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Seasonal Examination of Heavy Metal Levels in Muscle Tissues of European pilchard (*Sardina pilchardus*, Walbaum 1792)

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ABSTRACT

The sampling point of the research was determined as the shores of Çanakkale Strait, where fishing is dense. Seasonal sampling of European pilchard (*Sardina pilchardus*, Walbaum 1792) was made that caught by commercial fishing boats in Çanakkale Strait and rarely offered for consumption. Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) allows analysis of trace, minor and major concentration levels of the chemical element, was used to determine the amount of heavy metals in the samples. Analysis of Cadmium (Cd), Copper (Cu), Iron (Fe), Manganese (Mn), Lead (Pb) and Zinc (Zn) heavy metals in samples was performed by the United States Environmental Protection Agency (US EPA). Levels of Cd, Cu, Fe, Mn, Pb and Zn were determined in muscle tissues of European pilchard. In the samples, Cd values were detected only in autumn, Cu level was found highest in winter, Fe and Zn levels were highest in spring and the level of Mn was observed higher in winter. Pb has not been detected in fish muscle dough in any season. Mn level has been obtained above the limit values and Cd level was found under the offered limits. As a result, it has been determined that the heavy metal accumulation in the muscle tissues of European pilchard caught from Çanakkale Strait increased seasonally in parallel with the increase of pollution.

INTRODUCTION

Nowadays, aquatic ecosystem is damaged by persistent pollutants originated from agricultural and industry sectors. Heavy metal pollution has been identified as a concern in coastal areas due to discharges from industrial wastes, agricultural and urban wastewater. Heavy metal levels increase significantly in marine environment mainly because of anthropogenic activities (Sharifuzzaman et al., 2016). Fish and other aquatic organisms were not independent of environment in which they survive. This situation results in the complete extinction of species that are not tolerant to certain environmental factors, thus it causes structural and functional damage to organisms by interfering with the physiological and biochemical mechanisms. It is very important to determine the levels of heavy metals in foodstuffs, especially in sea food (Pörtner et al., 2005).

When the previous studies conducted to determine the metal accumulation in sea water in Çanakkale Strait are examined, it has been seen that these studies have relatively searched the sediment samples. Bat et al. (2019) evaluated the heavy metal pollution in water, sediment and polychaetes in Sinop coasts of Black Sea. Hg, Cd, Pb, Cu and Zn levels in water, sediment and polychaetes were determined and their anthropogenic effect on environment was evaluated. Metal concentrations in water were found as Zn> Cu> Pb> Cd> Hg, and the abundance of these metals in the sediment were respectively: Zn> Cu> Pb> Hg> Cd. Olgunoğlu et al. (2015) investigated the concentration of Cd, Pb, Cu, Zn and Fe in the gill and muscle tissues of four benthic fish species from the Northeastern part of Mediterranean region. Heavy metal concentrations in muscle tissues were found lowest while the highest levels were obtained for each species from the gills. Salam et al. (2019) stated that advanced agricultural activities and rapid industrialization cause pollution in aquatic areas. Heavy metals can eventually be transported from aquatic animals such as fish, shrimp, and crabs to human body through food chain. In this study, four fish species viz *Euthynnus affinis*, *Pampus argenteus*, *Descapterus macrosoma*, and *Leiognathus daura*, shrimp (*Fenneropenaeus indicus*) and crab (*Organisropenus indicus*) were used aim to detect the levels of heavy metal in muscle tissues.

Aquatic organisms can accumulate heavy metals from environment by various means including respiration, adsorption and ingestion (Sönmez et al., 2016). Particularly, fish accumulate heavy metals by internal organs, gills, skin and muscle. Human health is under threat with the consumption of fish and other seafood supplied from regions with excessive heavy metal concentration. Because heavy metals accumulate in various tissues over time and can be transferred to people through their food chain (Zhang et al., 2017). Therefore, in this study, it has been investigated that how the fish species that move from the industrially developed Marmara Region through the overcurrent system to the Dardanelles Strait and then to the Aegean Sea affected by the potential metal accumulation in their tissues. Çanakkale Strait is also exposed to a lot of ship wastes by transit ship passages. Our aim has been to determine the possible metal load in water and the extent of marine pollution and to investigate the accumulation of various heavy metals in fish that are frequently caught and consumed from this habitat.

