish and water along the Bulgarian coast.

Cetacean's Samples

The samples were collected by the transect method along previously selected parts of the coastline, located north of Varna and south of Burgas. For the purposes of the study, mostly extensive sandy beaches were selected. Only bone samples were collected, as all cetacean carcasses found precluded the collection of other tissue samples due to their advanced stages of decomposition. For the same reason, the body length of cetaceans was not measured (in some cases body parts are missing), but it should be noted that according to the visible remains, there are no new born individuals among them. All specimens were determined to species level according to "Guidelines for coordinated cetacean stranding response" (ACCOBAMS, Resolution 4.16).

Fish Samples

All fish species were caught by commercial fishing gear (gill nets and static nets) during November 2022 and March 2023. A total of 6 fish species, from the north and south area, were collected - 4 pelagic species Mediterranean horse mackerel (*Trachurus mediterraneus*), Atlantic bonito (*Sarda sarda*), Bluefish (*Pomatomus saltatrix*), European anchovy (*Engraulis encrasicolus*), and 2 bottom fish species - red mullet (*Mullus barbatus*), and family Gobies (Gobiidae). All specimens were determined to species level according to Karapetkova & Zhivkov (2010).

Water Sampling

The samples were taken from the surface water (at about 1 m depth) from the two survey points. The seawater samples was placed in glass bottles, stored in a refrigerated bag and sent for analysis to a licensed laboratory within 36 hours. A total 4 samples was collected - 2 from north and 2 from south. The results are expressed as mg/L.

Sampling Processing and Analysis

Each collected fish specimen was stored in clean plastic bag and freezed on −18°C. In the laboratory frozen fish samples were thawed at room temperature, and rinsed with distilled water to get rid of any trace metal residue on the external surface of the fish. It was prepared samples from muscles, bones and gills. Each tissue sample is prepare from multiple specimens (from several fish specimens).

Combined tissue samples were weighed and transferred to aluminum foil and processed by air-drying on 35°C for 36 hours. The cetacean's samples were stored in clean plastic bags (without preparations and chemicals) and then ground to a uniform mass weighing about 2 g.

About 1 gram of each biota sample is weighed, transferred to a flask for iodine number, moistened with 1-2 ml of bi distilled water and 10 ml of nitric acid and 5 ml of perchloric acid are added. The samples thus prepared are left in the cold for 24 hours to start the cold degradation process. After 24 hours, they are heated on a sand bath and evaporated to a moist residue. This wet residue is quantitatively transferred with 1N nitric acid in a test tube to a final volume of 10 ml. According to the same methods, a "zero" sample is prepared. The content of the pre-selected heavy metals was determined by ISP "OPTIMA 7000" Perkin-Elmer in the Atomic Absorption Spectrophotometry Laboratory. All the digested samples were analyzed three times for metals such as Cd, Cu and Pb using Atomic Absorption Spectrophotometer (AAS ZEEnit-700P). The results are expressed as a wet weight (ww).

Data Analysis

To estimate the concentration for each of the studied heavy metals in the samples the mean concentration (x) and the standard deviation (SD) were calculated. The test for normality was performed using *Kolmogorov–Smirnov test*, followed by comparison pair unpaired *t*-test, with which we evaluated the significance of the differences between heavy metals concentrations in the biota samples for the two monitored area - north and south coast. The *P*-value less than 0.05 was considered statistically significant ($P<0.05$). PAST statistical software was used for statistical analysis.

The bioaccumulation factor (BCF) is defined as:

$$\mathbf{BCF}_{water} = \mathbf{Cb}/\mathbf{C}_{water}$$

where **Cb** represents the heavy metal concentration in the living tissues and **C***water* represents the heavy metal concentrations in environment - water (Sijm & Hermens, 2000). We calculated BCF/*water* for fish and cetaceans. Bioaccumulation of heavy metals is confirmed when the 'Transfer Factor' (TF) is higher than **1** (Vrhovnik et al., 2013).

To calculate the extent of biomagnification in the food web, we used the biomagnification factor (BMF), which is described as:

$$\mathbf{BMF = CB/CD}$$

where **CB** is the chemical concentrations in the organism and the organism's diet **CD** - (Gobas & Morrison, 2000). The TF values **>1** indicate magnification.

Results

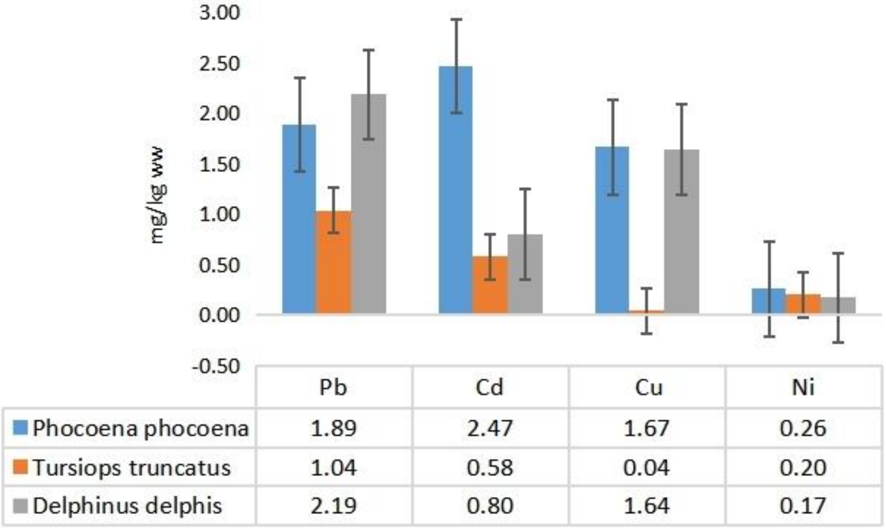
Heavy metal concentration in cetacean tissues

Four *Ph. phocoena,* three *D. delphis* and two *T. truncatus* individuals were found during the study period. The five study elements were found in all cetacean samples. The concentrations of Pb, Cd, Zn, Cu and Ni in the analyzed cetacean samples are presented in Appendix 1. Mean concentrations of heavy metals in different cetacean species are presented on Figure 2 and Figure 3.

