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Food safety aspects of possible chemical contamination in fish

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1. INTRODUCTION

Fish and seafood are ones of the major foods that most of us eat or encounter in our everyday life. The reason for its popularity is not only the high quality of protein source, but also the abundant level of other nutrients. The global consumption of fish and seafood has been increasing and even doubled in the past 50 years due to globalization (Guillen et al., 2019) as the global population rapidly increases and as we get to know more about its benefits. Especially in those areas where they have easy access to the sea or other water sources, fish and seafood are quite important sources of food and they have had a remarkably close relationship with them. And furthermore, they tend to have more varied kinds of traditional cuisines of fish, such as raw fish meals. However, just like we have many benefits of consumption of fish, there are also many risks and dangers of it, too. One of the biggest disadvantages can be the contamination of seafood, by chemical, microbiological, and toxicological substances. And therefore, in those areas where their meals are based more on fish or seafood can be affected by contamination more.

For example, heavy metal contamination with such as Arsenic (As), Cadmium (Cd), Lead (Pb), and Mercury (Hg) of water has been a significant issue in numerous areas of the world, and its awareness has been raised for many years. Although they are naturally occurring substances in the environment there are many other ways of such pollution. One of the major reasons is that they get emitted in the processes of industries either by accident or incident, through smoke, dust, disposal of contaminated water, and so on, and consequently contaminate the environment such as surface water, vegetation, and soil. These contaminations of the environment can reach the ecosystem, thus the food chain as well, and it causes the biological concentration of the contaminants. Certainly, the food chain of aquatic ecosystem is not an exception. In aquatic ecosystem, the contaminants get dissolved or form colloids or precipitations, and they get accumulated in the floor, where many aquatic organisms inhabit. Some organisms like mollusks have an ability to detoxify the heavy metals using the low molecular weight protein called metallothionein, but that also results in accumulation of the heavy metals in their tissues, which can be harmful when it comes for human consumption.

In Japan, after the World War two they went through an intensive economic growth that made the country become one of the largest economies back then, and thus the growth of the coal- and metal-related industries were quite notable as well. Itai-itai disease is one of the “four big pollution diseases of Japan”, and a well-known disease because of the contamination of water by cadmium and severe intoxication by that. Due to the wastewater contaminated with cadmium from mines in Toyama prefecture in Japan, the local river Jinzu river got contaminated and the river was used for fishing, drinking water and irrigation of the crops such as rice. People suffered from osteomalacia and kidney failure due to cadmium intoxication. Minamata disease is caused by organic mercury intoxication that causes central nervous system symptoms and is also one of the consequences of the industrial growth of Japan in the late 1950s. From these catastrophic events, the interest and awareness for the heavy metal contamination of our foodstuff have been arisen, and therefore, nowadays we do have to monitor the levels of them in the food and keep them as low as possible to avoid the tragic incidents to occur again.

Fish can take up such contaminants not only through their gastrointestinal organs but also by the filtration of the water with the gills and through their skin. And since they are naturally occurring substances, heavy metals can usually be found in fish, but it is quite crucial to regulate the limit of the amount that can be found in the fish for human consumption.

In this study, the aim is to revise the possible contaminants of fish for human consumption and evaluate the measured concentrations of heavy metals and other metals from European Seabass (*Dicentrarchus labrax*) that were originated from the Adriatic Sea in Croatia and purchased at a Hungarian fishery market, with the regulated official limit value.

2. REVIEW of LITERATURE

2.1. Advantages of fish consumption

Fish contains high quality of beneficial nutrients, and therefore it has important roles as a major source of nutrition in our general life as well as for other animals such as predatory birds and bears. The nutrients include macronutrients such as high-quality protein from its lean meat composed of some of the essential amino acids such as cysteine, glutamine, hydroxyproline, proline, and taurine (Lehel et al., 2021). Bioactive peptides, for example ACE inhibitory peptides, that we can get from fish also have important roles, such as antihypertensive effect, but there are also some with antioxidant, antimicrobial, and antiproliferative effects as well (Ryan et al., 2011).

Even though fish are rich in protein and low in fat, a significant proportion of omega-3 polyunsaturated fatty acid groups (n3PUFAs) is found in fish, especially oil rich fish, and this is one of the biggest benefits of regular consumption of fish meals. The n3PUFAs are eicosapentanoic acid (EPA) and decosahexanoic acid (DHA) for instance, and they are considered quite important for prevention of cardiovascular diseases such as coronary heart disease events (Ruxton, 2011). Furthermore, they also provide support for the immune cells i.e. phagocytosis by macrophages and neutrophils (Gutiérrez et al., 2019). However, even though they have an immense potential for more human health such as diabetes, cancer, mental illnesses, visual and neurological development, periodontal disease, and rheumatoid arthritis, Shahidi and Ambigaipalan (2018) reported that it is crucial to clinically investigate further because there are some findings of controversial effects of n3PUFA against those diseases as well. In fact, it is recommended that people should consume approximately 2 servings (2 portions of 140 g) of fish meals per week, which at least one of them should be oil rich fish i.e. herring, mackerel, salmon, trout, sardines, and tuna, in many countries such as the US and the UK (FDA 2022; CDC, 2023). Such consumption can contribute the 130% intake of GDA/RDA (Guideline daily amount/Recommended daily allowance) for n3PUFA, and the oil rich fish contains higher amount of n3PUFA (≧80 mg EPA, DHA/100 g) than other natural fish (≧40 mg EPA, DHA/100 g) according to Ruxton (2011).

Other than macronutrients, they also provide elevated levels and well-balanced number of micronutrients as well. Iodine, selenium, calcium, and phosphorus are quite abundantly found amongst the micronutrients in fish, whereas manganese, magnesium, and zinc are found at lesser level despite of their importance (Daschner, 2016). Usually, iron is found more in the head and viscera of fish, where we do not eat often, but some fish contains high level in edible parts such as small fish Mekong flying barb (*Esomus longimanus*), and the fish have an important role in Cambodia to contribute around 45% of daily requirement in women for example (Kawarazuka and Béné, 2011).

Vitamins are also rich in fish as micronutrients, but it also depends on which kind of fish and what part of fish we eat. For example, aquacultured fish can have a varied number of vitamins due to its different methods. However, oil rich fish can be the best source of vitamin D. Fish are dependent on dietary sources to obtain vitamin D since they cannot synthesize it, and in the aquatic environment, fish take it from plankton, in which vitamin D gets accumulated. When we consume fish meals, vitamin D is found with higher quantity in liver and fat tissues (Lock et al., 2010). The two-weekly intake of fish can provide around 34% of GDA/RDA for vitamin D according to Ruxton (2011), and considering the high amount of calcium and phosphorus, it is quite efficient to consume fish for the bone development in children for instance. Vitamin E is also crucial for our health as antioxidant agent, cell signaling, modulation of enzymatic activities, and gene expression, but also it is important for its shelf life as well since it takes part in prevention of lipid oxidation (Hamre, 2011). Another lipid soluble vitamin, Vitamin A, is not as significant as other vitamins in fish, but some freshwater small fish such as Mola carplet (Amblypharyngodon mola) in Bangladesh contains elevated level of vitamin A. However, the level of vitamin A in fish varies, and therefore it is not ideal to rely on fish to achieve the required amount of fish intake (Kawarazuka and Béné, 2011).

For the water-soluble vitamins, vitamin B_6 and B_{12} may be provided in an adequate amount from fish (Daschner, 2016), but as well as other vitamins, it also depends on the fish species and cultivated method.

2.2. Potential microbial and chemical contamination of fish

2.2.1 Parasites

Food contamination with parasites is still quite common worldwide, not only in developing countries but also in developed countries as well, even though food safety regulations are implemented in many countries regarding such contaminations. Amongst a lot of kinds of parasites that cause zoonotic infections, nematodes, cestodes, trematodes, and myxosporidia are especially important in food safety aspects (Lehel et al., 2021). In some countries, people traditionally eat raw fish meals such as sushi and sashimi in Japan, anchovy in Mediterranean countries, and ceviche in South American countries, and those meals had gotten quite popular among the other countries in the world as well due to the globalization. Thus, it is necessary to treat the fish by heating or freezing before serving as a meal and being consumed from the food safety aspect (Daschner, 2016), even though there is a chance that such treatments can alter the taste of the fish (Iwata et al., 2015).

One of the most known zoonotic parasite infections is called Anisakidosis by the *Anisakidae* family, which includes *Anisakis*, *Pseudoterranova*, and *Contracaecum* genera, but most of the observed cases are caused by *Anisakis simplex* (Buchmann and Mehrdana, 2016). According to Aibinu et al. (2019) the final hosts of Anisakid are cetaceans such as whales, seals and dolphins, and the intermediate hosts include crustaceans and mollusks. Krill (*Euphausiidae*) are the most important first intermediate host where the third stage larvae (L3) develop from the second stage larvae (L2), and lots of aquatic animals including fish ingest krill, which leads to paratenic infection of L3 to those animals. Humans are accidental hosts for Anisakid infections where L3 is ingested by consuming infected and undercooked or not well treated fish or seafood. If the L3 Anisakid gets ingested by cetaceans, they start to develop to become L4 stage, and thus adult stage later that lays eggs. The eggs get emitted into the environment with faeces, and they develop to the L2 stage there. In fish, *Anisakis* species can be found in the body cavity, muscles, and organs. *Pseudoterranova* species can be seen in muscles of fish, whereas *Contracaecum* species can be found in the liver, mesenteries, caecum, and body cavity mostly (Lehel et al., 2021). Anisakid infection can cause two major pathologies (Caramello et al., 2003). One is allergic reactions from mild symptoms to anaphylactic reactions with gastrointestinal symptoms. In case of mild symptoms, urticaria and angioedema can develop, but in case of severe reaction anaphylactic shock can occur, and since it is often associated with gastrointestinal symptoms it is also called ‘gastroallergic anisakiasis’. The allergic reaction can be initiated by the presence of Anisakid antigens, therefore, it does not necessarily mean the presence of alive Anisakid larvae in the food when allergic reactions are seen (Daschner et al., 2012). The other pathology is direct damage such as gut wall invasion, eosinophilic granuloma formation and perforation.

The most important cestode parasite from the food safety aspects of fish is *Diphyllobothrium* species, and they are widely distributed. The tapeworm species including *D. latum* and *D. nihonkaiense* infect humans and piscivorous birds and mammals as definitive hosts, via intermediate hosts such as freshwater and marine fish, especially anadromous species like salmonids. They usually cause asymptomatic infections in humans, but sometimes they cause abdominal pain or discomfort, diarrhea, fatigue, constipation, and pernicious anaemia. Since a number of infected people cannot realize the infection, they become patent and grow in the small intestines for decades becoming up to 12 m long, and thus, they find out by expulsion of the segments with the faeces. In case of heavy *D. latum* infection, the patient can develop megaloblastic anaemia due to vitamin B_{12}-intrinsic factor complex dissociation (Scholz et al., 2009).

Trematodes are also possibilities of fish contamination especially when it is not treated properly. *Clonorchis, Opisthorchis,* and *Paragonimus* species are ones of the most important trematodes affecting humans, where *Clonorchis* and *Opisthorchis* are liver flukes, and *Paragonimus* species infect lungs (Lehel et al., 2021). *Clonorchis sinensis, Opisthorchis viverrini*, and *O. felineus* infections from raw fish intake are widely distributed in the world, but especially in Asian regions, where raw cyprinid fish and shrimps are eaten. For instance, in Thailand millions of people are infected, and a lot more people are at risk of infection in Asian countries (Sripa et al., 2011). Their definitive hosts are not only humans, but also other mammals such as dogs, cats, pigs, badgers, minks, weasels, and rats. Their eggs get shed in the faeces of the infected mammals, and it infects freshwater snails like *Parafossarulus* and *Bithynia* (Rim, 2005). Eggs metamorphose into sporocysts, and they reproduce asexually. Cercariae then get shed into the water, and they infect fish by penetrating their skin. Cercariae become metacercariae in the muscle of infected fish, and this is the form that infects mammals including humans when they get eaten without being cooked or treated adequately. However, sometimes infection can occur by drinking water from a stream that is contaminated with dead infected fish, or accidental infection of metacercariae while handling the fish (Rim, 2005). Metacercariae encyst in the duodenum, and they migrate to the bile ducts, where they inhabit (Sripa et al., 2011). According to the study of Rim (2015), their infection to the bile ducts can cause pathogenesis such as mechanical blockage of the ducts, mucosal damages and desquamation caused by blood-sucking adult parasites, chemical action caused by metabolites of parasites, inflammatory lesions, and host's reaction. In heavy infections, pancreatic duct can get infected as well. Furthermore, in the most serious cases cancer such as cholangiocarcinoma development can be seen due to heavy infection.

Myxosporidia are microscopic parasites, and they have a complex life cycle which often includes fish species that are also for human consumption. For instance, *Myxobolus pavlovskii* is known to infect silver carp (*Hypophthalmichthys molitrix*) (Molnár, 1979), and *Kudoa septempunctata* infects olive flounder (*Paralichthys olivaceus*) (Matsukane et al., 2010). The number of *Kudoa septempunctata* infection is increasing in the past years in Japan, where people eat raw olive flounder as sashimi. Such infection can cause gastrointestinal symptoms including diarrhea and vomiting, but usually the symptoms resolve themselves and the patients recover within 24 hours after the beginning of the symptoms (Kawai et al., 2012). Furthermore, they can be killed by heat treatment or freezing, but myxosporidia species can cause pathological changes in fish as well, and therefore those fish cannot be supplied as food anymore (Matsukane et al., 2010).

