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Toxic and essential elements in freshwater and marine fish species from a central Italian market: assessment of consumers' health risks and benefits

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Abstract

The concentrations of selected toxic elements [mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As), inorganic As (iAs)] and essential elements [selenium (Se), zinc (Zn), manganese (Mn) and iron (Fe)] were determined in the muscles of freshwater (FF) and marine fish (MF) species collected from the market in Umbria and Marche regions (Italy). The consumers' exposure to all elements was determined, and a comprehensive benefit–risk assessment was conducted to assess the health impacts associated with fish consumption. The elements' concentration ranged as follows: Cd 0.01-0.01, Pb 0.01-0.02, Hg 0.04-0.12, As 0.07-11.42, Mn 0.21-0.56, Fe 2.35-6.79, Se 0.12-0.34, and Zn 5.76-4.77 mg/kg w/w. In all samples, Cd, Pb, and Hg were under the maximum allowed concentrations for safe human consumption in Europe. The Metal Pollution Index was estimated to be 0.014 for FF and 0.094 for MF, and the EDI for toxic elements ranged between 2.13E-05 and 2.64E-03 and for essential elements between 1.48E-03 and 1.75E-03 mg/kg bw/day for FF and MF, respectively. The contribution of fish to the daily reference intakes of essential elements set in the EC Reg. 1169/2011 was significant only for Se, which reached 15% of the threshold in both FF and MF; the Hg-Se balance was defined, revealing values >1 for the Se:Hg molar ratio and a positive Health Benefit Value for Se index. Risk was defined by the target hazard quotients that ranged from 0.0002 to 0.2471 and the hazard index with values of 0.0140 for FF and 0.3280 for MF, indicating the absence of non-carcinogenic risks. The carcinogenic risk and the margin of exposure both revealed no risk for carcinogenic effects for FF and low for MF. The results suggested that the consumption of MF species can pose a low, but still acceptable, carcinogenic risk due to iAs exposure and that both fish groups represent a significant source of Se, able to mitigate the toxic effect of Hg.

Introduction

Fishery products represent an important dietary sources of essential nutrients for human health; among these, unsaturated essential fatty acids (PUFAs), especially omega-3 PUFAs, are renowned for their health benefit (Chen *et al.*, 2022), but also vitamins (especially D and B2) and essential elements (EEs) such as selenium (Se), zinc (Zn), manganese (Mn) and iron (Fe) are crucial for maintaining a good health status (Khalili Tilami and Sampels, 2018). Although it is generally recognized that marine fish species contain higher amounts of essential nutrients, especially omega-3 PUFAs, some authors found that some freshwater fishes contribute significantly to the dietary intake of such beneficial substances (Özogul *et al.*, 2007; Li *et al.* 2011; Branciari *et al.*, 2020) These nutritional benefits promoted worldwide the release of specific dietary guidelines aimed to increase consumers' consciousness of the health benefits associated with fish and other fishery products' intake (Visseren *et al.*, 2021; European Commission, 2025a; USDA, 2025;). Consequently, the consumption of fish raised in many countries, granting these products a significant role in the human diet all over the world (FAO, 2024). The increased consumption, however, brings unintended consequences as, following the unchecked industrial activities, intensive farming and rapid population expansion, the amount of toxic contaminants released into the aquatic environments and able to accumulate and magnify in fish tissues significantly grows in recent years, posing potential risks to human health (Afonso *et al.*, 2017).

Among these toxic elements (TEs), heavy metals represent major environmental contaminants that commonly enter aquatic systems via numerous sources such as agricultural runoff, sewage disposal, power generation, and industrial waste discharge, combined with natural factors such as heavy rainfall (Wang, Luo, *et al.*, 2022). Once released in the aquatic environment, heavy metals such as mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) can accumulate within the tissues of aquatic organisms, and consequently, fish consumption can ultimately endanger human health (Genchi *et al.*, 2020; Wang, Cao, *et al.*, 2022; Wang, Luo, *et al.*, 2022).

This ambivalent scenario stimulated a greater interest in evaluating not only consumers' risks caused by fish consumption, as traditionally performed (Barone *et al.*, 2015; Rose *et al.*, 2015), but concurrently also the associated benefits for human health (Loring *et al.*, 2010; Chen *et al.*, 2022). The European Food Safety Authority (EFSA) states that where a food is recognized to have the potential to exert both health benefits and health risks, it is crucial for risk managers to be able to weigh the risks against the benefits on the basis of a qualitative and/or quantitative benefit-risk assessment (BRA) (EFSA, 2010, 2024).

