Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XII, 2023
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

EVALUATION OF HEAVY METALS CONCENTRATIONS IN THE BLACK SEA TURBOT AND ELEMENTS CORRELATION ANALYSIS

Ira-Adeline SIMIONOV[1, 2], Valentina CALMUC[1], Stefan-Mihai PETREA[1, 2], Alina ANTACHE[1, 2], Aurelia NICA[1, 3], Catalina ITICESCU[1, 3], Puiu-Lucian GEORGESCU[1, 3], Victor CRISTEA[4]

[1]"Dunarea de Jos" University of Galati, REXDAN Research Infrastructure, 98 George Cosbuc Street, Galati, Romania
[2]"Dunarea de Jos" University of Galati, Faculty of Food Science and Engineering, 47 Domneasca Street, Galati, Romania
[3]"Dunarea de Jos" University of Galati, Faculty of Sciences and Environment, 47 Domneasca Street, Galati, Romania
[4]"Dunarea de Jos" University of Galati, Cross-Border Faculty of Humanities, Economics and Engineering, 47 Domneasca Street, Galati, Romania

Corresponding author email: ira.simionov@gmail.com

Abstract

The Black Sea Turbot (BST) is one of the most valuable fish species exploited within the fisheries activities conducted in the Black Sea, due to market demand and high selling prices. However, due to the anthropogenic pressure exercised on the Black Sea, BST is prone to accumulate different contaminants such as heavy metals. The risk of heavy metals transfer to the human consumer, through fish consumption, is possible. Thus, constant evaluation of the biomass is needed in order to avoid consumer intoxication. It is well known that different metals manifest competing behaviour for binding spots when accumulating in biota. Therefore, the present study aims to evaluate the concentration of macro- (Ca, Mg, K, Na) and micro-elements (Fe, Zn, Cu, Ni, Cr, Mn, Co, Cd, Pb) in BST muscle tissue collected from the Romanian Black Sea sector and to determine the correlation relationship between them (Pearson coefficient). The following accumulation trend in BST muscle was identified: Na>K>Ca>Mg>Zn>Fe>Cu>Mn>Ni>Cd>Pb>Cr>Co.

***Key words**: Black Sea, correlation, heavy metals, turbot.*

INTRODUCTION

The high economic value of the marine flatfish turbot (*Scophthalmus maximus*, Pallas, 1814) is generally acknowledged due to market demand and its meat high nutritional value (Turan et al., 2019; Ivanova et al., 2021; Liu et al., 2021). Globally, the turbot is a target species for both the fisheries and the aquaculture sector (Khanaychenko & Giragosov, 2019; Ma et al., 2021; Massa et al., 2021).

In the Black Sea, the turbot represents one of the most valuable fish stocks for the fisheries activities conducted in all riparian countries (Giragosov & Khanaychenko, 2012; Firidin et al., 2020; Hulak et al., 2021). As well, from all riparian countries, it has been highlighted that Romania and Bulgaria have the most abundant turbot stocks, which are influenced by the presence of sandy habitats and rich prey items (Ulman et al., 2020). The nutritional value of turbot meat is assessed based on protein content, essential fatty acids, and also, macro- and microelements (Manthey-Karl et al., 2016).

The macro- and microelements profile of turbot meat depends heavily on the feeding regime of the fish (Pouil et al., 2016). In aquaculture production systems, the nutritional value of reared turbot meat can be easily controlled by using specialised pelleted fish feeds. However, in case of specimen obtained from fisheries activities, hence from natural and uncontrolled marine environments, the meat nutritional value depends on food availability and quality. Besides the uptake of essential elements from food, the turbot is prone to accumulate elements with toxic potential such as heavy metals with no essential role in fish metabolism (cadmium - Cd and lead - Pb). At the same time, it is well known that metals considered essential nutrients in living cells such as calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) can inhibit the uptake of heavy metals in fish by competing for binding spots when accumulating in biota (Marchetti, 2013). Therefore, the aim of the present study was to evaluate the macro- (sodium - Na, potassium - K, magnesium - Mg, calcium - Ca) and microelements with toxic potential (iron - Fe, zinc - Zn, copper - Cu, nickel - Ni, chromium - Cr, manganese - Mn and cobalt - Co) , and toxic trace elements lead - Pb, cadmium - Cd) profile of the Black Sea turbot meat - *Scophthalmus maeoticus* (Pallas, 1814).