2.2.2 Bacteria

Food spoilage and food poisoning are quite common problems we face in general life as well, and they often cause unpleasant smells, texture, and taste and serious health problems for us. Fish contamination with bacteria is not an exception, and since fish generally contain high levels of water and have ideal pH level, they are easy to grow on for bacteria and quite perishable. In fact, amongst food borne diseases in general, two thirds are caused by bacteria.

Vibrionaceae is an example of Gram-negative fermentative bacteria, and they can be the reason of spoilage of fish in the storage without any preservation process, meanwhile psychrotolerant Gram-negative bacteria such as *Pseudomonas* spp. and *Shewanella* spp. can grow on chilled fish. Even under CO_2 packing and NaCl treatment, respiratory bacteria such as lactic acid bacteria (*Lactobacillus* and *Carnobacterium)* can grow as well as *Photobacterium phosphorum* and *Enterobacteriaceae* (Gram and Dalgaard, 2002). Although in many cases it does not cause serious health issues by consuming them, some bacteria (*Bacillus aminophilus,* some *Salmonella* spp., *Clostridium perfringens*, *Morganella morganii etc.*) produce histamine by decarboxylation of histidine (Ienistea, 1971) in the dead fish muscles, especially Scombridae family fishes, such as sardine, tuna, and mackerel, and it can result in scombrotoxicosis (vomiting, diarrhea, and/or allergic reaction) (Lehane and Olley, 2000).

While food spoilage is easily detectable from its unpleasant odor due to the microbial activities and formation of amines, sulfides, alcohols, and organic acids etc., food poisoning is not as easy to detect from its appearance. However, food poisoning can cause severer symptoms.

Staphylococcus aureus is one of the most common bacterial causes of food poisoning, which develops gastroenteritis in patients with its staphylococcal enterotoxins (Le Loir et al., 2003). Even though the amount of data of *S. aureus* incidence in fishery products is limited (Simon and Sanjeev, 2007), it is quite important to control the safety of fish products because *S. Aureus* can be transmitted easily. In addition, *Clostridium botulinum* can also produce toxins in dead fish, and they can cause neuroparalytic symptoms by inhibiting exocytosis of neurotransmitters at the neuromuscular junctions. In *Vibrio* spp., *Vibrio cholera* is a causative agent of human cholera disease, and a lot of people get infected and die, especially in developing countries. Also, *Vibrio vulnificus* is known for its high mortality; they can cause acute gastroenteritis and septicaemia, and it can be highly fatal, however, *V. vulnificus* can be found mostly only from marine fish (especially shellfish) since they cannot live in freshwater (Baker-Austin and Oliver, 2018).

Other than those examples, there are much more bacterial species that cause food poisoning from fish consumption, such as *Clostridium perfringens, C. difficile, Listeria monocytogenes, Salmonella spp., E. coli, Campylobacter spp., Schigella spp.,*and *Yersinia spp.* (Lehel et al., 2021).

2.2.3 Viruses

Viral food poisoning caused by consumption of fish is not as common as parasitic and bacterial food poisonings. However, it is highly possible that food gets contaminated by viruses from infected humans to others during handling or processing them. In addition, shellfish can be contaminated with food borne viruses; Norovirus (Caliciviridae) (CDC, 2023) and Hepatitis A virus (WHO, 2023) that cause acute gastroenteritis and acute hepatitis respectively, and therefore, it is quite important to treat the food adequately especially when the food source sanity is questionable. According to the European Food Safety Agency (EFSA) (2022), Caliciviridae infection is one of the most common food-borne diseases in the EU.

2.2.4 Phycotoxins

In Japan, it is widely known that people eat raw fish as a meal such as sashimi, sushi, and other seafood cuisines. And therefore, they need to be more aware and cautious about possible contaminations of the raw fish. Apart from biological hazards such as bacteria and parasites, it is also necessary to be aware of biological toxins. For instance, Fugu fish (pufferfish) is a unique cuisine in Japan because it is often served raw, even though they are known to have lethal amount of toxins called tetrodotoxin. Only licensed cooks can serve them for such safety reasons, but occasionally the intoxication happens, nevertheless.

Phycotoxin is a kind of toxin that micro algae in the sea and freshwater produce, which is taken up by fish and other animals living in the water eventually and gets accumulated or vectored in those animals (Aligizaki and Nikolaidis, 2008; Yentsch and Incze, 1980). Phycotoxins can be classified as marine and freshwater toxins since the algae in different water types produce various kinds of toxins. In the sea water, it is common that shellfish tends to accumulate toxins that cause paralysis, diarrhea, amnesia, and neurotoxic effect, when they are consumed by humans (Lehel, 2002; Lehel, 2003). Paralytic shellfish poison (PSP) includes saxitoxin, neosaxitoxin, and gonyautoxin, and they cause inhibition of cellular influx of sodium ions in human bodies, and therefore results in paralysis. The toxins are produced for example by *Alexandrium*, *Gonyaulax*, *Gymnodium*, *Pyodinium* species of algae, and they get consumed by those types of shellfish that we frequently eat (Alam et al., 1982; Boyer et al., 1978; Genenah and Shimizu, 1981; Halstead, 1978; Narahashi, 1972; Schantz and Ghazarossian, 1975). There are many possibilities of intoxication by phycotosins that cause diarrhea, diarrhetic shellfish poisons (DSP), such as okadaic acid, dynophysistoxin, azaspiracids, pectenotoxins and so on. They are also produced by algae in the marine water but can be found in the common shellfish and also some crabs and fish as well, and they cause gastrointestinal symptoms in the consumers who got poisoned (EFSA, 2008; FAO, 2004; Honkanan et al., 1994; Murakami et al., 1982; Valdiglesias et al., 2013; Yasumoto et al., 1980). Amnesic shellfish poisons (ASP) such as domoic acid are neurotoxins that activate glutamate receptors and therefore make the cells die by the membrane depolarization. Clinical signs start with gastrointestinal signs and later neurological signs appear (headache, dizziness, disorientation, loss of short-term memory), and they can cause death in people who ate the shellfish or even some fish that have the toxins (Berman et al., 2002; Grant et al., 2010; Perl et al., 1990; Wright et al., 1989). Neurological signs can be seen in cases of poisoning by neurotoxic shellfish poisons (NSP) including brevetoxin, palytoxin, and cyclic imines as well. Brevetoxin affects upper respiratory system for example, by increased influx of sodium ions and changed resting potential, meanwhile palytoxin causes abnormal contractions by inhibiting sodium-potassium ATPase which results in myalgia (Deeds and Schwartz, 2010; Hughes and Merson, 1976; Rhodes and Munday, 2004; Watkins, 2008). Cyclic imines work on both muscarinic and nicotinic acetylcholine receptors and causes respiratory and muscular symptoms (Gill et al., 2003; Kharrat et al., 2008; Munday, 2008; Selwood et al., 2010).

Phycotoxins also undergo the food chain of fish as well as shellfish, and cause food poisoning in humans (Halstead, 1988). Toxins produced by *Gambierdiscus toxicus* are known for its neurotoxicity that initiates paralysis in consumers after the intake of fish such as tuna and mackerel, and ciguatoxin intoxication is the most common illness by marine biotoxin, according to ECDC (European Centre for Disease Prevention and Control) (De Fouw et al., 2001; Lehane, 2000; Lehane and Lewis, 2000; Lewis, 1992; Friedman et al., 2017). Other than neurological and muscular symptoms, gastrointestinal signs can be seen as well, and it can be fatal in severe cases. As mentioned before, tetrodotoxin is a toxin that can be found in fugu fish (*Tetraodon*, *Arothron* species) (Ahasan et al., 2004), and unlike ciguatoxins or other phycotoxins, it inhibits the influx of sodium ions into the cells, and therefore clinical signs such as numbness of mouth and motoric and general paralysis can be seen. In severe cases, respiratory depression, hypoxia, and hypotension occur and the patients die due to such reasons (Bentur et al., 2008; How et al., 2003; Torda et al., 1973).

In fresh water, accumulation of phycotoxin is not as common as it is in marine water, however they still do have toxicological effects on humans by direct contact exposures. Allergies to those toxins are possible, but more severe toxicities such as neurotoxicity and hepatotoxicity can occur as well though it is not as common as it could be (Carmichael, 1981; Carmichael and Mahmood, 1984; Falconer et al., 1983; Grauer and Arnold, 1961).

In scombroid fish (tuna, mackerel, sardine etc.), histamine can be detected because of microbial enzyme activities that decarboxylates histidine of those fish. Histamine intoxication can cause allergic reactions and clinical signs such as headache, dizziness, hypotension, and tachycardia, but usually the patients recover sooner or later (Ansdell, 2008; Arnold and Brown, 1978; Mcinerney et al., 1996; Wu et al., 1997).

2.2.5 Chemical contaminants

Xenobiotics are such chemical contaminants that are from non-natural sources, for example it includes synthesized chemical substances (Parkinson and Ogilvie, 2008). The major sources of xenobiotics are pesticides, residues of veterinary or medical products, especially when the fish are cultured in an artificial environment such as aquaculture farms. In addition, food additives are widely used all over the world for the mass consumptions of nowadays, and those substances are also considered as xenobiotics, though they are often harmless to human bodies, and they are strictly controlled by laws in Europe. However, it is also common to find other chemical contaminants from technological procedures such as processing plants as well. In marine environments, petroleum hydrocarbons and chlorobiphenyls are important examples which are results of human activities (Hawkes, 1980).

2.2.6 Heavy metals

In our daily intakes we can find many kinds of heavy metals including copper (Cu), zinc (Zn), manganese (Mn), nickel (Ni) and so on, and many of them are quite crucial for our body to function well and properly. However, if we take them excessively, it can counteract and cause problems to our body as well. Furthermore, as well as those heavy metals that we require, there are ones that are not needed for our health, such as mercury (Hg), cadmium (Cd), lead (Pb), and arsenic (As) for example. They are distributed in the nature and the environment, and it is possible that we consume them in a small quantity, however when they are consumed more than the safe amount or in a high concentration, it can become hazardous to our health even though it is relatively rare. The bioaccumulation of those substances is a good example of that.

Mercury can be primarily sourced in the earth, especially the mantle and the crust, and from those sources they get emitted to the atmosphere while the degassing of the earth. And furthermore, mercury that is trapped in the rocks can be released by the weather (Jonasson and Boyle, 1972). Nowadays it is significant that anthropogenic sources of mercury pollute the atmosphere. Since coal also contains high concentrations of mercury, combustion of it causes the emission of mercury into the atmosphere, which is the largest cause of mercury pollution. As well as the combustion of fossil fuels, discard of some materials such as batteries can result in the anthropogenic source of mercury (Jackson, 1997; Expert Panel on Mercury Atmospheric Processes, 1994). Mercury stays in the environment in different oxidation states, Hg^0 and Hg^{II} mostly. Hg^0 is a vaporous state that consists of more than 90% of atmospheric mercury, whereas

Hg^{II} is usually bound to other substances such as particles or ions, but they both are abundantly present in the atmosphere as well, especially higher concentration of Hg^{II} is found in the center of pollution (Jackson, 1997). Both natural and industrial emissions of mercury result in the deposition in the atmosphere, thus in the ocean as well, and in fact, the deposition in the ocean is mostly sourced from the atmosphere, besides the direct deposition from the natural sources. Hg^0, that makes up the high fraction of atmospheric mercury is converted to Hg^{II} by oxidation when it is deposited into the ocean, and furthermore converted to the organic mercury, methylmercury, or reversed to Hg^0, which can be evaded to the atmosphere, in the water. Reduction to Hg^0 and methylation of Hg^{II} influence each other's reaction in a competitive way, in which reduction occurs dominantly in the presence of oxygen such as the water near the surface, compared to methylation. However, since the anthropogenic pollution of mercury has elevated the amount of mercury level in the atmosphere by industrialization, the amount of methylmercury has increased as well (Mason et al., 1994). The balance of the deposition and evasion of mercury in the ocean is of high importance because methylmercury, which has potential neurotoxic effects when it is consumed by human, can be deposited in the marine animals, and therefore causes bioaccumulation (Liu et al., 2020). As well as bioaccumulation, biomagnification of methylmercury has to be considered in marine animals for human consumption. According to Mason et al. (1995), methylmercury is more efficiently transferred from phytoplankton to zooplankton in the food web compared to inorganic mercury, and furthermore, in the sea water the bioaccumulation of methylmercury in the planktivorous fish is 16 times greater than inorganic mercury.