The concentration of Cd, Cu and Ni was highest in *Ph. phocoena* and of Pb and Zn in *D. delphis.* The distribution of the average concentration of elements in cetaceans follows the following pattern: Zn > Pb > Cd > Cu > Ni. The metal concentration in samples from the two studied areas is shown in Figure 4. No significant difference was found between the concentration of the elements in the samples collected in the Northern and Southern Black Sea coasts (P>0.05), (Table 1).

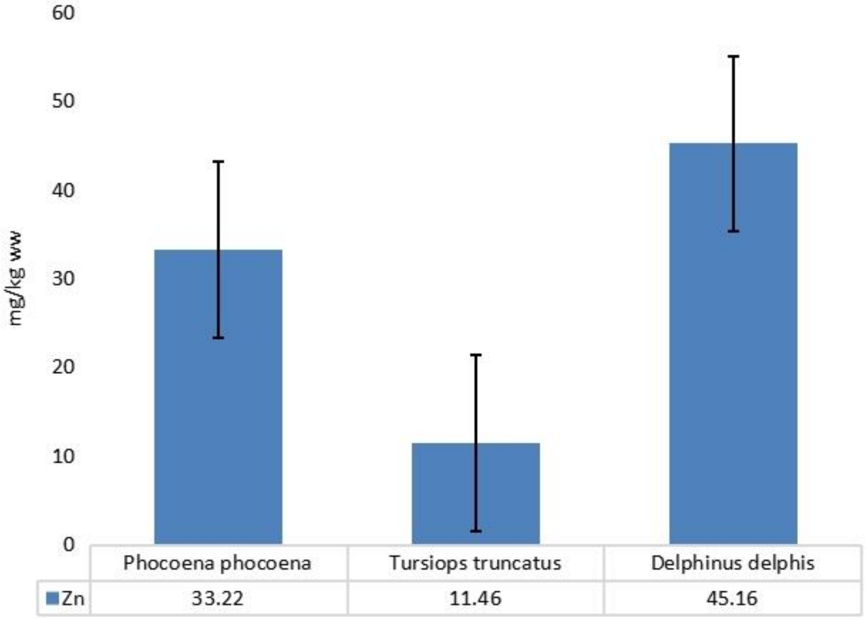
Heavy Metal Concentration in Fish Tissues

The results for the individual fish tissue samples are given in Appendix 2 and Appendix 2.1. The comparison between the two study area showed higher values of Zn and Cd in the samples from the Northern



	Pb	Cd	Cu	Ni
Phocoena phocoena	1.89	2.47	1.67	0.26
Tursiops truncatus	1.04	0.58	0.04	0.20
Delphinus delphis	2.19	0.80	1.64	0.17

Figure 2. Mean values and SD of heavy metals (Pb, Cd, Cu, Ni) concentrations in cetaceans samples by species (mg/kg ww).



	Phocoena phocoena	Tursiops truncatus	Delphinus delphis
Zn	33.22	11.46	45.16

Figure 3. Mean values and SD of heavy metals for Zn concentrations in cetaceans samples by species (mg/kg ww).

Black Sea coast (Figure 5), and the concentration values of Pb, Ni and Cu were higher in the samples from South. However, there was no statistically significant difference between concentration of any of the studied elements in the fish samples from the two studied area (P>0.05) (Table 1). A comparison was made between the concentrations of heavy metals in the different fish species, generally (from both regions) (Figure 6).

The presence of Pb and Cd was found in all studied fish species from the Bulgarian Black Sea, with Pb values from 0.77 to 24.74 mg/kg ww (maximum in *E. encrasicolus*). Regarding Cd, values is from 0.10 to 0.47 mg/kg ww, which exceeded the permissible norm and was the highest in the samples of gobies. The highest concentration of Ni was found in Gobiidae samples (0.50 mg/kg ww), and the lowest results in *M. barbatus* (0.17 mg/kg ww). Zinc was lowest in Gobiidae samples (0.89 mg/kg ww) and highest in *E. encrasicolus* (19.46 mg/kg ww). Regarding Cu, the highest concentration was observed in *T. mediterraneus* (2.41 mg/kg ww). The descending order of mean levels of elements in fish tissues was as follows: Zn > Pb > Cu > Ni > Cd.

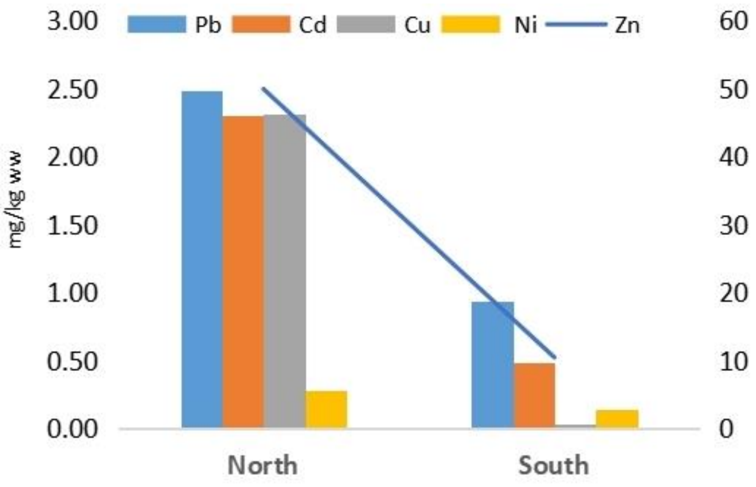


Figure 4. Mean heavy metals concentrations (Pb, Cd, Zn, Cu, Ni) in cetaceans samples (mg/kg ww) from the North and South Black Sea coast.