MATERIALS AND METHODS

The BST specimen (*n=21*) were caught by commercial fishing, using specialized gillnets, in the Romanian coastal waters of the Black Sea, Sf. Gheorghe, Tulcea county.

Figure 1. Sample collection from the studied biological material (*Scophthalmus maeoticus)*

The biological material was stored in polyethylene bags and kept on ice until transportation to the laboratory within the REXDAN Research Infrastructure, where samples of muscle tissue were processed in triplicate (Figure 1).
The biometric measurements were determined for each fish specimen and the results are presented as average ± standard deviation in Table 1.
All the extracted samples were subjected to the digestion process with suprapure reagents (nitric acid and hydrogen peroxide) and the final aqueous solution was analysed by inductively coupled plasma with mass spectrometry (ICP-MS) with the Perkin Elmer NexION 2000 equipment (Figures 2 and 3).

Table 1. Biometric measurements of the biological material

Indicator	Value
Total length (cm)	37 ± 2.75
Total weight (g)	1.4 ± 0.12

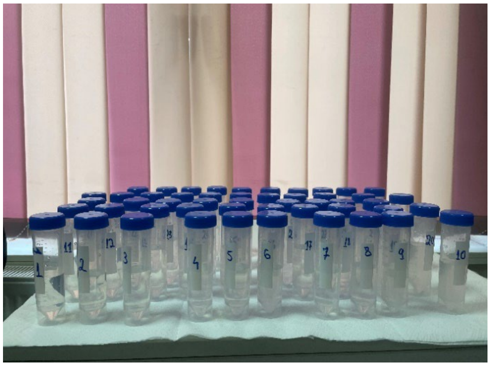

Figure 2. Sample preparation through microwave digestion

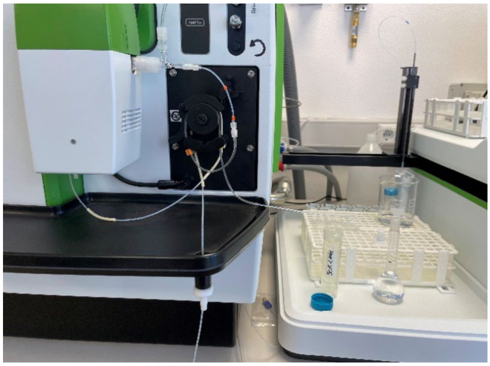

Figure 3. Sample analysis through ICP-MS technique

The following elements were quantified in the muscle tissue of the Black Sea turbot: Na, K, Mg, Ca, Fe, Zn, Cu, Ni, Cr, Mn, Pb, Cd and Co.

RESULTS AND DISCUSSIONS

The concentration of macroelements in the turbot muscle registered the following decreasing trend Na>K>Ca>Mg (Figure 4). As it is expected, the highest concentration was registered in case of the macroelement Na (735.96 ± 115 μg/g fresh weight). Na is involved in the normal function of the muscle by maintaining an adequate blood pressure (Din et al., 2015; Stoyanova, 2018).