MATERIAL AND METHODS

ICP-OES Element Determination

Sampling was done four times (Spring-April, 2020, Summer-June, 2020, Autumn-September, 2020, Winter-January, 2021) throughout the year. Muscle tissue (meat part) required for the detection of heavy metal accumulation, was taken from the dorsal fin approximately 2 cm below. In the study, samples with an average length of 15.4 cm and a weight of 35.7 g were used.

Before heavy metal analysis, pre-treatment process, fresh samples were freeze-dried at -55˚C for 72 hours with lyophilizer. In this analysis, Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), which allows the analysis of trace, minor and major concentration levels of the chemical element was used to determine the amount of heavy metals in samples (Fallah et al., 2011). Analysis of Cd, Cu, Fe, Mn, Pb and Zn were performed by EPA (2007) method. Pre-treatment and analysis were carried out by Çanakkale Onsekiz Mart University Science and Technology Application and Research Center.

Statistical evaluation was made according to the seasonal heavy metal accumulation comparatively. The heavy metal contents of the samples were evaluated considering the risk factors and the environment which they grown.

All data sets were subjected and analyzed for homogeneity of variances and normality test. One-way ANOVA was adopted to get a comparative outlook of heavy metals with respect to European pilchard in single tissue. Statistical analysis indicated the significant values for processed data ($p<0.05$). For the ANOVA post hoc test, Tukey was applied for pairwise mean comparisons where homogeneity of variance was established. Statistical analysis has been done with the help of SPSS 19.0 for Windows (Table 1).

RESULTS

In our study, Cd, Cu, Fe, Mn, Pb and Zn levels in muscle tissues taken from *Sardina pilchardus* from Çanakkale Strait were determined by ICP-OES technique. Average values were determined according to the seasons. Results are given in the following table. Prior to analysis, the ICP-OES instrument was calibrated using standard solutions 0.1, 0.25, 0.5, 1, and 2 mg/L for each element. It was also used as a quality control solution in a 0.1 mg/L solution. To show the sensitivity of the method, LOD (limit of detection) study was conducted for each parameter and the values obtained because of the studies were given in Table 1.

Table 1. LOD and wave length values

	Cd	Cu	Fe	Mn	Pb	Zn
LOD (ppb)	5	5	5	5	5	5
Wave length (nm)	226.5	324.7	259.9	257.6	220.3	213.8

Cadmium values of European pilchard tissues were determined between April 2020 and January 2021. Cd could not be detected in the samples in spring, summer, and winter seasons. Cd value was only determined as 0.01 (μg/g) in autumn shown in Table 2. It has been determined that the value of Cu in spring was noted as 0.04 (μg/g), in autumn it was observed as 0.03 (μg/g) and in winter it was found as 0.05 (μg/g). During summer, Cu was not detected. In the comparison made based on seasons, the highest value for European pilchard was found in winter samples (Table 2).

Fe value in spring was recorded as 68.22 (µg/g), in autumn, it was 54.36 (µg/g), and the value in winter was 61.38 (µg/g). Fe value in summer determined as 50.74 (µg/g). In the comparison made on the basis of seasons, the highest value for European pilchard was found in spring samples (Table 2).

Mn value in spring was mentioned as 2.81 (µg/g), the value of Mn in autumn determined as 3.65 (µg/g), and the value in winter was as 5.42 (µg/g). In summer, Mn value was determined as 2.80 (µg/g). During the comparison made on the basis of seasons, the highest Mn value for European pilchard was observed in those samples which are taken in winter (Table 2).

Pb content of this species was not detected during seasonal evaluation (Table 2).

Zn value of spring samples was noted as 74.18 (µg/g), in autumn, it was recorded as 44.11 (µg/g) while the value in winter samples was determined as 59.42 (µg/g). The Zn value in summer was determined as 47.38 (µg/g). During the comparison made on the basis of seasons, the highest Zn value for European pilchard was found in spring samples (Table 2).

While the accumulation of heavy metals in the muscle tissue of the European pilchard, which tried to determine seasonally, was expected to differ, the differences between the elements such as Cd and Pb that could not be determined, and the elements that were very close to each other in some seasons and could not be statistically significant were ignored. Because the undetectable Cd and Pb are related to the minimum value to be read in the adjustment of the device, they have a very low probability of accumulation. At the same time, it shows that although there is negativity in terms of statistical significance among the elements detected at very close values in some seasons, it should be taken into account that it is above the limit value.