When performing a BRA is important to investigate the associations between real-life human-specific exposures and health outcomes, adverse and beneficial (EFSA, 2010). Is to consider, indeed, that although a regular consumption of most fish species should not lead to adverse health effects for the average consumer, some case-specific aspects such as the choice of species consumed, the peculiar consumption habits, and the meal size, are pivotal factors to consider in order to adequately assess the health benefits and risks of fish consumption (Domingo, 2016).

The objective of this study was to conduct a BRA considering a specific scenario, related to the consumption of different freshwater and marine fish species by the adult population of central Italy (Umbria and Marche regions), and considering as risk the ingestion of TEs (Pb, Hg, Cd, As) and as benefit the intake of EEs (Se, Mn, Zn, Fe). Furthermore, in consideration of consolidated evidence that Se potently antagonizes the Hg-induced toxicity (Ralston *et al*., 2016; Ralston *et al*., 2019; Grgec *et al*., 2020), the Hg-Se balance was also addressed in accordance with EFSA Scientific Opinion (EFSA 2024a), which considers "the probability of a reduction of an adverse health effect" as a specific beneficial effect.

Materials and Methods

Samples collection and preparation

A total of 50 samples were collected, chosen from the species available in the central Italian (Umbria-Marche region) local fish shops and supermarkets. The freshwater and marine fish species analyzed are reported in Table 1.

Muscle tissue is the primary part of fish species consumed by humans, and therefore it was selected to evaluate both the elements concentration and the potential health risks and benefits. The samples were prepared as already described by Ciriaci *et al*. (2025), by removing the non-edible parts using plastic cutlery and containers to avoid metal contamination. Approximately 100 g of muscle tissue was finely homogenized, 1.0±0.2 g of sample was weighed into Teflon vessels, and 6 mL HNO_3 and 2 mL of H_2O_2 were added (Ciriaci *et al*., 2025). The digestion was performed by a microwave system (Milestone-Ethos1-HPR1000) using the following temperature program: heating to 200 °C in 15 min, holding at 200 °C for 15 min, venting for 60 min. The obtained digestate was 1:5 diluted with ultrapure water in plastic vessels, ready for instrumental analysis following the UNI EN 13804:2013 (UNI, 2013) and UNI EN 13805:2014 (UNI, 2014).

Elements determination and Metal Pollution Index

The eight targeted elements, As, Cd, Hg, Fe, Mn, Se, Pb, and Zn, were simultaneously analyzed by means of a validated method suitable for the multi-determination of nineteen different elements, as already described by Ciriaci *et al*. (2025). Briefly, the instrumental analyses were performed by Inductively Coupled Plasma-Mass Spectrometry on an iCAP-RQ (Thermo Fisher) equipped with an ASX-560 autosampler (Teledyne CETAC Technologies). Samples were introduced into the instrument via a peristaltic pump, equipped with a quartz Meinhard MicroMist U-Series nebulizer and a quartz cyclonic spray chamber. The internal standard was automatically dispensed through a mixing block. High-purity argon (99.999%) was employed as the plasma torch, nebulizer, and auxiliary gas, while helium (99.99%) was used as the collision gas in KED mode (SM1). Quantification was achieved using external calibration curves comprising at least four points in addition to the blank (0 μg/kg). The experimental quantification limits (LOQ) were 0.003 mg/kg for Cd, Pb; 0.010 mg/kg for Hg; 0.020 mg/kg for As; 0.10 mg/kg for Mn, Se; 2.0 mg/kg for Fe, Zn.

The Metal Pollution Index (MPI) was calculated to quantify the overall contamination level adopting Eq. 1:

$$MPI = (C1 * C2 \dots * Cn)^{1/n} \qquad \text{[Eq. 1]}$$

Where *C1, C2*... up to *Cn* are the concentrations of each TE in the fish tissue, and *n* is the number of TEs. This metric allows for the comparison of the total contents of TEs among fish species and tissues as a higher MPI value reflects a higher level of pollution (Töre *et al*., 2021; Varol *et al*., 2022).

Estimated daily intake of metals

Estimation of dietary intake of these elements from consumption of the analyzed fish was further carried out by administering an online questionnaire to residents of the Umbria and Marche region, aiming to gather detailed information on dietary habits concerning both marine and freshwater fishery products, focusing on categories of food consumed, frequency, and typical portion sizes. The collected data will facilitate a comprehensive analysis of eating patterns in this specific population, providing valuable insights regarding potential health risks or benefits associated with their dietary choices.

Consistent with previous studies (Chen *et al*., 2002; Traina *et al*., 2019), it was assumed that the ingested dose corresponds to the absorbed dose and that cooking had no effect on the elemental composition of the fish. This assumption allows for standardized estimation and comparison of elemental intake and associated risks and benefits.

