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Possible Metal Burden of Potentially Toxic Elements in Rainbow Trout (*Oncorhynchus mykiss*) on Aquaculture Farm

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Abstract: Aquaculture products are important parts of human nutrition due to their healthy components; however, they may contain elements that are potentially toxic to consumers. The aim of the study was to detect the concentration of As, Cd, Hg, and Pb by inductively coupled plasma optical emission spectroscopy in rainbow trout (Oncorhynchus mykiss) originated from an aquaculture farm in Italy. The amount of As was 1.65 ± 0.49 mg/kg; however, the total As cannot be evaluated due to the absence of official regulation. The calculated EDI and THQ for the inorganic As content were below the reference values. The level of Cd was higher than the regulated limit in 30% of the samples; however, its EDI and THQ were below the recommended limit. The concentration of Hg was below the regulated maximum limit in all the samples. The detected amount of Pb was above the maximum limit in 10% of the samples; however, EDI and THQ were below the reference dose and recommended value. Despite higher concentrations of Cd and Pb than the official regulated limit, HI was below 1 for adults; thus, the consumption of the investigated rainbow trout is safe, but it was above the official regulated limit for children, resulting in a slight risk, particularly during long-term intake.

Keywords: freshwater fish; heavy metals; arsenic; risk assessment; public health; estimated daily intake; target hazard quotient; hazard index

Key Contribution: Fish and fishery products are healthy to human consumers based on their natural components (e.g., proteins, essential amino acids); however, they can contain different chemical contaminants (e.g., heavy metals) originated from the environment or during the housing and nutrition of the animals.

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1. Introduction

Basically, 75% of the world's most valuable marine fish stocks are either overfished or have already reached the maximum quantity of fish that can be harvested ecologically, which can influence or disturb the given ecosystem, so the annual catch/capture cannot be increased any further. World fish consumption increased from 45 to 75 million tons between 1973 and 2000, and FAO estimates that an additional 40 million tons of fish will be needed by 2030 to maintain current food levels [1].

The global fish production in 2020 was 178 million tons, which was below the 179 million tons of 2018. Finfish (freshwater, diadromous, and marine fish) had a share of 76 percent of the total in 2020, 51% of the total sourced from capture production, and 49% from aquaculture production [2]. Per capita fish consumption was estimated at 20.5 kg in 2018, which meant a 3.1% increase from 1960 to 2017 [3]. Generally, half of the fish consumed by the population is farmed, but this proportion was only 9% in 1980 [4].

Aquaculture production is developing rapidly in all parts of the world, due to the increasing demand for fishery products and the scarcity of marine fish stocks. However, the producers must comply with the increasingly strict community and national standards regarding product quality, environmental protection, and animal health [5,6].

The procedures used in aquaculture systems (e.g., fishpond management, flow-through or recirculation systems, caged fish rearing in natural waters, e.g., lake, river or its estuary, sea, etc.) must be energy-efficient and efficient in terms of water and waste management. On the other hand, the consumer must be provided with safe food (fish species, fishery products), and the given system should not cause a decrease in the quality of the fish meat, but it is expected that the taste, composition, and nutritional value of the fish meat will improve in the various aquaculture systems [7–9].

Rainbow trout (*O. mykiss*) is one of the most common farmed freshwater species in Europe; approximately 1000 million individuals are kept for meat production, representing 1.6% of the total value of internationally traded fish products in 2018 [10].

Salmonids are commonly farmed in concrete basins or ponds with flow-through aquaculture systems, where water passes through the system, and after the treating process, it is finally discharged back to the aquatic environment. In this system, water supplies the oxygen and transports waste out of the system. Farms can be connected to a river as a chain, using the former farm outflowing water as incoming supply [11].

It was found in different species (European carp [*Cyprinus carpio*], bighead carp [*Hypophthalmichthys nobilis*]) that the feeding diet has a higher impact on the sensory and chemical characteristics of fish flesh than other breeding or husbandry factors. Furthermore, the composition and control of the feed used in fish production is of particular importance, since, in addition to possible water pollution, fish can absorb chemical pollutants through the feed [11].

Protein content is a crucial nutrient in fish compound feed, as fish species require a higher protein level in feed compared to other farm animals, partly because most fish species live and feed as predators, and—as a special characteristic of fish—they use amino acids as a source of energy [12].

Since feed quality greatly influences fish meat appearance and its nutritive value, it is recommended that feed producers take necessary measures to ensure nutritional quality and protect feedstuff from contamination [13].

Contamination of feedstuff or overdosing mineral mix in fish feed can be a source of toxic effects, even in the case of essential dietary ingredients. Some raw materials tend to be a high source of heavy metals, typically fish meal or krill meal. Using alternative protein sources, heavy metal burden can be reduced in fish diet and fish muscle as well [14]. Glencross et al. (2020) also found that using alternative protein sources in feed formulations can lead to a decreased level of heavy metals in fish and fish muscle [14].

Investigating commercially available fish meal products in southwest Nigeria, Bernard and Adetola (2022) were highly concerned about cadmium concentration in fish meal. Regarding the heavy metal bioaccumulation capacity of fish, the authors that recommend feed producers pay more attention to the monitoring program on raw materials [15].

A study in Iran found that the accumulation of heavy metals in rainbow trout (*O. mykiss*) was influenced by water, feed, and sediment. Regarding water, factors like solubility and water temperature should also be considered. It was also found that the concentration of heavy metals in fish and their diet are closely related, and deep sediments contain large amounts of heavy metals; thus, benthos is the largest source of heavy metals, especially for benthivore species [16].

The quality of water has an impact on the quality of fish. As water supply for fish farming is usually derived from the rivers or canals of the area, their contamination by hazardous chemicals or trace elements can have an impact on fish quality. For this reason, changing water continuously is recommended to prevent increasing levels of trace elements and their settling in the sediments. Furthermore, trace elements can derive from feed produced using raw materials contaminated with undesirable substances, such as heavy metals. Basically, fish can uptake metals, including potentially toxic elements, through ingestion, their gills, or skin from the environment [17–20], i.e., from the water; these elements may be dissolved, suspended, and emulsified in it, or even present as solid particles [21–23], and bioaccumulate in the muscle tissue, posing a potential risk for human consumption. As the bioaccumulation capacity of a fish is correlated with its ability to digest feed ingredients, fish in early life stage contain less trace metals in their tissues [24].

However, manganese, strontium, and zinc levels may be higher in the muscle of juveniles [25], iron in the flesh and the gills, and the mercury, strontium, and zinc in the liver [26].

1.1. Legislation

The European Green Deal and the Farm to Fork Strategy declare the importance of farmed seafood production as a major source of protein for food and feed and for reducing carbon footprint. The European Green Deal also emphasizes the fight against water pollution as a fundamental challenge for a high-quality and healthy seafood supply. Inland aquaculture is regarded as a sustainable form of food production in the case of species such as common carp (*C. carpio*), rainbow trout (*O. mykiss*), and emerging species such as European catfish (*Silurus glanis*) and pikeperch (*Sander lucioperca*) [27].

Based on the Commission Regulation No 2023/915, the maximum level for lead and cadmium is 0.3 and 0.05 mg/kg wet weight (ww) in the muscle meat of fish, and for mercury it is 0.3 mg/kg ww in Salmo and Oncorhynchus species (except *Salmo trutta*) and 0.5 mg/kg ww in fishery product [28].

