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RESEARCH ARTICLE

Assessing the human health risk of Baltic Sea sea trout (*Salmo trutta* L.) consumption

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Abstract. The current study assessed health risks posed by exposure to metals from the consumption of Baltic Sea trout (*Salmo trutta* L.). Concentrations of essential minerals in sea trout muscles were determined and compared with the physiological requirements of these minerals in Polish nutrition standards, recommended dietary allowances (RDA), and estimated average requirements (EAR). Chemical analyses indicated that sea trout was rich in phosphorus, calcium, magnesium, sodium, and potassium. The pattern significance gradation of the element concentrations was as follows: P>Ca>Mg>Na>K>Fe>Zn>Cu>Se>Mn>Co. Sea trout consumption can provide a considerable portion of the RDA of copper, magnesium, phosphorus, calcium, iron, and zinc. The ranking order of the mean toxic element concentrations in sea trout muscle tissues was As>Pb>Cd>Hg. Potential risk estimated with the hazard quotient (HQ) indicated that most metals posed no health risk because they did not exceed reference doses at HQ < 1. For carcinogenic and non-carcinogenic effects, the maximum allowable rates of sea trout consumption were sufficiently high to ensure human health. According to these data, the consumption of farmed sea trout from the Baltic Sea in the northern region of Pomerania, Poland did not pose a risk to human health.

Keywords: fish consumption, sea trout, Baltic Sea, health risk, human health risk assessment, consumption limits, target hazard quotients (THQs)

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Introduction

The Baltic Sea is one of the world's most intensively exploited seas with constant traffic and densely populated catchment areas. Large rivers flow into the Baltic Sea from highly industrialized countries, and its coasts are used intensively for recreation. Many economic activities associated with the sea generate income for the inhabitants of this region (Krek et al. 2018, Raudsepp et al. 2019). Simultaneously, the Baltic Sea is a unique ecosystem, and its specific geographic and hydro-morphological conditions contribute to this uniqueness. The Baltic Sea is connected to the ocean by the narrow Danish straits, which inhibits water exchange. Therefore, compared to most oceans and seas, salinity is very low, and this leads to a unique mix of marine and freshwater species that have adapted to these specific conditions. Nevertheless, such special conditions render species and biotopes susceptible to changes in environmental conditions; therefore, the fragile ecosystem of the Baltic Sea needs attention and protection (Johannesson and André 2006). The Baltic Sea is severely impacted by pollution that is considered the main environmental problem in Europe. Much effort has been made over the past 30 years to reduce present and future human pollution and to eradicate accumulated pollutants from the sea. While there have been some improvements, the main challenge remains to find ways to balance protecting the fragile Baltic Sea ecosystem with its diversified economic uses (HELCOM 2010, 2018). Dietz et al. (2021) reported that over the past few decades the Baltic Sea has regenerated considerably with respect to Hg concentrations in local species, while it still has a legacy of elevated Hg levels from high industrial and agricultural exploitation in its drainage basin and a slow water turnover regime. This also results from the remobilization of accumulated pollutants from the land (Saniewska et al. 2014) and sediments (Beldowski et al. 2009). The potential health risks attributable to dioxins in Baltic fish have also decreased by more than half in the past decade (Tuomisto et al. 2020).

There are many types of fish migrations in the Baltic Sea. The best known are anadromous salmonid migrations. Atlantic salmon, *Salmo salar* L. and sea trout *Salmo trutta* L. spend their adult lives in the sea, but they spawn in rivers and streams. Fry spend a year or two in fresh water and then descend the rivers into the sea. Sea trout inhabit the Baltic, White, Barents, North, and Norwegian seas and in the Atlantic Ocean surrounding Iceland and along the Iberian Peninsula, and they undertake spawning migrations up the rivers that flow into these seas and oceans (Torniainen et al. 2017, Jacobson et al. 2020).

In recent years, global fish consumption has increased significantly because of its nutritional and therapeutic benefits (El-Moselhy et al. 2014, Ullah et al. 2017). Fish is the most important source of high-quality protein and accounts for about 17% of animal protein and 6.7% of all protein consumed by the world's population (FAO 2016). In addition to being a rich source of protein, fish provides high contents of essential fats, vitamins, and minerals (Medeiros et al. 2012, Ullah et al. 2017). Since fish is an important component of the human diet, it is often considered the most suitable target among aquatic ecosystem bioindicators of pollutants (Abdel-Baki et al. 2011). Moreover, metal contents in fishes can indicate bioaccumulation throughout the food chain (Pintaeva et al. 2011). For example, consuming just one serving (140 g) of Baltic cod, *Gadus morhua* L. exposes the consumer to 51% of the daily acceptable Cd intake (Jarosz-Krzemińska et al. 2021).

Water pollution and fish contamination are acute and chronic health hazards, even in remote marine areas, and they can have devastating consequences for the environment and human health. Heavy metal toxicity is a serious threat and is associated with several health risks (Jaishankar et al. 2014). Because of their high toxicity, persistence, and tendency to accumulate in water and sediments, heavy metals and metalloids in higher concentrations are extremely poisonous for living organisms (Has-Schön et al. 2006, Pratush et al. 2018). Food chain contamination with heavy metals and toxic ions almost always follows the cyclical order of industry, atmosphere, soil, water, phytoplankton, zooplankton, fish, and then humans (Has-Schön et al. 2006).

Thus, the aims of our study were: 1) to determine the concentration of essential minerals, heavy metals, and arsenic in muscle tissues of sea trout obtained from a coastal zone in the southern Baltic Sea (Pomeranian Voivodeship, northern Poland); 2) to compare the concentration of these minerals with physiological requirements of Polish nutrition standards, recommended dietary allowances (RDA), and estimated average requirements (EAR); 3) to analyze the non-carcinogenic and carcinogenic risk levels of exposure to heavy metals and arsenic by assessing estimated target hazard quotients (THQ), total target hazard quotients (STHQ), and carcinogenic risk (CR).

Materials and Methods

Fish

The sea trout were purchased from September 2016 to February 2017 from local fishermen working Ustka harbour (54°34'43''N 16°52'09''E), Pomeranian Voivodeship, northern Poland. Fish was purchased twelve times from local fishermen, and sampling was performed every two weeks. Ustka is a town in the central Pomerania region of northwestern Poland with 17,100 inhabitants (2001). It has also been part of Słupsk County in Pomeranian Voivodeship since 1999. Each of the 188 fishes was collected and wrapped in individual polyethylene bags for transport to the Department of Zoology and Animal Physiology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk and the Department of Medical Biology and Biochemistry, Department of Ecology and Environmental Protection, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń (Poland).