Minamata disease is a disease that was discovered first in Japan in 1956. Minamata is a name of the place that the disease was found, and the city has a bay called Minamata Bay, which is located in Kumamoto prefecture in the southern Japan. After this tragic outbreak of the disease in Minamata, more than 2000 people in the area have been identified as Minamata disease, however, a lot more people are still not identified yet by the government and suffering from the chronic neurological symptoms. This incident was revealed to be caused by the contaminated materials discharged to the bay from a factory that changed the cocatalyzer. The change resulted in a higher amount of methylmercury to be discharged. A decade later, another similar incident had happened in the central Japan, along the Agano River in Niigata prefecture, and more than 700 people were diagnosed as Minamata disease so far (Ministry of Environment of Japan, 2021). Pathological changes can be seen in the central nervous system such as cerebrum and cerebellum and in the peripheral nerves as well. According to Eto (2000), cerebral edema and demyelination can be observed in the perivascular region, whereas in cerebellum granulocytes were lost in spite of well-preserved Purkinje cells. Pathological signs in the peripheral nerves are found more prominently in chronic cases. According to those pathological alterations, clinical symptoms including sensory disturbances, visual field constriction, impaired muscle movements, muscle weakness can occur. Besides the clinical symptoms, deposition of mercury in the organs could be seen histopathologically as well, such as epithelial cells of proximal convoluted tubules in the kidneys, parenchymal tissues and Kupffer cells of liver, and neurons and glial cells in the brain of affected patients (Eto, 2000). Those catastrophic events that happened in Japan during the industrialization had led us to be more aware of the possible toxic effects of contaminated fish with heavy metals (Castro-González and Méndez-Armenta, 2008). According to ATSDR (2008), Cadmium can be found in the environment naturally as well, for example as cadmium ore, which is also called greenockite. In the ore they are found to be cadmium sulfide, and their dispersion to the environment is highly associated with zinc production, mining, and smelting. Cadmium is used by us mainly in metal plates, nickel-cadmium batteries, phosphate fertilizers, and plastic stabilizers in the industry for example. The dispersed cadmium stays in the atmosphere, and eventually falls onto the ground, for instance, by the rain. Cadmium particles deposited in the ground and water system can be taken up by plants, and therefore enter the food chain. In fact, Itai-itai disease that occurred during 1910s to 1970s in Japan was caused by the contamination of the earth for the agricultural products such as rice. The contaminated water with cadmium, lead, and zinc from Kamioka mine in Gifu prefecture had been released to the Jinzu River, and sequently discharged to the soil (Ministry of Environment of Japan, 1973). Itai-itai patients had gotten renal damage, osteomalacia, endocrinological alterations, and depletion of calcium, and therefore fracture and distortion of bones and osteoporosis as well, and they caused mainly middle-aged to elderly women to suffer. Furthermore, according to Genchi et al. (2020), the intestinal absorption of cadmium can be increased by the less body store of iron. Regarding seafood, crustaceans, mollusks, and shellfish are considered as additional sources of cadmium, and some of them are also known as hyper-accumulators, and their maximally permissible concentrations (MPC) are established (Satarug, 2018). Furthermore, in case of fish, there is research about the cadmium accumulation in carp (*Cyprinus carpio*), and it shows that accumulation of cadmium is more prominent in kidneys than liver, meanwhile the accumulation in muscles was only significant after a certain amount of time, and it was still lower than the kidneys and the liver. In addition, the research suggests that the accumulation is correlated with the concentration of cadmium exposure due to the higher level of accumulation seen in higher concentration (De Conto Cinier et al., 1999).

We can encounter lead very easily in our life, such as toys, paints, and other products even though the level of lead has been regulated these days in order to decrease the exposure to our body. However, lead is also in the environment including the atmosphere, water, dust, and soil, and therefore our food, especially in urban areas or industrialized areas due to the dust from cars for example (Body et al., 1991; Flora et al., 2006). Also due to its non-biodegradability, they can stay in the environment persistently. And furthermore, lead toxicity has been known and researched well, and nowadays we know that there is no such level of lead that can be advantageous to our health, but they can cause oxidative stress in the body (Flora et al., 2012). In case of acute toxicity, which is rarer than chronic exposure, patients show clinical signs of muscle pain, headache, vomiting, and central nervous signs such as seizures and coma. Chronic cases are much more common due to daily exposure to lead in the environment, and it can cause mainly central nervous signs such as encephalopathy, delirium, convulsions, and coma (Flora et al., 2006). Other than those clinical signs, people with constant exposure to lead may have problems in hematopoietic, hepatic, and renal functions (Kalia and Flora, 2005). Lead in live fish is generally higher level than cadmium and mercury, but it does not exceed 10 μg/g body weight (Jezierska and Witeska, 2006).

Arsenic is precisely a metalloid, that has both metal and non-metal characteristics. It is present in the environment as well in the form of inorganic and organic, which have distinctive characteristics in various aspects. Just like other heavy metals that were mentioned, they can be found in the environment not only naturally, but also due to the anthropogenic activities since it has been used on many occasions e.g. glass, textile, and paper manufactures, herbicide, pesticide, fungicide, and mostly in the process of treating wood to prevent rotting. According to ATSDR (2007), inorganic arsenic, such as arsenites and arsenates, is more toxic than organic ones, and has carcinogenic effect, however, nowadays humans are more prone to be exposed to organic arsenics than inorganic arsenics in daily life for example from agricultural products, fish, and shellfish. In the case of fish and shellfish, it is known that arsenobetaine and arsenocholine are the forms that the organic arsenic is found mostly, and the former has roles in osmoregulation in fish. Those organic arsenic compounds in fish are considered much less toxic (Abernathy et al., 2003), however, according to Uneyama et al. (2007), the level of inorganic arsenic in seafood seems to be the highest amongst our dietary intake, but meanwhile, Ministry of Agriculture, Forestry, and Fisheries of Japan (2019) published statistics that show that inorganic arsenic intake is mostly from rice in Japan, and they have discovered that rice contains higher fraction of inorganic arsenic amongst its total arsenic contents than other food products. The research of Has-Schön et al. (2006) shows that the muscle of fish contains a higher concentration of arsenic. On the other hand, since it is present in the groundwater, the carcinogenic effect of arsenic can cause cancer in places where people consume the groundwater (Smith et al., 2000). Due to sulfhydryl group-containing proteins being altered arsenic compounds, it causes other clinical signs in human bodies as well (Thomas et al., 2001). In acute cases, patients show gastrointestinal signs such as diarrhea, vomiting, and colic, and cardiovascular system and central nervous system can be disturbed. Chronic exposure causes dermatological signs such as hyperkeratinization on the palms and soles, and neurological signs due to peripheral and central nervous system damage (Castro-González and Méndez-Armenta, 2008).

2.3. Food-toxicological and food safety aspects

Food is something that we encounter every day, and it is necessary and enjoyable at the same time in our life, and we can say that it forms a huge part of our society. Not only for human beings, but also for animals, food (feed) is the basis to keep them alive and healthy, and it is important especially in livestock animals for human consumption because their health status can directly affect human health as well. Nowadays, human population has grown more than 8 billion, and of course, all of us need food in daily basis, and the amount of food we need in a day is growing as well, even though it varies in different countries, economy, religion, and many other factors. Therefore, it is always crucial to keep an eye on the safety of food to deliver safe food to every one of us, especially since the industrialization and globalization made a massive amount of products travel all over the world and go through a lot of processes and circumstances during the transport etc., and that also means that hazardous materials in the products can travel throughout the world as well. Furthermore, civilization, society, culture, and a lot more evolutions of human history were strongly connected and depended on the concept of food, and therefore, the forms and trends of food have been changing a lot as well. As we evolve as human beings, we always search for ways to live more conveniently and efficiently, and it is a natural instinct as living things with such high intelligence, however, such changes for a convenience may initiate new problems as well. For example, in the history of mass production of crops and thus increased demand of higher efficiency, humans invented many kinds of chemical products such as pesticides in order to grow more crops without loss. They have helped society grow more rapidly, but later started to reveal its side effects for long time exposure to humans and to the environment as well. In order to control and prevent such hazard, EU has legislations, for example, Commission implementing regulation (EU) 2020/2235 and Commission implementing regulation (EU) 2020/2236 are for animal health certificates including fishery products for the entry into EU and within EU, respectively. In this chapter, I will assess food safety from the aspect of legislations of European Union regarding to fish and seafood production.

According to Deardorff (1991), over 50 species of helminthic parasites are known to cause zoonotic infections from fish, bivalves, crabs, cray fish, and snails. Anisakidosis is a famous one, like introduced in the last chapter, and the infection can occur when the products are not well treated before consumption, even though the risk of human infection of those helminths overall is low. Health authorities have tried to control the safety of their own countries' fishery products before, but due to globalization and increased amount of imported and exported products between countries, it is necessary that each country cooperates to the others at international levels in order to keep the products safe, and especially developing countries can get more information and instructions to follow that way. And therefore, to make the international cooperation possible, besides the application of higher level of education, international organizations such as FAO and WHO suggest HACCP (hazard analysis and critical control points), so it is easier to follow the international standards of control (Northrop-Clewes and Shaw, 2000). In Europe, in 2018 EFSA has reviewed and published an article about three important food-borne infections of parasites which are *Toxoplasma gondii*, *Cryptosporidium* spp., and *Echinococcus* spp. (EFSA 2018). In those infections, fishery products do not take a large part usually, but according to the article, there are data that shows contamination of mollusks with *T. gondii* and *Cryptosporidium*, however, the data is not sufficient and clear. In addition, other parasitic diseases for fish such as white spot disease ro leeches that are not really of concern for infecting humans have to be controlled. In EU, Commission regulation (EC) 2074/2005 states the obligations of visual inspections on fishery products for detection of ectoparasites. In case of bacterial contamination of food, *Campylobacter* and *Salmonella* are the most common food-borne bacterial infections in Europe in 2021 according to EFSA (2022), and *Salmonella* contamination is common in fishery products as well. In shellfish, they can be a source of food-borne infection of *Campylobacter* as well (Tenius et al., 1997). Commission regulation (EC) 2073/2005 of EU describes microbiological criteria for foodstuff, and therefore includes the regulation of limits of *Salmonella* and *E. coli* that can be detected in shellfish. Histamine is regulated by the same commission regulation, 2073/2005, specifically for the fish species associated with a high amount of histidine. Marine biotoxins content of bivalve molluscs such as PSP, ASP, DSP, and NSP are regulated in 2019/627 EU Regulation (EC) 853/2004. In order to prevent such intoxication of the biotoxins, it is important to follow the risk assessment by sampling and analyzing the phycotoxins and risk management by prohibiting the live animals that are likely contaminated to go to consumers (O'Mahony, 2018).

2.4. Official regulation of heavy metals

2.4.1 Mercury

For the mercury level that can be detected in our food, the European Commission has set the maximum level in the Commission regulation (EC) No. 1881/2006 of 19 December 2006 at first (Commission regulation, 2006). This was set in order to keep the contaminant levels in foodstuff as low as reasonably achievable (ALARA), since mercury can cause serious health problems when it is consumed in high amounts. As regards mercury, the EFSA adopted on 24 February 2004 an opinion related to mercury and methylmercury in food and endorsed the provisional tolerable weekly intake (PTWI) of 1.6 µg/kg b.w. Methylmercury is the chemical form of most concern and can make up more than 90 % of the total mercury in fish and seafood. Taking into account the outcome of the SCOOP-task 3.2.11, EFSA concluded that the levels of mercury found in foods, other than fish and seafood, were of lower concern. The forms of mercury present in these other foods are mainly not methylmercury and they are therefore considered to be of lower risk, according to the official journal of EU in 2006. However, CONTAM panel of EFSA has lowered the PTWI level of methylmercury from 1.6 µg/kg b.w. to 1.3 µg/kg b.w. due to new studies finding out that n3PUFA may counteract negative effects from methylmercury exposure (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012).

In addition, the maximum levels that can be detected from the foodstuff were set in Commission regulation (EC) No. 1881/2006 as well, however, it was only divided into two categories; the muscle meat of some specific fishes such as tuna (*Thunnus* species, *Euthynnus* species, *Katsuwonus pelamis*), and other fishery products. The maximum allowed levels of mercury were 1.0 mg/kg wet weight and 0.5 mg/kg wet weight, respectively. However, according to Commission regulation (EU) 2023/915 of 25 April 2023 in the newly released official journal of the EU, the fishery products and bivalve mollusks are divided into three categories, with an extra category of cephalopods and so on to the previous two categories. The maximum level of mercury in the new category products is 0.3 mg/kg, while the other two categories stay the same according to the official Journal of the EU in 2023 (Commission regulation, 2023).

The PTWI of methylmercury from seafood in Japan is also set as 1.6 µg/kg b.w. as of 2015 according to Food Safety Commission of Japan, and the maximum level is also 0.3 mg/kg w.w. (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2015). Additionally, FSCJ (Food Safety Commission of Japan) has stated that the fetuses are considered as a high-risk group, which has importance of pregnant women to be aware of it.

2.4.2 Cadmium

In 2010 CONTAM panel of EFSA was ordered by European Commission to confirm whether the existing PTWI level and PTMI (provisional tolerable monthly intake) level for cadmium are appropriate, and therefore, CONTAM has assessed it and published a statement that confirms the levels 2.5 µg/kg b.w. and 25 µg/kg b.w. are appropriate, respectively (EFSA, 2011). In 2003 Ministry of Health, Labour, and Welfare of Japan asked the Food Safety Commission of Japan (FSCJ) to assess the PTWI level of cadmium, and in 2008 the commission has published that PTWI of cadmium can be set 7 µg/kg b.w. according to their assessment of the impact of the substance to human health and the possible exposure level of people in Japan (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2009).

Maximum levels in food are stated in Commission regulation (EC) 1881/2006 as well as for mercury, and the latest version, Commission regulation (EU) 2023/465 was released in March, as well as Commission regulation (EU) 2023/915. In the newest version, the classification of the fish species is different from the initial act, which is 1) mackerel, tuna, and bichique, 2) bullet tuna, and 3) anchovy, swordfish, and sardine. However, there was not a significant change in the number of the maximum level values. The values in the newest version are 0.05-0.25

mg/kg for fishes depending on the species, 0.5 mg/kg for crustaceans, and 1.0 mg/kg for bivalves and cephalopods. Japan is stating the maximum levels of cadmium in seafood according to Codex Alimentarius Commission (CAC), which are 2.0 mg/kg for bivalves and cephalopods (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2019). It is more important in Japan to focus on the level of cadmium in rice, since it is one of the major foods that is consumed in Japan. In addition, due to the characteristics of cadmium, it is more concerned to be detected in the soils, thus in the crops.

2.4.3 Lead

As well as mercury and cadmium, the maximum level of lead in EU is indicated in the same commission regulations, and the latest update shows that the value can be 0.3 - 1.5 mg/kg, depending on the product. They changed the value for cephalopods from the initial act, from 1.0 mg/kg to 0.3 mg/kg. Due to its harmful effect on the neurodevelopment, Joint FAO/WHO Expert Committee on Food Additives (JECFA) has released an evaluation in 2011, and stated that it is not possible to establish a PTWI that is considered health protective, and they withdrew the previously stated PTWI level (25 µg/kg b.w.). EU and Japan are following that decision (EFSA, 2010; Ministry of Agriculture, Forestry, and Fisheries of Japan, 2022). Furthermore, in Japan, they keep investigating the level of lead in food, but so far in the seafood products, the value has been lower than quantitation limit in ten years between 2005 and 2014, and therefore, it is considered safe (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2023).