Toxic effects and toxic levels of essential elements or contaminants, such as heavy metals, are highly dependent on their chemical form. The most important and hazardous non-essential metals for animal health and food safety are arsenic, cadmium, lead, and mercury. Legislative maximum levels usually define the total concentration of the element. While the organic form of arsenic (e.g., arsenobetain) in finfish is non-toxic, unlike its inorganic form, organic mercury (e.g., methylmercury), having the highest concern of heavy metal compounds, is more toxic than its inorganic chemical form [14].

1.2. Heavy Metals as Environmental Pollutants

Potentially toxic elements pose particularly high risk for the environment, as they are not degraded or eliminated from the ecosystem and finally can be accumulated in living organisms and metabolized mostly to more toxic, rarely to less toxic, chemical forms [29,30]. Regarding the aquatic ecosystem, sediments have an outstanding role in the bioaccumulation of pollutants [31,32]. Fish, as sentinels for biomonitoring, are one of the generally used species for water pollution, not only because of the risk they pose to humans as food originating from water, but also as a delicate organism; they can respond to even a low level of water pollution as well [33–35]. Since toxic elements have hematological, biochemical, and ionoregulatory effects, changing of these parameters can be used in environmental biomonitoring systems [29,33,36,37]. In aquatic ecosystems, heavy metals are one of the most significant pollutants in terms of persistence [36]. Heavy metal residues in the flesh of fish can originate both from natural and anthropogenic sources. Heavy metals or other elements can finally bioaccumulate in the fish body, resulted in human health risk. The degree of human health risk posed by fish consumption is influenced by different factors, such as physiochemical properties of metallic compounds, environmental contamination level, and the length of exposure; it is also influenced by human characteristics (e.g., age, sex, etc.) [38]. Milošković et al. (2022) found that especially Al, Mn, and Zn, and to a lesser extent Cu and Fe, significantly correlated with the physicochemical parameters of the environment [37]. Demirak et al. (2022) reported that, according to their study conducted in

European eels (*Anguilla anguilla*), physiological needs and metabolic activities in different habitats have a significant effect on metal accumulation [39]. Furthermore, accumulation levels are also influenced by metal, fish species and the organ measured [40,41]. Different seasonal patterns in heavy metal concentration—especially cadmium and lead—in the fish's body or in its organs may result from seasonal pollution in the catchment, as reported in the study conducted by Farkas et al. (2000) [42]. Nikolić et al. (2022) investigated the levels of heavy metals in three different species (European chub [*Squalius cephalus*], cactus roach [*Rutilus virgo*], pikeperch [*S. lucioperca*]) and found that the highest levels were generally detected in the gill followed by the liver and the gonads, and the lowest ones were measured in the muscle [36].

1.2.1. Arsenic

The most common sources of arsenic (As) pollution are the mining industry and agriculture, contributing to its increased level in soil and water. It occurs as a mixture of different chemical forms of the element, the trivalent inorganic form, which has the most toxic effects in living organisms, and organic arsenicals, which are usually less toxic [33].

Generally, the concentration of As is decreased in freshwater ecosystems; however, it is biomagnified in marine aquatic ecosystems of a higher trophic level [43].

As is classified as a carcinogenic element, causing oxidative stress, and can affect the biotransformation methylation processes, as well as the metabolism of other essential metals. It binds to the thiol and sulfhydryl groups of proteins, inhibiting their physiological function, causing developmental abnormalities, perinatal death, and growth retardation in rodent species [29]. Arsenic intoxication results in increased risks of cancer in organs, such as skin, lungs, bladder, and kidney; however, it can manifest in other symptoms like skin disorders, e.g., hyperkeratosis or pigmentation changes [44,45]. It has harmful effects on the gastrointestinal tract, cardiovascular system, lungs, skin, liver, kidneys, and the hematopoietic system, especially in the case of chronic exposure. Since the fish are continuously exposed to water pollution, they can biotransform the toxic form of As to a less toxic form. Research conducted in fingerling rainbow trout (*O. mykiss*) found that the tolerance to chronic exposure of As seems to be increased with temperature, while in invertebrates, the opposite is true [33].

1.2.2. Cadmium

Cadmium (Cd) occurs in the environment mostly from agricultural sources, because of the intensive use of fertilizers. It can bioaccumulate in plants, which mechanism is enhanced by phosphate-containing fertilizers, acidic soils, and industrial wastewater used for irrigation, but biomagnification in aquatic ecosystems is not a characteristic of it. Based on its structural similarity and physicochemical features, it can replace Zn, Ca, and other metal elements in proteins, altering their structure and function. Cadmium causes oxidative stress, increases lipid peroxidation, and changes the lipid composition of membranes. Reactive oxygen radicals created by Cd can lead to decreased DNA synthesis and DNA damage, and it has been proven to cause cancer in humans. It is distributed throughout the body, but more than half of the total amount accumulates in the kidneys and liver, and to a lesser extent in the muscle. In fish (e.g., rainbow trout), it is mainly concentrated in the gills and kidneys, and smaller levels of cadmium can be measured in the liver [29,44]. Cadmium is a non-essential element and has no biological role in the human body, but it has a highly toxic potential, especially in the case of long-lasting exposure, even at very low concentration. It can bioaccumulate in the human body, causing pulmonary, hepatic, skeletal, reproductive, and renal damage, and even cancer [45].

1.2.3. Mercury

Mercury (Hg) can derive from natural sources, such as volcanic activity or soil erosion, or even anthropogenic sources, such as industrial or mining activities. The most toxic form is its organic form, methylmercury [46]. Inorganic mercury is strongly bound to sediment or organic matter in natural waters and soils. In the aquatic environment, bacteria methylate the inorganic compounds to organic methylmercury in the sediment, due to its good lipophilic properties, and thus, it enters the food chain. Mercury has biomagnification potential in the aquatic ecosystem at all trophic levels [43]. The highest mercury levels can be measured in long-lived predatory fish (e.g., swordfish [*Xiphias gladius*], shark spp.), freshwater pike (*Esox lucius*), and perch (*Perca fluviatilis*) living in the oceans [29,39]. The accumulation of Hg in fish increases with the age and size of the animal [26,33,47]. It can be found in high concentration in the kidneys, spleen, and liver, or to a decreasing extent in the gills, genitals, brain, and muscles [26]. However, some authors showed that the highest level of mercury was detected in the flesh of fish [35,47–54]. The main point of attack of inorganic mercury compounds is the kidney, while the target organ of organic mercury compounds is primarily the nervous system [29]. Mercury poses a high health risk for humans consuming fish or fishery products, as the element is distributed at a high concentration in aquatic environments. Living organisms of aquatic ecosystems convert the inorganic form to organic methylmercury, which binds to the sulfhydryl group of the tissue and bioaccumulates at a higher trophic level of the aquatic food chain [40]. The toxicity of Hg to humans depends on its chemical form, dose, and rate and method of exposure. The main route of exposure is ingested food or water, and inhalation to a lesser extent. Mercury has several harmful effects on the human body, involving the central nervous system, cardiovascular system, kidneys, and embryo damage. Besides humans, mercury has negative effects on animals' reproductive capacity (reduced fertility, change in hatching time, impaired embryo development) [55].