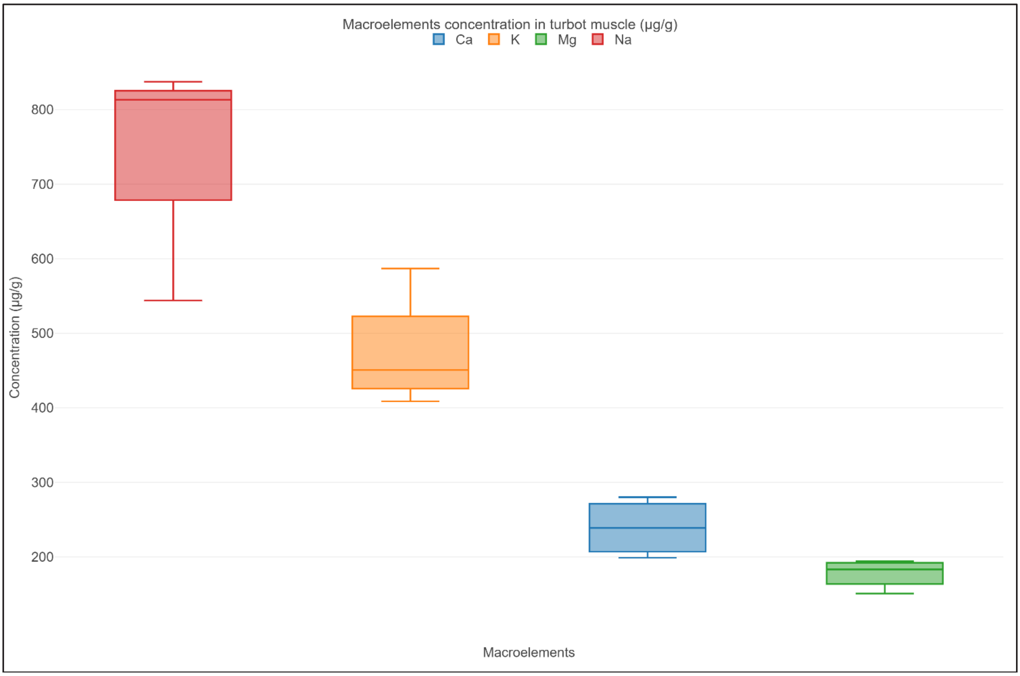

Figure 4. Boxplot representation of macroelements concentration in turbot muscle

Following, the second highest concentration was registered in case of K concentration (474.29 ± 67 µg/g fresh weight). As well as Na, K is involved in the contraction of the muscles, and at the same time, in responsible for maintaining the fish osmoregulation balance (Lall & Kaushik, 2021; Wen et al., 2021; Presas-Basalo, 2022).

Further on, the Ca concentration in the muscle of Black Sea turbot registered an average value of 239.22 ± 33 µg/kg. According to (Hrynevych et al., 2022), there is a positive correlation between Ca accumulation in fish muscle and water temperature.

The Zn concentration in the muscle tissue of the studied biological material registered an average value of 9.01 ± 0.4 µg/g fresh weight (Figure 5). Considering Fe concentration, the analysis of muscle tissue of Black Sea turbot indicated a value of 1.93 ± 0.33 µg/g fresh weight (Figure 5).

Fe and Zn are essential elements involved in fish metabolism. For instance, Fe is part of the haemoglobin protein, which is responsible for the transport of oxygen, while Zn is part of several metalloenzymes in fish (Zhao et al., 2014; Silva et al., 2019; Abd-Elhamed et al., 2021.

The concentration of Cu registered an average value of 0.21 ± 0.06 µg/g fresh weight in the muscle tissue of the studied biological material, while Mn registered a mean value of 0.13 ± 0.01 µg/g fresh weight (Figure 6). As well as Fe, Cu is an essential element of fish erythrocytes (Kamunde et al., 2002; Malhotra et al., 2020).

At the same time, Mn is involved in enzymatic activities and anti-oxidant processes (Antony Jesu Prabhu et al., 2019; Zhou et al., 2022).

Regarding the concentration of Ni, the mean registered value in the muscle of the Black Sea turbot was 0.07 ± 0.02 µg/g fresh weight. In case of Cd and Pb concentrations, the average value in the muscle was 0.02±0.001 µg/g fresh weight and 0.01 ± 0.001 µg/g fresh weight respectively. Ni essentiality in fish has been speculated, however no evidence has been found to support that hypothesis (Muyssen et al., 2011; Blewett & Leonard, 2017). Therefore, it could be considered that trace amounts of Ni, Cd and Pb, which have no biological role in the fish organism can negatively influence fish growth and welfare.