Table 2. Seasonal heavy metal values (µg/g) for *S. pilchardus*

Heavy metals \ Season	Spring	Summer	Autumn	Winter	Limit values
Cd	ND	ND	0.01	ND	0.01
Cu	0.04±0.01	ND A*, D*	0.03±0.008 B*, F*	0.05±0.014 C*, E*	0.02
Fe	68.22±8.21	50.74±7.64 A****, D****	54.36±7.85 B***, F**	61.38±8.12 C**, E**	ND
Mn	2.81±0.60	2.80±0.56 A*, D****	3.65±0.84 B**, F***	5.42±1.13 C****, E****	1.0
Pb	ND	ND	ND	ND	0.3
Zn	74.18±13.41	47.38±7.65 A****, D*	44.11±6.88 B****, F***	59.42±7.74 C****, E***	50

Note: The maximum limit value for Mn (mg/kg), for the other elements the values given by WHO (2002) were considered (ND: Not defined), (Mokhtar et al., 2009). Data are given as mean ± SD. *p>0.05, **p<0.05, ***p<0.001, ****p<0.0001. Group comparison: A: spring and summer, B: spring and autumn, C: spring and winter, D: summer and autumn, E: summer and winter, F: autumn and winter.

DISCUSSION

Heavy metal levels in muscle tissues of the Sardines showed variation seasonally. Cd was detected only in samples which collected in autumn. Cu has been detected in all seasons except, summer. Fe has been found in all seasons. Statistically, Fe values were determined the highest in spring and the lowest in summer season according to different seasons. Mn was determined in all seasons and the seasonal ranking was determined the highest in winter and the lowest in summer. Pb has detected in all seasons. Zn has been determined in all seasons and determined the highest in spring and the lowest in autumn. In similar studies conducted on fish samples collected from the coasts of Turkey and other countries, it was observed that there are regional differences in the accumulation of heavy metals in fish species consumed as food.

Wastewater is released because of industrial establishments, buildings, agriculture and animal husbandry practices, the operations of energy generating power plants and contains chemicals that adversely affect health, is one of the most important sources of pollution (Dündar et al., 2012). Heavy metal pollution may be higher in places where streams flow into the sea as compared to other regions (Wojciechowska et al., 2019). It is expected that there will be more heavy metal accumulation in their bodies, as the rate of fish consumption of people living in regions with coasts to the Black Sea, is higher than the rate of fish consumption of people living in regions with no coasts (Küçük, 2015). Elderwish et al. (2019) found a strong correlation in the positive direction of metals in some stations of their seasonal examined the accumulation of heavy metals in waters of the western Black Sea coast of Turkey. Engel et al. (1981) stated that metals are found in aquatic environments absorbed by organic or inorganic compounds, free ions, and particulate matter. In this way, after some of the heavy metals enter the water, some of them are carried directly to the organisms and some to the sediment (Kaptan, 2014). Recently studies on marine pollution have increased considerably and the data obtained that the pollution is increasing day by day according to the results of the research conducted both on global basis. Pollution in the seas disrupts the aquatic ecosystem, many sea creatures, especially fish, exposed to deteriorating and accumulating elements in their tissues. Heavy metals can reach people with consuming seafood and this can cause chronic diseases (Abalaka et al., 2020). Çanakkale Strait is also highly polluted by ship crossing every year. The threat to human health of fish caught by hunting needs to be researched and clarified. Studies show that contamination is increasing and is at very high levels in some locations of Çanakkale Strait. Study results are not compatible with the results of previous studies in terms of the determined heavy metal in species (Bat et al., 2015; Culha et al., 2016).

Culha et al. (2016) investigated metal contamination in *Holothuria tubulosa* (sea cucumber) and sediments in Çanakkale Strait. This study was carried out to determine the concentrations of some trace metals (Cd, Cu, Pb, Ni, Zn, and Fe) in *H. tubulosa* (Gmelin, 1788) belonging to Echinoderm species in three different locations. The statistical difference between seasonally determined trace metals in muscle tissue of *H. tubulosa* was significant ($p<0.05$). Culha et al. (2016) obtained that there are also significant differences between seasonal trace elements accumulated by muscle tissues (Cd, Pb, Fe, Mn, Zn and Cu) of *H. tubulosa*. In this study, as far as the Zn is concerned, the highest value was determined in spring. In addition, when evaluated in terms of pollution, most of the heavy metal obtained results are below the limit values and they have changed accordingly to seasons.