1.2.4. Lead

Lead is a non-essential environmental pollutant element resulting in serious human health risks, like neuro- and nephrotoxicity, increased risk of heart disease, damaged lung function, and several further health damage [45]. Earlier usage as a fuel additive resulted in lead being the most significant pollutant due to its highly persistent properties. In aquatic environments, lead is found more in algae and benthic organisms than in carnivorous fish at higher trophic levels. In the body, lead most likely binds to proteins and changes their function, inhibiting them, or mimics the effect of calcium; it can replace zinc in various enzymes, and causes oxidative stress. Lead inhibits the function of several enzymes, thus causing disturbances in hem synthesis [29].

2. Materials and Methods

2.1. Materials

Samples (10 g of flesh of fish) were taken from 40 rainbow trout (*O. mykiss*) to measure and to assess the potential health risk for the consumer. They were collected at a local fishery market in Hungary. However, the fish investigated originated from an aquaculture farm, Treviso County, Veneto Region, Italy.

Laboratory tests were taken to determine the arsenic, cadmium, mercury, and lead content of the samples.

2.2. Methods

2.2.1. Analytical Process

During laboratory circumstances, the samples were ground and homogenized (Potter S, B. Braun Biotech International GmbH, Melsungen, Germany), and then they were placed in plastic bags labelled for identification and stored in a freezer (So-Lo, Ultra-Lo Freezer, Model C85-9, Environmental Equipment Co., Inc., Cincinnati, OH, USA) at −70 °C until processing.

A standard quantity (0.5 g) from each sample was weighed and placed into CEM MARS XPreSS Teflon tubes (CEM Matthes, NC, USA), then 5 mL of analytical-grade nitric acid (69 m/m%, Aristar, VWR International Ltd., Leicestershire, UK) and hydrogen peroxide (30 m/m%, Normapur, VWR International Ltd., Leicestershire, UK) was mixed with the samples and it was destroyed using the CEM MARS6 microwave detector (CEM Corporation, Matthews, NC, USA). The ramp was 35 min, the temperature 200 °C, the hold 50 min, and the energy 1700 W during the measurement. Finally, the samples were filled up with 25 mL of deionized water, and the analysis was performed after twofold dilution. The two blank (1 for calibration, 1 for blind sample) and the quality control (QC) samples proceeded through a similar process.

Calibration was performed with ICP mono- (VWR International Ltd., Leicestershire, UK) and multi-element (Perkin Elmer Inc., Shelton, CT, USA) standards.

Argon gas with a purity of 4.6 was used for the measurements (Messer Hungarogáz Ltd., Budapest, Hungary).

A 1 mg/L yttrium solution (VWR International Ltd., Leicestershire, UK) and a 0.25 mg/L gold solution (VWR International Ltd., Leicestershire, UK) were used as internal standards to stabilize the mercury content.

For the control (QC sample), mussel tissue (ERM-CE278k) and tuna fish (ERM-CE464) purchased from European Commission, Joint Research Centre, Geel, were used as a standard reference material.

Heavy metal concentrations were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES) type Perkin Elmer Optima 8300 DV (Perkin Elmer, Shelton, CT, USA).

2.2.2. Method for Validation

To evaluate the reliability of the sample preparation and the analytical method, different validation parameters were determined based on the regulations in force (Table 1) [56]). The limits of detection (Limit of Detection LOD; or limit of decision, CCα) and the limits of quantification (Limit of Quantification LOQ; or ability of detection, CCβ) were defined as three times and ten times the standard deviation (SD) of the blank samples, respectively.

Table 1. Results of validation.

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	63,870	0	0.999529	0.17	0.05	8.4	−10.9
Mercury	194.168	10,030	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.

Precision was calculated as the relative SD of the signals from ten replicates of the same sample. Trueness was determined by analyzing standard tissue materials (mussel tissue ERM-CE278k and tuna fish ERM-CE464). After that, solutions of the four investigated elements with known concentration (50 µg/kg each) were added to the certified reference materials and the results of these measurements were compared and evaluated and expressed in percentages.

Precision was accepted below 20%; trueness was adequate if the deviation of the measured parameter did not exceed ±15%. Linearity was evaluated by the equations of the calibration curves. The matrix effect was not studied, since the Y solution used as an internal standard provided compensation. The results of the QC samples and the recovery percentages are summarized in Table 2.

Table 2. Outcomes of quality control (QC) measurements.

Element	Certified Value (mg/kg)	Measured Value (mg/kg)	LOD (mg/kg)	Recovery (%)
		ERM-CE287k (Mussel tissue)		
Arsenic	6.70	6.87 ± 0.08	0.50	102.5
Cadmium	0.34	0.36 ± 0.02	0.05	106.5
Lead	2.18	2.00 ± 0.11	0.20	91.7
		ERM-CE464 (Tuna fish)		
Mercury	5.24	5.14 ± 0.08	0.50	98.1

2.2.3. Exposure Calculation

Based on the detection concentrations of the investigated metals (As, Cd, Hg, Pb), the provisional tolerable intake (PTI), the estimated daily intake (EDI), the target hazard quotient (THQ), and the hazard index (HI) were calculated.

Provisional Tolerable Intake (PTI)

The Provisional Tolerable Weekly Intake (PTWI) for Cd and Pb and Provisional Tolerable Monthly Intake for Cd were calculated using the detected concentrations, which were multiplied by the average annual fish consumption per person. Based on the EFSA data, the average annual fish consumption per person is 23.97 kg/year/person in the European Union; thus, the 65.7 g/day/person value was applied during the calculation [57]. The value obtained was divided by 70 kg body weight, which is the average human body weight for the European adult population [58], and multiplied by seven (days/week) or by seven and four (days/weeks/month), depending on whether calculating the dietary weekly or—in case of Cd—monthly intake of the measured elements by consuming fishery products equivalent to the sample.

These calculated intake values were compared to the recommended provisional tolerable weekly (PTWI) or monthly (PTMI) intakes set by the World Health Organization (WHO) [59–61].

The European Food Safety Agency's (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) set a tolerable weekly intake (TWI) for cadmium of 2.5 μg/kg bw. The PTMI of 25 μg/kg bw set by Joint FAO/WHO Expert Committee on Food Additives (JECFA) corresponds to a weekly intake of 5.8 μg/kg, which two parameters are clearly not consistent. The reason for the discrepancy is that the assessments of the EFSA's CONTAM Panel and the JECFA are worked out by different methodological approaches. For lead, a previously set PTWI of 25 μg/kg was withdrawn, because this value is not considered safe by EFSA, as there is no safe threshold for critical lead exposure that induced harmful effects, especially nephrotoxicity in adult people and developmental neurotoxic damage in the fetus and in young children [62].

Estimated Daily Intake (EDI)

EDI value was calculated by the following equation [63]:

$$\mathrm{EDI} = (\mathrm{C} \times \mathrm{Cons})/\mathrm{BW} \tag{1}$$

where C is the concentration of the metals (arsenic, cadmium, lead, mercury) (mg/kg) in the investigated sample; Cons means the daily average fish consumption (65.7 g/day/person) [42]; and BW is average human body weight (70 kg).

The calculated EDI values were compared to the dietary heavy metal reference doses [49,62,64–67].

Target Hazard Quotient (THQ)

THQ value was calculated using the following equation [68]:

$$THQ = EDI/RfD \quad (2)$$

During the calculation of THQ, EDI value was divided by reference dose (RfD) in the case of each metal (inorganic As: 0.3 µg/kg, Cd: 1 µg/kg, Hg: 0.3 µg/kg, Pb: 0.16 µg/kg for adult, 0.26 µg/kg for children) [49,62,64–67].

If the THQ value is below 1, the metal concentration induces no harmful effect; however, if the value is above 1, it indicates risk to the human consumer.