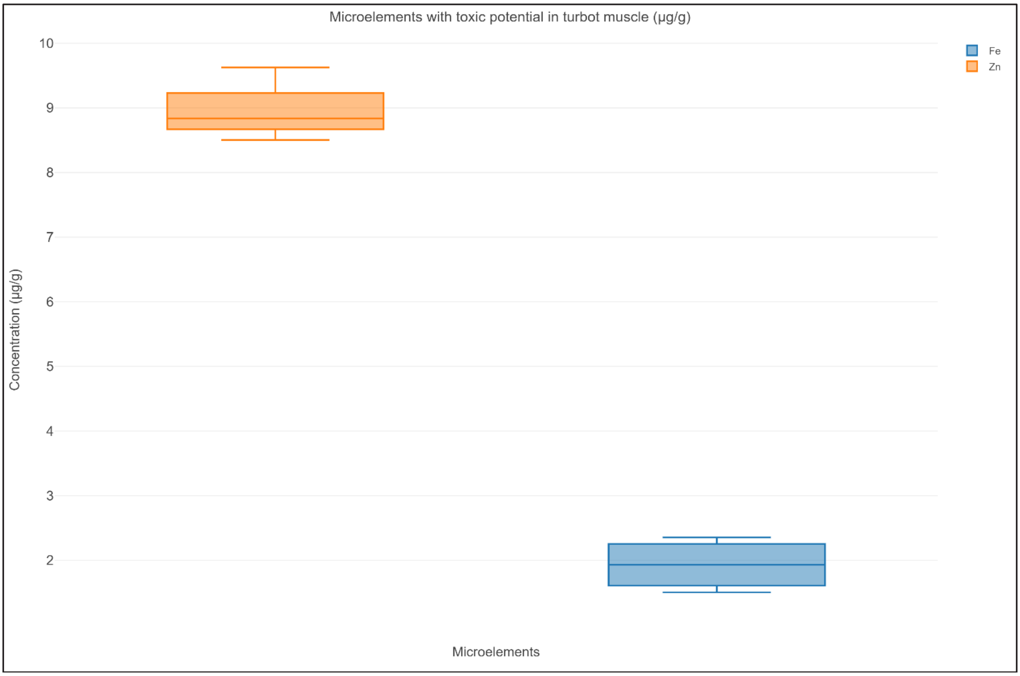

Figure 5. Boxplot representation of microelements concentration in turbot muscle

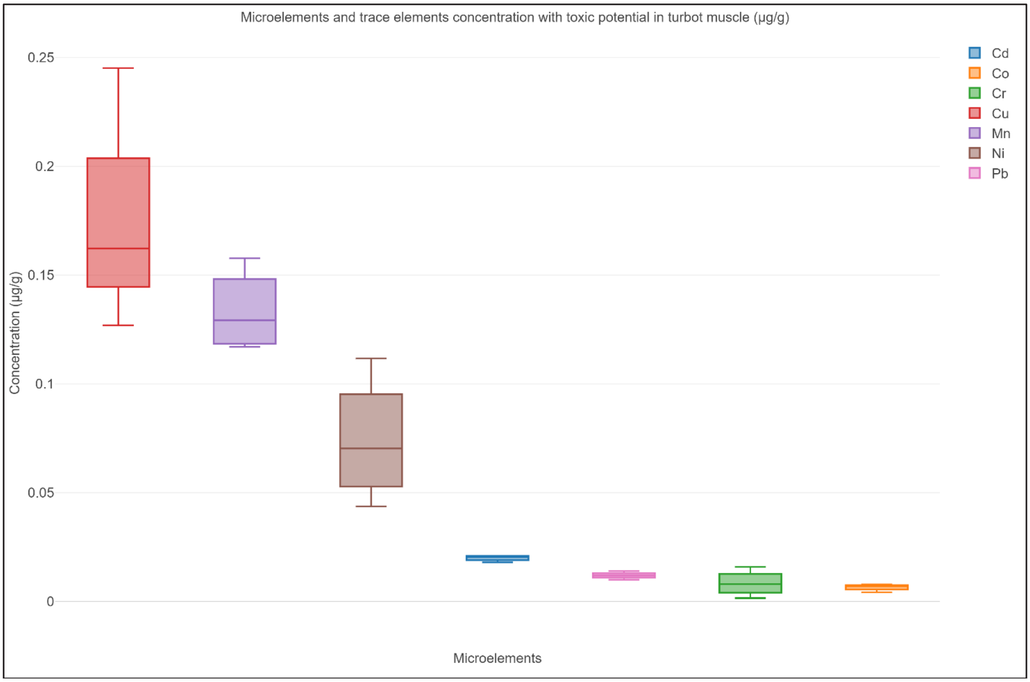

Figure 6. Boxplot representation of microelements concentration in turbot muscle

The lowest concentrations of elements in the muscle tissue of the Black Sea turbot were registered in case of Cr and Co respectively, with mean values of 0.008 ± 0.00 µg/g fresh weight and 0.007 ± 0.00 µg/g fresh weight respectively (Figure 6).

Chromium is boots insulin activity and holds an essential role in the glucose metabolism, whilst cobalt is part of B_{12} vitamin (Blust, 2011; Zhang et al., 2022).

The correlation matrix (Figure 7) highlights the positive relationship between the following elements: Cr-Co (0.57), Ni-Mn (0.59), Ni-K (0.97), Cu-K (0.8), Zn-Cr (0.81), Fe-Cr (0.81), Cu-Cr (0.63), Cu-Ni (0.74), Zn-Ca (0.75), Na-Ca (0.87), Fe-Ca (0.54), Fe-Cu (0.9), Na-Cu (0.57), Zn-Cu (0.73), Ca-Zn (0.75), Zn-Fe (0.95), Na-Fe (0.71).

As well, in Figure 7 it can be observed the following negative correlations between elements in the muscle tissue of the Black Sea turbot:

Mn-Co (-0.87), K-Cd (-0.93), K-Mg (-0.89), Cr-Pb (-0.64), Cr-Mn (-0.68), Ca-Pb (-0.86), Ca-Co (-0.6), Cu-Pb (-0.66), Cu-Cd (-0.91), Cu-Mg (-0.9), Zn-Pb (-0.97), Na-Pb (-0.9), Fe-Pb (-0.89), Fe-Cd (-0.66), Fe-Mg (-0.66).

Further on, as it can be observed in Figure 8, the PCA matrix confirms the positive and negative correlations between elements. Withal, the PCA analysis explains 77.1% of the data, which are distributed in 2 groups.

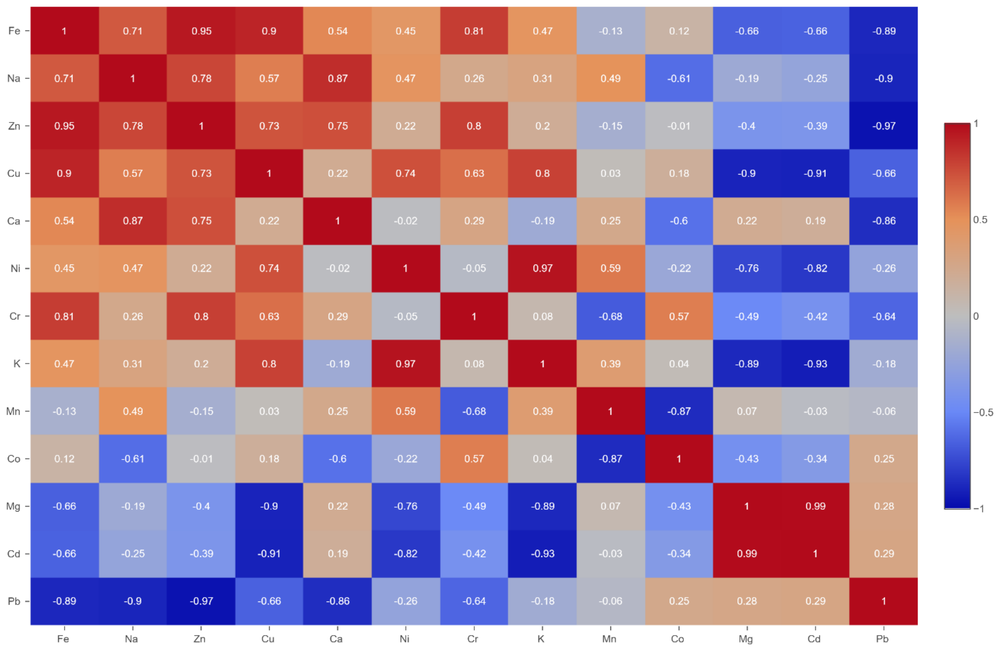

Figure 7. Correlation matrix of elements in the muscle tissue of the Black Sea turbot