The muscle tissues used in chemical analyses were sampled from above the lateral line near the dorsal fin. The fish muscle tissue samples were weighed directly into acid-washed Teflon vessels. Next, a fragment of muscle tissue was removed from each sample and chopped into pieces with a steam-cleaned stainless steel knife. The muscle tissues were then washed with deionized water, air-dried to remove extra water, and subsequently homogenized in a food processor; 100 g test portions were stored at -20°C.

Analytical methods

Sample digestion

Concentrations of elements were determined after the samples were digested with the following procedure: samples of up to 100 mg dry weight were weighed and placed in calibrated 25 ml test tubes washed and rinsed with double-distilled water. Briefly, 1.5 ml pure 65% concentrated nitric acid (Sigma-Aldrich, Poznań, Poland) was added to each sample to make a clear solution according to standard procedures (Berghof GmbH), and the tubes were left to stand at room temperature for 20-24 h. Next, 0.5 ml 62% perchloric acid was added, and the contents of each tube were mixed before being placed in a Berghof Speedwave MWS-2 digestion system electrically heated to 400°C, fitted with a regulator and temperature gauge, and a microwave pressure digestion unit with built-in in situ temperature measurement. The mixtures were heated at 100°C for 1 h, at 150°C for another hour, and then at 200°C for the final hour (according to the standardized method of the system) until approximately 0.2 ml of a colorless clear solution was obtained. After cooling, each mixture was topped up to 5 ml with double-distilled water, stirred, and poured in its

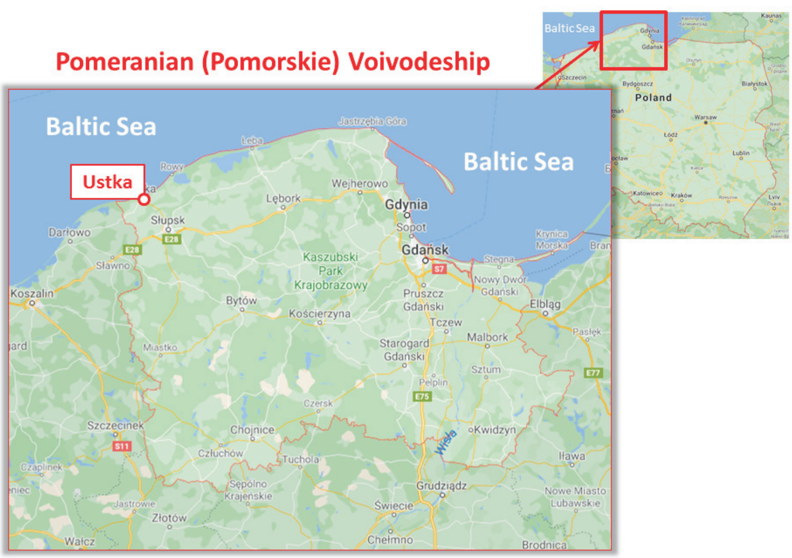

Figure 1. Map of sampling location in Ustka (Pomeranian Voivodeship, Poland).

entirety into tightly sealable, absolutely neutral polyethylene Nalgene containers (Nalge Nunc Int., USA, www.nalgene.com), according to the standardized method by Berghof GmbH. In each case, all glassware and plastic containers were washed in pure 65% nitric acid for analysis (Sigma-Aldrich) to remove adsorbed metals.

Samples were prepared in a Class 100 clean hood to prevent contamination by atmospheric particulates. Digestion was performed with microwave methods using the Discovery SPD (CEM, Matthews, NC). After digestion, samples were analyzed for trace minerals using an Agilent 7900 ICP-MS, which is a quadrupole mass spectrometry system equipped with a helium collision mode.

Prior to digestion, all samples were vortexed thoroughly to provide a homogeneous matrix for digestion. Samples were immediately pipetted to prevent settling before the samples were removed. A sample volume of 250 μL was dispensed into an acid-washed glass microwave digestion vessel. Reagent blanks were prepared by adding deionized water in place of the samples. The blank test was a mixture of nitric acid (V) and water subjected to digestion conditions together with the samples. A volume of 300 μL concentrated nitric acid (HNO_3) (Ultrex purity, Fisher), 200 μL concentrated hydrochloric acid (HCl) (Ultrex, Fisher), and 100 μL of non-stabilized 30% hydrogen peroxide (H_2O_2) solution (Ultrex, Fisher) was added to each vial containing samples. Deionized water was added to provide a final volume of 2.0 mL. The samples were then sealed, placed in the microwave, and digested as described below:

Microwave digestion program for samples

Max Temp (°C)	Ramp (min)	Hold (min)	Max Pressure (PSI)	Power (W)	Stir rate
200	07:00	09:00	300	200	Medium

Whole blood digestion microwave program

Max Temp (°C)	Ramp (min)	Hold (min)	Max Pressure (PSI)	Power (W)	Stir rate
200	07:00	09:00	400	200	Medium

At the end of digestion, all samples were removed from the microwave and allowed to cool to room temperature. In the clean hood, samples were quantitatively transferred to acid-washed 15 mL polypropylene sample tubes, spiked with a multi-element internal standard to provide a final concentration of 10 ng mL^{-1} Indium, Scandium, and Praseodymium, and diluted to the final volume with deionized (DI) H_2O. Samples were stored in a monitored refrigerator at a nominal temperature of 4°C until analysis.

The digestion method was applied to all elements, and there was no separate digestion. We confirmed experimentally the effectiveness of this method and good mercury recovery.

Microwave digestion with concentrated nitric acid and hydrogen peroxide was used to decompose dried muscle tissue, which was weighed into reaction vessels. Eight mL of 69-70% Baker Instra Analyzed grade nitric acid was added with 2 mL of 30% Chem. Lab. Analyst. grade hydrogen peroxide. The samples were microwaved for 5 min at 190°C (ramping time 25 min), then 5 min at 200°C (ramping time 5 min), and finally 5 min at 210°C (ramping time 5 min) to ensure the total decomposition of organic matter. The digested solution was moved to disposable calibrated tubes and filled up to 50 mL with deionized 0.05 μS cm^{-1} water.