Hazard Index (HI)

The sum of THQ values of the investigated metals (As, Cd, Hg, Pb) is used to calculate the hazard index (HI) to evaluate the potential risk of adverse health effects of the mixture of metals, by the following formula [69]:

$$HI = THQ_{As} + THQ_{Cd} + THQ_{Hg} + THQ_{Pb} \quad (3)$$

In this equation, THQAs means the calculated value for inorganic arsenic, which is generally 5% in aquatic animals [65].

If the HI is below 1.0, it is unlikely that there will be adverse effects, while if the HI is above 10, it indicates high risk.

2.2.4. Statistical Evaluation

Statistical analysis was carried out with Microsoft Excel and R program (version 3.3.2.).

During the statistical evaluation, half of the LOD value was used in the case of those samples in which the concentration of metals was below the limit of detection [70].

3. Results

A summarized description of the samples' measured and calculated values is presented in Table 3.

Table 3. Measured concentration (mg/kg), dietary heavy metal reference values (µg/kg/day), and the calculated estimated daily intake of metals (µg/kg/day).

	Total As	**As (Inorganic, 5% of Total As)**	**Cd**	**Hg**	**Pb**
LOD	0.5	ND[1]	0.05	0.50	0.20
Maximum Limit (ML) (mg/kg)	ND[1]	ND[1]	0.05	0.50	0.30
Measured concentration (mg/kg)					
Average ± SD	1.65 ± 0.49	0.08 ± 0.02	0.03 ± 0.02	<0.50	0.16 ± 0.16
Minimum (measured)	0.54	0.03	0.05	<0.50	0.24
Maximum (measured)	2.79	0.14	0.07	<0.50	0.85
Ratio of sample above the LOD (%)	100	NA	30	0	15
Ratio of sample above the ML (%)	NA	NA	30	0	10
Estimated daily intake (µg/kg)					
Reference dose (µg/kg)	ND[1]	0.3	1	0.3	0.16 (adult) 0.26 (children)
Average ± SD	1.55 ± 0.46	0.08 ± 0.02	0.03 ± 0.01	<0.47	0.15 ± 0.15
Minimum (measured)	0.51	0.03	0.05	ND[2]	0.23
Maximum (measured)	2.62	0.13	0.06	ND[2]	0.80
Ratio of sample above the reference value (%)	NA	0	0	0	15 (children) 10 (adult)

ND[1] = no data exist; ND[2] = no data, because all of the concentrations are <LOD; NA = not applicable.

3.1. Arsenic

In our study, arsenic concentrations were above the LOD in all samples with an average concentration of 1.65 ± 0.49 mg/kg. These values are the measured total arsenic contents in our samples, as the applied method was not able to distinguish between organic and inorganic forms. As the two chemical forms of arsenic have different toxicological features, it is important to calculate the inorganic proportion as well. The average ratio of inorganic arsenic compounds in aquatic organisms is 5% [65]. The organic forms are mainly found as arsenobetain in fish muscle tissue, which is not toxic for the consumers [71].

The maximum residue level is set neither at the EU nor the national level. As inorganic arsenic compounds pose a risk for the consumer, we calculated inorganic arsenic level, taking 5% of the measured total arsenic content, and found that the mean level of inorganic arsenic was 0.08 ± 0.02. Assessing the exposure, EDI was also calculated by the equation above, and was compared to the 0.3 µg/kg bw/day reference dose of inorganic arsenic. EDI values were found to be 100% below the reference value (Table 3).

The target hazard quotient for inorganic arsenic was 0.26, on average.

3.2. Cadmium

Twelve of the samples contained cadmium at or above the LOD level; thus, 70% of the total had cadmium less than 0.05 mg/kg. These samples were involved in the calculation process with half of the ML (0.025 mg/kg) value. Since both LOD and ML set by EU regulation are 0.05 mg/kg, 30% of the samples were at or above, and 70% of the samples were under the ML value at the same time [28].

Estimated daily intake was found 0.03 µg/kg bw/day on average, which was far from 1 µg/kg bw/day as the reference dose for cadmium. THQ as the ratio of EDI and reference dose was thus found to be 0.03.

The average calculated PTMI and PTWI was 0.90 ± 0.39 µg/kg bw and 0.23 ± 0.10 µg/kg bw, respectively, based on the detected concentration. Comparing them with the officially recommended PTMI and PTWI parameters, such as 25 µg/kg bw and 2.5 µg/kg bw [60], more than twenty-eight and ten times higher difference was found.

3.3. Mercury

Among the 40 samples of the investigated fish, there was not any which contained mercury above the 0.5 mg/kg LOD, which meant, at the same time, that all were under the ML value (0.5 mg/kg) set by EU regulation [28].

PTWI value of mercury (5 µg/kg bw) has been established by JECFA [72], but this was withdrawn in 2011, and another one has not been established since then; however, the Committee recommended the PTWI value for inorganic mercury to be 4 µg/kg bw [60].

Using these data, calculations of EDI, PTWI, or THQ parameters would not be relevant.

3.4. Lead

The limit of detection of the applied method was 0.2 mg/kg, which was exceeded by only six samples, with the highest being a lead value of 0.85 mg/kg. Four of these six were above the 0.3 mg/kg ML value. Taking half LOD at samples under the 0.2 mg/kg limit, an average of 0.15 µg/kg bw/day EDI was calculated. The target hazard quotient must be calculated for children and adults separately, as there are two different reference doses distinguished for these age groups. The reference dose for children is lower, at 0.16 µg/kg bw/day, while for adults, it is 0.26 µg/kg bw/day [62,66]. THQ was found to be below 1 in both cases, but it was just slightly less than 1 (0.93 µg/kg bw/day) for children, while for adults, it was 0.57 µg/kg bw/day.

3.5. Total Metal Exposure

To assess the potential risk of adverse health effects posed by a meal equal to the samples, hazard index was calculated, adding up THQ values as determined above. Using this formula and considering the special aspects detailed at the three measured metal elements, the calculated HI value was 1.22 for children and 0.86 for adults.

As the HI value for adults is below 1.0, it means that it is unlikely that there will be adverse effects for consumers, while for children, it indicates a slight risk.

4. Discussion

The main source of heavy metal pollution in fish and fishery products derives from the water environment or dietary sources [16,73]. Several studies are conducted to investigate the difference between the possible health risk of fish or fishery products from different sources (fish farms or wild).

As mentioned above, the samples measured in this study are derived from an Italian aquaculture fish farm. Italy represents 14% of the EU's fish production, where more than 50% of the production comes from fisheries, where rainbow trout is the most widely farmed species [74]. Cammilleri et al. (2023) reported Cd, Pb, and Hg levels measured in 151 samples of farmed and wild fish of different species (gilt-head bream [*Sparus aurata*], European seabass [*Dicentrarchus labrax*], shi drum [*Umbrina cirrosa*]) from the south Mediterranean [75]. Mercury was detected only in *S. aurata,* with an average of 0.06 ± 0.13 mg/kg and 0.03 ± 0.03 mg/kg for farmed and wild samples, respectively. Although farmed samples' average Hg content is slightly higher than that of wild species, the authors did not find significant differences in Pb, Cd, and Hg levels when comparing wild and farmed fish samples. In conclusion, they found that the level of toxic metals in fish depends more on the pollution of the site than the type of production. The method Cammilleri et al. applied has lower LOD than the analytical method used in this study; thus, the values of the two studies cannot be compared, but taking 0.06 mg/kg concentration as the average Hg level in farmed fish samples, EDI can be calculated as 0.05 μg/kg bw/day, which is far lower than RfD (0.3) for Hg.