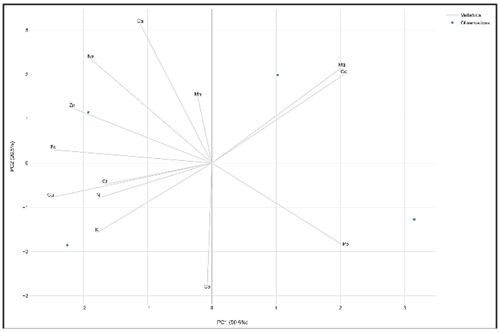

Figure. 8. Principal component analysis of elements in the Black Sea turbot

CONCLUSIONS

The main conclusion of this research is that the Black Sea turbot is an important source of essential macronutrients such as Ca, Mg, K, Na and micronutrients such as Fe, Zn, Cu, Mn, Cr and Co. At the same time, the Black Sea turbot can also be a source of non-essential elements, with toxic potential in the human diet, such as Ni, Cd and Pb.

The following accumulation trend in Black Sea turbot muscle was identified: Na>K>Ca>Mg>Zn>Fe>Cu>Mn>Ni>Cd>Pb>Cr>Co.

Competition between elements has been identified through correlation and PCA analysis, especially between K and Cd, Mg and Cu, Ca and Pb, Na and Pb.

ACKNOWLEDGEMENTS

The present research was supported by the project An Integrated System for the Complex Environmental Research and Monitoring in the Danube River Area, REXDAN, SMIS code 127065, co-financed by the European Regional Development Fund through the Competitiveness Operational Programme 2014-2020, contract no. 309/10.07.2021.

This work was supported by the Fondo Proserpina S.R.L., grant number 2506/2022, "The impact of heavy metals and microplastics from aquatic organisms on human health".

REFERENCES

Abd-Elhamed, M. (2021). Applying Nano-technology in tilapia Nutrition: Influence of Iron and Zinc nanoparticles as dietary supplementary on biological performance and body composition of Oreochromis niloticus fry. *Mediterranean Aquaculture Journal*, *8*(1), 30–41. https://doi.org/10.21608/maj.2021.225673

Antony Jesu Prabhu, P., Silva, M. S., Kröeckel, S., Holme, M.-H., Ørnsrud, R., Amlund, H., Lock, E.-J., &Waagbø, R. (2019). Effect of levels and sources of dietary manganese on growth and mineral composition of post-smolt Atlantic salmon fed low fish meal, plant-based ingredient diets. *Aquaculture*, *512*, 734287. https://doi.org/10.1016/j.aquaculture.2019.734287

Blewett, T. A., & Leonard, E. M. (2017). Mechanisms of nickel toxicity to fish and invertebrates in marine and estuarine waters. *Environmental Pollution*, *223*, 311–322. https://doi.org/10.1016/j.envpol.2017.01.028

Blust, R. (2011). 6—Cobalt. In C. M. Wood, A. P. Farrell, & C. J. Brauner (Eds.), *Fish Physiology* (Vol. 31, pp. 291–326). Academic Press. https://doi.org/10.1016/S1546-5098(11)31006-0

Din, N., Nazeer, N., Masood, Z., & Ullah, A. (2015). *The Levels of Some Selected Metals in Muscle's Tissues of Three Commercially Important Edible Fishes Collected from the Fish Market of Quetta City in Balochistan Province*.

Firidin, S., Ozturk, R. C., Alemdag, M., Eroglu, O., Terzi, Y., Kutlu, I., Duzgunes, Z. D., Cakmak, E., & Aydin, I. (2020). Population genetic structure of turbot (*Scophthalmus maximus* L., 1758) in the Black Sea. *Journal of Fish Biology*, *97*(4), 1154–1164. https://doi.org/10.1111/jfb.14487

Giragosov, V., &Khanaychenko, A. (2012). The State-of-Art of the Black Sea Turbot Spawning Population off Crimea (1998-2010). *Turkish Journal of Fisheries and Aquatic Sciences*, *12*(5), 377–383.