Instruments and reagents

The ICP-MS technique was used for trace element quantitative analyses. An Agilent 7500ce ISP-MS apparatus was fitted with a micro-mist nebulizer, a Peltier cooled double pass spray chamber, and a peristaltic pump. Argon 5.0 (99.999% purity) was used as the carrier gas. The apparatus was also fitted with a torch with a shield torch system to reduce secondary discharge, off-axis ion lenses, a reaction/collision chamber with hydrogen 6.0, and helium 6.0 (purity 99.9999%) as reaction/collision gasses to eliminate interferences. The vacuum system consisted of a rotary pump and a turbo-molecular pump. A quadrupole with hyperbolic rods was the mass separator. The detector permitted working in digital and analog modes, which facilitated work through nine orders of magnitude.

The concentration of each element (μg g^{-1} of dry weight) was determined and the calibration curve method was used to estimate the concentration of the elements. Three replications were performed for each sample. Three-point calibration curves were calculated with the weighted method. Standard curves were prepared using standardized Merck samples. The following standards were used for the analysis of individual elements: KNO_3; $Mg(NO_3)_2$; $NaNO_3$; $Ca(NO_3)_2$; $As(NO_3)_2$; $Zn(NO_3)_2$; $Cu(NO_3)_2$; $Fe(NO_3)_3$; $Co(NO_3)_2$; $Mn(NO_3)_2$; $Cd(NO_3)_2$; $Pb(NO_3)_2$.

Quality control programs

All determinations were made in the presence of ^{45}Sc, ^{89}Y, and ^{159}Tb as internal standards to minimize the matrix effect and ensure long-term stability. The procedure was also performed for blank samples as a control of contamination. Simultaneously, for every series of samples, certified reference material (NCS ZC73016 chicken) from the China National Analysis Center for Iron and Steel was used to meet quality control requirements. Recoveries ranging from 90 to 110% were achieved for this material, and the uncertainty of the measurement was established at 10%.

There were many zeros in the list that indicate values below detection limits. Markings in the table with a value of 0 can be replaced with detection limits, although this is not quite the correct approach. This is acceptable assuming that these are objects with the lowest concentration of a given element, and perhaps in this group there were anomalies more often. An important observation arises in cases in which there are small variations in concentrations that indicate a certain degree of stability, and they are not interesting from the point of view of the analysis of various dependencies. Generally, in cases in which there were many zeros indicating values below detection limits, it was reasonable to consider these elements in scientific considerations.

Biological sample digestion in the microwave oven in hermetically sealed Teflon containers did not pose a risk of volatile Hg losses. After mineralization, the containers were cooled to room temperature, and only then was the analyte transferred to the measuring vessel. The problem with mercury is that in an environment such as nitric acid, mercury reduction can occur in the presence of reducing agents. Mercury adsorption on hydrophobic surfaces of Teflon vessels used for mineralization can also be a problem, but this can be the source of the memory effect that is quite typical of mercury. It is possible to check the effect of mineralization in the furnace on recovery by adding mercury from the standard in several test tubes. For comparison, it is possible to analyze samples with standard additions that are not subjected to mineralization since the rest, i.e. the amount of acid and the final volume of the sample analyzed, would be the same; it was certain that mineralization in no way led to analyte loss; the recoveries were very good (close to 100%). The recovery level was highly reproducible, and, therefore, the microwave oven mineralization method was acceptable. Mineralization was performed in sealed Teflon containers; after mineralization, they were cooled to avoid losses. The low pH of the solution after digestion and quantitative transfer to volume effectively prevented Hg losses. The influence of the reaction on Hg losses was investigated by Rosain and Wai (1973).

The mercury mineralization process itself did not cause any significant losses of this element. The first calibration standard on the curve was 50 ng L^{-1}. The mercury recovery from samples fortified at this level for samples after mineralization was almost 100%. For samples analyzed without mineralization, the recoveries were worse. The template without template was also mineralized so that the final concentration after transfer to 25 mL was 10 ng L^{-1}, 25 ng L^{-1}, and 50 ng L^{-1}. Here, too, the recoveries were fairly good.

The addition of an internal standard was used to monitor the conditions. The device, receiving the signal from the internal standard, calculated the results continually to eliminate the influence of the environment (e.g., changes in flow caused by reduced hole diameter in the cone from calcium deposits).

The results of element concentrations were mostly not within ranges reported in the literature

(Kabata-Pendias and Mukherjee 2007, Ping et al. 2009, Kabata-Pendias and Pendias 2010, Gall et al. 2015, Ullah et al. 2017, A-Javier 2018, Solihat et al. 2019, European Commission Directorate-General Joint Research Centre 2019, Kabata-Pendias and Szteke 2019) or those reported on the basis of them, so it was difficult to identify certain regularities in possible methodological errors during the analyses.

The analytical procedure of preparing samples was the same as that for the material analyzed. Simultaneously, we used standardized samples obtained from Merck, i.e., each particular quality standard for each specific chemical element. We obtained adequate analytical assurance, i.e., the standard reference material recoveries were within acceptable limits for the particular chemical elements analyzed. Thus, the differences between the results of the analysis conducted using certified reference material were within acceptable limits (Ullah et al. 2017, A-Javier 2018, European Commission Directorate-General Joint Research Centre 2019, Solihat et al. 2019). The reference materials used in the analysis of the chemical element concentrations were also used in papers published by Kabata-Pendias and Mukherjee (2007), Ping et al. (2009), Kabata-Pendias and Pendias (2010), Gall et al. (2015), and Kabata-Pendias and Szteke (2019).

Statistical analysis and health risk assessment

The results are presented as means ± SEM (n = 188) and expressed in mg per kg wet weight (ww). Both maximum and minimum concentrations of chemical elements in muscle tissues were determined to compare them with levels in edible fish filets as stated in the Codex Alimentarius (FAO 1995), Commission Regulation (EC) No 1881/2006 and Commission Regulation (EC) No 466/2001 (EC 2006, later amended in 2014 and 2015), and Polish nutrition standards (Jarosz et al. 2017).

The actual daily intake of nutrients from human fish product consumption in comparison with ADI was assessed according to FAO Fisheries Circular No 825 (Food Safety Regulation Applied to Fish Major Importing Countries, 1998), Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, Commission Regulation (EC) No 466/2001 of 8 March 2001 on the maximum levels for certain contaminants in foodstuffs, and the Regulation of the Minister of Health of Poland, 13.01.2003 on the maximum levels of chemical and biological contaminants in food, food ingredients, permitted additives, processing aids, or on the surface of food (Journal of Laws 2003. No. 37, item 326 with later amendments) to establish the same maximum levels of Hg, Cd, and Pb, i.e. 0.500, 0.050, and 0.300 mg kg^{-1} ww, respectively.