Majlesi et al. (2019) measured a rainbow trout (*O. mykiss*) sample where the mean concentration of Hg, Cd, and Pb was 0.02, 0.11, and 1.07 mg/kg, respectively; these parameters, except for Hg, are higher than they were in our study [16]. The authors found a significant correlation between heavy metal accumulation in fish tissue and dietary source and water supply used by the fishery.

Okbah et al. (2018) measured 0.40–0.97 mg/kg Cd level in samples of five fish species (bogue [*Sparus boops*], black-barred halfbeak [*Hemiramphus far*], round sardinella [*Sardinella aurita*], brushtooth lizardfish [*Saurida undosquamis*], chub mackerel [*Scomber japonicus*]) originating from the Northern African coast (Tripoli Port, Libya), values of which were about ten or twenty times higher than our findings [76]. Despite all these values, and the fact of industrial activities on-shore and off-shore in Tripoli Port, all the measured concentrations of potentially toxic elements (Zn, Cu, Fe, Cd) were surprisingly below the limits recommended by WHO.

In addition to fish tissue or feed, Habib et al. (2024) measured the concentration of potentially toxic elements in water as a habitat of wild and farmed fish production sites in Pakistan [77]. Their concentration trend was Zn > Pb > Cu > Cd > Cr, which is similar to our study regarding Cd and Pb levels in fish samples. They also found mostly higher levels of heavy metals in samples from wild fish, with higher levels of Cd and Pb in the water than the WHO standard level. Although EDI and THQ values were higher in wild fish, HI was lower than 1 for both groups of fish samples. Statistical analysis suggests a positive correlation between heavy metal concentration in fish tissue and the water's heavy metal content. Regarding these data, farmed fish pose lower health risk to humans than wild fish.

Measuring Zn, Cu, As, Cr, and Cd levels, samples from Bangladesh's four fish farms showed HI = 0.08 values in three different fish species (Pangas catfish [*Pangasius pangasius*], rohu [*Labeo rohita*], Nile tilapia [*Tilapia nilotica*]), which parameters suggest that the samples do not pose any health risk for human consumption [78]. These HI values are significantly lower than HIs in our study, although the measured metal elements are not the same in the two studies.

Fallah et al. (2011) also intended to measure the differences between farmed and wild rainbow trout (*O. mykiss*) heavy metal and trace element accumulation in edible fish tissues, gathering samples from 12 Iranian fishery sites [73]. The authors found that liver had significantly higher heavy metal and trace element concentrations compared to the muscle in farmed or wild fish, and a slightly higher proportion of samples exceeded the regulatory level in wild fish (45.8%) than in farm fish (41.6%). A similar pattern has been shown regarding Pb level in the two different production methods, as 50% of farmed fish samples and 62.5% of wild fish samples were above the EU's regulatory limit. Hg and As concentrations were lower than the legislated limits in all of the measured samples. Confirming the previously cited authors, variations in concentrations were suggested to be derived from the different environmental and dietary conditions.

A study conducted in Pakistan analyzed Cd, Cr, and Pb level in carp fish from fish farms and a fish market (rohu [*L. rohita*], mrigal carp [*Cirrhinus mrigala*], silver carp [*Hypophthalmichthys molitrix*], grass carp [*Ctenopharyngodon idella*]). The highest level of heavy metals was Pb, with a mean concentration of 0.33 ± 0.01 mg/kg, which is twice as high as in our study. In contrast, Cd concentration measured significantly less than in our rainbow trout samples, with a level of 0.01 ± 0.00 mg/kg [79].

Calculating the risk posed by fish consumption can result in significantly different outcomes, as annual fish consumption in different human populations varies across a wide range among coastal or inland countries [16,80].

5. Conclusions

Potentially toxic elements can be widely found in the natural environment, and their concentrations may be increased by anthropogenic activities. Besides water as a habitat, dietary sources can also affect the heavy metal contamination of fish in the human diet.

As the average fish consumption may be several times higher in the maritime countries than the European average and depends on many factors, e.g., eating habits and populations, this risk assessment—and risk assessments in general—must be evaluated in the given circumstances.

Numerous studies have assumed that fishery products from fish farms are safer than fish from wild captures, but this theory is based on the knowledge of the biomagnification and bioaccumulation phenomenon in aquatic organisms and necessarily relies on the controllability of the environmental parameters in an aquaculture farm and adequate quality control measurements during production.

The concentration of cadmium and lead were higher than the officially regulated limit in 30% and 10% of the samples, respectively; however, the calculated EDI values did not exceed the recommended daily intake. Thus, the investigated fish could be declared safe for human consumption; it cannot induce any potential hazard to the consumers.

Despite the low number of above-the-limit concentrations in the case of Cd and Pb, samples are not objectionable for consumption by adult human consumers considering HI value; however, it induces a slight risk in children for prolonged, long-term intake.

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Institutional Review Board Statement: The investigation was performed on fish which were purchased from a fishery market as food; thus, based on regulation, it is NOT an experiment on animals. Permission is not needed.

Data Availability Statement: The data used to support the findings of this study can be made available by the corresponding author uSpon request.