The estimated daily intake (EDI), expressed in µg/kg body weight (bw)/day, was calculated for each element across all fish species using the following formula [Eq. 2] for an average adult population:

$$EDI = \frac{C * Ac}{bw} \qquad \text{[Eq. 2]}$$

Where *C* is the mean concentration of the metal in fish muscle tissue (mg/kg wet weight, ww), *Ac* represents the average fish consumption per person (12.3 g/day for freshwater fish, 15.8 g/day for marine fish as resulted from the distributed questionnaire), *bw* is the average adult body weight (70 kg), as referenced in Varol and Sünbül (2020).

In the mean metal concentration estimation, left-censored data were managed using a substitution method with the middle-bound approach, as outlined by Varol *et al.* (2022). This approach improves the accuracy of EDI estimates by addressing non-detectable values in a standardized manner.

Quantitative health benefit-risk assessment

The BRA is an essential scientific approach used to evaluate the balance of beneficial and harmful compounds that may coexist within a particular food item (EFSA, 2010, 2024). By assessing both the risks and benefits within the same dietary intake range, BRA enables a comprehensive view of how a food contributes to health, supporting informed decision-making for consumers, industries, and policymakers (Roila *et al*., 2021; Tijhuis *et al*., 2012).

Contribution to the established daily reference intakes

In this study, the benefit assessment of fishery product consumption focuses primarily on the intake of certain EEs for human health, specifically Mn, Fe, Zn, and Se. For each fish species, the mean levels of these elements were compared with the established daily reference intakes for adults as set by the European Union (Reg. EU 1169/2011) (European Parliament and Council of the European Union, 2011). Contributions to daily nutrient intake were then calculated and expressed as a percentage. According to European Union standards, any food providing at least 15% of the nutrient reference values (NRVs) per 100 g can be classified as a “significant source of minerals” (Reg. EU 1169/2011).

The Hg-Se balance

The nutritional essentiality of Se has been recognized since 1957 (Schwarz and Foltz, 1957), but the relevance of tissue Hg-Se ratios concerning Hg toxicity was not understood until it was discovered that Se-dependent enzymes, critical for brain and kidney function, are inhibited by Hg (Wada *et al*.,

1976). As a result, evaluating fish Hg content without considering their Se levels is insufficient to accurately assess the potential risks and benefits of fish consumption (Barone *et al.*, 2021). The molar ratio (Se:Hg, μmol g−1) was calculated individually for each fish species, dividing Se and Hg concentrations by their respective molecular weights (Hg: 200.59; Se: 78.96) (Barone *et al.*, 2021). Generally speaking, an excess of Se relative to Hg offers a possible defense against the detrimental effects of Hg. In particular, the Se protective effect against Hg toxicity happens when the Se:Hg ratio, calculated as a molar ratio, exceeds 1. Ralston *et al.* (2016), aiming to improve Hg risk assessment, introduced a refined equation of the Health Benefit Value for Se index (HBV_{Se}) that reflects the effects of dietary Hg exposure and Se intake. Evidence shows that besides Hg and Se concentrations in fish tissue, the Se:Hg molar ratio and the HBV_{Se} index are useful in the interpretation of Hg toxicity (Grgec *et al.*, 2020).
The HBV_{Se} index quantifies the relative molar proportions of Se and Hg in food, providing a measure of the interaction between dietary Hg exposure and Se intake. The HBV_{Se} is calculated according to the reported formula [Eq. 3], taking into account the relative amount of Se available in food ([Se - Hg]/Se) and the total amount of Hg and Se present in the food (Se + Hg).

$$HBV_{Se} = \left(\frac{Se-Hg}{Se}\right) * (Se + Hg). \qquad \text{[Eq. 3]}$$

The sign of the index indicates whether the food would increase or decrease Se status: positive values indicate a molar excess of Se over Hg, while negative results reflect a molar excess of Hg over Se. Furthermore, the scale of the value proportionally reflects the Se excess or scarcity associated with the dietary exposure considered.

On the risk assessment side, the health risks associated with consumption of TEs, namely As (particularly inorganic As or iAs), Cd, Hg, Pb, were evaluated using the multiple indexes (Rahman *et al.*, 2026) described as follows.