Table 1. A paired sample *t* test that was used to observe the significant differences in heavy metal concentrations in cetaceans and fish between two monitored area - North and South. The *P*-value less than 0.05 was considered statistically significant (P<0.05).

Samples	Pb	Cd	Zn	Cu	Ni
Cetaceans	*t*=1.244	*t*=0.937	*t*=0.307	*t*=0.906	*t*=0.068
	P=0.115	*P*=0.181	*P*=0.381	*P*=0.189	*P*=0.472
Fish	*t*=0.322	*t*=1.024	*t*=0.257	*t*=0.585	*t*=0.524
	P=0.374	*P*=0.158	*P*=0.399	*P*=0.308	*P*=0.302

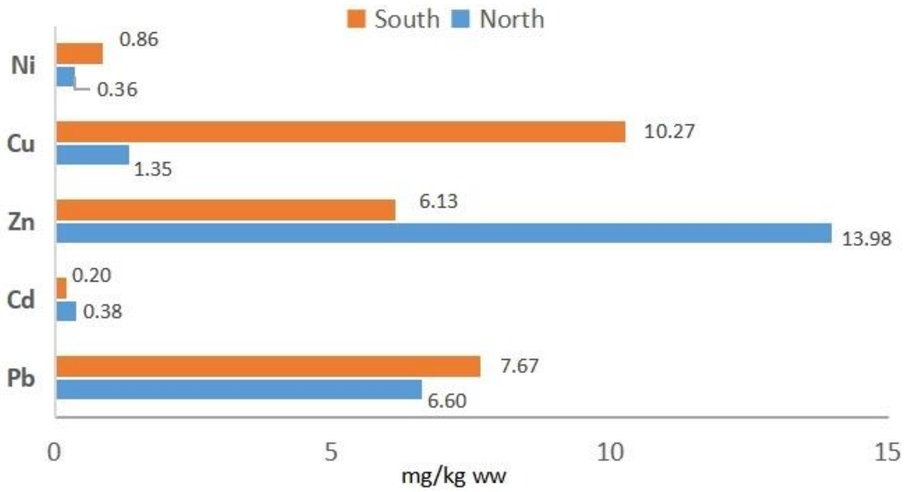


Figure 5. Mean concentrations of heavy metals (Pb, Cd, Zn, Cu, Ni) (mg/kg ww) in all fish species from the North and South Bulgarian Black Sea.

Heavy Metal Concentration in Seawater

The highest metal concentrations in seawater were found for Zn (0.032 mg/L) and the lowest of 0.00011 mg/L for Cd in the northern area (Table 2). According to the national legislation, for the mean concentrations determined for Pb, Cd, Zn, Cu, and Ni, in the seawater body, all value complies with norms.

Accumulation and Biomagnification

The BCF*water* value indicated that cetaceans accumulate Cd, Pb and Zn (TF>1), and fish accumulate Pb and Cd (TF>1), (Figure 7). Most of the studied elements show a tendency to accumulate from the environment in the biota.

The Biomagnification factor (BMF) results indicate biomagnification in all cetacean samples for the elements Cd and Zn (BMF for Cd=5.15, BMF Zn=3.22). In comparing the individual species BMF is positive for Cd, Zn and Cu in all three species. In *Ph. phocoena* BMF is hight for Cd and Cu, and at *D. delphis* for Zn (Figure 8).

Discussion

Environmental problems related to the pollution of the Black Sea are important not only for the Bulgarian coast, but also for all the countries along the Black Sea (Bat et al., 2018). The Bulgarian Black Sea is divided on northern and southern region by physical-geographical end climatic features (Penin, 2007), as well as by the seasonal distribution of fish species (Zlateva et al., 2022). In the southern region, tourism is more developed, various sources of pollution also come from the ports and the lack of a sufficiently well-built sewage network in small towns. In the north, agriculture predominates and different pollutants come from the infusion of one of Europe's largest rivers, the Danube, into the sea (Ministry of Agriculture Food and Forestry, 2020).

Bioaccumulation and long-term exposure to contaminants can be expected to pose a threat to organisms at the upper levels of the food chain, such as cetaceans (Das et al., 2004). However, the limited research on heavy metal accumulation in cetaceans in the Black Sea leaves many gaps on the subject, and only long-term studies would fill them. The current study presents new data on the accumulation of five heavy metals (Pb, Cd, Zn, Cu and Ni) in the bones of three cetacean species. The focus is on these five elements for several reasons: their frequent presence in many studies and the possibility of comparison, their role in animal metabolism and harmful effects (Çelik & Oehlenschläger, 2004; Delgado-Suarez et al., 2023). However, it should be noted that comparison of results often is difficult due to different sample processing and calculation methods. There are currently no established norms, levels or time periods for accumulation of heavy metals in cetaceans, and accumulation always depends on many factors (Bossart, 2011; Kehring et al., 2016).