2.4.4 Arsenic

Due to the fact that they discovered that rice and Hijiki seaweed tend to contain both prominent level of total arsenic and higher fraction of inorganic arsenic, which is more toxic than organic arsenic, in the total arsenic contents, Japan focuses more on the arsenic level in rice and the seaweed. Japan does not set a maximum level of arsenic specifically in fish and other seafood, but they keep the investigation of the levels in food and coordinate with CAC in order to help them set the level. For instance, the level of inorganic arsenic measured in rice has been up to 0.25 – 0.60 mg/kg in the past ten years, and up to 17 – 130 mg/kg in Hijiki seaweed in 2006-2008 (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2022). Likewise, the maximum level of arsenic is not set for seafood products in EU either, though it is set for cereals, rice products, fruit juice, baby food, and salt.

PTWI was once set as 15 µg/kg b.w. by JECFA, but since a wide range of adverse effects had been reported even with lower levels of arsenic, thus, the PTWI level was withdrawn (WHO/JECFA, 2011), and both EU and Japan follow that change.

3. MATERIALS and METHODS

3.1. **Materials**

The samples of European Seabass (*Dicentrarchus labrax*) were collected on a local fishery product market in Hungary, but the fish are originated from Adriatic Sea, Croatia (FAO Fishing area 37.2.1). A total of 40 fish were examined to determine their heavy metal and arsenic contents.

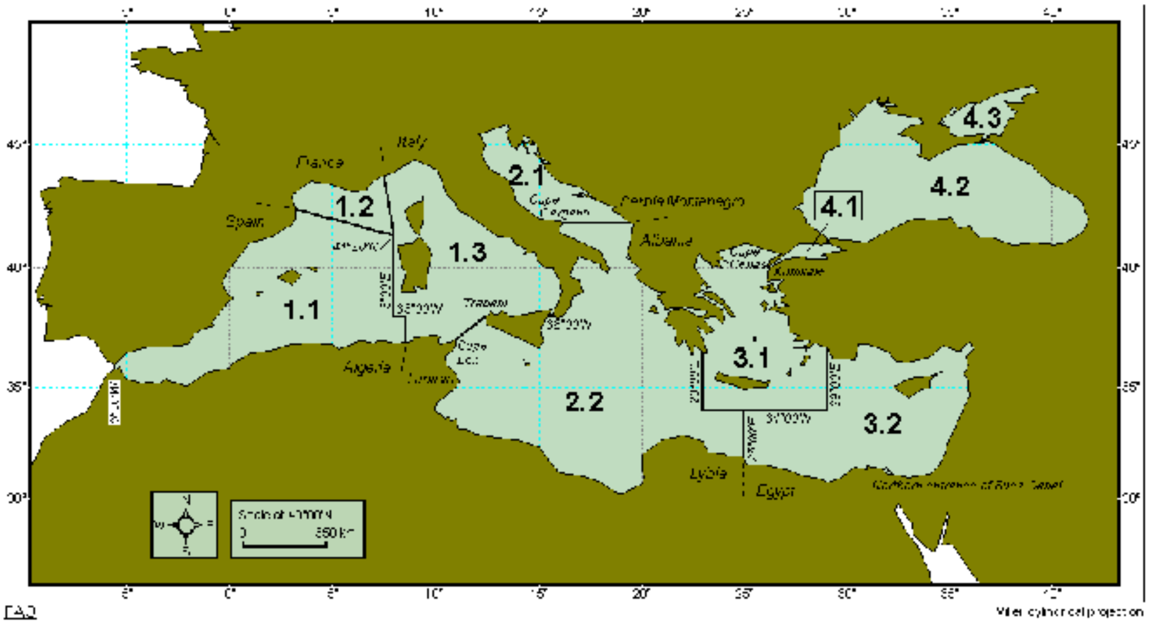
3.1.1 Origin – Central Mediterranean (Subarea 37.2)

All marine waters bounded, to the west, by a line running from Cape Bon (Ras el Tib) (at 37°08' N latitude; 11°00'E longitude) in a northeasterly direction to Trapani (38°02'N latitude; 12°32'E longitude); thence in a southeasterly direction along the coast of Sicily to a point on the northeast coast of Sicily at latitude 38°00'N; thence across the Strait of Messina due east to the southwest coast of Italy and bounded, to the east, by a line running from the northern bank of the Corinth Canal across the said Canal and in a southerly direction along the coast of the Peloponnese to a point at longitude 23°00'E; thence due south to latitude 34°00'N; thence due east to longitude 25°00'E; thence due south to the coast of Libya (FAO, 2023).

3.1.2 Origin – Adriatic Sea (Division 37.2.1)

The waters of the Adriatic Sea north of a line running from the Albanian northern frontier on the east coast of the Adriatic Sea due west to Cape Gargano at 41°49'N latitude and 16°12'E longitude on the coast of Italy (FAO, 2023).

Fig. 1 MEDITERRANEAN AND BLACK SEA (Major Fishing Area 37)



© FAO 2023. MEDITERRANEAN AND BLACK SEA (Major Fishing Area 37). Fisheries and Aquaculture Division [online]. Rome. [Cited Wednesday, October 18th, 2023]. https://www.fao.org/fishery/en/area/37/en

3.2. Analytical method

3.2.1. Reagents and analytical standards

For the preparation of samples, we utilized hydrogen peroxide (30% mass/mass, Normapur, VWR International Ltd., Leicestershire, UK) and nitric acid (69% mass/mass, Aristar, VWR International Ltd., Leicestershire, UK), both of trace analysis quality. To clean all laboratory glassware and plastic tools, a 0.15M hydrochloric acid solution (37% mass/mass, Aristar, VWR International Ltd., Leicestershire, UK) was employed, followed by rinsing with deionized water generated by a Purite Select Fusion 160 BP water purification system (Suez Water Ltd., Thame, UK). Calibration was carried out using ICP multi-element standards (Perkin Elmer Inc., Shelton, USA) and mono-element standards (VWR International Ltd., Leicestershire, UK) for quantitative ICP measurement. Quality control (QC) standards were prepared from standard bovine liver (NIST SRM 1577c, NIST, Gaithersburg, Maryland, USA), and measurements utilized Argon gas with a purity of 4.6 (Messer Hungarogáz Ltd., Budapest, Hungary).

3.2.2. Sample preparation

A metal-free instrument was employed to section the longitudinal back muscle samples from sardines. Following cutting and homogenization using a Potter S device (B. Braun Biotech International GmbH, Melsungen, Germany), the prepared samples were deposited into appropriately labeled plastic bags and stored at -70°C in a So-Low Ultra-Low Freezer (Model C85-9, Environmental Equipment Co. Inc., Cincinnati, USA) until analysis.

For each sample, precisely 0.5 g was measured into a CEM MARS XPreSS Teflon vessel (CEM Matthews, North Carolina, USA). Hydrogen peroxide and nitric acid, both at 5 ml, were introduced, initiating the decomposition process in a CEM MARS6 microwave digestion system (CEM Corporation, Matthews, North Carolina, USA). The decomposition parameters were as follows: Ramp: 35 min; temperature: 200°C; hold: 50 min; energy: 1700 W. The resulting solution was adjusted to 25 ml with deionized water, and ICP-OES was employed for analysis following a double dilution with deionized water. A 1 mg/l Y solution (VWR International Ltd., Leicestershire, UK) served as an internal standard, and a 0.25 mg/l Au solution (VWR International Ltd., Leicestershire, UK) was used for Mercury content stabilization. The preparation of blank and quality control (QC) samples followed a similar procedure.

3.2.3. Instrumentation

The analysis of heavy metals was conducted using a Perkin Elmer Optima 8300 DV (Perkin Elmer, Shelton, USA) Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) instrument, with the following measurement parameters: RF generator: 40 MHz solid-state, flat plate plasma technology, free running; RF power: 1300 W; Nebulizer type: (BURGENER PEEK MIRA MIST); Plasma gas flow rate: 12 dm^3/min; Nebulizer gas flow rate: 0.7 dm^3/min; Auxiliary gas flow rate: 0.2 dm^3/min; Observation height: 15 mm. The detected wavelengths for each element can be found in Table 1.

3.2.4. Validation of the analytical method

To evaluate the effectiveness of sample preparation and the reliability of the analytical method, we established various validation parameters in accordance with relevant guidelines (Commission Decision, 2002).

Limits of quantitation (LOQ) and limits of detection (LOD) were determined as three and ten times the standard deviation of the signals from blank samples, respectively. Precision was assessed as the relative standard deviation of signals obtained from ten replicates of the same sample. Trueness was determined by initially analyzing certified reference material (standard bovine liver NIST SRM 1577c). Subsequently, trueness was further verified by adding a solution of the four target elements with known concentrations (50 µg/kg each) to the same certified reference material, comparing the results, and evaluating the analysis outcomes. Percentages were used to express both precision and trueness. Trueness was considered acceptable if the deviation of the measured parameter did not exceed ±15%, while precision values were deemed acceptable if below 20%.

Linearity was assessed through the equations of the calibration curves. The study did not include an examination of the Matrix effect since the Y solution used as an internal standard provided compensation. The certified Cd content in the reference sample was above the LOD of the method, allowing for direct measurement. The recovery values and standard deviations are presented in Table 1.

Table 1 Validation Results

Element	Wavelength of detection (nm)	Calibration curve parameters			Limit of quantitation (mg/kg)	Limit of detection (mg/kg)	Precision (%)	Trueness (%)
		Equation (y=a·x+b)(1)		(2)				
		a	**b**	**r**				
Arsenic	188.979	1287	0	0.999828	1.67	0.50	12.7	13.6
Cadmium	228.802	63870	0	0.999529	0.17	0.05	8.4	-10.9
Mercury	194.168	10030	0	1.000000	1.67	0.50	12.3	8.1
Lead	220.353	6520	0	0.999813	0.67	0.20	3.5	-8.4

(1) (where 'y' means the signal of the target element at the given concentration level; 'x' means the concentration)
(2) regression coefficient

In Table 2, the certified values of As, Hg, and Pb served as the relevant limits of detection (LODs). To scrutinize these parameters, quality control (QC) samples were spiked, adding an extra 0.05 mg/l of each element (equivalent to 5.0 mg/kg in the original sample). The same internal standard was consistently applied. The overall acceptability of sample preparation was determined based on the recoveries of all measured elements falling within the acceptable range. To further assess the measurement reliability of these elements, an alternative perspective was considered. The "percentage of the spiked QC sample" was calculated by dividing the measured results of the spiked sample by the theoretical results (certified value + 5.0 mg/kg) and multiplying by 100. These spiked QC samples underwent the same sample preparation process as all other samples. This percentage, in our view, can serve as an indicator of the method's trueness for these elements, aligning with the criteria set in the 2002/657/EC Commission Decision.

The limits of detection for the heavy metals were determined as 0.5 for Arsenic, 0.05 for Cadmium, 0.5 for Mercury, and 0.2 for Lead. These values represent the minimum quantities of the substances that can be distinguished from the absence of that substance.

Table 2 outcomes of quality control (QC) measurements, presented in milligrams per kilogram (mg/kg)

Element	Certified value	Measured value (without spike)	Measured (spiked with qc samples)	LOD	Percentage of the spiked qc sample	Recovery (%)
Arsenic	0.019	N.d.	5.120±0.180	0.500	102.0	N.a.
Cadmium	0.097	0.095±0.006	N.a.	0.050	N.a.	98.2
Mercury	0.005	N.d.	5.260±0.195	0.500	105.1	N.a.
Lead	0.063	N.d.	4.890±0.265	0.200	96.6	N.a.

n.d.=not detectable; N.A.=not available

3.3. Statistical analysis and evaluation

The concentrations of Arsenic, Cadmium, Lead, and Mercury in European Seabass samples were subjected to a comparison through a one-way ANOVA test. The statistical analysis was conducted using the R statistical program (version 3.1.3). Samples with concentrations below the limit of detection (LOD) were excluded from the analysis. It is noteworthy that all measured concentrations of mercury in the European Seabass samples were found to be below the LOD. Consequently, these results were not subjected to statistical evaluation.

3.4. Exposure calculation

The Provisional Tolerable Daily, Weekly, or Monthly Intake (PTDI, PTWI, and PTMI) was determined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Japan's government (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2019) based on their maximum amount that cannot induce damage to health during long-life uptake to protect a consumer’s health. However, some of them have been withdrawn, thus, instead of it, the estimated daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) were calculated for each metal tested in our investigation. During the calculation and comparison, the dietary reference values (RfD, reference dose) were used (EFSA, 2009; CONTAM, 2010; CONTAM, 2012; Chamannejadian et al., 2013).

According to EFSA data, the average consumption of fisheries and aquaculture products per person per year in EU-28 is 23.97 kg/person/year. Meanwhile, Japan has almost the doubled value of fisheries and aquaculture products consumption compared to EU countries, which appeared to be 46.74 kg/person/year (European Commission, 2023; OECD-FAO, 2019). And therefore, the daily consumption value was obtained as 65.7 g/person/day and 128.1 g/person/day by calculating for those EU-28 countries and Japan, respectively.

3.4.1. Estimated Daily Intake (EDI)

EDI was calculated from the values of the heavy metals in the samples multiplied by the daily fish consumption of EU-28 countries and Japan (65.7 g/person/day and 128.1 g/person/day), and then these values were divided by average human body weight (b.w.), which is estimated as 60 kg here. Furthermore, for the values of cadmium, the weekly intake is estimated by multiplying the EDI values by seven, and therefore, it is compared to the weekly tolerable (PTWI) amount that is set by WHO and Japan’s government (Ministry of Agriculture, Forestry, and Fisheries of Japan, 2019).