Target hazard quotient
The target hazard quotient (THQ) assesses the non-carcinogenic health risk and is determined as follows [Eq. 4], considering for each TE a defined reference dose (RfD) (Li *et al.*, 2021):

$$THQ = \frac{Ef*Ed*Ac*C}{RfD*bw*At_{noncancer}} * 10^{-3} \qquad \text{[Eq. 4]}$$

Additionally, since consumers are exposed to all the TEs simultaneously in the present dietary study, the total THQ, also known as the Hazard Index (HI), was employed and defined as follow [Eq. 5] (Varol and Sünbül, 2020):

$$HI = \sum_{n}^{i} THQn \qquad \text{[Eq. 5]}$$

where *Ef* is the exposure frequency (350 day/year), *Ed* is exposure duration (26 years), *Ac* average fish consumption (12.3 g/day for freshwater fish, 15.8 g/day for sea fish), *C* refers to the mean metal concentration in fish muscle (mg/kg ww), *RfD* is Reference dose (mg/kg/day), *bw* indicates the average body weight (70 kg), $At_{noncancer}$ is the averaging time for non-carcinogens (365×26 days). Concerning results interpretation, THQ or HI values <1.0 indicate that consumers are unlikely to face non-carcinogenic health effects, whereas values >1.0 reflect a potential risk of such effects for targeted consumers (Varol *et al.*, 2022; USEPA, 2021).

Cancer risk
Cancer risk (CR) refers to the increased probability of an individual developing cancer as a result of exposure to a carcinogen. For the purpose of the present study, only iAs (established as 3% of total As) was considered carcinogenic, although Cd was classified by the International Agency for

Research on Cancer (IARC) as a carcinogenic by inhalation (Group 1) there is not enough evidence to classify it as carcinogenic by oral ingestion (IARC, 2012a, 2012b; Genchi *et al.*, 2020; Charkiewicz *et al.*, 2023). CR for iAs was calculated with the following formula [Eq. 6] (Ciriaci *et al.*, 2025):

$$CR = \frac{Ef * Ed * Ac * C * Csf}{bw * At_{cancer}} * 10^{-3} \qquad \text{[Eq. 6]}$$

where *Ef* is the exposure frequency (350 day/year), *Ed* is exposure duration (26 years), *Ac* average fish consumption (12.3 g/day for freshwater fish, 15.8 g/day for sea fish), *C* refers to the mean metal concentration in fish muscle (mg/kg ww), *Csf* is the oral carcinogenic slope factor from the Integrated Risk Information System (1.5 µg/g/day for iAs), *bw* indicates the average adult body weight (70 kg), At_{cancer} is the averaging time for carcinogens (365 × 70 days) (Varol *et al.*, 2022).

Margin of exposure

For studies assessing the dietary exposure to genotoxic and carcinogenic xenobiotic present in foodstuff, the definition of the margin of exposure (MOE) for consumers is suggested by the EFSA (2005, 2025). The MOE is a risk assessment metric used to evaluate potential safety concerns arising from the presence of toxic substances in food and, although it does not provide an exact quantification of risk, it offers an indication of the level of health concern and supports decision-makers in prioritizing interventions (EFSA 2024a). The MOE is determined by calculating the ratio between the benchmark dose lower confidence limit (BMDL, the dose at which a small but measurable adverse effect is observed) and the estimated exposure level of a given population (EFSA, 2024; Roila *et al.*, 2021). Since EFSA identifies the increased incidence of skin cancer linked to iAs exposure as the most critical adverse effect, this study adopted the related BMDL corresponding to 0.06 µg/kg bw/day (EFSA, 2024). As a rule, lower MOE values correspond to higher level of risk for consumers and according to recent human studies, an MOE of ≤1 suggests a dietary exposure level to iAs that may be associated with an elevated risk of skin cancer (EFSA, 2024).

Uncertainty analysis

Uncertainty analysis represents the process of identifying limitations in scientific assessments and evaluating their repercussion for scientific conclusions with the purpose to ascertain a transparent dietary BRA and to allow consumers, industries, and policymakers to make appropriate decisions (EFSA, 2018). Dietary exposure uncertainties were assessed according to EFSA's tiered method, applying the first tier "qualitative analysis of uncertainties" (EFSA, 2006, 2018). This approach entailed outlining the main uncertainty sources characterizing this exposure assessment, with consideration of their direction, magnitude, and ultimately the overall combined impact on the assessment (EFSA, 2006).

Results and Discussion

Concentration of elements in marine and freshwater fish

Mean values of the element concentration measured in targeted fish species are reported in Table 2. As was detected (>LOQ) in 60% of FF and in 100% of MF samples, Cd in 0% of FF and in 30% of MF, Hg in 70% and 100%, while Pb in 60% and 74% of FF and MF samples, respectively. Concerning the EEs, Fe, Mn, and Se registered a detection rate of 50% in FF samples and in MF of 70%, 22% and 100%, respectively. Zn was quantified in 70% of FF samples and in 100% of MF ones.