The values obtained for the cetaceans (Appendix 1) exhibit a high degree of variability in metal accumulation, as a function of species (Frodello & Marchand, 2001). The concentration of the element Zn in bone tissue was the highest, and for Ni was the lowest in all studied samples. These results are consistent with

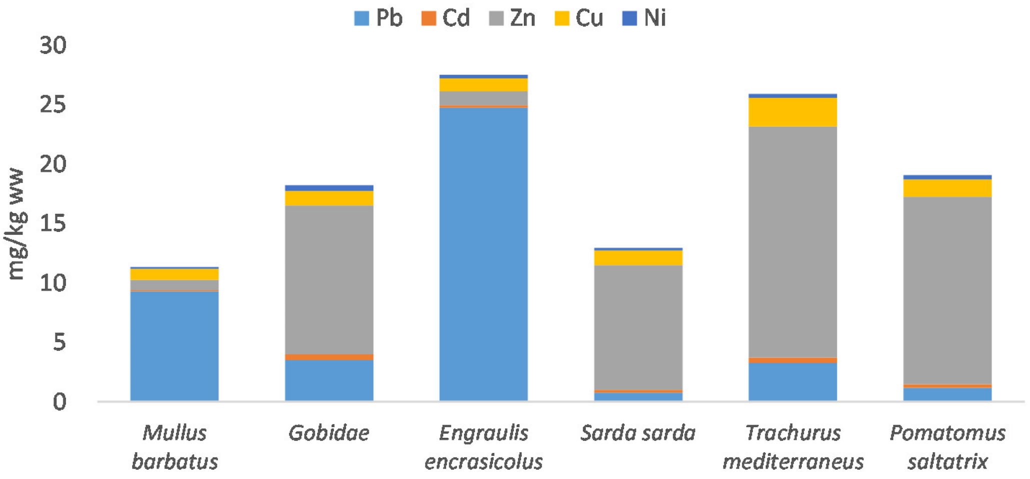


Figure 6. Mean concentrations of heavy metals (Pb, Cd, Zn, Cu, Ni) (mg/kg ww) by fish species (generally from both region).

Table 2. Heavy metal concentrations in seawater mg/L (mean) from Bulgarian Black Sea coast.

Sampling area	Pb	Cd	Zn	Cu	Ni
North	0.0014	0.00011	0.032	0.0037	0.0018
South	0.00088	0.00016	0.028	0.0038	0.0016

other studies where, for various cetacean species the highest concentrations were found for Zn and the lowest for Ni (Delgado-Suarez et al., 2023). Looking at individual species, Zn levels were highest in *D. delphis* and lowest for *T. truncatus*. The similar high levels of Zn have been observed for *D. delphis* from the coast of Portugal (Monteiro et al., 2016). This difference in zinc concentration between *D. delphis* and *T. truncatus* is likely due to species affiliation (Law, 1994) or some other factor that can not clarify without additional data of age, sex, etc. The concentration of Pb in the bones of *D. delphis* from the Mediterranean Sea is reported from 0.82 to 1.20 mg/kg and from 0.60 to 1.11 mg/kg for *T. truncatus* (Frodello & Marchand, 2001). In the present study, Pb concentrations showed higher values for *D. delphis* (2.19 mg/kg), and similar to those of *T. truncatus* from the Mediterranean - (1.04 mg/kg). The availability of Pb varies according to a number of environmental factors, as endogenous factors strongly infuence metal kinetics and effects (Foulkes, 1995). However, the presence of Pb and Cd is a cause for concern in any case, as even small concentrations can have negative consequences (Çelik & Oehlenschläger, 2004; Borrell et al., 2015).

The mean concentrations of metals determined in the samples of *Ph. phocoena* from the Bulgarian Black Sea coast in the present study are lower than those measured for the same species five years ago (Evtimova et al., 2019). Differences were observed for the elements Pb and Zn (Pb - 1.89 mg/kg in the present study and 13.8 mg/kg study five years ago) as well as for Zn (33.22 mg/kg and 294.8 mg/kg respectively). Differences in zinc levels between studies may be due to the fact that the 2017 sample consisted primarily of young and neonates individuals aged 0 to 3 years, while the present samples lacked neonates entirely. According to the literature, high concentrations of Zn are observed in young individuals and neonates (Wagemann et al.,

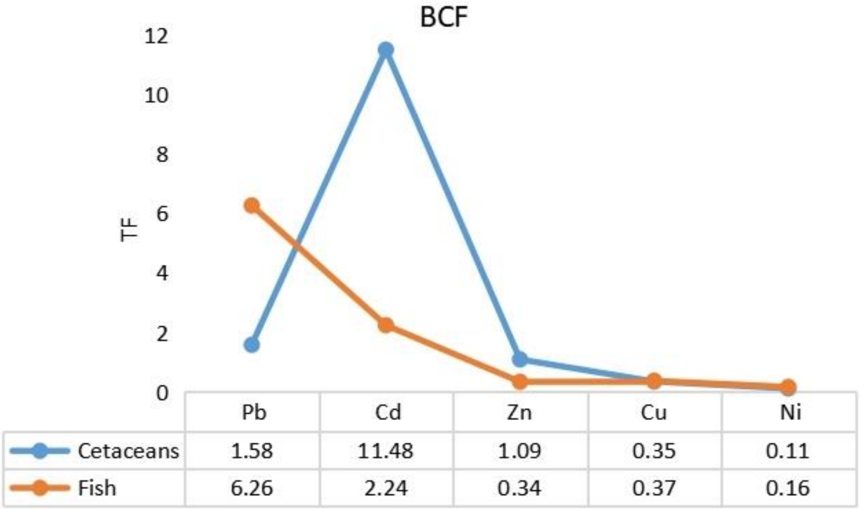


Figure 7. Bioaccumulation factor BCF*water* in cetacean and fish. TF > 1 indicate accumulation.