Hrynevych, N., Svitelskyi, M., Solomatina, V., Ishchuk, O., Matkovska, S., Sliusarenko, A., Khomiak, O., Trofymchuk, A., Pukalo, P., &Zharchynska, V. (2022). Acclimatization of fish to the higher calcium levels in the water environment. *Potravinarstvo Slovak Journal of Food Sciences*, *16*, 101–113. https://doi.org/10.5219/1732

Hulak, B., Leonchyk, Y., Maximov, V., Tiganov, G., Shlyakhov, V., &Pyatnitsky, M. (2021). The current state of the turbot, (Linnaeus, 1758), population in the northwestern part of the Black Sea. *Fisheries & Aquatic Life*, *29*(3), 164–175. https://doi.org/10.2478/aopf-2021-0018

Ivanova, P., Dzhembekova, N., Atanassov, I., Rusanov, K., Raykov, V., Zlateva, I., Yankova, M., Raev, Y., &Nikolov, G. (2021). Genetic diversity and morphological characterisation of three turbot (*Scophthalmus maximus* L., 1758) populations along the Bulgarian Black Sea coast. *Nature Conservation*, *43*, 123–146. https://doi.org/10.3897/natureconservation.43.64195

Kamunde, C., Grosell, M., Higgs, D., & Wood, C. M. (2002). Copper metabolism in actively growing rainbow trout (Oncorhynchus mykiss): Interactions between dietary and waterborne copper uptake. *Journal of Experimental Biology*, *205*(2), 279–290. https://doi.org/10.1242/jeb.205.2.279

Khanaychenko, A. N., &Giragosov, V. E. (2019). Morphological features of the Black Sea turbot (*Scophthalmus maeoticus*) during the period of embryonic development. *Marine Biological Journal*, *4*(4), Article 4. https://doi.org/10.21072/mbj.2019.04.4.06

Lall, S. P., & Kaushik, S. J. (2021). Nutrition and Metabolism of Minerals in Fish. *Animals : An Open Access Journal from MDPI*, *11*(9), 2711. https://doi.org/10.3390/ani11092711

Liu, W., Wang, Q., Mei, J., &Xie, J. (2021). Shelf-Life Extension of Refrigerated Turbot (*Scophthalmus maximus*) by Using Weakly Acidic Electrolyzed Water and Active Coatings Containing Daphnetin Emulsions. *Frontiers in Nutrition*, *8*. https://www.frontiersin.org/articles/10.3389/fnut.2021.696212

Ma, A., Huang, Z., Wang, X., Xu, Y., & Guo, X. (2021). Identification of quantitative trait loci associated with upper temperature tolerance in turbot, *Scophthalmus maximus*. *Scientific Reports*, *11*(1), Article 1. https://doi.org/10.1038/s41598-021-01062-3

Malhotra, N., Ger, T.-R., Uapipatanakul, B., Huang, J.-C., Chen, K. H.-C., & Hsiao, C.-D. (2020). Review of Copper and Copper Nanoparticle Toxicity in Fish. *Nanomaterials*, *10*(6), 1126. https://doi.org/10.3390/nano10061126

Manthey-Karl, M., Lehmann, I., Ostermeyer, U., &Schröder, U. (2016). Natural Chemical Composition of Commercial Fish Species: Characterisation of Pangasius, Wild and Farmed Turbot and Barramundi. *Foods*, *5*(3), Article 3. https://doi.org/10.3390/foods5030058

Marchetti, C. (2013). Role of Calcium Channels in Heavy Metal Toxicity. *ISRN Toxicology*, *2013*, 184360. https://doi.org/10.1155/2013/184360