The average values largely differ among elements and fish product groups, with the highest levels shown by As in MF (0.066 mg/kg) and the lowest by Cd in FF (0.002 mg/kg, 100% of undetected samples). Regulation (EU) 915/2023 set maximum limits (ML) for Cd, Pb and Hg in fish and fish product, and all the levels measured in the present study for the three TE were below the respective MLs, for all the considered species.

The comparison with other studies in the literature reveal multiple scenarios as the elements concentration in fish tissue is affected by several factors namely the specie-specific feeding habits, the element considered, the ecological characteristic of the ecosystem and more importantly the pollution pressure that insists on each water body (Aziz *et al*., 2023; Zahran *et al*., 2025). In a previous study, Branciari *et al*. (2020) reported Pb, Hg, Cd and As equal to 0.012, 0.095, 0.003, 0.077 mg/kg, respectively, in important fishes of Lake Trasimeno (Umbria region, central Italy); in line with the results reported in the present study. Merola *et al*. (2021) studied the metal contamination of brown trout from two rivers located in Abruzzo region (Italy) revealing levels of TEs similar to the one here reported with average values of 0.10 mg/kg for As, 0.01 mg/kg for Cd, 0.01 mg/kg for Pb and 0.04 mg/kg for Hg. Varol and Sünbül (2020) reported almost 100-fold higher (average 1.3 mg/kg) levels for As in two freshwater fish species caught in three reservoirs in Turkey, while Cd and Pb were comparable to the levels of the present study (average 0.0014 μg/kg, and 0.064 mg/kg). The Hg was not analyzed by Varol and Sünbül (2020). Contrarily, Peycheva *et al*. (2022) referred that As was undetected in 100% of samples while Cd was 0.036 mg/kg and Pb 0.216 mg/kg in freshwater fish species from Lake Mandra and Lake Bourgas (Bulgaria), both an order of magnitude higher that the samples considered in this study. On the other hand, another study from Bangladesh reported average concentrations (mg/kg) of heavy metals in studied freshwater fish samples of 0.22 mg/kg for Pb and of 0.03 mg/kg for Cd, similarly to our results (Akter *et al*., 2021). For instance Han et al., 2021 published mean concentrations of As, Cd, Cr, Hg and Pb were 0.783, 0.009, 0.031, 0.043 mg/kg for marine fish from Zhejiang region (China), slightly different from those reported in Table 1. Łuczyńska and Paszczyk (2019) reported a similar scenario of metal concentration in FF species in Lakes of Warmia and Mazury Region (Poland) with the exception of Hg, that resulted in higher values (0.065-0.346 mg/kg ww) compared to those of this study. The values reported are in line with those previously published by Ciriaci *et al*. (2025) for MF species from central Italy. Dugo *et al*. (2006) found higher levels of Cd and Pb, similar of Se and lower for Zn analyzing sea bass tissues from Tyrrhenian Sea and Sicilian Sea (Italy). Another study on the contamination of Atlantic, blue, and chub mackerel purchased in the Jagalchi fish market of Korea revealed slightly lower contamination of Hg and slightly higher one for Pb (Bae *et al*., 2011).

Metal pollution index

MPI provides a quantitative indicator of the overall burden of TEs, representing a reliable and precise metric for evaluating heavy-metal contamination in foods. In this study the MPI values is 0.014 and 0.094 for freshwater and marine fishes, respectively. Higher MPI denotes greater contamination and, generally, the here presented results are lower than those reported in the literature (Łuczyńska *et al*., 2018; Sofoulaki *et al*., 2019), showing that the fish available in the Umbrian market is characterized by low levels of heavy metal. Is interesting to highlight that marine fish species resulted slightly more contaminated than freshwater fish ones, as already discussed for elements concentration in fish muscle. The comparisons of MPI values obtained from different studies must acknowledge that the index calculation can vary across studies, because the metals included in the calculation is not always consistent.

Estimated daily intake of metals

The questionnaire distributed to the target Umbria and Marche population revealed that marine fish consumption was on average 15.8 g/die while freshwater fish accounted for a lower proportion of the consumers' diets with values of 12.3 g/day, for a total of 28.1 g/die of consumed fish. This value is lower than the Italian average consumption, estimated to be 31 kg *per capita* for the year 2022 (European Commission, 2022); however, the difference may arose from the fact that in the latter value all fishery products are included, also bivalve mollusks, gasteropods, cephalopods and crustaceans, while in the present study only targeted fish species are considered; furthermore Umbria is a landlocked region, not touched by the sea, which may influence the consumption habits of inhabitants.