To assess the potential risk of the dietary intake of mineral elements and heavy metals from fish consumption, the recommended dietary allowances (RDAs) and EAR were used according to Polish nutrition standards (Jarosz et al. 2017). The food and nutrition board of the Institute of Medicine suggests that the RDAs or the adequate intakes (AIs) can be used as goals for individual intake, as they are set to meet the needs of 97-98% of individuals in a group.

The analysis of the data was based also on USEPA (1986, 1989, 2000, 2010), i.e. Risk Assessment Guidance for Superfund, Human Health Evaluation Manual, EPA/540/1-89/002, Risk-Based Concentration Table, and Risk-based Concentration Table.

Estimated average daily dose and estimated daily intake

The estimated average daily dose (ADD) of each heavy metal was calculated as in Wang et al. (2005) using 70 years as the exposure duration and equivalent to the average lifetime and 365 days as the exposure frequency. The estimated daily intake (EDI) of each heavy metal was calculated according to Saha and Zaman (2013).

Non-carcinogenic risk

The non-carcinogenic health risks associated with the consumption of fish were assessed based on THQs. The THQ values linked with fish consumption were assessed for each heavy metal. The calculations were performed using the standard assumption for an integrated USEPA risk analysis (USEPA, 1989; Ahmed et al., 2015). The oral reference doses (RfDs) were based on 0.001, 0.0003, 0.004, 1.5, 0.02, and 0.04 mg kg^{-1} BW d^{-1} for Cd, As, Pb, Cr, Ni, and Cu, respectively (USEPA, 2010). RfDs were based on 0.004, 0.001, 0.003, and 0.0005 mg kg^{-1} bw d^{-1} for Pb, Cd, As, and Hg respectively. RfDs are an estimate of the daily exposure to which the human population can be continually exposed over a lifetime without an appreciable risk of deleterious effects. If THQ is less than 1, the exposed population is unlikely to experience obvious adverse effects. If the THQ is equal to or higher than 1, there is a potential health risk (Wang et al. 2005), and appropriate interventions and protective measures should be taken.

Combined risk of multiple heavy metals

Reports indicate that exposure to two or more pollutants can result in additive and/or interactive effects (Hallenbeck 1993, Saha and Zaman 2013), and the total THQ (TTHQ) of heavy metals for individual foodstuffs is treated as the mathematical sum of the THQ value of each metal:

TTHQ (individual foodstuff) = THQ (toxicant 1) + THQ (toxicant 2) +…+ THQ (toxicant n).

Carcinogenic risk

For carcinogens, the risks were estimated as the incremental probability of an individual developing cancer over lifetime exposure to a potential carcinogen (i.e., incremental or excess individual lifetime cancer risk (USEPA 1989)). The acceptable risk levels for carcinogens range from 10^{-4} (the risk of developing cancer over a human lifetime is 1 in 10,000) to 10^{-6} (the risk of developing cancer over a human lifetime is 1 in 1,000,000). The equation used for estimating the target cancer risk (lifetime cancer risk) was calculated according to USEPA (1989).

CR indicates an increased likelihood of an individual developing life-threatening cancer caused by exposure to a potential carcinogen. The level used to assess the risk of cancer (USEPA 2000) was as follows:

$$CR = ADD \times CSF,$$

where CR – carcinogenic risk; ADD – average daily dose; CSF – carcinogenic factor tilt.

CSF is the carcinogenic slope factor of 0.0085 mg kg^{-1} day^{-1} for Pb, 0.38 mg kg^{-1} bw d^{-1} for Cd and 1.5 mg kg^{-1} bw d^{-1} for As (USEPA 2010).

Results and Discussion

The mean contents of minerals (manganese, iron, copper, zinc, magnesium, calcium, cobalt, sodium, selenium, phosphorus, potassium) with a range of minimum and maximum values in the sea trout muscle tissue are summarized in Table 1. The chemical analyses indicated that sea trout muscle tissues were rich in phosphorus, calcium, magnesium, sodium, and potassium at mean respective concentrations of 147.10 ± 8.051 mg kg^{-1}; 129.873 ± 15.124 mg kg^{-1}; 122.689 ± 3.011 mg kg^{-1}; 103.579 ± 6.153 mg kg^{-1}; and 98.346 ± 1.433 mg kg^{-1}. Concentrations of the following were low in the samples tested: iron –

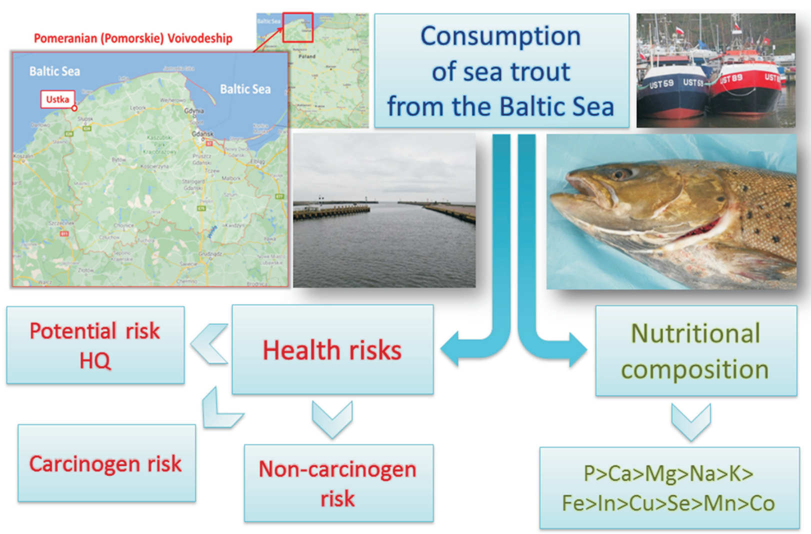

Figure 2. Assessment of the potential risk of the dietary intake of mineral elements and heavy metals from fish consumption.