Küpeli et al. (2014) evaluated trace element levels in the muscle tissues of fish species collected from three different locations in Sakarya. Trace element levels of several fish species collected from the waters of Sakarya were determined below the limit values provided by Turkey Food Codex (TFC) (Anonymous, 2002), the Food and Agriculture Organization (FAO, 2018) and the World Health Organization (WHO, 2002). Rajeshkumar & Li (2018) examined the bioaccumulation of heavy metals in fish species in Meiliang Bay-Taihu Lake, China. Bioaccumulation of heavy metal (Cr, Cu, Cd and Pb) content was determined in *Cyprinus carpio* and *Pelteobagrus fluvidraco*, which were caught from Meiliang Bay-Taihu Lake, a large, shallow, and eutrophic location. The results showed that the content of Cr, Cu, Cd, and Pb in the edible parts of the two fish species was much lower than the Chinese Food Health Criteria (Rajeshkumar & Li, 2008).

Bat et al. (2015) studied the heavy metal levels in four commercial fish from the Black Sea coast of Sinop (Turkey). The heavy metal concentrations were quite below of the limit values. Therefore, these heavy metal values in the total body load can be considered negligible. Sönmez et al. (2016) determined the heavy metal accumulation in some economical fish species caught from the coastal regions of Kastamonu. This study was carried out to determine heavy metal accumulation in whiting (*Merlangius euxmus*), bonito (*Sarda sarda*), horse mackerel (*Trachurus trachurus*), and red mullet (*Mullus barbatus*). As a result, Fe, Cu, Ni and Zn data were determined within acceptable limits within the framework of the Turkish Food Codex, European Union directives and WHO standards, while cadmium and lead were determined above these limits. According to the results obtained from this study, only Mn related results were found above the limit values for all seasons, while Zn was found to be quite high in spring. It would be beneficial to repeat the analyses in different fish species obtained from the same locations. Because the differences in results of other studies and we obtained them due to the collection of samples from different regions. Restrictions can be listed as hunting in a wide area, the variety of species highly consumed, and the variety of elements determined for analysis. In this case, it is important to investigate the issue more comprehensively conducted projects in terms of both meeting the nutritional needs for future and health.

Bawuro et al. (2018) examined the bioaccumulation of heavy metals in some fish tissues in Geriyo Lake, Adamawa State, Nigeria. Bioaccumulation of heavy metals (Zn, Pb, Cd, and Cu) was determined in the liver, gills, and muscle tissues in benthic and pelagic fish species collected over a period of two seasons. According to the obtained data, there was no threat to human consumption and for public health. These levels may be due to anthropogenic inputs, since they were interpreted as there was no industrial activity around the lake.

Ibemenuga et al. (2019) investigated the bioaccumulation of some heavy metals in three different organs of fish that have been grown in the Niger River (Nigeria). In this study, the bioaccumulation of cadmium, zinc, lead, and mercury in the gills, muscles, and intestines of *Tilapia zillii*, *Malapterurus electricus,* and *Clarias gariepinus* taken from the Niger River was investigated. The results obtained in this study revealed that the Niger River was polluted with varying levels by Zn and Cd. The proposed control measures include public disclosure of the giving up anthropogenic activities that can lead to water pollution.

CONCLUSION

The negative effects of heavy metals, which have a high probability of mixing water, on fishery products, are a very rich food source in terms of protein and have an importance in nutrition, should not be overlooked. Wastes left to nature consciously or unconsciously can reach all organisms through the food chain. The criterion values of the authorities should be taken into consideration in the release of heavy metals, which reach from the first to the last step of the food chain and cause various health problems by investigate the correlation between metal concentrations in water and metal accumulation in fish caught from areas. For this reason, the accumulation and toxic effects of some heavy metals cause the growth and survival rates of fish larvae to decrease. In future, this problem will affect the natural fish populations, endangering sustainability and also affect the welfare of the societies with the increase of unhealthy food. Safe food and nutrition are of great importance for our health. For this reason, protecting nature is one of our most important duties that we should pay attention to both in terms of food safety as well as leaving a healthy environment for our future generations.

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Compliance with Ethical Standards

Authors' Contributions

Author LCİ designed the study, ŞÖ wrote the first draft of the manuscript, LCİ and ŞÖ managed the structure of manuscript. Both authors have read and approved the final shape of manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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