The EDI for each TE and EE was calculated multiplying the average consumption for the analytical concentration in the samples and is reported in Figure 1. The EDI of iAs was determined assuming that it accounted for the 3 % of the total As concentration (EFSA, 2009; Traina *et al.*, 2019). The overall EDI for the considered elements was of 1.50 µg/kg bw/day for freshwater fish and 4.40 µg/kg bw/day for marine fish, this difference is a result of both higher consumption and higher elements' levels in marine fish. However, as represented in Figure 1, results show that the mentioned difference is mainly imputable to TEs (reported in the red shades in Figure 1) as the ∑EDI-TE for MF was 2.64 µg/kg bw/day and for FF 0.02 µg/kg bw/day; in particular the As content (and consequently the iAs) resulted two orders of magnitude greater in MF than FF. On the other hand, MF and FF contributed equally to the intake of EEs, with values of 1.75 and 1.48 µg/kg bw/day, respectively. These results suggest that the target consumers, in the context of the dietary habits identified by the questionnaire, are more likely exposed to harmful xenobiotic when consuming MF. This result seems to contrast with other studies suggesting that consumers of FF may be particularly at risk, since freshwater ecosystems often show higher levels of heavy metals than marine systems (Aborisade *et al.*, 2024), largely due to the limited water turnover (Chidammodzi and Muhandiki, 2015).

However, is crucial to consider that inland water bodies are characterized by very different contamination pattern that reflect a largely diverse risk potential for consumers, rendering the study of specific consumption scenarios for defined population/subpopulation, of utmost importance to perform a thorough risk assessment.

Is to be noticed that the majority of the FF available in the Umbria region markets, and therefore consumed by the local population, is caught in Trasimeno Lake, renowned to be characterized by low pollutant pressure (Branciari *et al.*, 2020; Roila *et al.*, 2023). The study from Han *et al.* (2021) highlighted different values of EDI for As, Cd, Hg and Pb equal respectively to 1.214, 0.014, 0.048 and 0.067 µg/kg bw/day, due to marine fish consumption by the population in Zhejiang, China. Torabi *et al.* (2023), in their study concerning health risk assessment of heavy metals in MF caught from the northwest Persian Gulf, registered EDI values ranging from 0.0-to 3.0 for Ni, from 0.0 to 0.03 for Pb and 0.0 to 0.12 for Cd expressed as µg/day for a 70-kg person.

Contribution of essential elements to the daily reference intakes

The contributions (%) of Fe, Mn, Se, and Zn coming from fish to the NRVs were calculated for both fish categories (Figure 2). Mn contribution was the lowest, with an average of 1.03% for FF and of 2.80% for MF; Fe and Zn reference values were covered by 1.68-4.85% and 4.77-5.76%, for FF and MF, respectively. Figure 2 shows that the threshold reported in EC Reg. 1169/2011 and used to define a specific food item as significant source of minerals (>15%), was reached for Se in both fish groups, registering the maximum contributions: 22.53% and 61.41% of NRV for FF and MF, respectively. Overall, MF contribute to NRVs more than FF, for all the EEs investigated, with the exception of Zn. Similarly, Ciriaci et al., 2024 reported Se as one of the major contributor to NRVs, albeit in the mentioned studies other elements reached the threshold.

The Hg-Se balance

The toxicological effects of Hg exposure may be mitigated by dietary Se intake; however, estimations of Se intake alone may not accurately reflect the health benefit/risk if the Se-Hg interaction is not carefully considered. Numerous studies have shown that Se not only reduces the uptake of Hg, but also mitigates its toxicity in a variety of animal species, including fish and humans (Cabañero *et al.*, 2007; Peterson *et al.*, 2009)

Although the precise mechanisms are still unclear, the majority of them entail the production of Hg–Se complexes (Zhang *et al.*, 2014), which are scarcely bioavailable and help in removing and excreting Hg by demethylation (Barone *et al.*, 2021). Studying the relationships between these two factors is critical when looking at the health issues related to Hg exposure, particularly as a consequence of fish consumption, primary route for general human population.

An excess of Se respect to Hg can help mitigate the harmful effects of Hg. As already reported, when the molar Se:Hg ratio is greater than 1, Se is thought to exert a protective influence against Hg toxicity (Peterson *et al.*, 2009), representing a specific health benefit for consumers. In this study, in both fish species groups, the Se:Hg molar ratios are greater than 1, being 7.76 for FF and 7.45 for MF. Similar values have been reported by Barone *et al.* (2021) for gilthead seabream (6.44). It has been also reported that Se:Hg molar ratio dependents on a combination of several factors such as fish size, season and location of sampling, decreasing with increasing trophic level and organism age and size (Grgec *et al.*, 2020). However, to develop more reliable food safety assessments, the HBV_{Se} has been introduced as an innovative and trustworthy indicator for evaluating the risks associated with Hg exposure (Ralston *et al.*, 2016) and in our studies HBV_{Se} values were of 0.011 for FF and 0.029 for MF. Considering these results, where Se:Hg molar ratios were above 1 and the HBV_{Se} index positive, the consumption of the analyzed species can be regarded as safe. Nonetheless, given the public health implications, Ralston *et al.* (2016) recommend that the precautionary principle should still be applied, especially when the HBV_{Se} values, although positive, are relatively low.