From the PTWI values for each heavy metal, the acceptable levels of EDI can be stated 0.19 µg/kg b.w./day for mercury, 2.14 µg/kg b.w./day for arsenic, 0.36 or 1.0 µg/kg b.w./day for cadmium in EU member states and Japan respectively, and 3.57 µg/kg b.w./day for lead.

3.4.2. Target Hazard Quotient (THQ)

THQ is a parameter to evaluate the probability of the adverse effect on the human body when they are exposed to a certain hazardous trace element through diet (ATSDR, 2022). It describes the non-cancer risk of contaminants by the ratio of exposed dose and the reference dose (RfD) (United States Environmental Protection Agency, 2023). This value can be calculated with the equation:

$$\sum \mathrm{THQ} = \sum \mathrm{EDI}/\mathrm{RfD}$$

where THQ is the hazard quotient, EDI is the estimated dietary daily intake in mg/day and RfD is the reference dose in mg/day. Furthermore, THQ value less than 1 usually indicates that the concentration is not harmful, while a THQ value greater than 1 indicates that it has potential harm to human body (US-EPA, 2023; Barreca et al., 2023), and thus THQ value below 1 is acceptable.

3.4.3. Hazard Index (HI)

In order to evaluate the potential risk of adverse effects on human health from a mixture of toxic heavy metals the HI was calculated as the sum of THQ for each metal:

$$\text{HI} = \text{THQAs} + \text{THQCd} + \text{THQPb}$$

When HI is less than 1.0, it is unlikely that there will be obvious adverse effects, while HI greater than 10 indicates high risk and chronic or even acute effect (Djedjibegovic et al., 2020), and therefore, if HI value was less than 10, it is acceptable.

4. RESULT

The concentrations of heavy metals investigated in the samples are shown in Table 3 and Table 4.

Table 3. Concentrations of heavy metals in European Seabass (Dicentrarchus labrax) collected in Fishery market in Hungary

SAMPLE IDENTIFICATION	AS	CD	HG	PB
	mg/kg	mg/kg	mg/kg	mg/kg
F1	0.50	<0.05	<0.5	0.60
F2	1.44	<0.05	<0.5	0.24
F3	0.99	<0.05	<0.5	<0.2
F4	1.70	<0.05	<0.5	0.88
F5	1.06	<0.05	<0.5	0.23
F6	<0.5	<0.05	<0.5	0.24
F7	1.76	0.058	<0.5	0.21
F8	2.42	<0.05	<0.5	<0.2
F9	0.93	<0.05	<0.5	<0.2
F10	0.85	<0.05	<0.5	<0.2
F11	0.74	<0.05	<0.5	<0.2
F12	3.25	0.095	<0.5	0.35
F13	1.34	0.050	<0.5	<0.2
F14	0.50	<0.05	<0.5	<0.2
F15	0.89	<0.05	<0.5	<0.2
F16	0.88	0.053	<0.5	0.34
F17	1.02	<0.05	<0.5	<0.2
F18	1.51	0.051	<0.5	0.43
F19	1.14	<0.05	<0.5	0.58
F20	0.68	0.057	<0.5	0.20
F21	2.57	0.082	<0.5	<0.2
F22	4.09	0.121	<0.5	<0.2
F23	3.56	0.091	<0.5	<0.2
F24	1.12	<0.05	<0.5	<0.2
F25	2.52	0.067	<0.5	<0.2
F26	3.07	0.077	<0.5	<0.2
F27	<0.5	0.073	<0.5	<0.2
F28	2.60	0.087	<0.5	<0.2
F29	1.16	<0.05	<0.5	<0.2
F30	1.47	0.051	<0.5	<0.2

F31	1.44	<0.05	<0.5	<0.2
F32	0.93	<0.05	<0.5	<0.2
F33	0.71	<0.05	<0.5	<0.2
F34	<0.5	<0.05	<0.5	<0.2
F35	1.52	<0.05	<0.5	<0.2
F36	1.19	<0.05	<0.5	<0.2
F37	0.50	<0.05	<0.5	<0.2
F38	1.23	<0.05	<0.5	<0.2
F39	1.81	<0.05	<0.5	<0.2
F40	1.14	<0.05	<0.5	<0.2

Table 4 Concentrations of potentially toxic elements found in the investigated European Seabass (Dicentrarchus labrax) (mg/kg w.w.)

Concentration	**Total As**	**Inorganic As (5% of total)**	**Cd**	**Hg**	**Pb**
Average ± SD	1.44 ± 0.90	0.07 ± 0.05	0.06 ± 0.02	< 0.50	0.25 ± 0.14
Minimum (measured)	0.50	0.03	0.05	NA	0.20
Maximum (measured)	4.09	0.20	0.12	NA	0.88
LOD	0.50	0.03	0.05	0.50	0.20
Official maximum limit	-	-	0.05	0.50	0.30

NA = Not Applicable, because 95% of the detected concentration is <LOD.

4.1. Arsenic

The arsenic concentrations of 37 samples out of 40 samples (92.5%) were above LOD. The mean concentration of arsenic in the valid samples was 1.44 ± 0.90 mg/kg of wet weight (w.w.) (Table 3; Table 4). Although the detected arsenic level was the total arsenic level, it is known that inorganic arsenic has more toxic effects in human body, and furthermore, 95% of arsenic found in fish is known as organic arsenic that is used for their osmoregulation and they get eliminated easily from human body (Abernathy et al., 2003). And therefore, it is not regulated neither in EU nor in Japan. However, since high level of inorganic arsenic content can still cause harmful effects on human health, we calculated inorganic arsenic value as 5% of the detected total arsenic level, and the mean level of inorganic arsenic was 0.07 ± 0.05 (Table 4).

The calculations for EDI of the arsenic in EU and Japan were done according to the results in Table 4, and they were compared to the reference value of arsenic (0.3 µg/kg b.w./day). EDI in

EU countries appeared to be 100% below the reference value, however, EDI of 10 samples in Japan exceeded the reference value (10%).

PTWI is nowadays not regulated by WHO/JECFA either, since we cannot estimate the value of arsenic that is considered healthy (EFSA, 2009), and therefore, here we do not calculate the estimated weekly intake of arsenic from the samples.

Table 5 Dietary inorganic arsenic reference value (µg/kg b.w./day) and the calculated estimated daily intake of inorganic arsenic (µg/kg b.w./day)

Inorganic As (5% of total As)	**EU-28**	**Japan**
Reference value	0.30	0.30
Estimated daily intake		
Average	0.08	0.15
Minimum (LOD)	0.03	0.06
Minimum (measured)	0.03	0.06
Maximum (measured)	0.22	0.43
Ratio of samples above the reference value (%)	0	10

4.2. Cadmium

The cadmium concentrations detected in the 14 samples out of 40 samples (35%) were above LOD, and at the same time, above the maximum limit that is regulated by EU (0.05 mg/kg w.w.) as well (Table 3; Table 4). The average concentration of cadmium was 0.06 ± 0.02 mg/kg w.w.. The EDI calculations can be seen in Table 6, which shows that the concentrations detected in the samples do not exceed the reference value of daily intake for cadmium (1.0 µg/kg b.w./day). Since different PTWI values are set by EU and JECFA for, which are applied in the EU countries and Japan respectively, the estimated weekly intake is calculated and compared to each PTWI value (Table 7). Both calculations for EU and Japan showed that the weekly intake level would be less than PTWI value with the collected samples.

Table 6 Dietary cadmium reference value (μg/kg b.w./day) and the calculated estimated daily intake of cadmium (μg/kg b.w./day)

Cadmium	EU-28	Japan
Reference value	1.0	1.0
Estimated daily intake		
Average	0.07	0.13
Minimum (LOD)	0.05	0.11
Minimum (measured)	0.05	0.11
Maximum (measured)	0.13	0.26
Ratio of samples above the reference value (%)	0	0

Table 7 PTWI values for cadmium (μg/kg b.w./week) and the calculated estimated weekly intake of cadmium (μg/kg b.w./week)

Cadmium	EU-28	Japan
PTWI	2.5	7.0
Calculated weekly intake		
Average	0.49	0.91
Minimum (measured)	0.35	0.77
Maximum (measured)	0.91	1.82
Ratio of samples above PTWI value (%)	0	0

4.3. Mercury

Although all the data from the samples were below LOD value, here we calculate the parameters using the value 0.5 mg/kg w.w.. According to Table 8 and Table 9, the values of EDI and EWI are over the reference value and PTWI respectively, both in European countries and Japan, however, in order to investigate the actual concentrations of mercury, we would need a further investigation for the accurate concentrations because we calculated them from the estimated maximum value (0.5 mg/kg w.w.).

THQ of mercury was calculated assuming it is 0.5 mg/kg w.w. as well, and the values for European countries and Japan were 1.83 and 35.6 respectively, and they are both above 1, and that indicates the possible risk to human health by intake of the fish. However, these values are not reliable for further calculation because we do not know the specific concentrations from the samples, and therefore, it will not be included in the calculation of HI.

Table 8 Dietary mercury reference value (µg/kg b.w./day) and the calculated estimated daily intake of mercury (µg/kg b.w./day)

Mercury	EU-28	Japan
Reference value	0.3	0.3
Estimated daily intake		
Average	0.55	10.68
Minimum (LOD)	0.55	10.68
Minimum (measured)	0.55	10.68
Maximum (measured)	0.55	10.68
Ratio of samples above the reference value (%)	100	100

Table 9 PTWI values for mercury (µg/kg b.w./week) and the calculated estimated weekly intake of mercury (µg/kg b.w./week)

Mercury	EU-28	Japan
PTWI	1.6	1.6
Calculated weekly intake		
Average	3.85	74.73
Minimum (measured)	3.85	74.73
Maximum (measured)	3.85	74.73
Ratio of samples above PTWI value (%)	100	100

4.4. Lead

According to Table 3 and Table 4, 27.5% (11 samples/40 samples) of the investigated samples contained lead concentrations that are above LOD level, and 15% (6 samples/40 samples) were above the maximum limit level as well (Table 3; Table 4). The mean concentration of lead was 0.25 ± 0.14 mg/kg w.w. in the examined samples. Table 8 shows EDI levels obtained from the results, and for both EU and Japan, 100% of the results showed the exceeded level compared to the reference level for adults. However, since the reference level for children is higher, only 3 samples (7.5%) were above the reference level for children in EU, meanwhile none of them appeared to be above the reference level for children in the calculation for Japan.

PTWI for lead is withdrawn as well as arsenic, and therefore, we do not compare the concentrations here.

Table 10 Dietary lead reference value (µg/kg b.w./day) and the calculated estimated daily intake of lead (µg/kg b.w./day)

Lead	EU-28	Japan
Reference value	0.16 (adults) 0.26 (children)	0.16 (adults) 0.26 (children)
Estimated daily intake		
Average	0.27	0.53
Minimum (LOD)	0.22	0.43
Minimum (measured)	0.22	0.43
Maximum (measured)	0.96	1.88
Ratio of samples above the reference value (%)	100 (adults) 7.5 (children)	100 (adults) 0 (children)

4.5. THQ and HI

As it is described in the 3.4. Exposure calculation chapter, THQ and HI were calculated based on the results of the sample investigation (Table 9). The result indicates that Pb level of the samples can have a potential to cause harm to human body, both in adults and children in both EU member states and Japan. However, according to HI calculation, these samples would not cause any severe harm or chronic/acute effect since they would not exceed the value of 10.

Table 11 THQ values for arsenic, cadmium, and lead and HI values from calculated THQ calues

THQ	EU-28	Japan
As	0.27	0.50
Cd	0.07	0.13
Hg	1.83	35.6
Pb	1.69 (adults)	3.31 (adults)

	1.04 (children)	2.04 (children)

HI	EU-28	Japan
THQAs + THQCd + THQPb	2.03 (adults) 1.38 (children)	3.94 (adults) 2.67 (children)

5. DISCUSSION

From the results, we can see that some samples (14 samples and 6 samples respectively) contained higher cadmium and lead levels than the official maximum limit level (arsenic: NA, cadmium: 0.05 mg/kg w.w., mercury: 0.50 mg/kg w.w., lead: 0.30 mg/kg w..w.), however, only the EDI of lead for adults was significantly exceeded the reference level as well, both in EU and Japan. This can indicate the level of lead in the samples was concerning for our health, however, it is not exceeding the calculated acceptable level from previously stated PTWI. Meanwhile, arsenic levels in the samples showed that EDI of 10% of the samples are over the reference level in Japan since people eat almost twice as much as people in EU, however, it is not exceeding the reference level for EU so it can be considered not to be harmful. Nevertheless, it is still important for us to monitor the level of lead and arsenic to keep it low because there is no tolerable level that we can get benefit. Although the calculated values of EDI of mercury were over the acceptable level and also over the reference value, none of mercury levels in the samples were over LOD, and thus, we can estimate that the risk of mercury intake is still at a low level though we would need a further examination in order to investigate the actual concentrations, and we can say that the European Seabass samples we took from a market in Hungary, originally from Adriatic Sea in Croatia (FAO Fishing area 37.2.1), are safe for human consumption in regards of mercury contamination.