Table 1
Concentrations of elements in sea trout muscles and percentage of mineral intakes for adults compared to the mean concentration in 100 g of sea trout consumed (n = 188)

Elements	Concentration (M ± m) (mg kg^{-1})	Range of values (min-max) (mg kg^{-1})	Percentage of mineral intake, (%)
Manganese, Mn	0.029 ± 0.002	0.0038 – 0.202	53.72
Iron, Fe	1.244 ± 0.082	0.1882 – 7.58	58.51
Copper, Cu	0.559 ± 0.072	0.009 – 5.28	72.34
Zinc, Zn	0.833 ± 0.060	0.123 – 5.27	57.98
Magnesium, Mg	122.689 ± 3.011	27.0 – 219.0	53.72
Calcium, Ca	129.873 ± 15.124	11.3 – 204.0	72.87
Cobalt, Co	0.0005 ± 0.000	0.0001 – 0.0025	67.02
Sodium, Na	103.579 ± 6.153	6.82 – 286.0	52.12
Selenium, Se	0.036 ± 0.004	0.0019 – 0.308	65.96
Phosphorus, P	147.10 ± 8.051	12.9 – 636.0	57.98
Potassium, K	98.346 ± 1.433	11.3 – 204.0	48.94

1.244 ± 0.082 mg kg^{-1}; zinc – 0.833 ± 0.060 mg kg^{-1}; copper – 0.559 ± 0.072 mg kg^{-1}; selenium – 0.036 ± 0.004 mg kg^{-1}; manganese – 0.029 ± 0.002 mg kg^{-1}; cobalt – 0.0005 ± 0.000 mg kg^{-1} (Table 1). The pattern significance gradation of element concentrations was as follows: P> Ca>Mg> Na>K> Fe> Zn> Cu> Se>Mn>Co.

Since a wide range of values (from minimum to maximum) of the content of both micro- and macronutrients in sea trout muscle was identified, the percentage of individual mineral intake compared to mean concentrations in 100 g of consumed sea trout was also calculated. The results indicated that the concentration of minerals in most of the samples tested was lower than the mean values. Thus, the human intake of minerals from consuming 100 g of sea trout compared to mean values was 72.87% of calcium, 72.34% of copper, 67.02% of cobalt, and 65.96% of selenium. The value for other minerals, except potassium, exceeded 50% (Table 1).

Fish consumption is distributed unevenly around the world, with continental, regional, and national differences, and also differs with regard to income. Per capita fish consumption ranges from less than 1 kg to more than 100 kg annually. Geographical differences are also evident within countries, and consumption is generally higher in coastal areas (FAO 2006).

The current study analyzed the actual intakes of micro- and macroelements with a daily consumption of 100 grams of sea trout and whether or not the nutritional requirements of adults (mean bodyweight of 70 kg) aged 18 and older were met. The human requirement for nutrients derived from foods was assessed according to Polish nutrition standards (Jarosz et al. 2017) by comparing the actual intake of nutrients with the RDA and EAR (Table 2). According to the Food and Nutrition Board of the Institute of Medicine, RDA reflects average daily intakes that are sufficient to meet the nutrient requirements of nearly all (97-98%) healthy people from particular gender and life stage groups (Institute of Medicine 1998). The RDA for a nutrient is the value used as the goal for dietary intake by healthy individuals. EAR is the daily intake value of a nutrient that is estimated to meet the nutrient requirements of half of the healthy individuals in life stage and gender groups. The potential intake of chemical elements through sea trout consumption was calculated based on the average actual daily intake with regard to existing standard levels (RDA and EAR), energy conversion factors, definitions, and Polish nutrition standards (Jarosz et al. 2017).

The potential and actual intake of micro- and macroelements by adults (both males and females) who consume 100 g of sea trout with regard to RDA and EAR are presented in Tables 2 and 3. This comparison shows that the potential mineral intake is relatively low. Consuming 100 g of sea trout can provide a considerable intake of copper (62.22% of the RDA

Table 2
Actual mineral intake through consumption of 100 g of sea trout compared to the recommended dietary allowances (RDA) (n = 188)

Elements	Recommended dietary allowances (RDA*), (mg day^{-1} person^{-1})		Actual intake of minerals from 100 g of product, (mg day^{-1} person^{-1})	Potential intake of minerals according to RDA*, (%)	
	males	females		males	females
Manganese, Mn	2.3	1.8	0.0029	0.12	0.16
Iron, Fe	10.0	18.0	0.124	12.44	6.91
Copper, Cu	0.9	0.9	0.056	62.22	62.22
Zinc, Zn	11.0	8.0	0.083	7.54	10.37
Magnesium, Mg	420.0	320.0	12.269	29.21	38.34
Calcium, Ca	1000.0	1200.0	12.987	12.99	10.82
Selenium, Se	5.5	5.5	0.004	0.65	0.65
Phosphorus, P	700.0	700.0	14.710	21.01	21.01

*RDA according to Polish nutrition standards (Jarosz et al. 2017).

for both men and women), magnesium (38.34% and 29.21% for women and men, respectively), phosphorus (21.01% for men and women), calcium and iron (12.99% and 10.82% and 12.44% and 6.91% for men and women, respectively), and zinc (10.37% and 7.54% for women and men, respectively) compared to the RDA according to Polish nutrition standards (Jarosz et al. 2017) and the EU Commission Directive (2008). A low percentage of selenium (0.65% of the RDA for women and men) and manganese (0.16% and 0.12% for women and men, respectively) intake was noted with the consumption of 100 g of sea trout (Table 2). These trace elements were present in a low concentration in sea trout and probably did not significantly affect its nutritional value.

The potential intake of minerals from 100 g of sea trout in comparison with EAR is presented in Table 3. The highest potential intake of copper (80.0%) with the consumption of 100 g of sea trout among men and women was noted (Table 3). This amount of product provides 35.05% and 46.29% of the EAR for magnesium, 25.36% for phosphorus, 20.73% for iron, and 16.23% for calcium intakes for men and women. This fish can only be a supplement to other dietary items since selenium and manganese were found in small quantities in the samples (0.79% of estimated average requirements for selenium, and 0.12% and 0.16% for manganese intakes for men and women, respectively (Table 3).