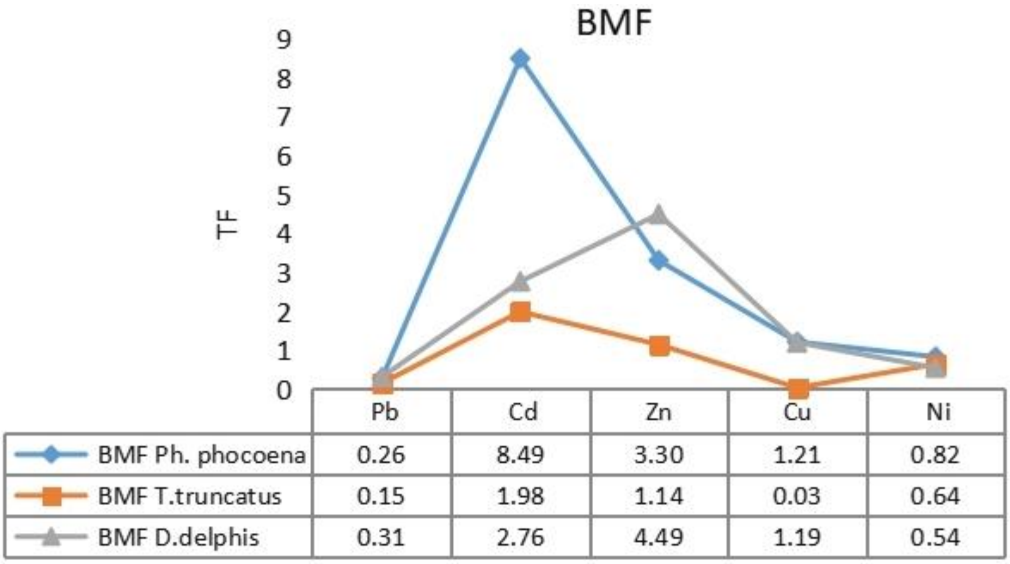


Figure 8. Biomagnification Factor (BMF) by cetaceans species. TF values >1 indicate biomagnification.

1984), since this element is known to be in high levels in tissues undergoing rapid growth (Caurant & Triquet, 1995). Additionally, in the *Ph. phocoena* higher concentrations of Pb and Cd were found compared to *D.delphis* and *T.truncatus*. This is probably related to the main habitats of the species - coastal waters (Birkun et al., 2014), and it can be assumed that it is most exposed to harmful anthropogenic factors in this area (Jitar et al., 2015).

Metals pass into cetaceans either directly through the contact with the ambient water and/or food (Spry & Wood, 1988). The results of the fish samples also show the presence of all the investigated elements and are indicative of the significant variations among fish species along the Bulgarian Black Sea coast. Accumulation of Pb and Cd was observed in all fish species (from 0.56 to 28.98 mg/kg for Pb and from 0.08 to 0.87 mg/kg for Cd), and can explains their presence in cetaceans. No significant differences were found between the presence of heavy metals in different fish species from the northern and southern area. This is probably due to the fact that most species are highly mobile and migrate from south to north (Zlateva et al., 2022) and due to the lack of statistical differences in seawater samples (in environment). The highest content of Pb was recorded in the samples of *E. encrasicolus*, and of Cd in Gobiidae. The high Pb content in *E. encrasicolus* is a cause for concern, as the species is one of the main food bases not only for cetaceans but also for other predatory fish (Tonay et al*.,* 2007; Krivokhizhin & Birkun, 2009).

BCF results show that cetaceans tend to accumulate heavy metals from the environment, BCF confirmed for Cd and Pb (Cd - TF = 1.48, and lowest for Zn - TF = 1.09), and the other heavy metals (Cu, Ni) showed no accumulation. Fish also mainly accumulate from the environment Pb, followed by Cd and none of the other heavy metals (Zn, Cu, Ni). The accumulation of metals in fish tissues depends on the degree of environmental pollution and the diet of the species (Kalantzi et al., 2013). Various studies have shown that heavy metal concentrations for fish living in the same environmental conditions are constant, with the exception of predatory fish (Jitar et al., 2015). Other authors maintain that overall BCF has higher values for plankton and zoobenthos and lower values for fish (Tao et al., 2012). The bioaccumulation factor calculated for fish may in some cases be irrelevant due to their mobility in water, with heavy metal concentrations being higher for benthic than for pelagic species (Copat et al., 2013). So in this case, this statement can also apply to cetaceans as motile species.

BMF in cetaceans is confirmed for the elements Cd, Zn and Cu. The tendency for essential metals, as a Zn to accumulate in a higher concentration than non-essential metals can be explained by the fact that they are actively involved in metabolism (Elnabris et al., 2013). The highest values of biomagnification of heavy metals are observed at *Ph. Phocoena*, and lowest in *T.truncatus*. Obviously, different species tend to accumulate different concentrations of heavy metals as well. The high levels of accumulation in *Ph. Phocoena* can be explained not only by habitat, but also by diet. The diet of *Ph. phocoena* often includes benthic species such as Gobiidae, unlike the other two species (Tonay et al., 2007; Krivohizhin & Birkun, 2009). According to Oros & Gomoiu (2012), benthic fish generally accumulate higher concentrations of heavy metals than pelagic fish, which is explained by their association with the sediment substrate. In our case, the samples of Gobiidae also showed a high value of some metals, as Pb and Ni. However, to date, more samples and data on age, sex, etc. are needed before firm conclusions can be drawn, but in any case the results show that cetaceans tend to accumulate heavy metals along the Black Sea food chain and can be used as a reliable bioindicator.