Massa, F., Aydın, I., Fezzardi, D., Akbulut, B., Atanasoff, A., Beken, A. T., Bekh, V., Buhlak, Y., Burlachenko, I., Can, E., Carboni, S., Caruso, F., Dağtekin, M., Demianenko, K., Deniz, H., Fidan, D., Fourdain, L., Frederiksen, M., Guchmanidze, A., Yücel-Gier, G. (2021). Black Sea Aquaculture: Legacy, Challenges & Future Opportunities. *Aquaculture Studies*, *21*(4). https://www.aquast.org/abstract.php?lang=en&id=524

Muyssen, B. T. A., Brix, K. V., DeForest, D. K., & Janssen, C. R. (2011). Nickel essentiality and homeostasis in aquatic organisms. *Environmental Reviews*. https://doi.org/10.1139/a04-004

Pouil, S., Warnau, M., Oberhänsli, F., Teyssié, J.-L., Bustamante, P., &Metian, M. (2016). Influence of food on the assimilation of essential elements (Co, Mn, and Zn) by turbot *Scophthalmus maximus*. *Marine Ecology Progress Series*, *550*, 207–218. https://doi.org/10.3354/meps11716

Presas-Basalo, F. X. (2022). Potassium Homeostasis and Fish Welfare in Coupled Aquaponic Systems. *Fisheries and Aquaculture Journal*, *13*(2), 1–5.

Silva, M. S., Sele, V., Sloth, J. J., Araujo, P., &Amlund, H. (2019). Speciation of zinc in fish feed by size exclusion chromatography coupled to inductively coupled plasma mass spectrometry – using fractional factorial design for method optimisation and mild extraction conditions. *Journal of Chromatography B*, *1104*, 262–268. https://doi.org/10.1016/j.jchromb.2018.11.010

Stoyanova, S. (2018). *Investigation of macroelements in the muscle of four marine fish species*. *6*(2), 219–222.

Turan, C., Ivanova, P. P., Raykov, V. S., Gurlek, M., Erguden, D., Yaglioglu, D., Karan, S., Dogdu, S. A., Uyan, A., Ozturk, B., Nikolov, V., Dobrovolov, I., Khanaychenko, A., &Giragosov, V. (2019). Genetics Structure Analysis of Turbot (Scophthalmus maximus, Linnaeus, 1758) in the Black and Mediterranean Seas for Application of Innovative Management Strategies. *Frontiers in Marine Science*, *6*. https://www.frontiersin.org/articles/10.3389/fmars.2019.00740

Ulman, A., Zengin, M., Demirel, N., & Pauly, D. (2020). The Lost Fish of Turkey: A Recent History of Disappeared Species and Commercial Fishery Extinctions for the Turkish Marmara and Black Seas. *Frontiers in Marine Science*, *7*. https://www.frontiersin.org/articles/10.3389/fmars.2020.00650

Wen, Z.-Y., Qin, C.-J., Lv, Y.-Y., Li, Y.-P., Zou, Y.-C., Guo, S.-T., & Shi, Q. (2021). Homeostasis Regulation by Potassium Channel Subfamily K Member 3 (KCNK3) in Various Fishes. *Frontiers in Marine Science*, *8*. https://www.frontiersin.org/articles/10.3389/fmars.2021.816861

Zhang, Y., Luo, J., Zhu, T., Zhang, X., Jin, M., Jiao, L., Meng, F., Figueiredo-Silva, C., Hong, Y., & Zhou, Q. (2022). Dietary chromium could improve growth, antioxidant capacity, chromium accumulation in tissues and expression of genes involved into glucose and lipid metabolism in juvenile mud crab *Scylla paramamosain*. *Aquaculture Reports*, *23*, 101088. https://doi.org/10.1016/j.aqrep.2022.101088

Zhao, L., Xia, Z., & Wang, F. (2014). Zebrafish in the sea of mineral (iron, zinc, and copper) metabolism. *Frontiers in Pharmacology*, *5*. https://www.frontiersin.org/articles/10.3389/fphar.2014.00033

Zhou, M., Zhang, Y., Wang, J., Shi, Y., & Puig, V. (2022). Water Quality Indicator Interval Prediction in Wastewater Treatment Process Based on the Improved BES-LSSVM Algorithm. *Sensors*, *22*(2), Article 2. https://doi.org/10.3390/s22020422