In our study, we also analyzed the actual intake of micro- and macronutrients in the daily consumption of 80 g of fish and the nutrients provided to children with an average body weight of 48 kg (aged 7-18) and the daily consumption of 60 g of fish and the nutrients provided to children with an average body weight of 19 kg (aged 6 and under). The actual intake of minerals was also calculated taking into account the average values of micro- and macroelements in sea trout with a daily consumption of 80 g of fish and the nutrients provided to children with an average body weight of 48 kg (aged 7-18), and the consumption of 60 g of fish and the nutrients provided to children with an average body weight of 19 kg (aged 6 and under) according to Abubakar et al. (2015) (Table 4). The actual intake of micro- and macroelements in the body of children of different ages (aged 7-18 and 6 and under) consuming a certain amount of sea trout and the potential intake of minerals specified by RDA and EAR and reported by Jarosz et al. (2017) were calculated and are presented in Tables 4 and 5. In our study, the standard RDA and EAR values were indicated within ranges from lower to higher since these indicators were considered for children of different ages (Tables 5 and 6). The highest values of the actual intake of minerals from sea trout according to the RDA were for copper (8.25% for children aged 6 and under and 6.43-5.0% for children aged 7-18) and magnesium (7.55-2.39%

Table 3
Potential mineral intake with 100 g of sea trout compared to the estimated average requirements (EAR) (n = 188)

Elements	Estimated average requirements (EAR)*, (mg kg^{-1})		Potential mineral intake according to EAR*, (%)	
	males	females	males	females
Manganese, Mn	2.3	1.8	0.12	0.16
Iron, Fe	6.0	6.0	20.73	20.73
Copper, Cu	0.7	0.7	80.00	80.00
Zinc, Zn	9.4	6.8	8.83	12.21
Magnesium, Mg	350.0	265.0	35.05	46.29
Calcium, Ca	800.0	800.0	16.23	16.23
Selenium, Se	4.5	4.5	0.79	0.79
Phosphorus, P	580.0	580.0	25.36	25.36

*EAR according to Polish nutrition standards (Jarosz et al. 2017)

Table 4
Actual intake of minerals with sea trout consumption by children of different ages

Elements	Actual intake of minerals from 80 g of product by children aged 7-18, (mg day^{-1} $person^{-1}$)	Actual intake of minerals from 60 g of product by children 6 and under, (mg day^{-1} $person^{-1}$)
Manganese, Mn	0.0023	0.0017
Iron, Fe	0.099	0.075
Copper, Cu	0.045	0.033
Zinc, Zn	0.067	0.050
Magnesium, Mg	9.810	7.360
Calcium, Ca	10.390	7.792
Selenium, Se	0.0029	0.0022
Phosphorus, P	11.768	8.826

for children aged 7-18 and 5.66% for children 6 and under) (Table 5). The actual intake of the other minerals with the consumption of fish by children of different ages compared to recommended intakes was insignificant.

The results of calculating the actual intake of minerals from sea trout compared to EAR according to consumption norms for children of different ages were similar to the previous ones. High intakes were noted for copper (9.0-6.43% and 11.0% for children aged 7-18 and 6 and under, respectively) and magnesium (8.92-2.88% and 6.69% for children aged 7-18 and 6 and under, respectively) (Table 6). Iron intakes were 2.47-1.24% and 1.87% of EAR, while those of phosphorus were 2.35-1.12% and 2.15% for children aged 7-18 and 6 and under, respectively (Table 6). The actual intakes of the other minerals when children of various ages consumed sea trout according to EAR were insignificant (Table 6). Therefore, consuming this fish provided adults and children with the greatest intakes of copper and magnesium.

The hygienic regulation of the xenobiotic contents in foods requires compliance with two standards: maximum permissible concentration (MPC) and the maximum permissible level (MPL) in individual products and also acceptable daily intake (ADI). These standards are the basis for performing hygienic monitoring of chemical levels in food raw materials and finished products. MPC and MPL standards are the criteria for the safety of individual food products, while ADI reflects dietary standards for populations.

The concentrations of heavy metals, i.e., cadmium, lead, mercury, and arsenic, were determined in the sea trout samples tested and were compared with MPCs of metals in the product (Table 7). No values exceeding MPCs compared to the mean concentration were detected in the fish samples. The concentration of heavy metals was lower than the

Table 5
Potential mineral intake with the consumption of 80 g and 60 g of sea trout by children aged 7-18 and 6 and under, respectively, compared to recommended dietary allowances (RDA) (n = 188)

	Recommended dietary allowances (RDA*) (mg day^{-1} person^{-1})		Potential intake of minerals according to RDA*, (%)	
Elements	children 7-18	children 6 and under	children 7-18	children 6 and under
Manganese, Mn	1.5-2.2	1.5	0.15-0.10	0.11
Iron, Fe	10-12	10	0.99-0.82	0.75
Copper, Cu	0.7-0.9	0.4	6.43-5.0	8.25
Zinc, Zn	5-11	5	1.34-0.61	1.0
Magnesium, Mg	130-410	130	7.55-2.39	5.66
Calcium, Ca	1000-1300	1000	1.04-0.80	0.78
Selenium, Se	30-55	30	0.01-0.005	0.007
Phosphorus, P	600-1250	500	1.96-0.94	1.76

Table 6
Potential mineral intake with the consumption of 80 g and 60 g of sea trout by children aged 7-18 and 6 and under, respectively, compared to the estimated average requirements (EAR) (n = 188)

	Estimated average requirements (EAR)*, (mg kg^{-1})		Potential mineral intakes according to EAR*, (%)	
Elements	children 7-18	children 6 and under	children 7-18	children 6 and under
Manganese, Mn	1.5-2.2	1.5	0.15-0.10	0.11
Iron, Fe	4-8	4	2.47-1.24	1.87
Copper, Cu	0.5-0.7	0.3	9.0-6.43	11.0
Zinc, Zn	4-8.5	4	1.67-0.79	1.25
Magnesium, Mg	110-340	110	8.92-2.88	6.69
Calcium, Ca	800-1100	800	1.3-0.94	0.97
Selenium, Se	23-45	23	0.013-0.006	0.009
Phosphorus, P	500-1050	410	2.35-1.12	2.15

*EAR according to Polish nutrition standards (Jarosz et al. 2017).

Table 7
Comparison of the concentration of heavy metals and arsenic in sea trout with the maximum permissible concentration of metals in the product (n = 188)

Toxic elements	Concentration (M ± m) (mg kg^{-1})	Range of values (min-max) (mg kg^{-1})	Maximum permissible concentration (MPC*) of metals in the product (mg kg^{-1})
Cadmium, Cd	0.0012 ± 0.0001	0.00003 – 0.0103	0.05
Lead, Pb	0.0062 ± 0.0014	0.0008 – 0.2184	0.2
Mercury, Hg	0.0011 ± 0.000	0.0006 – 0.0372	0.5
Arsenic, As	0.0189 ± 0.0009	0.0009 – 0.0739	4.0

* The Maximum Permissible Concentration (MPC) is the maximum quantity of an injurious substance per unit volume (air, water, or other liquid) or weight (for example, food products) to which daily exposure for an indefinite period does not cause any pathological deviations or unfavorable hereditary changes in offspring.