Conclusions

The present study analyzed the presence and levels of five heavy metals (Pb, Cd, Zn, Cu, Ni) in bones of the three cetacean species and six fish species in the Bulgarian Black Sea. Among the metals, the highest average concentration was observed for Zn in all studied samples (cetaceans, fish and seawater). BCF*water* was higher for cetaceans for Cd, Pb, and Zn, while for fish the same trend was registered for Pb and Cd. The BMF have shown that, as the largest predator in the Black Sea food chain, cetaceans preferentially accumulate Cd and Zn (TF>1).The distribution of the concentration of heavy metals follows the following pattern: Zn > Pb> Cd> Cu> Ni (in cetaceans) and Zn > Pb > Cu > Ni > Cd (in fish).

Finally, it is of great importance that we continue to monitor cetacean strandings and use them to indicate pollution levels in the sea. The future establishment of a database and regulatory framework for Black Sea species will be useful in studying this issue. In addition to studying heavy metals accumulation, it will be very interesting to study how all these elements affect the population.

Ethical Statement

All cetacean samples were taken with a permit issued by the Bulgarian Ministry of Environment and Water.

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Author Contribution

All steps in the preparation of this article are entirely the work of the author.

Conflict of Interest

The author declare that have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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References

ACCOBAMS Guidelines, Resolution 4.16. Emergency task force: Guidelines for a coordinated cetacean stranding response during mortality events caused by infectious agents and harmful algal blooms, compiled by Marie-Françoise Van Bressem. https://www.accobams.org/wpcontent/uploads/2016/06/ACCOBAMS_MOP4_Res.4.16.pdf

Ali, H., & Khan, E. (2019). Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—Concepts and implications for wildlife and human health. Human and Ecological Risk Assessment: An International Journal, 25(6), 1353–1376. https://doi.org/10.1080/10807039.2018.1469398

Bat, L., & Arici, E. (2016). Health risk assessment of heavy metals in *Sarda sarda* Bloch, 1793 for people through consumption from the Turkish Black Sea coasts. International Journal of Zoology Research, 1: 01-07.

Bat, L., Şahin, F., & Öztekin, A. (2018).Toxic Metals in *Nereis Diversicolor* Müller, 1776 From Inner Shores in Sinop Peninsula of the Black Sea as Bio–Indicator Species. Pakistan Journal of Marine Sciences, 27: 11-20.

Bat, L., Öztekin, A., Arici, E., & Şahin, F. (2020). Health risk assessment: heavy metals in fish from the southern Black Sea. Foods and Raw Materials 8:115-124.

Birkun, A., Northridge, S., Willsteed, E., James, F., Kilgour, C., Lander, M., & Fitzgerald, G. (2014). Adverse Fisheries Impacts on Cetacean Populations in the Black Sea. Final report to the European Commission, Brussels, 347 p.

Boon, J.P., Oostingh, I., Van der Meer, J., & Hillebrand, M. (1994). A model for the bioaccumulation of chlorobiphenyl congeners in marine mammals. European Journal of Pharmacology: Environmental Toxicology and Pharmacology, 270: 237-251. https://doi.org/10.1016/0926- 6917(94)90068-X.

Borrell, A., Clusa, M., Aguilar, A., & Drago, M. (2015). Use of epidermis for the monitoring of tissular trace elements in Mediterranean striped dolphins (S*tenella coeruleoalba*). Chemosphere, 122: 288–294. https://doi.org/10.1016/j.chemosphere.2014.10.080.

Bossart, G. (2011). Marine mammals as Sentinel species for oceans and human health. Veterinary Pathology, 48: 676-690. https://doi.org/10.1177/0300985810388525

Bushuyev, S. (2000). Depletion of forage reserve as a factor limiting population size of Black Sea dolphins. Pp. 437 - 452 in: Ecological safety of coastal and shelf areas and a composite utilization of shelf resources. Proc. Marine Hydrophysical Institute, Sevastopol. (in Russian).

Caurant, F.,& Triquet, A. (1995). Cadmium contamination in Pilot Whales Globicephala melas: Source and potential hazard to the species. Marine Pollution Bulletin, 30: 207-210.

Çelik, U., & Oehlenschläger, J. (2004). Determination of zinc and copper in fish samples collected from Northeast Atlantic by DPSAV. Food Chemistry 87(3):343–347. https://doi.org/10.1016/j.foodchem.2003.11.018

Commission regulation (EC) No1881/2006 of 19 December 2006, setting maximum levels for certain contaminants in foodstuffs. https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDFCopat, Copat, C., Arena, G., Fiore, M., Ledda, C., Fallico, R., Sciacca, S., & Ferrante, M. (2013). Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Consumption advisories. Food and Chemical Toxicology, 53: 33-37. https://doi.org/10.1016/j.fct.2012.11.038

Das, K., Debacker, V., Pillet, S., & Bouquegneau, J.M. (2002). Heavy metals in marine mammals (eds), Toxicology of Marine Mammals, 135-167 p.

Das, K., Holsbeek, L., Browning, J., Siebert, U., Birkun, A., & Jean-Bouquegneau, M. (2004). Trace metal and stable isotope measurements (δ13C and δ15N) in the harbour porpoise *Phocoena phocoena relicta* from the Black Sea. Environmental Pollution , 131 (2): 197-204.