Conflicts of Interest: The author Melinda Plachy was employed by the company Bonafarm-Bábolna Feed Ltd., Hungary. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. FAO (Food and Agriculture Organization of the United Nations). *Integrated Agriculture-Aquaculture: A Primer*; FAO Fisheries Technical Paper, No. 407; FAO/ICLARM/IIRR: Rome, Italy, 2001; 149p; Available online: http://www.fao.org/DOCREP/005/Y1187E/Y1187E00.HTM (accessed on 25 March 2024).
2. FAO (Food and Agriculture Organization of the United Nations). *World Food and Agriculture—Statistical Yearbook*; FAO: Rome, Italy, 2022. [CrossRef]
3. FAO (Food and Agriculture Organization of the United Nations). The State of World Fisheries and Aquaculture 2020. In *Sustainability in Action*; FAO: Rome, Italy, 2020. [CrossRef]
4. FAO (Food and Agriculture Organization of the United Nations). *Aspects of FAOs Policies, Programmes, Budget and Activities Aimed at Contributing to Sustainable Development*; Document to the Ninety-Fourth Session of the FAO Council, Rome, 15–25 November 1988; FAO: Rome, Italy, 1988; CL94/6.
5. Beveridge, M.C.M.; Phillips, M.J.; Macintosh, D.J. Aquaculture and the environment: The supply of and demand for environmental goods and services by Asian aquaculture and the implications for sustainability. *Aquacult. Res.* **1997**, *28*, 797–807. [CrossRef]
6. Focardi, S.; Corsi, I.; Franchi, E. Safety issues and sustainable development of European aquaculture: New tools for environmentally sound aquaculture. *Aquacult. Int.* **2005**, *13*, 3–17. [CrossRef]
7. Frankic, A.; Hershner, C. Sustainable aquaculture: Developing the promise of aquaculture. *Aquacult. Int.* **2003**, *11*, 517–530. [CrossRef]
8. Halberg, N.; van der Werf, H.M.G.; Basset-Mens, C.; Dalgaard, R.; de Boer, I.J.M. Environmental assessment tools for the evaluation and improvement of European livestock production systems. *Livest. Prod. Sci.* **2005**, *96*, 33–50. [CrossRef]
9. Wurts, W.A. Sustainable Aquaculture in the Twenty-First Century. *Rev. Fish. Sci.* **2000**, *8*, 141–150. [CrossRef]
10. Giménez-Candela, M.; Saraiva, J.L.; Bauer, H. The legal protection of farmed fish in Europe—Analysing the range of EU legislation and the impact of international animal welfare standards for the fishes in European aquaculture. *Derecho Anim. (Forum Anim. Law Stud.)* **2020**, *11*, 65–118. [CrossRef]
11. SustainAqua. Integrated Approach for a Sustainable and Healthy Freshwater Aquaculture. *SustainAqua Handbook—A Handbook for Sustainable Aquaculture.* 2009. Available online: https://haki.naik.hu/sites/default/files/uploads/2018-09/sustainaqua_handbook_en.pdf (accessed on 25 March 2024).
12. Radhakrishnan, G.; Yashwanth, B.S.; Shivkumar, S.; Sidramappa, M.V.; Nevil, P.; Prathik, M.R.; Pradeep, K. Dietary protein requirement for maintenance, growth, and reproduction in fish: A review. *J. Entomol. Zool. Stud.* **2020**, *8*, 208–215.
13. Sarkar, M.M.; Rohani, M.F.; Hossain, M.A.R.; Shahjahan, M. Evaluation of Heavy Metal Contamination in Some Selected Commercial Fish Feeds Used in Bangladesh. *Biol. Trace Element. Res.* **2022**, *200*, 844–854. [CrossRef]
14. Glencross, B.D.; Baily, J.; Berntssen, M.H.G.; Hardy, R.; MacKenzie, S.; Tocher, D.R. Risk assessment of the use of alternative animal and plant raw material resources in aquaculture feeds. *Rev. Aquacult.* **2020**, *12*, 703–758. [CrossRef]
15. Bernard, E.; Adetola, O. Nutritional Composition and Heavy Metal Contamination of Prominent Fishmeal Samples. *J. Fish. Aquat. Sci.* **2022**, *17*, 1–8. [CrossRef]
16. Majlesi, M.; Malekzadeh, J.; Berizi, E.; Toori, M.A. Heavy metal content in farmed rainbow trout in relation to aquaculture area and feed pellets. *Foods Raw Mater.* **2019**, *7*, 329–338. [CrossRef]
17. Abarshi, M.M.; Dantala, E.O.; Mada, S.B. Bioaccumulation of heavy metals in some tissues of croaker fish from oil spilled rivers of Niger Delta region, Nigeria. *Asian Pac. J. Trop. Biomed.* **2017**, *7*, 563–568. [CrossRef]
18. Jorgensen, L.A.; Pedersen, S. Trace metals in fish used for time trend analysis and as environmental indicators. *Mar. Pollut. Bull.* **1994**, *28*, 24–32. [CrossRef]
19. Malik, N.; Biswas, A.K.; Qureshi, T.A.; Borana, K.; Virha, R. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. *Environ. Monit. Assess.* **2010**, *160*, 267–276. [CrossRef] [PubMed]
20. Weber, P.; Behr, E.R.; Knorr, C.D.L.; Vendruscolo, D.S.; Flores, E.M.M.; Dressler, V.L.; Baldisserotto, B. Metals in the water, sediment, and tissues of two fish species from different trophic levels in a subtropical Brazilian river. *Microchem. J.* **2013**, *106*, 61–66. [CrossRef]
21. Agbozu, I.E.; Ekweozor, K.E.; Opuene, K. Survey of heavy metals in the catfish *Syndontis claris*. *Int. J. Environ. Sci. Technol.* **2007**, *4*, 93–97. Available online: https://www.bioline.org.br/pdf?st07012 (accessed on 25 March 2024). [CrossRef]
22. Linnick, P.M.; Zubenko, I.B. Role of bottom sediments in the secondary pollution of aquatic environments by heavy-metal compounds. *Lakes Reserv. Res. Manag.* **2000**, *5*, 11–21. [CrossRef]
23. Mager, E.M.; Grosell, M. Effects of acute and chronic waterborne lead exposure on the swimming performance and aerobic scope of fathead minnows (*Pimephales promelas*). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2011**, *154*, 7–13. [CrossRef] [PubMed]