MPC of metals in food in all sea trout samples, except for lead, an excess of which was found in one sample (0.2184 mg kg^{-1}), which accounted for 0.53% of all samples tested. According to these results, the ranking order of the mean concentration of heavy metals in sea trout muscle tissues was As (0.0189 mg kg^{-1}) > Pb (0.0062 mg kg^{-1}) > Cd (0.0012 mg kg^{-1}) > Hg (0.0011 mg kg^{-1}).

EDI of heavy metals by adults, children aged 7-18, and children aged 6 and under from the consumption of sea trout are presented in Table 8. The highest EDI of heavy metals by adults, children aged

Table 8

Estimated daily intake of heavy metals by an adults, children aged 7-18, and children aged 6 and under with sea trout consumption

Toxic elements	Estimated daily intake of heavy metals from 100 g of product by adults, (mg day^{-1} $person^{-1}$)	Estimated daily intake of heavy metals from 80 g of product by children aged 7-18, (mg day^{-1} $person^{-1}$)	Estimated daily intake of heavy metals from 60 g of product by children aged 6 and under, (mg day^{-1} $person^{-1}$)
Cadmium, Cd	0.00012	0.000096	0.000072
Lead, Pb	0.00062	0.000496	0.000372
Mercury, Hg	0.00011	0.000088	0.000066
Arsenic, As	0.0019	0.001512	0.001134

Table 9

Estimated target hazard quotients (THQ), total target hazard quotients (ΣTHQ), and carcinogenic risk (CR) of each metal from sea trout consumption

Toxic metals	Average daily dose (ADD) (mg kg^{-1} d^{-1})	Oral reference dose (RfD) (mg kg^{-1} d^{-1})	Target hazard quotients (THQ)	Total target hazard quotients (ΣTHQ)	Carcino-genic slope factor (CSF) (mg kg^{-1} day^{-1})	Carcino-genic risk (CR)
Cadmium, Cd	2.86 10^{-8}	0.001	2.86 10^{-5}	1.29 10^{-2}	0.38	1.09 10^{-8}
Lead, Pb	4.79 10^{-8}	0.004	1.20 10^{-3}		0.0085	4.07 10^{-8}
Mercury, Hg	1.06 10^{-6}	0.0005	2.12 10^{-3}		-	-
Arsenic, As	2.88 10^{-5}	0.003	9.6 10^{-3}		1.5	4.32 10^{-5}

7-18, and children aged 6 and under were noted for As at 0.0019, 0.001512, and 0.001134 mg day^{-1} $person^{-1}$, respectively (Table 9).

Non-carcinogenic risk assessment is usually performed to determine the health effects of pollutants constituting potential hazards (Ullah et al. 2017). Health risks are also assessed by determining the THQ of each heavy metal and the hazard index (HI). THQ estimates the non-carcinogenic risk level of consuming specific pollutants present in products, while HI is the sum of THQs and estimates global risks related to consuming products (Khemis et al. 2017).

The results in Table 9 reveal that the THQ value of each metal was less than 1, suggesting that individuals would not experience significant health risks with the intake of each heavy metal alone by consuming sea trout. The ΣTHQ was also less than 1 and indicated that there was no considerable health hazard from consuming sea trout or from exposure to a combination of the four metals tested (As, Pb, Cd, Hg). In the current study, the major risk factor was As at 74.22%, followed by Hg (16.33%), Pb (9.23%), and Cd (0.22%).

The ΣTHQ was also lower than 1, which indicated the combined metals did not pose a risk to consumers. The CR was 1.09 10^{-8} and 4.07 10^{-8} for Cd and Pb, and 4.32 10^{-5} for As, which indicated a negligible CR for Cd and Pb and an acceptable one for As. CR values lower than 10^{-6} are usually regarded as negligible, while those above 10^{-4} are unacceptable, and values between 10^{-6} and 10^{-4} are regarded as within an acceptable range (USEPA 2010). In the present study, the CRs for Cd, Pb and As were in the acceptable range and indicated that the risk of cancer-related exposure to these heavy metals from fish consumption was acceptable and negligible (Table 10). Consequently, consuming sea trout from the Baltic Sea by adults does not pose health risks or considerable health hazards. The estimated THQ, ΣTHQ, and CR associated with each metal for children aged 7-18 and 6 and under consuming sea trout are presented in Tables 10 and 11. The calculated CR in the group of children aged 7-18 was 8.76 10^{-8}

Table 10
Estimated target hazard quotients (THQ), total target hazard quotients (ΣTHQ), and carcinogenic risk (CR) of each metal from sea trout consumption (children aged 7-18)

Toxic metals	Average daily dose (ADD), (mg kg^{-1} d^{-1})	Oral reference dose (RfD), (mg kg^{-1} d^{-1})	Target hazard quotients (THQ)	Total target hazard quotients (ΣTHQ)	Carcino-genic slope factor (CSF), (mg kg^{-1} day^{-1})	Carcino-genic risk (CR)
Cadmium, Cd	2.0 10^{-6}	0.001	2.0 10^{-3}	1.87 10^{-2}	0.38	7.6 10^{-7}
Lead, Pb	1.03 10^{-5}	0.004	2.58 10^{-3}		0.0085	8.76 10^{-8}
Mercury, Hg	1.83 10^{-6}	0.0005	3.66 10^{-3}		-	-
Arsenic, As	3.15 10^{-5}	0.003	1.05 10^{-2}		1.5	4.73 10^{-5}

Table 11
Estimated target hazard quotients (THQ), total target hazard quotients (ΣTHQ), and carcinogenic risk (CR) of each metal from sea trout consumption (children aged 6 and under)

Toxic metals	Average daily dose (ADD), (mg kg^{-1} d^{-1})	Oral reference dose (RfD), (mg kg^{-1} d^{-1})	Target hazard quotients (THQ)	Total target hazard quotients (ΣTHQ)	Carcino-genic slope factor (CSF), (mg kg^{-1} day^{-1})	Carcino-genic risk (CR)
Cadmium, Cd	3.79 10^{-6}	0.001	3.79 10^{-3}	3.55 10^{-2}	0.38	1.44 10^{-6}
Lead, Pb	1.96 10^{-5}	0.004	4.9 10^{-3}		0.0085	1.67 10^{-7}
Mercury, Hg	3.47 10^{-6}	0.0005	6.94 10^{-3}		-	-
Arsenic, As	5.97 10^{-5}	0.003	1.99 10^{-2}		1.5	8.96 10^{-5}

for Pb and 7.6 10^{-7} for Cd; hence, the CR was insignificant or minimal, while the CR for As was 4.73 10^{-5}, which was an acceptable CR (Table 11). In the group of children aged 6 and under, the CR was 1.67 10^{-7} for Pb, which was minimal, while the CR for Cd was 1.44 10^{-6} and and for As it was 8.96 10^{-5} for As, which were acceptable (Table 11). Thus, the results of the study indicated acceptable CR for adults and children linked with the daily consumption of sea trout containing heavy metals in the concentrations above.