Delgado-Suarez, I., Lozano-Bilbao, E., Hardisson, A., Paz, S., & Gutiérrez, Á.J. (2023). Metal and trace element concentrations in cetaceans worldwide: A review. Marine Pollution Bulletin 192:115010. https://doi.org/10.1016/j.marpolbul.2023.115010. Epub 2023 May 9. PMID: 37167666.EC

Directive 2006, Directive 2006/1881/EC the Comission of the European Communities Setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). Official Journal of the European Union, 364/5, 20.12.2006.

Elnabris, K., Muzyed, S., & El-Ashgar, N. (2013). Heavy metal concentrations in some commercially important fishes and their contribution to heavy metals exposure in Palestinian people of Gaza Strip (Palestine). Journal of the Association of Arab Universities for Basic and Applied Sciences, 13: 44-51. https://doi.org/10.1016/j.jaubas.2012.06.001

Evtimova, V., Parvanov, D., Grozdanov, A., Tserkova, F., Zlatkov, B., Vergilov, V., Sivilov, O., Yordanov, S., & Delov, V. (2019). Heavy metals in bones from Harbour Porpoises *Phocoena phocoena* from the Western Black Sea Coast. Zoo Notes, 136:1-4.

FAO (1983). Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products; FAO Fishery Circular 1983 No. 464; FAO: Rome, Italy, 1983 pp. 5-100.

Foulkes, E. (1995). Metal disposition: An analysis of underlying mechanisms. In Metal toxicology, ed. R. A. Goyer, C. D. Klaassen, & M. P. Waalkes, 3–29. San Diego: Academic Press.

Frodello, J.P., & Marchand, B. (2001). Cadmium, copper, lead, and zinc in five toothed whale species of the Mediterranean Sea. International Journal of Toxicology, 20 (6): 339-343.

https://doi.org/10.1080/109158101753333613

Frodello, J.P., Viale, D., & Marchand, B. (2002). Metal concentrations in the milk and tissues of a nursing *Tursiops truncatus* female. Marine Pollution Bulletin, 44 (6): 551-554. https://doi.org/10.1016/S0025-326X(02)00067-X

Garcia-Cegarra, A., Jung, J., Orrego, R., Padilha, J., Malm, O., Ferreira-Braz, B., Santelli, R., Pozo, K., Pribylova, P., Alvarado-Rybak, M., Azat, C., Kidd, K., Espejo, W., Chiang, G., & Bahamonde, P. (2021). Persistence, bioaccumulation and vertical transfer of pollutants in long-finned pilot whales stranded in Chilean Patagonia. Science of The Total Environment, 770:145259. https://doi.org/10.1016/j.

Gerpe, M., Rodríguez, D., Moreno, V., Bastida, R., & De Moreno, J. (2002). Accumulation of Heavy Metals in Franciscanas from Buenos Aires Province, Argentina. Latin American Journal of Aquatic, 1: 95-106. https://doi.org/10.5597/lajam00013

Gobas, F., & Morrison, H. (2000). Bioconcentration and biomagnification in the aquatic environment. In: Boethling R, Mackay D (eds), Handbook of property estimation methods for chemicals: environmental and health sciences. Lewis, Boca Raton, 189-231 pp.

Jaishankar, M., Tenzin, T., Anbalagan, N., Mathew, B., & Beeregowda, K. (2014). Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology, 7 (2):60-72. https://doi.org/10.2478/intox-2014-0009

Jakimska, A., Konieczka, P., Skóra, K., & Namieśnik, J. (2011). Bioaccumulation of Metals in Tissues of Marine Animals, Part II: Metal Concentrations in Animal Tissues. Polish Journal of Environmental Studies, 20:1127-1146.

Jitar, O., Teodosiu, C., Nicoara, M., Plavan, G., & Nicoara, M. (2013). Study of Heavy Metal Pollution and Bioaccumulation in the Black Sea Living Environment. Environmental Engineering and Management Journal, 12(2):271-276. https://doi.org/10.30638/eemj.2013.032

Jitar, O., Teodosiu, C., Oros, A., Plavan, G., & Nicoara, M. (2015). Bioaccumulation of heavy metals in marine organisms from the Romanian sector of the Black Sea. New Biotechnology 32 (3):369-378. https://doi.org/10.1016/j.nbt.2014.11.004

Karapetkova, M., & Zhivkov, M. (2010). The fishes of Bulgaria. Gaia-Libris Press, 248 pp. (In Bulgarian).

Kehring, H., Hauser-Davis, R., Seixas, T., Pinheiro, A., Paula, A., & Di Beneditto, A. (2016). Mercury species, selenium, metallothioneins and glutathione in two dolphins from the southeastern Brazilian coast: Mercury detoxification and physiological differences in diving capacity. Environmental Pollution, 213:785-792. https://doi.org/10.1016/j.envpol.2016.03.041

Kryvokhizhin, S., & Birkun, A. (2009). Diet spectrum of Black Sea cetaceans. Marine Ecology J. 8:67 -78 (in Ukrainian).

Law, R. (1994). Collaborative UK marine mammal project: Summary of data produced 1988–1992. Fisheries Research Technical Report 97, 42 p.

Lozano-Bilbao, E., Alcázar-Treviño, J., Alduán, M., Lozano, G., Hardisson, A., Rubio, C., Gonzalez-Weller, D., Paz, S., Carrillo, M., & Gutierrez, A.J. (2021). Metal content in stranded pelagic vs deep-diving cetaceans in the Canary Islands. Chemosphere, 285:131441. https://doi.org/10.1016/j.chemosphere.2021.131441. Epub 2018 Jan 9. PMID: 29329095.