24. Mannzhi, M.P.; Edokpayi, J.N.; Durowoju, O.S.; Gumbo, J.; Odiyo, J.O. Assessment of selected trace metals in fish feeds, pond water and edible muscles of *Oreochromis mossambicus* and the evaluation of human health risk associated with its consumption in Vhembe district of Limpopo Province, South Africa. *Toxicol. Rep.* **2021**, *8*, 705–717. [CrossRef]
25. Djikanović, V.; Skorić, S.; Jarić, I.; Lenhardt, M. Age-specific metal and accumulation patterns in different tissues of nase (*Chodrostoma nasus*) from the Medjuvršje Reservoir. *Sci. Total Environ.* **2016**, *566–567*, 185–190. [CrossRef]
26. Nikolić, D.; Skorić, S.; Janković, S.; Hegediš, A.; Djikanović, V. Age-specific accumulation of toxic metal(loid)s in northern pike (*Esox lucius*) juveniles. *Environ. Monit. Assess.* **2021**, *193*, 229. [CrossRef] [PubMed]
27. COM 2021. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions; Strategic Guidelines for a More Sustainable and Competitive EU Aquaculture for the Period 2021 to 2030. COM/2021/236 Final. Available online: https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:52021DC0236 (accessed on 25 March 2024).
28. Commission Regulation (EC) No 2023/915 on setting maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. *Off. J. Eur. Union L* **2023**, *119*, 103–157. Available online: http://data.europa.eu/eli/reg/2023/915/oj (accessed on 25 March 2024).
29. Lehel, J.; Lányi, K.; Laczay, P. Food safety significance of heavy metal contamination in foods of animal origin. *Magy. Állatorv. Lapja* **2016**, *138*, 99–112. Available online: https://real-j.mtak.hu/20206/2/mal_2016_138_2_.pdf (accessed on 1 May 2020). (In Hungarian).
30. Das, S.; Sultana, K.W.; Ndhlala, A.R.; Mondal, M.; Chandra, I. Heavy Metal Pollution in the Environment and Its Impact on Health: Exploring Green Technology for Remediation. *Environ. Health Insights* **2023**, *17*, 11786302231201259. [CrossRef] [PubMed]
31. Shahidul Islam, M.; Tanakan, M. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. *Mar. Pollut. Bull.* **2004**, *48*, 624–649. [CrossRef] [PubMed]
32. Sojka, M.; Jaskuła, J. Heavy Metals in River Sediments: Contamination, Toxicity, and Source Identification-A Case Study from Poland. *Int. J. Environ. Res. Public Health* **2022**, *19*, 10502. [CrossRef] [PubMed]
33. Kumari, B.; Kumar, V.; Sinha, A.K.; Ahsan, J.; Ghosh, A.K.; Wang, H.; DeBoeck, G. Toxicology of arsenic in fish and aquatic systems. *Environ. Chem. Lett.* **2017**, *15*, 43–64. [CrossRef]
34. Mancera-Rodríguez, N.J.; Galiano, D.R.; López-Montoya, A.J.; Llorent-Martínez, E.J.; Molina-García, L.; Azorit, C. Common carp as an ecological indicator of environmental pollution in reservoirs of southern Spain: Inferring the environmental risks of anthropogenic activities. *Environ. Sci. Pollut. Res.* **2024**, *31*, 36192–36206. [CrossRef] [PubMed]
35. Nikolić, D.; Poleksić, V.; Skorić, S.; Tasić, A.; Stanojević, S.; Rašković, B. The European Chub (*Squalius cephalus*) as an indicator of reservoirs pollution and human health risk assessment associated with its consumption. *Environ. Pollut.* **2022**, *310*, 119871. [CrossRef]
36. Nikolić, D.; Skorić, S.; Mićković, B.; Nikčević, M.; Smederevac-Lalić, M.; Djikanović, V. Accumulation of 25 elements in gills, liver, gonads, and muscle of European chub (*Squalius cephalus*), Cactus roach (*Rutilus virgo*), and pikeperch (*Sander lucioperca*) from *Zlatar reservoir* (Serbia). *Environ. Sci. Pollut. Res.* **2022**, *29*, 50271–50280. [CrossRef]
37. Milošković, A.; Stojković Piperac, M.; Kojadinović, N.; Radenković, M.; Đuretanović, S.; Čerba, D.; Milošević, D.; Simić, V. Potentially toxic elements in invasive fish species Prussian carp (*Carassius gibelio*) from different freshwater ecosystems and human exposure assessment. *Environ. Sci. Pollut. Res.* **2022**, *29*, 29152–29164. [CrossRef]
38. Mukherjee, J.; Saha, N.C.; Karan, S. Bioaccumulation pattern of heavy metals in fish tissues and associated health hazards in human population. *Environ. Sci. Pollut. Res.* **2022**, *29*, 21365–21379. [CrossRef] [PubMed]
39. Demirak, A.; Keskin, F.; Silm, M.; Özdemir, N.; Yıldız, D.; Bernotas, P.; Öğlü, B. Bioaccumulation and health risk assessment of heavy metals in European eels taken from Lakes Köyceğiz (Turkey) and Võrtsjärv (Estonia). *Environ. Sci. Pollut. Res.* **2022**, *29*, 1620–1633. [CrossRef] [PubMed]
40. Rahmanikhah, Z.; Esmaili-sari, A.; Bahramifar, N. Total mercury and methylmercury concentrations in native and invasive fish species in Shadegan International Wetland, Iran, and health risk assessment. *Environ. Sci. Pollut. Res.* **2020**, *27*, 6765–6773. [CrossRef] [PubMed]
41. Naeem, S.; Ashraf, M.; Babar, M.E.; Zahoor, S.; Ali, S. The effects of some heavy metals on some fish species. *Environ. Sci. Pollut. Res.* **2021**, *28*, 25566–25578. [CrossRef] [PubMed]
42. Farkas, A.; Salánki, J.; Varanka, I. Heavy metal concentrations in fish of Lake Balaton. *Lakes Reserv. Res. Manag.* **2000**, *5*, 271–279. [CrossRef]
43. Saidon, N.B.; Szabó, R.; Lehel, J.; Budai, P. Trophic Transfer and Biomagnification Potential of Environmental Contaminants (Heavy Metals) in Aquatic Ecosystems. *Environ. Pollut.* **2023**, *340*, 122815. [CrossRef] [PubMed]
44. Sabbir, W.; Rahman, M.Z.; Halder, T.; Khan, M.N.; Ray, S. Assessment of heavy metal contamination in fish feed available in three districts of South Western region of Bangladesh. *Int. J. Fishe. Aquatic Stud.* **2018**, *6*, 100–104. Available online: https://www.researchgate.net/publication/323701174_Assessment_of_heavy_metal_contamination_in_fish_feed_available_in_three_districts_of_South_Western_region_of_Bangladesh (accessed on 25 March 2024).
45. Islam, M.M.; Avha, N.J.; Ahmed, S.; Akbor, M.A.; Islam, M.S.; Mostafiz, F.; Habibullah-Al-Mamun, M. Trace metals and organochlorine pesticide residues in imported fishes in Bangladesh and human health risk implications. *Environ. Sci. Pollut. Res.* **2022**, *29*, 17499–17512. [CrossRef]