Target hazard quotient and hazard index

Although THQ and HI do not yield a precise estimate of health risk for the exposed population, they serve as indicators of the potential hazards associated to individual contaminants (THQ) and of their cumulative effects (HI). Results reported in Table 3 show that for both food categories THQ and HI values are <1.0, indicating, as already reported, that the sub-population targeted in the present study is not exposed to a significant non-carcinogenic health risk due the consumption of fish from the regional market. However, it is interesting to highlight that the HI referred to MFs is 10-fold higher than that of FF and the main contributors to this value are iAs for MF and Hg for FF. In FF, the lowest THQ value was registered for As and the highest for Hg, while in MF, the highest value was for iAs and the lowest for Pb.

A study on FF caught from Lake Hawassa reports THQ_{Cd} between 0.14 and 0.16, THQ_{Pb}=0.001, THQ_{As} between 0.07 and 0.18, higher compared to those found in the present study, but still under the threshold; also the reported HIs were generally higher (0.067-0.155) than those here reported for FF, but still on the safe level (Melake *et al.*, 2022). Peycheva *et al.* (2022) reported a THQ_{Cd} 0.003-0.010, a THQ_{Pb} 0.007-0.011 for fish species from Bulgarian lakes. Barone *et al.* (2015) referred that the THQ_{Hg} and THQ_{Cd} were lower than 1 for all targeted marine species with values ranging from 0.04 to 0.80. Comparable results were also reported by Torabi *et al.* (2024) for Persian Gulf fish consumption with average THQ_{Cd} of 0.05 and THQ_{Pb} of 0.04 with a total HI of 0.13.

Carcinogenic risk and margin of exposure

As is identified worldwide as a chemical of major public-health concern, and for population with no occupational exposure, diet constitutes the principal exposure route with contaminated drinking water, fish and seafood as main contributors (WHO, 2019; Hackethal *et al.*, 2023). iAs is one of the As species present in foodstuff and is classified as carcinogenic to humans by several international authorities, based on epidemiological evidence of increased cancers of the skin, lung, and urinary bladder (EFSA, 2009, 2024b; IARC, 2012a; WHO, 2001). The iAs carcinogenic risk for an average consuming adult was estimated by the lifetime CR; the MOE was performed to further characterize the priority of risk management measures (Hackethal *et al.*, 2023).

As reported in Table 4, the iAs CR for FF was below the established threshold of 10^{-6} indicating that the consumption of the species considered in the present study poses no carcinogenic risk for the consumers, however the CR for MF exceeds 10^{-5} indicating a low, still acceptable, carcinogenic risk. Similarly, Varol *et al.* (2020) reported that CR values in some FF cough in Turkish reservoirs were between 10^{-4} and 10^{-6} suggesting a low carcinogenic risk. Concerning MOE, the value estimated in this study for MF is slightly lower than the threshold defined for iAs by EFSA (≤1) demonstrating that the consumers can be exposed to an increased risk of iAs harmful effects trough the consumption of these fishery products (EFSA, 2024b). This consideration is not applicable to

FF since the MOE value is much higher than the EFSA threshold, indicating the absence of a increased risk of skin cancer (Table 4). Okoye *et al.* (2022) reported MOE values for Nigerian adults consuming fish from Niger Delta with values ranging from 42.4 to 280, highlighting a low risk. In the 2021 EFSA re-assessed the chronic dietary exposure of the European population to iAs, taking into account the most recent occurrence data in food, confirming the relevance of terrestrial foods but also concluding that 'fish and other seafood' are among the most relevant sources of iAs exposure in certain countries (EFSA, 2021). On 2023, the EFSA adopted a scientific opinion updating the risk assessment on iAs in food, highlighting the need to lower population exposure by setting maximum levels for fish and other seafood, which significantly contribute to overall exposure (EFSA, 2024b). The values extrapolated for the purpose of the study (data not shown) are under the ML for fish and fish product set by Commission Regulation (EU) 2025/1891 (European Commission, 2025b).