Even though PTWI levels of arsenic and lead had been withdrawn by JECFA, we can calculate the estimated weekly intake (EWI) from the calculated EDI and compare them with previously published PTWI levels (arsenic: 15 µg/kg b.w., lead: 25 µg/kg b.w.) (WHO/JECFA, 2011). EDI calculated from the average of arsenic levels in the samples were 0.08 and 0.15 µg/kg b.w./day respectively for EU and Japan, and from this, EWI can be estimated as 0.56 and 1.05 µg/kg b.w./week respectively. In addition, the EWI values calculated from the maximum concentration of arsenic in the samples are 1.54 µg/kg b.w./week and 3.01 µg/kg b.w./week. The previously published PTWI for arsenic by JECFA in 1988 was 15 µg/kg b.w./week, so we can say that it is below the value (WHO/JECFA, 1988). Likewise, the average EWI values of lead for EU and Japan from the samples indicates 1.89 and 3.71 µg/kg b.w./week respectively, and they are lower than 25 µg/kg b.w./week, which was set by JECFA before (WHO/JECFA, 1999). Furthermore, EWI values calculated from the maximum concentration of lead detected in the samples show

6.72 and 13.61 μg/kg b.w./week respectively, and again this is lower than the previous PTWI value.

EDI values calculated from the concentrations of inorganic arsenic in the samples were mostly below the reference value, except for some samples for the consumption in Japan. 10% of the samples showed that EDI values would be above the reference value, though it is still not above the acceptable level calculated from previously stated PTWI, and the calculated EWI values would not exceed the PTWI value both in EU and Japan. However, since inorganic arsenic can cause dermatological and neurological problems in case of chronic toxicity, it is important to keep it as low as possible in our diet and keep monitoring the concentration in food.

Although both the EDI and EWI values for cadmium did not show any excess than the reference value, many samples contained the concentration above the official maximum limit level, which is 0.05 mg/kg w.w.. Unlike arsenic and lead, cadmium has PTWI set by WHO/JECFA, EU, and Japan, and the calculated EWI did not exceed the values in both EU and Japan. In addition, EDI value was also below the calculated acceptable level. From this we can say that it is not concerning in terms of acute or chronic toxicity from the intake of the fish, but we need to keep monitoring and make sure it would not reach the level that the consumption can cause harmful effects in humans. However, according to De Conto Cinier et al. (1999), cadmium tends to accumulate in kidneys and liver of fish, and it is less likely to cause cadmium toxicity by eating the fish meat (muscles). In fact, Kljaković et al. (2002) studied the concentrations of cadmium and lead in muscles and liver of fish from the Adriatic Sea, and the result showed the concentrations were higher in the liver. As itai-itai disease was caused by ingestion of crops that accumulated cadmium by growing with the water contaminated with high level of cadmium, it is also important to keep an eye on the concentration of water such as in the irrigation. And furthermore, since bioaccumulation can happen in animals too, water contamination can lead to bioaccumulation of cadmium in fish and especially shellfish as well (Genchi et al., 2020).

6 samples of all 40 investigated samples (15%), or 6 samples out of 11 samples above LOD level (54%) contained lead concentrations above the official maximum limit value, and 100% of the calculated EDI was above the reference value for adults, meanwhile only 7.5% and 0% of EDI were above the reference value for children in EU and Japan, respectively. And although the level was not significantly high and not dangerous because the number of samples above the limit was quite few, this can still indicate the possible risk of lead toxicity from the fish, especially for adults. In case of lead toxicity, besides the neurological problems, it can also cause

reproductive problems as well, in both males and females. It may be more severe in females, especially pregnant women, which causes miscarriage, prematurity, lower birth weight, and developmental issues during the childhood of the kid (Wani et al., 2015). And therefore, it is required to monitor the lead level of water and fish for human consumption and keep it as low as possible.

According to the study of Perugini et al. (2014), the concentrations of heavy metals in muscles of Red mullet (*Mullus barbatus*), European hake (*Merluccius merluccius*), Blue whiting (*Micromesistius poutassou*), and Atlantic mackerel (*Scomber scombrus*) from central Adriatic Sea in Italy shows similar results for the concentration of cadmium (average 0.07 mg/kg w.w.) and mercury (average 0.45 mg/kg w.w.). However, arsenic concentration (average 41.17 mg/kg w.w.) seemed much higher than our study and lead level (average 0.05 mg/kg w.w.) seemed lower.

Although the ranges are quite large, the research of Bilandžić et al. (2011) shows that the heavy metal concentrations of anchovy (*Engraulis encrasicholus*), mackerel (*Scomber japonicus*), red mullet (*Mullus surmuletus*) and picarel (*Spicara smaris*) from the Croatian waters of the Adriatic Sea in 2008 and 2009 were more or less similar to our results. The mean heavy metal levels (arsenic, cadmium, mercury, and lead respectively) measured in the fishes were 1.90 mg/kg w.w., 0.003 mg/kg w.w., 0.07 mg/kg w.w., and 0.02 mg/kg w.w., and they are below the maximum limit levels.

The heavy metal accumulation, detoxification, and biotransformation can take place mainly in liver of fish (Weber et al., 2013), and therefore it is more accurate to measure the concentration from the liver in order to investigate the actual environmental levels, however, their muscles are of importance in terms of human consumption.

Lehel et al. (2023) have investigated the heavy metal concentration of tuna fish (*Thunnus albacares*) from Indian Ocean, which were sold in a market in Hungary, and they figured out that arsenic, cadmium, and mercury levels were considered safe due to their concentrations and calculated EDI levels, but the concentrations of lead seemed higher in 40% of the samples than the maximum limit, and EDI was above the reference values.

Sardina pilchardus fish was investigated by Plachy et al. (2022), and they were caught in the Atlantic Northeast (fishing area 27). In this study, the concentrations of arsenic, cadmium, and mercury seemed not hazardous as well, but the concentrations of lead in the fish were higher than the maximum limit in 73% of the samples. This result is similar to the study by Lehel et al. (2023).

The study by Sepe et al. (2003) on six fish species collected from the Adriatic Sea coast investigated the concentrations of cadmium, chromium, lead, and vanadium, and it shows that cadmium and lead levels were at safe levels (Cd: 3.1 - 20.2, Pb: 11.4 - 45.9 µg/kg fresh weight), and they were lower than the levels that were detected in our study.

It is also known that shellfish tend to accumulate heavy metals in their tissues, and Jureša and Blanuša (2003) found that the lead and cadmium concentrations were higher in shellfish (mussels, *Mytilus galloprovincialis*) than other fish such as hake (*Merluccius merluccius*) and mackerel (*Scomber scombrus*).

According to Copat et al. (2012), the fish caught in the petrochemical area in Mediterranean Sea were more contaminated with higher heavy metal concentrations, and bioaccumulation of mercury was observed in the Sicily Channel. However, although some concentrations were over the limit, PTWI and THQ showed unlikeliness of hazard.

Makedonski et al. (2017) have investigated the heavy metal concentrations of fish from the north-east coast of the Black Sea and discovered that the maximum concentrations of arsenic with 1.10 mg/kg w.w., lead wirh 0.08 mg/kg w.w., and mercury with 0.12 mg/kg w.w., which are quite similar to our study.

The study of Renieri et al. (2019) about cadmium, lead, and mercury concentrations of gilthead seabream and seabass from aquaculture sites of Aegean Sea and Cretan Sea demonstrates that seabass accumulates higher level of mercury and lower level of cadmium than seabream. Furthermore, they showed that lead accumulation has a tendency of being affected by seasonality, and mercury accumulation is affected more by location.

Overall, the investigated heavy metal concentrations were not too high that can trigger any toxic effects to humans. However, the lead level of 15% of the samples was over the maximum limit, and 100% of calculated EDI was over the reference value. Therefore, it is necessary to keep monitoring since other studies show similar results as well. In addition, as it was discussed already, bioaccumulation and biomagnification can occur in the ecosystem, and thus, it leads to chronic ingestion of those heavy metals (Ali and Khan, 2018).

6. SUMMARY

The aim of this study was to reevaluate the possible contaminants of fish for human consumption and analyze the measured concentrations of mercury, cadmium, lead, and arsenic which can cause toxic effects on human body, from European Seabass (*Dicentrarchus labrax*) that are sold in a local market in Hungary. The investigated 40 samples of the European Seabass were originated from Adriatic Sea, Croatia (FAO Fishing area 37.2.1). The samples were investigated with Perkin Elmer Optima 8300 DV (Perkin Elmer, Shelton, USA) Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) instrument to investigate the concentration of the elements that are mentioned above. According to the result of the investigation and the discussion, the detected concentrations of arsenic (1.44±0.90 mg/kg w.w.), cadmium (0.06±0.02 mg/kg w.w.), mercury (<0.50 mg/kg w.w.), and lead (0.25±0.14 mg/kg w.w.) in the fish meat and the calculated values such as EDI, EWI, and HQ were not considered dangerous for regular consumption of the fish though the lead level seemed a little high compared to the official maximum limit and reference values.

Those heavy metals are originated from the nature, but also from anthropogenic origins such as industrial processes, and it can contaminate natural resources such as water, and thus, plants, planktons, and animals living in the water or ingesting the water. Bioaccumulation and biomagnification can occur in the ecosystem, and when it arrives to the dishes for human consumption, the level of the heavy metals can reach high and cause health issues such as neurological, skeletal, renal, hepatic, and cardiovascular diseases. The prominent incidents caused by industrial contamination of the local water system and accordingly chronic intake of heavy metal of the local people were itai-itai disease and Minamata disease that happened in Japan in the late 20th century.

However, the result of our investigation on the European Seabass (*Dicentrarchus labrax*) from the Adriatic Sea in Croatia did not show high levels of heavy metals that would be considered to cause any harmful effect on human health, neither in EU nor in Japan.

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8. REFERENCES

Abernathy, C.O., Thomas, D.J., Calderon, R.L., 2003: Health Effects and Risk Assessment of Arsenic. *The Journal of Nutrition*, 133. p. 1536S-1538S.

Ahasan, H. a. M.N., Mamun, A.A., Karim, S.R., Bakar, M.A., Gazi, E.A., Bala, C.S., 2004: Paralytic complications of puffer fish (tetrodotoxin) poisoning. *Singapore Medical Journal*, 45. p. 73–74.

Aibinu, I.E., Smooker, P.M., Lopata, A.L., 2019: Anisakis Nematodes in Fish and Shellfish-from infection to allergies. *International Journal for Parasitology: Parasites and Wildlife*, 9. p. 384–393.

Alam, M., Oshima, Y., Shimizu, Y., 1982: About gonyautoxins-I, -II, -III, and -IV. *Tetrahedron Letters*, 23. p. 321–322.

Ali, H., Khan, E., 2018: Bioaccumulation of non-essential hazardous heavy metals and metalloids in freshwater fish. Risk to human health. *Environmental Chemistry Letters*, 16. p. 903–917.

Ansdell, V., 2008: Travel Medicine: Food-borne Illness. *Elsevier*. p. 475–483.

Arnold, S.H., Brown, W.D., 1978: Advances in Food Research: Histamine (?) Toxicity from Fish Products. Vol. 24. *Elsevier*. p. 113–154.

ATSDR, n.d.: Calculating Hazard Quotients and Cancer Risk Estimates. [WWW Document] URL: https://www.atsdr.cdc.gov/pha-guidance/conducting_scientific_evaluations/epcs_and_exposure_calculations/hazardquotients_cancerrisk.html. Accessed: 12 October 2023.

Baker-Austin, C., Oliver, J.D., 2018: Vibrio vulnificus: new insights into a deadly opportunistic pathogen: Vibrio vulnificus review. *Environmental* Microbiology, 20. p. 423–430.

Barreca, S., Orecchio, Santino, Orecchio, Silvia, Abbate, I., Pellerito, C., 2023: Macro and micro elements in traditional meals of Mediterranean diet: Determination, estimated intake by population, risk assessment and chemometric analysis. *Journal of Food Composition and Analysis 123*. p. 105541.

Bentur, Y., Ashkar, J., Lurie, Y., Levy, Y., Azzam, Z.S., Litmanovich, M., Golik, M., Gurevych, B., Golani, D., Eisenman, A., 2008: Lessepsian migration and tetrodotoxin poisoning due to Lagocephalus sceleratus in the eastern Mediterranean. *Toxicon*, 52. p. 964–968.

Berman, F.W., LePage, K.T., Murray, T.F., 2002: Domoic acid neurotoxicity in cultured cerebellar granule neurons is controlled preferentially by the NMDA receptor Ca2+ influx pathway. *Brain Research*, 924. p. 20–29.

Bilandžić, N., Đokić, M., Sedak, M., 2011: Metal content determination in four fish species from the Adriatic Sea. *Food Chemistry*, 124. p. 1005–1010.

Body, P.E., Inglis, G., Dolan, P.R., Mulcahy, D.E., 1991: Environmental lead: A review. *Critical Reviews in Environmental Control*, 20. p. 299–310.

Boyer, G.L., Schantz, E.J., Schnoes, H.K., 1978: Characterization of 11-hydroxysaxitoxin sulphate, a major toxin in scallops exposed to blooms of the poisonous dinoflagellate Gonyaulax tamarensis. *Journal of the Chemical Society, Chemical Communications*, p. 889.

Buchmann, K., Mehrdana, F., 2016: Effects of anisakid nematodes Anisakis simplex (s.l.), Pseudoterranova decipiens (s.l.) and Contracaecum osculatum (s.l.) on fish and consumer health. *Food and Waterborne Parasitology*, 4. p. 13–22.

C. M. Yentsch, L. S. Incse, 1980: Accumulation of algal biotoxins in mussels. *Developments in Aquaculture and Fisheries Science*, p. 223–246.

Caramello, P., Vitali, A., Canta, F., Caldana, A., Santi, F., Caputo, A., Lipani, F., Balbiano, R., 2003: Intestinal localization of anisakiasis manifested as acute abdomen. *Clinical Microbiology and Infection*, 9. p. 734–737.

Carmichael, W. W., Mahmood, N. A., 1984: Seafood Toxins: Toxins from Freshwater Cyanobacteria. Ragelis, E.P. (Ed.), ACS Symposium Series. Washington, D.C., American Chemical Society. vol. 262. p. 377–389.