Makedonski, L., Peycheva, K., & Stancheva, M. (2017). Determination of heavy metals in selected black sea fish species. Food Control, 72: 313-318. https://doi.org/10.1016/j.foodcont.2015.08.024

Ministry of Agriculture Food and Forestry (2020). Annual report on the state and development of agriculture, 324 pp.

Monteiro, S., Pereira, A., Costa, E., Torres, J., Oliveira, I., Bastos-Santos, J., Araújo, H., Ferreira, M., Vingada, J., & Eira, C. (2016). Bioaccumulation of trace element concentrations in common dolphins (*Delphinus delphis*) from Portugal. Marine. Pollution. Bulletin. 113:400–407. https://doi.org/10.1016/j.marpolbul.2016.10.033.

Oros, A., & Gomoiu, M. (2012). A review of metal bioaccumulation levels in the Romanian Black Sea biota during the last decade – a requirement for implementing marine strategy framework directive (descriptors 8 and 9). Journal of Environmental Protection and Ecology, 13: 1730-1743.

Penin, R. (2007). Natural physical geography of Bulgaria, (eds) Sofia, Bulgaria: Bulvest 2000 Press pp. 231-237 (in Bulgarian).

Prodanov, K., Mikhailov, K., Daskalov, G., Maxim, C., Chashchin, A., Arkhipov, A., & Özdamar, E. (1997). Environmental impact on fish resources in the Black Sea. In E. Özsoy and A. Mikaelyan (eds), Sensitivity to change: Black Sea, Baltic Sea and North Sea (Dordrecht, The Netherlands: Springer-Science+Business Media, B.V. pp. 163-181. https://doi.org/10.1007/978-94-011-5758-2_14

Radulescu, V. (2023). Environmental Conditions and the Fish Stocks Situation in the Black Sea, between Climate Change, War, and Pollution. Water, 15:1012. https://doi.org/10.3390/w15061012

Shukla, S., Mbingwa, G., Khanna, S., Dalal, J., Sankhyan, D., Malik, A., & Badhwar, N. (2023). Environment and health hazards due to military metal pollution: A review. Environmental Nanotechnology, Monitoring and Management, 20: 100857. https://doi.org/10.1016/j.enmm.2023.100857

Sijm, D.T., & Hermens, J.L. (2000). Internal Effect Concentration: Link Between Bioaccumulation and Ecotoxicity for Organic Chemicals. In: Beek, B. (eds) Bioaccumulation – New Aspects and Developments . The Handbook of Environmental Chemistry, vol 2J. Springer, Berlin, Heidelberg. https://doi.org/10.1007/10503050_2.

Spry, D., & Wood, C. (1988). Zinc influx across the isolated perfused head preparation of rainbow trout (*Salmo gairdneri)* in hard and soft water. Canadian Journal of Fisheries and Aquatic Sciences, 45:2206–2215.

Stancheva, M., Peycheva, K., Makedonski, L., & Rizov, T. (2010). Heavy metals and PCBs level of bluefish (*Pomatomus saltatrix*) from Bulgarian black sea waters. Ovidius University Annals of Chemistry, 21(1): 41–48.

Tao, Y., Yuan, Z., Xiaona, H., & Wei, M. (2012). Distribution and bioaccumulation of heavy metals in aquatic organisms of different trophic levels and potential health risk assessment from Taihu lake, China. Ecotoxicology and Environmental Safety, 81: 55–64. https://doi.org/10.1016/j.ecoenv.2012.04.014

Tonay, A., Dede, A., Ayaka, A., Ozturk, A., & Ozturk, B. (2007). Stomach content of harbor porpoises (*Phocoena phocoena)* from the Turkish western Black Sea in spring and early summer. Rapp. Comm. Int. Mer. Medit. 38: 615-616.

Vrhovnik, P., Arrebola, J.P., Serafimovski, T., Dolenec, T., Smuc, N.R., Dolenec, M., & Mutch, E. (2013). Potentially toxic contamination of sediments, water and two animal species in lake Kalimanci, FYR Macedonia: relevance to human health. Environmental Pollution, 180:92-100. https://doi.org/10.1016/j.envpol.2013.05.004.

Wagemann, R., & Muir, G. (1984). Concentrationsof heavy metals and organochlorines in marine mammals of northern waters: overview and evaluation. Canadian technical report of fisheries and aquatic sciences, 1279:97.

Xiong, X., Qian, Z., Mei, Z., Wu, J., Hao, Y., Wang, K., Wu, C., & Wang, D. (2019). Trace elements accumulation in the Yangtze finless porpoise (*Neophocaena asiaeorientalis asiaeorientalis*) - A threat to the endangered freshwater cetacean. The Science of The Total Environment, 686: 797-804. https://doi.org/10.1016/j.scitotenv.2019.06.031

Yoshino, K., Mori, K., Kanaya, G., Kojima, S., Henmi, Y., Matsuyama, A., & Yamamoto, M. (2020). Food sources are more important than biomagnification on mercury bioaccumulation in marine fishes. Environmental Pollution, 262: 113982. https://doi.org/10.1016/j.envpol.2020.113982

Zlateva, I., Raykov, V., Slabakova, V., Stefanova, E., & Stefanova, K. (2022). Habitat suitability models of five keynote Bulgarian Black Sea fish species relative to specific abiotic and biotic factors. Oceanologia, 64:665-674. https://doi.org/10.1016/j.oceano.2022.06.002