46. Maury-Brachet, R.; Gentes, S.; Dassié, E.P.; Feurtet-Mazel, A.; Vigouroux, R.; Laperche, V.; Gonzalez, P.; Hanquiez, V.; Mesmer-Dudons, N.; Durrieu, G.; et al. Mercury contamination levels in the bioindicator piscivorous fish *Hoplias aïmara* in French Guiana rivers: Mapping for risk assessment. *Environ. Sci. Pollut. Res.* **2020**, *27*, 3624–3636. [CrossRef]
47. Polak-Juszczak, L. Distribution of organic and inorganic mercury in the tissues and organs of fish from the southern Baltic Sea. *Environ. Sci. Pollut. Res.* **2018**, *25*, 34181–34189. [CrossRef] [PubMed]
48. Jezierska, B.; Witeska, M. The metal uptake and accumulation in fish living in polluted waters. In *Soil and Water Pollution Monitoring, Protection and Remediation*; Twardowska, I., Allen, H.E., Häggblom, M.M., Stefaniak, S., Eds.; NATO Science Series; Springer: Dordrecht, The Netherlands, 2006; Volume 69. [CrossRef]
49. Jovičić, K.; Nikolić, D.M.; Višnjić-Jeftić, Ž.; Đikanović, V.; Skorić, S.; Stefanović, S.M.; Lenhardt, M.; Hegediš, A.; Krpo-Ćetković, J.; Jarić, I. Mapping differential elemental accumulation in fish tissues: Assessment of metal and trace element concentrations in wels catfish (*Silurus glanis*) from the Danube River by ICP-MS. *Environ. Sci. Pollut. Res.* **2015**, *22*, 3820–3827. [CrossRef] [PubMed]
50. Dušek, L.; Svobodová, Z.; Janoušková, D.; Vykusová, B.; Jarkovský, J.; Šmíd, R.; Pavliš, R. Bioaccumulation of mercury in muscle tissue of fish in the Elbe River (Czech Republic): Multispecies monitoring study 1991–1996. *Ecotoxicol. Environ. Saf.* **2005**, *61*, 256–267. [CrossRef] [PubMed]
51. Nikolić, D.; Skorić, S.; Poleksić, V.; Rašković, B. Sex-specific elemental accumulation and histopathology of pikeperch (*Sander lucioperca*) from Garaši reservoir (Serbia) with human health risk assessment. *Environ. Sci. Pollut. Res.* **2021**, *28*, 53700–53711. [CrossRef] [PubMed]
52. Nikolić, D.; Skorić, S.; Rašković, B.; Lenhardt, M.; Krpo-Ćetković, J. Impact of reservoir properties on elemental accumulation and histopathology of European perch (*Perca fluviatilis*). *Chemosphere* **2020**, *244*, 125503. [CrossRef]
53. Łuczyńska, J.; Paszczyk, B.; Łuczyński, M.J. Fish as a bioindicator of heavy metals pollution in aquatic ecosystem of Pluszne Lake, Poland, and risk assessment for consumer's health. *Ecotoxicol. Environ. Saf.* **2018**, *153*, 60–67. [CrossRef]
54. Rašković, B.; Poleksić, V.; Skorić, S.; Jovičić, K.; Spasić, S.; Hegediš, A.; Vasić, N.; Lenhardt, M. Effects of mine tailing and mixed contamination on metals, trace elements accumulation and histopathology of the chub (*Squalius cephalus*) tissues: Evidence from three differently contaminated sites in Serbia. *Ecotoxicol. Environ. Saf.* **2018**, *153*, 238–247. [CrossRef] [PubMed]
55. Novotna Kruzikova, K.; Siroka, Z.; Jurajda, P.; Harustiakova, D.; Smolikova, Z.; Kubicek, M.; Svobodova, Z. Mercury content in fish from drinking-water reservoirs in the Morava River Basin (Czech Republic). *Environ. Sci. Pollut. Res.* **2022**, *29*, 17394–17405. [CrossRef]
56. Commission Regulation. No. 333/2007/EC of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. *Off. J. Eur. Union L* **2007**, *88*, 29–38. Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:088:0029:0038:EN:PDF (accessed on 3 January 2020).
57. European Commission: Food, Farming, Fisheries. Oceans and fisheries: Consumption. Available online: https://oceans-and-fisheries.ec.europa.eu/facts-and-figures/facts-and-figures-common-fisheries-policy/consumption_en (accessed on 15 February 2024).
58. EFSA (European Food Safety Authority). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA J.* **2012**, *10*, 2985. [CrossRef]
59. JECFA-776. Evaluation of Certain Food Additives and Contaminants, 33rd Report of Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series 776. Geneva. 1989. Available online: https://www.who.int/publications/i/item/9241207760 (accessed on 3 January 2020).
60. JECFA-959. Evaluation of Certain Food Additives and Contaminants, 72nd Report of Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series 959. Geneva. 2011. Available online: https://www.who.int/publications/i/item/9789241209595 (accessed on 3 January 2020).
61. JECFA-960. Geneva. Evaluation of Certain Food Additives and Contaminants, 73rd Report of Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series 960. 2011. Available online: https://www.who.int/publications/i/item/9789241209601 (accessed on 3 January 2020).
62. EFSA (European Food Safety Authority). Scientific opinion on lead in food. *EFSA J.* **2010**, *8*, 1570. [CrossRef]
63. Chamannejadian, A.; Sayyad, G.; Moezzi, A.; Jahangiri, A. Evaluation of estimated daily intake (EDI) of cadmium and lead for rice (*Oryza sativa* L.) in calcareous soils. *Iran. J. Environ. Health Sci. Engin.* **2013**, *10*, 28. [CrossRef] [PubMed]
64. EFSA (European Food Safety Authority). Scientific opinion on arsenic in food. *EFSA J.* **2009**, *7*, 1351. [CrossRef]
65. EFSA (European Food Safety Authority). Scientific opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. *EFSA J.* **2009**, *980*, 1–139. [CrossRef]
66. Wong, C.; Roberts, S.M.; Saab, I.N. Review of regulatory reference values and background levels for heavy metals in the human diet. *Regul. Toxicol. Pharmacol.* **2022**, *130*, 105122. [CrossRef] [PubMed]
67. Parang, H.; Esmaeilbeigi, M. Total mercury concentration in the muscle of four mostly consumed fish and associated human health risks for fishermen and non-fishermen families in the Anzali Wetland, Southern Caspian Sea. *Reg. Stud. Mar. Sci.* **2022**, *52*, 102270. [CrossRef]
68. Barreca, S.; Orecchio, S.; Orecchio, S.; Abbate, I.; Pellerito, C. Macro and micro elements in traditional meals of Mediterranean diet: Determination, estimated intake by population, risk assessment and chemometric analysis. *J. Food Compos. Anal.* **2023**, *123*, 105541. [CrossRef]

69. Djedjibegovic, J.; Marjanovic, A.; Tahirovic, D.; Caklovica, K.; Turalic, A.; Lugusic, A.; Omeragic, E.; Sober, M.; Caklovica, F. Heavy metals in commercial fish and seafood products and risk assessment in adult population in Bosnia and Herzegovina. *Sci. Rep.* **2020**, *10*, 13238. [CrossRef] [PubMed]
70. Tekindal, M.A.; Erdoğan, B.D.; Yavuz, Y. Evaluating Left-Censored Data Through Substitution, Parametric, Semi-parametric, and Nonparametric Methods: A Simulation Study. *Interdiscipl. Sci. Comput. Life Sci.* **2015**, *9*, 153–172. [CrossRef]
71. Bosch, A.C.; O'Neill, B.; Sigge, G.O.; Kerwath, S.E.; Hoffman, L.C. Heavy metals in marine fish meat and consumer health: A review. *J. Sci. Food Agric.* **2016**, *96*, 32–48. [CrossRef]
72. JECFA-505. Evaluation of Certain Food Additives and the Contaminants Mercury, Lead, and Cadmium: 16th Report of the Joint FAO/WHO Expert Committee on Food Additives, Technical Report Series 505. Geneva. 1972. Available online: https://www.who.int/publications/i/item/9241205059 (accessed on 3 January 2020).
73. Fallah, A.A.; Saei-Dehkordi, S.S.; Nematollahi, A.; Jafari, T. Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (*Oncorhynchus mykiss*) using ICP-OES technique. *Microchem. J.* **2011**, *98*, 275–279. [CrossRef]
74. D'Agaro, E.; Gibertoni, P.; Esposito, S. Recent Trends and Economic Aspects in the Rainbow Trout (*Oncorhynchus mykiss*) Sector. *Appl. Sci.* **2022**, *12*, 8773. [CrossRef]
75. Cammilleri, G.; Calabrese, V.; Accordino, L.; Pantano, L.; Migliazzo, A.; Galluzzo, F.G.; Ferrantelli, V. Toxic metals and total lipids comparison between wild and farmed fish of South mediterranean. *Nat. Prod. Res.* **2023**, *37*, 2232–2242. [CrossRef] [PubMed]
76. Okbah, M.A.; Dango, E.A.S.; El Zokm, G.M. Heavy metals in Fish Species from Mediterranean Coast, Tripoli Port (Libya): A comprehensive assessment of the potential adverse effects on human health. *Egypt. J. Aquat. Biol. Fish.* **2018**, *22*, 149–164. Available online: https://journals.ekb.eg/article_19514_77d1588b729d9e1c3e17fe309a729723.pdf (accessed on 25 March 2024). [CrossRef]
77. Habib, S.S.; Naz, S.; Fazio, F.; Cravana, C.; Ullah, M.; Rind, K.H.; Attaullah, S.; Filiciotto, F.; Khayyam, K. Assessment and Bioaccumulation of Heavy Metals in Water, Fish (wild and Farmed) and Associated Human Health Risk. *Biol. Trace Element. Res.* **2024**, *202*, 725–735. [CrossRef] [PubMed]
78. Resma, N.S.; Meaze, A.M.H.; Hossain, S.; Khandaker, M.U.; Kamal, M.; Deb, N. The presence of toxic metals in popular farmed fish species and estimation of health risks through their consumption. *Phys. Open* **2020**, *5*, 100052. [CrossRef]
79. Chatta, A.; Muhammad, K.; Zahid, M.; Asif, A. Heavy metal (cadmium, lead, and chromium) contamination infarmed fish: A potential risk for consumers' health. *Turk. J. Zool.* **2016**, *40*, 248–256. [CrossRef]
80. Lehel, J.; Papp, Z.; Bartha, A.; Palotás, P.; Szabó, R.; Budai, P.; Süth, M. Metal Load of Potentially Toxic Elements in Tuna (*Thunnus albacares*)—Food Safety Aspects. *Foods* **2023**, *12*, 3038. [CrossRef]