Carmichael, W.W., 1981: The Water Environment: Freshwater Blue-Green Algae (Cyanobacteria) Toxins - A Review. Boston, MA, Springer US. p. 1–13.

Castro-González, M.I., Méndez-Armenta, M., 2008: Heavy metals: Implications associated to fish consumption. *Environmental Toxicology and Pharmacology*, 26. p. 263–271.

CDC, n.d.: Norovirus. [WWW Document] URL: https://www.cdc.gov/norovirus/index.html. Accessed: 15 April 2023.

Chamannejadian, A., Sayyad, G., Moezzi, A., Jahangiri, A., 2013: Evaluation of estimated daily intake (EDI) of cadmium and lead for rice (Oryza sativa L.) in calcareous soils. *Journal of Environmental Health Science and Engineering*, 10. p. 28.

Copat, C., Bella, F., Castaing, M., Fallico, R., Sciacca, S., Ferrante, M., 2012: Heavy Metals Concentrations in Fish from Sicily (Mediterranean Sea) and Evaluation of Possible Health Risks to Consumers. *Bulletin of Environmental Contamination Toxicology*,88. p. 78–83.

Daschner, A., 2016: Fish and Fish Oil in Health and Disease Prevention: Risks and Possible Health Effects of Raw Fish Intake. *Elsevier*. p. 341–353.

Daschner, A., Cuéllar, C., Rodero, M., 2012: The Anisakis allergy debate: does an evolutionary approach help? *Trends in Parasitology*, 28. p. 9–15.

De Conto Cinier, C., Petit-Ramel, M., Faure, R., Garin, D., Bouvet, Y., 1999: Kinetics of cadmium accumulation and elimination in carp Cyprinus carpio tissues. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 122. p. 345–352.

De Fouw, J. C., Van Egmond, H. P., Speijers, G. J. A., 2001: Ciguatera fish poisoning: a review (Report No. 388802021). Rijksinstituut voor Volksgezondheid en Milieu, The Netherlands.

Deardorff, T.L., 1991: Epidemiology of marine fish-borne parasitic zoonoses. *Southeast Asian Journal of Tropical Medicine and Public Health*, 22. p. 146–149.

Deeds, J.R., Schwartz, M.D., 2010: Human risk associated with palytoxin exposure. *Toxicon*, 56. p. 150–162.

Djedjibegovic, J., Djedjibegovic, J., Marjanovic, A., Tahirovic, D., Caklovica, K., Turalic, A., Lugusic, A., Omeragic, E., Sober, M., Caklovica, F., 2020: Heavy metals in commercial fish and seafood products and risk assessment in adult population in Bosnia and Herzegovina. *Scientific Reports*, 10. p. 13238.

EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Cacciò, S., Chalmers, R., Deplazes, P., Devleesschauwer, B., Innes, E., Romig, T., van der Giessen, J., Hempen, M., Van der Stede, Y., Robertson, L., 2018: Public health risks associated with food-borne parasites. *EFSA Journal*, 16. 12. 5495.

EFSA Panel, 2008: Marine biotoxins in shellfish - okadaic acid and analogues - Scientific Opinion of the Panel on Contaminants in the Food chain. *EFSA Journal*, 589. p. 1–62.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009: Cadmium in food. *EFSA Journal*, 7. 3. 980.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009: Scientific Opinion on Arsenic in Food. *EFSA Journal*, 7. 10. 1351.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010. Scientific Opinion on Lead in Food. *EFSA Journal*, 8. 4. 1570.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2011: Statement on tolerable weekly intake for cadmium. *EFSA Journal*, 9. 2. 1975.

EFSA Panel on Contaminants in the Food Chain (CONTAM), 2012: Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA Journal*, 10. 12. 2985.

Egmond, H.P. van, Apeldoorn, M.E. van, Speijers, G.J.A., 2004: Marine biotoxins, FAO food and nutrition paper. Rome, Food and Agriculture Organization of the United Nations.

Eto, K., 2000: Minamata disease. *Neuropathology*, 20. p. 14–19.

European Commission, n.d.: Consumption. [WWW Document] URL: https://oceans-and-fisheries.ec.europa.eu/facts-and-figures/facts-and-figures-common-fisheries-policy/consumption_en. Accessed: 20 July 2023.

European Food Safety Authority, European Centre for Disease Prevention and Control, 2022: a. The European Union One Health 2021 Zoonoses Report. EFS2, 20.

European Food Safety Authority, European Centre for Disease Prevention and Control, 2022: b. The European Union One Health 2021 Zoonoses Report. EFS2, 20.

Expert Panel on Mercury Atmospheric Processes. (Mercury Atmospheric Processes: A Synthesis Report No. TR-104214), 1994: Electric Power Research Institute.

FAO: Mediterranean and Black Sea (Major Fishing Area 37). [WWW Document] URL: https://www.fao.org/fishery/en/area/37/en. Accessed: 10 October 2023.

Falconer, I.R., Runnegar, M.T.C., Beresford, A.M., 1983: Evidence of liver damage by toxin from a bloom of the blue-green alga, Microcystis aeruginosa. *Medical Journal of Australia*, 1. p. 511–514.

FDA: Advice about Eating Fish. [WWW Document] URL: https://www.fda.gov/food/consumers/advice-about-eating-fish. Accessed: 22 July 2023.

Flora, G., Gupta, D., Tiwari, A., 2012: Toxicity of lead: a review with recent updates. *Interdisciplinary Toxicology*, 5. p. 47–58.

Flora, S.J.S., Flora, G., Saxena, G., 2006: Lead: Environmental occurrence, health effects and management of lead poisoning. *Elsevier*. p. 158–228.

Food Safety Risk Assessment Related to Methylmercury in Seafood (No. kya20040723175), 2005: The Food Safety Commission, Japan The Contaminant Expert Committee.

Friedman, M., Fernandez, M., Backer, L., Dickey, R., Bernstein, J., Schrank, K., Kibler, S., Stephan, W., Gribble, M., Bienfang, P., Bowen, R., Degrasse, S., Flores Quintana, H., Loeffler, C., Weisman, R., Blythe, D., Berdalet, E., Ayyar, R., Clarkson-Townsend, D., Swajian, K., Benner, R., Brewer, T., Fleming, L., 2017: An Updated Review of Ciguatera Fish Poisoning: Clinical, Epidemiological, Environmental, and Public Health Management. *Marine Drugs*, 15. p. 72.

Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A., Catalano, A., 2020: The Effects of Cadmium Toxicity. *International Journal of Environmental Research and Public Health*, 17. p. 3782.

Genenah, A.A., Shimizu, Y., 1981: Specific toxicity of paralytic shellfish poisons. *Journal of Agricultural and Food Chemistry*, 29. p. 1289–1291.

Gill, S., Murphy, M., Clausen, J., Richard, D., Quilliam, M., MacKinnon, S., LaBlanc, P., Mueller, R., Pulido, O., 2003: Neural Injury Biomarkers of Novel Shellfish Toxins, Spirolides: A Pilot Study Using Immunochemical and Transcriptional Analysis. *NeuroToxicology*, 24. p. 593–604.

Gram, L., Dalgaard, P., 2002: Fish spoilage bacteria – problems and solutions. *Current Opinion in Biotechnology*, 13. p. 262–266.

Grant, K.S., Burbacher, T.M., Faustman, E.M., Grattan, L., 2010: Domoic acid: Neurobehavioral consequences of exposure to a prevalent marine biotoxin. *Neurotoxicology and Teratology*, 32. p. 132–141.

Grauer, F.H., Arnold, H. L., 1961: Seaweed Dermatitis: First Report of a Dermatitis-Producing Marine Alga. *Archives of Dermatological Research*, 84. p. 720.

Guillen, J., Natale, F., Carvalho, N., Casey, J., Hofherr, J., Druon, J.-N., Fiore, G., Gibin, M., Zanzi, A., Martinsohn, J.Th., 2019: Global seafood consumption footprint. *Ambio*, 48. p. 111–122.

Gutiérrez, S., Svahn, S.L., Johansson, M.E., 2019: Effects of Omega-3 Fatty Acids on Immune Cells. *International Journal of Molecular Sciences*, 20. p. 5028.

Halstead, B.W., 1988: Poisonous and venomous marine animals of the world, 2. rev. ed. ed, A International Oceanographic Foundation selection. Princeton, N.J., Darwin Pr.

Hamre, K., 2011: Metabolism, interactions, requirements and functions of vitamin E in fish: Vitamin E in fish. *Aquaculture Nutrition*, 17. p. 98–115.

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. p. 545–551.

Hawkes, J. W., 1980: The Effects of Xenobiotics on Fish Tissues: Morphological Studies. Federation Proceedings, 39. no. 14. p. 3230–36.

Honkanan, R.E., Codispoti, B.A., Tse, K., Boynton, A.L., 1994: Characterization of natural toxins with inhibitory activity against serine/threonine protein phosphatases. *Toxicon*, 32. p. 339–350.

How, C.-K., Chern, C.-H., Huang, Y.-C., Wang, L.-M., Lee, C.-H., 2003: Tetrodotoxin poisoning. *The American Journal of Emergency Medicine*, 21. p. 51–54.

Hughes, J.M., Merson, M.H., 1976. Fish and Shellfish Poisoning. *The New England Journal of Medicine*, 295. p. 1117–1120.

Ieniştea, C., 1971: Bacterial Production and Destruction of Histamine in Foods, and Food Poisoning Caused by Histamine. *Nahrung*, 15. p. 109–113.

Iwata, K., Fukuchi, T., Yoshimura, K., 2015: Is the Quality of Sushi Ruined by Freezing Raw Fish and Squid? A Randomized Double-Blind Trial with Sensory Evaluation Using Discrimination Testing. *Clinical Infectious Diseases*, civ057.

Jackson, T.A., 1997: Long-range atmospheric transport of mercury to ecosystems, and the importance of anthropogenic emissionsa critical review and evaluation of the published evidence. *Environmental Reviews*, 5. p. 99–120.

Jezierska, B., Witeska, M., 2006: Soil and Water Pollution Monitoring, Protection and Remediation: The Metal Uptake and Accumulation in Fish Living in Polluted Waters, Twardowska, I., Allen, H.E., Häggblom, M.M., Stefaniak, S. (Eds.). Dordrecht, Springer Netherlands. Vol. 69. p. 107–114.

Jonasson, I. R., Boyle, R. W., 1972: Geochemistry of mercury and origins of natural contamination of the environment. *The Canadian Mining and Metallurgical Bulletin*, 65. 717. p. 32–39.

Jureša, D., Blanuša, M., 2003: Mercury, arsenic, lead and cadmium in fish and shellfish from the Adriatic Sea. *Food Additives and Contaminants*, 20. p. 241–246.

K. Aligizaki, G. Nikolaidis, 2008: Morphological identification of two tropical dinoflagellates of the genera Gambierdiscus and Sinophysis in the Mediterranean Sea. *Journal of Biological Research – Thessaloniki*, 9. p. 75–82.

Kalia, K., Flora, S.J.S., 2005: Strategies for Safe and Effective Therapeutic Measures for Chronic Arsenic and Lead Poisoning. *Journal of Occupational Health*, 47. p. 1–21.

Kawai, T., Sekizuka, T., Yahata, Y., Kuroda, M., Kumeda, Y., Iijima, Y., Kamata, Y., Sugita-Konishi, Y., Ohnishi, T., 2012: Identification of Kudoa septempunctata as the Causative Agent of Novel Food Poisoning Outbreaks in Japan by Consumption of Paralichthys olivaceus in Raw Fish. *Clinical Infectious Diseases*, 54. p. 1046–1052.

Kawarazuka, N., Béné, C., 2011: The potential role of small fish species in improving micronutrient deficiencies in developing countries: building evidence. *Public Health Nutrition*, 14. p. 1927–1938.

Kharrat, R., Servent, D., Girard, E., Ouanounou, G., Amar, M., Marrouchi, R., Benoit, E., Molgó, J., 2008: The marine phycotoxin gymnodimine targets muscular and neuronal nicotinic acetylcholine receptor subtypes with high affinity. *Journal of Neurochemistry*, 107. 4. p. 952-963.

Kljaković Gašpić, Z., Zvonari, T., Vrgo, N., Odak, N., Bari, A., 2002: Cadmium and lead in selected tissues of two commercially important fish species from the Adriatic Sea. *Water Research*, 36. p. 5023–5028.

Lehane, L., 2000: Ciguatera update. *Medical Journal of Australia*, 172. p. 176–179.

Lehane, L., Lewis, R.J., 2000: Ciguatera: recent advances but the risk remains. *International Journal of Food Microbiology*, 61. p. 91–125.

Lehane, L., Olley, J., 2000: Histamine fish poisoning revisited. *International Journal of Food Microbiology*, 58. p. 1–37.

Lehel, J., 2002: Poisoning of animal origin. Review article 1. General aspects. *Magyar Állatorvosok Lapia*, 124. p. 754–760.

Lehel, J., 2003: Poisoning of animal origin. Review article 2. Poisoning by sponges, coelenterates, annelid worms, molluscs and echinoderms. *Magyar Állatorvosok Lapja*, 125. p. 244–251.

Lehel, J., Papp, Z., Bartha, A., Palotás, P., Szabó, R., Budai, P., Süth, M., 2023: Metal Load of Potentially Toxic Elements in Tuna (Thunnus albacares)—Food Safety Aspects. *Foods*, 12. p. 3038.

Lehel, J., Yaucat-Guendi, R., Darnay, L., Palotás, P., Laczay, P., 2021: Possible food safety hazards of ready-to-eat raw fish containing product (sushi, sashimi). *Critical Reviews in Food Science and Nutrition*, 61. p. 867–888.