Heavy metal concentrations in waters, sediments, and the muscles of various fish species in Europe are reported in the literature. Usydus et al. (2005) determined the total arsenic content in the muscles of the major Baltic fish species of herring, *Clupea harengus* L., sprat, *Sprattus sprattus* (L.) cod, and flounder, *Platichthys flesus* (L.) caught in different areas of the Baltic Sea. They also evaluated whether flounder As content depended on fish size. Since flounder is a fairly stationary, it might be a good indicator species for comparisons of area-dependent degrees of pollution. The As levels did not exceed this level in any of the samples. The highest mean As content (0.78 mg kg^{-1} ww) was noted in sprat, while the lowest (0.26 mg kg^{-1} ww) was recorded in flounder. The muscle As contents differed significantly among the species studied, but there were no significant interspecific differences between cod and flounder. Eastern Baltic flounder had significantly higher As contents than their did their conspecifics caught in the central and western Baltic. As contents in flounder 30-40 cm in length were significantly higher than those recorded in those measuring 20-30 cm and those smaller than 20 cm (Usydus et al. 2005). In a later study, cod and hagfish, *Myxine glutinosa* L. muscles were confirmed to accumulate arsenic-based warfare agents (Niemikoski et al. 2020).

Sanderson et al. (2009) assessed the potential indirect human health risks of consuming fishes contaminated with chemical warfare agents at a dumpsite area east of Bornholm where roughly 11,000 tons of these agents were dumped during German disarmament after World War II. Moreover, elevated frequencies of lesions on fish caught at a chemical warfare agent dumpsite in the Mediterranean Sea were observed. The fish from the Mediterranean dumpsite had elevated total As concentrations in their muscles, and elevated total As levels were also observed in sediments (Sanderson et al. 2009).

Fakhri et al. (2021) conducted a systematic global review, meta analysis, and health risk assessments of rainbow trout, Oncorhynchus mykiss (Walbaum and brown trout fillet consumption over a period of 37 years. Meta analysis revealed that potentially harmful elements in trout fillets included 19,996.64 μg kg^{-1} Fe; 1,834.75 μg kg^{-1} Co; 772.21 μg kg^{-1} Cu; 335.78 μg kg^{-1} Ni; 290.46 μg kg^{-1} Se; 226.20 μg kg^{-1} Cr; 178.11 μg kg^{-1} Pb; 77.40 μg kg^{-1} Hg; 19.40 μg kg^{-1} Cd; and 3.66 μg kg^{-1} inorganic As. The non-carcinogenic risk assessment indicated that the lowest and highest HI in adults was in Pakistan (0.0012) and Turkey (0.2388), while in children it was in Pakistan (0.0057) and Turkey (1.114), respectively. The non-carcinogenic risk was acceptable for adult consumers in all countries (HI > 1 value), but the non-carcinogenic risk for children was unacceptable in Turkey. The country ranking order of CR for inorganic As in adults was China (1.44 10^{-6}) > Iran (9.1 10^{-8}) > Turkey (4.45 10^{-8}) > Portugal (9.04 10^{-10}). The CR was at the threshold for adult consumers in China (CR < 10^{-6}) (Fakhri et al. 2021).

Many studies revealed that the risk from trace elements and potentially harmful elements to consumers of farmed and wild rainbow trout in some countries was minimal. Varol and Sünbül (2017) compared heavy metal levels of farmed and escaped farmed rainbow trout and assessed the health risks associated with their consumption. Of ten metals, only Co and Fe levels in escaped rainbow trout were significantly higher than those in farmed rainbow trout. The metal levels in farmed and escaped rainbow trout were below the maximum permissible limits. The EDI of each metal in both farmed and escaped farmed rainbow trout were much lower than the respective tolerable daily intake. The THQs for individual metals and ΣTHQ for combined metals were lower than 1 in both farmed and escaped rainbow trout, indicating no health risk for humans (Varol and Sünbül 2017). Heshmati et al. (2019) assessed concentrations of Pb, Cd, Hg, Ni, Fe, Zn, and Cu in farmed rainbow trout muscle, feed, and water samples collected from Hamadan Province, Iran, and they also assessed the risk of ingesting these metals by consuming this fish species. The risk assessment indicated the intake of the potentially toxic elements analyzed when consuming rainbow trout posed no health risk to consumers. The rainbow trout muscles analyzed were not considered to bioaccumulate potentially toxic elements (Heshmati et al. 2019).

Conclusions

1. The pattern significance gradation of element concentrations in Baltic sea trout (P>Ca>Mg>Na>K>Fe>Zn>Cu>Se>Mn>Co) and the human intake of minerals when 100 g of fish was consumed in comparison with mean values (Ca: 72.9%, Cu: 72.3%, Co: 67.02%, Se: 65.9%) revealed that metal concentrations in sea trout were below maximum permissible levels for human consumption as set by international food standards.
2. The actual intake of minerals from sea trout according to the RDA was the highest for copper (8.25% for children aged 6 and under and 6.43-5.0% for children aged 7-18) and magnesium (7.55-2.39% for children aged 7-18 and 5.66% for children aged 6 and under). The actual intake of other minerals with sea trout consumption by children of different ages according to the estimated average requirements was insignificant. Consumption of this fish can provide adults and children with the greatest intakes of copper and magnesium.
3. The EDI of toxic metals based on actual sea trout consumption was minimal compared to the RDA (the ranking order of mean heavy metal concentrations in sea trout muscles was As>Pb>Cd>Hg). There was no considerable health hazard from sea trout consumption and exposure to As, Pb, Cd, or Hg.
4. The results of the current study indicated that CR was acceptable for adults and children with regard

to the daily consumption of sea trout containing heavy metals in the concentrations determined. For carcinogenic and non-carcinogenic effects, the maximum allowable rates for consumption of sea trout were sufficient to ensure human health. According to this data, the consumption of sea trout from Baltic Sea did not pose a risk to human health.

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