Le Loir, Y., Baron, F., Gautier, M., 2003: Staphylococcus aureus and food poisoning. *Genetics and Molecular Research*, 2. p. 63–76.

Lewis, R.J., 1992: Ciguatoxins are potent ichthyotoxins. *Toxicon*, 30. p. 207–211.

Liu, M., Xiao, W., Zhang, Q., Shi, L., Wang, X., Xu, Y., 2020: Methylmercury Bioaccumulation in Deepest Ocean Fauna: Implications for Ocean Mercury Biotransport through Food Webs. *Environmental Science & Technology Letters*, 7. p. 469–476.

Lock, E.-J., Waagbã, R., Wendelaar Bonga, S., Flik, G., 2010: The significance of vitamin D for fish: a review. *Aquaculture Nutrition*, 16. p. 100–116.

Makedonski, L., Peycheva, K., Stancheva, M., 2017: Determination of heavy metals in selected black sea fish species. *Food Control*, 72. p. 313–318.

Mason, R.P., Fitzgerald, W.F., Morel, F.M.M., 1994: The biogeochemical cycling of elemental mercury: Anthropogenic influences. *Geochimica et Cosmochimica Acta*, 58. p. 3191–3198.

Mason, R.P., Reinfelder, J.R., Morel, F.M.M., 1995: Bioaccumulation of mercury and methylmercury. *Water, Air & Soil Pollution,* 80. p. 915–921.

Matsukane, Y., Sato, H., Tanaka, S., Kamata, Y., Sugita-Konishi, Y., 2010: Kudoa septempunctata n. sp. (Myxosporea: Multivalvulida) from an aquacultured olive flounder (Paralichthys olivaceus) imported from Korea. *Parasitology Research*, 107. p. 865–872.

Mcinerney, J., Sahgal, P., Vogel, M., Rahn, E., Jonas, E., 1996: Scombroid Poisoning. *Annals of Emergency Medicine*, 28. p. 235–238.

Ministry of Agriculture, Forestry, and Fisheries of Japan: メチル水銀の概要及びリスク管理措置. [WWW Document] URL: https://www.maff.go.jp/j/syouan/tikusui/gyokai/g_kenko/busitu/pdf/mmhg.pdf. Accessed: 10 September 2023.

Ministry of Agriculture, Forestry, and Fisheries of Japan: 食品中のカドミウムに関する我が国の基準値. [WWW Document] URL: https://www.maff.go.jp/j/syouan/nouan/kome/k_cd/04_kijyun/02_nat.html. Accessed: 16 September 2023.

Ministry of Agriculture, Forestry, and Fisheries of Japan: 食品中のカドミウムに関する国際基準値. [WWW Document] URL: https://www.maff.go.jp/j/syouan/nouan/kome/k_cd/04_kijyun/01_int.html. Accessed: 12 September 2023.

Ministry of Agriculture, Forestry, and Fisheries of Japan: 食品からのヒ素の摂取量. [WWW Document] URL: https://www.maff.go.jp/j/syouan/nouan/kome/k_as/exposure.html#4. Accessed: 08 September 2023.

Ministry of Agriculture, Forestry, and Fisheries of Japan: 食品に含まれるヒ素の実態調査. [WWW Document] URL: https://www.maff.go.jp/j/syouan/nouan/kome/k_as/occurrence.html. Accessed: 24 August 2023.

Ministry of Agriculture, Forestry, and Fisheries of Japan: 食品中の鉛に関する情報. [WWW Document] URL: https://www.maff.go.jp/j/syouan/nouan/kome/k_pb/index.html. Accessed: 10 September 2023.

Ministry of Environment of Japan: 第 3 節 イタイイタイ病とカドミウム汚染. [WWW Document] URL: https://www.env.go.jp/policy/hakusyo/s48/1226.html. Accessed: 20 July 2023.

Ministry of Environment of Japan: 第 8 節 環境保健対策. [WWW Document] URL: https://www.env.go.jp/policy/hakusyo/r03/html/hj21020608.html. Accessed: 20 July 2023.

Molnár, K., 1979: Myxobolus pavlovskii (Achmerov, 1954) (Myxosporidia) infection in the silver carp and bighead. *Acta Veterinaria Academiae Scientiarum Hungaricae Tomus,* 27. p. 207–216.

Munday, R, 2008: Seafood and Freshwater Toxins: Toxicology of Cyclic Imines: Gymnodimine, Spirolides, Pinnatoxins, Pteriatoxins, Prorocentrolide, Spiro-Prorocentrimine, and

Symbioimines. Botana, L. M. (Ed.). Pharmacology, Physiology, and Detection. 2nd ed. CRC Press. p. 581–594.

Murakami, Y., Oshima, Y., Yasumoto, T., 1982: Identification of okadaic acid as a toxic component of a marine dinoflagellate Prorocentrum lima. *Nippon Suisan Gakkaishi*, 48. p. 69–72.

Narahashi, T., 1972: Mechanism of action of tetrodotoxin and saxitoxin on excitable membranes. *Federation Proceedings*, 31. p. 1124–1132.

Northrop-Clewes, C.A., Shaw, C., 2000: Parasites. *British Medical Bulleti*n, 56. p. 193–208.

O'Mahony, M., 2018. EU Regulatory Risk Management of Marine Biotoxins in the Marine Bivalve Mollusc Food-Chain. *Toxins*, 10. p. 118.

Parkinson, A.,hake Ogilvie, B.W., 2008: Casarett and Doull's toxicology. the basic science of poisons. Biotransformation of xenobiotics. 7th ed. New York, McGraw-Hill Medical. p.161-304.

Perl, T.M., Bédard, L., Kosatsky, T., Hockin, J.C., Todd, E.C.D., Remis, R.S., 1990: An Outbreak of Toxic Encephalopathy Caused by Eating Mussels Contaminated with Domoic Acid. *The New England Journal of Medicine*, 322. p. 1775–1780.

Perugini, M., Visciano, P., Manera, M., Zaccaroni, A., Olivieri, V., Amorena, M., 2014: Heavy metal (As, Cd, Hg, Pb, Cu, Zn, Se) concentrations in muscle and bone of four commercial fish caught in the central Adriatic Sea, Italy. *Environmental Monitoring and Assessment*, 186. p. 2205–2213.

Plachy, M., Bartha, A., Budai, P., Palotás, P., Lehel, J., 2022: Toxic elements in Sardina pilchardus and food toxicological significance. *Food Additives & Contaminants,* Part B 15. p. 212–220.

Renieri, E.A., Safenkova, I.V., Alegakis, A.K., Slutskaya, E.S., Kokaraki, V., Kentouri, M., Dzantiev, B.B., Tsatsakis, A.M., 2019: Cadmium, lead and mercury in muscle tissue of gilthead seabream and seabass: Risk evaluation for consumers. *Food and Chemical Toxicology*, 124. p. 439–449.

Rhodes, L. L., Munday, R, 2004: Palytoxins: a risk to human health? In: *The 20th Marine Biotoxin Science Workshop*. 2004, Wellington, New Zealand. New Zealand Food Safety Authorities.

Rim, H.-J., 2005: Clonorchiasis: an update. *Journal of Helminthology*., 79. p. 269–281.

Ruxton, C.H.S., 2011: The benefits of fish consumption: Benefits of fish consumption. *Nutrition Bulletin*, 36. p. 6–19.

Ryan, J.T., Ross, R.P., Bolton, D., Fitzgerald, G.F., Stanton, C., 2011: Bioactive Peptides from Muscle Sources: Meat and Fish. *Nutrients*, 3. p. 765–791.

Satarug, S., 2018: Dietary Cadmium Intake and Its Effects on Kidneys. *Toxics*, 6. p. 15.

Sepe, A., Ciaralli, L., Ciprotti, M., Giordano, R., Funari, E., Costantini, S., 2003: Determination of cadmium, chromium, lead and vanadium in six fish species from the Adriatic Sea. *Food Additives and Contaminants*, 20. p. 543–552.

Schantz, E.J., Ghazarossian, V.E., 1975: Paralytic poisons from marine dinoflagellates. In: *Proceedings of the First International Conference on Toxic Dinoflagellate Blooms*. 1975, Massachusetts Science and Technology Foundation; Massachusetts Institute of Technology. p. 267–275.

Scholz, T., Garcia, H.H., Kuchta, R., Wicht, B., 2009: Update on the Human Broad Tapeworm (Genus Diphyllobothrium), Including Clinical Relevance. *Clinical Microbiology Reviews*, 22. p. 146–160.

Selwood, A.I., Miles, C.O., Wilkins, A.L., Van Ginkel, R., Munday, R., Rise, F., McNabb, P., 2010: Isolation, Structural Determination and Acute Toxicity of Pinnatoxins E, F and G. *Journal of Agricultural and Food Chemistry*, 58. p. 6532–6542.

Shahidi, F., Ambigaipalan, P., 2018: Omega-3 Polyunsaturated Fatty Acids and Their Health Benefits. *Annual Review of Food Science and Technology*, 9. p. 345–381.

Simon, S.S., Sanjeev, S., 2007: Prevalence of enterotoxigenic Staphylococcus aureus in fishery products and fish processing factory workers. *Food Control*, 18. p. 1565–1568.

Smith, A.H., Lingas, E.O., Rahman, M., 2000: Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull World Health Organ*, 78. p. 1093–1103.

Sripa, B., Bethony, J.M., Sithithaworn, P., Kaewkes, S., Mairiang, E., Loukas, A., Mulvenna, J., Laha, T., Hotez, P.J., Brindley, P.J., 2011: Opisthorchiasis and Opisthorchis-associated cholangiocarcinoma in Thailand and Laos. *Acta Tropica*, 120. p. S158–S168.

Teunis, P., Havelaar, A., Vliegenthart, J., Roessink, G., 1997: Risk assessment of Campylobacter species in shellfish: identifying the unknown. *Water Science and Technology*, p. 35.

Torda, T.A., Sinclair, E., Ulyatt, D.B., 1973: Puffer Fish (Tetrodotoxin) Poisoning Clinical Record and Suggested Management. *Medical Journal of Australia*, 1. p. 599–602.

Uneyama, C., Toda, M., Yamamoto, M., Morikawa, K., 2007: Arsenic in various foods: Cumulative data. *Food Additives and Contaminants*, 24. p. 447–534.

US-EPA: Regional Screening Levels (RSLs) – Generic Tables. [WWW Document] URL: https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables. Accessed: 05 October 2023.

Valdiglesias, V., Prego-Faraldo, M., Pásaro, E., Méndez, J., Laffon, B., 2013: Okadaic Acid: More than a Diarrheic Toxin. *Marine Drugs*, 11. p. 4328–4349.

Wani, A.L., Ara, A., Usmani, J.A., 2015: Lead toxicity: a review. *Interdisciplinary Toxicology*, 8. p. 55–64.

Watkins, S.M., 2008: Neurotoxic Shellfish Poisoning. *Marine Drugs*, 6. p. 430–455.

Weber, P., Behr, E.R., Knorr, C.D.L., Vendruscolo, D.S., Flores, E.M.M., Dressler, V.L., Baldisserotto, B., 2013: Metals in the water, sediment, and tissues of two fish species from different trophic levels in a subtropical Brazilian river. *Microchemical Journal*, 106. p. 61–66.

World Health Organisation/Evaluation of the Joint FAO/WHO Expert Committee on Food Additives JECFA

WHO, n.d.: Hepatitis A. [WWW Document] URL: https://www.who.int/news-room/fact-sheets/detail/hepatitis-a. Accessed: 25 August 2023.

Wright, J.L.C., Boyd, R.K., Freitas, A.S.W.D., Falk, M., Foxall, R.A., Jamieson, W.D., Laycock, M.V., McCulloch, A.W., McInnes, A.G., Odense, P., Pathak, V.P., Quilliam, M.A., Ragan, M.A., Sim, P.G., Thibault, P., Walter, J.A., Gilgan, M., Richard, D.J.A., Dewar, D., 1989: Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. *Canadian Journal of Chemistry*., 67. p. 481–490.

Wu, M.L., Yang, C.C., Yang, G.Y., Ger, J., Deng, J.F., 1997: Scombroid fish poisoning: an overlooked marine food poisoning. *Veterinary and Human Toxicology*, 39. p. 236–241.

Yasumoto, T., Oshima, Y., Sugawara, W., Fukuyo, Y., Oguri, H., Igarashi, T., Fujita, N., 1980: Identification of Dinophysis fortii as the causative organism of diarrhetic shellfish poisoning. *Nippon Suisan Gakkaishi*, 46. p. 1405–1411.

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Thesis progress report for veterinary students

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Department: Food Hygiene

Thesis title: **Food safety aspects of possible chemical contamination in fish**

Consultation – 1st semester

Timing				Topic / Remarks of the supervisor	Signature of the supervisor
	day	month	year		
1.	15	02	2022	Discussion of the basic topics and structure of the thesis	
2.	23	02	2023	Search for literature, Review of literary processing	
3.	15	05	2023	Review of parts of Literature	
4.	-	-	-	Discussion and evaluation of results	
5.	-	-	-	Discussion/Conclusions chapter overview	

Grade achieved at the end of the first semester: **fair/medium (3)**

Consultation – 2nd semester

Timing				Topic / Remarks of the supervisor	Signature of the supervisor
	day	month	year		
1.	25	09	2023	Control of the literature part	
2.	19	10	2023	Discussion of Materials and Methods	
3.	27	10	2023	Discussion and control of results and calculated parameters	

4.	03	11	2023	Check the Discussion section	
5.	07	11	2023	Similarity report	
5.	14	11	2023	Final correction	

Grade achieved at the end of the second semester: **excellent (5)**

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

……………………………………………………………...
signature of the supervisor
/József LEHEL/

Signature of the student: …………………………………

Signature of the secretary of the department: …………………….…………

Date of handing the thesis in……….. ………………….....