Uncertainty analysis

The main uncertainties associated with the dietary exposure estimations refer to the impact of using the substitution method to handle the left-censored data and to the not considered effect of food preparation on elements and especially on the iAs levels. Another source of uncertainty is related to the presence of common consumers' biases when answering food consumption questionnaires. Among these, the "social desirability bias" when the respondents tend to give answers that are socially acceptable or viewed positively, such as the over-report healthy foods; the "recall error / memory bias" where the participants may not accurately remember what or how much they ate but also "the portion size misestimating", causing distortions in nutrient intake estimates, have been considered the most relevant bias of this study (Arija *et al*., 2015; Kirkpatrick *et al*., 2019). The qualitative analysis of the direction and magnitude of uncertainties revealed that the combined effect of the identified uncertainties might lead to an overall moderate overestimation of mean elements exposure among the targeted population, with no impact on overall BRA considerations.

Conclusions

There is broad scientific evidence sustaining the benefits and potential risks of consuming fish, and this issue becomes even more important when involves harmful chemicals such as Hg and iAs. Concentrations of TEs in fish is species specific with higher values for marine fishes, mainly as a consequence of higher levels of Hg.

Information regarding Hg, Se, and Se/Hg balance in commercial fish are crucial for a thorough assessment of fish consumption safety. Our results indicate that both fish groups represent a significant source of Se according to EC Reg.1169/2011, furthermore Se content in fish seems enough to mitigate the potentially toxic effects of Hg.

Benefit risk assessment methods indicated that non-carcinogenic effects due to the intake of TEs *via* consumption of fish species are not expected while an acceptable carcinogenic risk is attributable to marine fish species. These results confirm that no relevant health risks are expected for the targeted population in relation to the consumption habits registered. The outcomes also highlight the importance of developing specific dietary exposure assessments and related benefit risk assessments considering types and frequency of fish consumption, average population but also vulnerable sub-population groups, such as young children, elderly and women during the reproductive period. In light of the above, it is important to consider that a constant overall monitoring of TEs is crucial to assess the presence of potential human health risks.

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Table 1. Analyzed samples from central Italy market.

Categories	Common name	Scientific name	N° sample
Freshwater fish	Striped Catfish	*Pangasianodon hypophthalmus*	5
	Rainbow trout	*Oncorhynchus mykiss*	6
	Sea trout	*Salmo trutta L*	3
	Eel	*Anguilla anguilla*	3
	Golden fish	*Carassius auratus*	4
	Largemouth bass	*Micropterus salmoides*	3
	Perch	*Perca fluviatilis*	2
	Carp	*Cyprinus carpio*	3
		ToT. sample	29
Marine fish	Common sole	*Solea vulgaris*	3
	European plaice	*Pleuronectes platessa*	5
	Surmullet	*Mullus surmuletus*	3
	Red mullet	*Mullus barbatus*	4
	Red gurnard	*Aspitrigla cuculus*	3
	Red scorpionfish	*Scorpaena scrofa*	2
	Atlantic mackerel	*Scomber scombrus*	5
	European seabass	*Dicentrarchus labrax*	4
	Blue whiting	*Micromesistius poutassou*	6
	Gilthead sea bream	*Sparus aurata*	2
		ToT. sample	37

Table 2. Toxic (TEs) and essential elements (EEs) concentration (mg/kg ww) in the analyzed freshwater and marine fish species: mean value (median).

	Freshwater fish	Marine Fish
TEs		
As	0.066 (0.040)	11.417 (2.919)
Cd	0.002 (0.002)	0.005 (0.002)
Hg	0.040 (0.042)	0.115 (0.090)
Pb	0.011 (0.005)	0.013 (0.005)
EEs		
Fe	2.351 (1.728)	6.793 (2.780)
Mn	0.206 (0.076)	0.560 (0.264)
Se	0.124 (0.083)	0.338 (0.322)
Zn	5.757 (3.601)	4.768 (4.635)

Table 3. Target Hazard Quotient (THQ) and % contribution (in bracket) of each element to total Hazard Index (HI).

	THQ (*% Contribution to HI*)					HI
	As	iAs	Cd	Hg	Pb	
Freshwater fish	0.0002	0.0011	0.0003	0.0120	0.0005	0.0140
	(*1.14*)	(*7.97*)	(*1.80*)	(*85.36*)	(*3.37*)	
Marine fish	0.0353	0.2471	0.0010	0.0437	0.0008	0.3280
	(*10.76*)	(*75.35*)	(*0.32*)	(*13.33*)	(*0.24*)	

Table 4 Carcinogenic risk (CR) and Margin of Exposure (MOE) of inorganic As, associated to the consumption of freshwater and marine fish species by Umbrian population.

	Freshwater fish	Marine Fish
CR	0.0000002	0.0000413
MOE	171.62	0.78

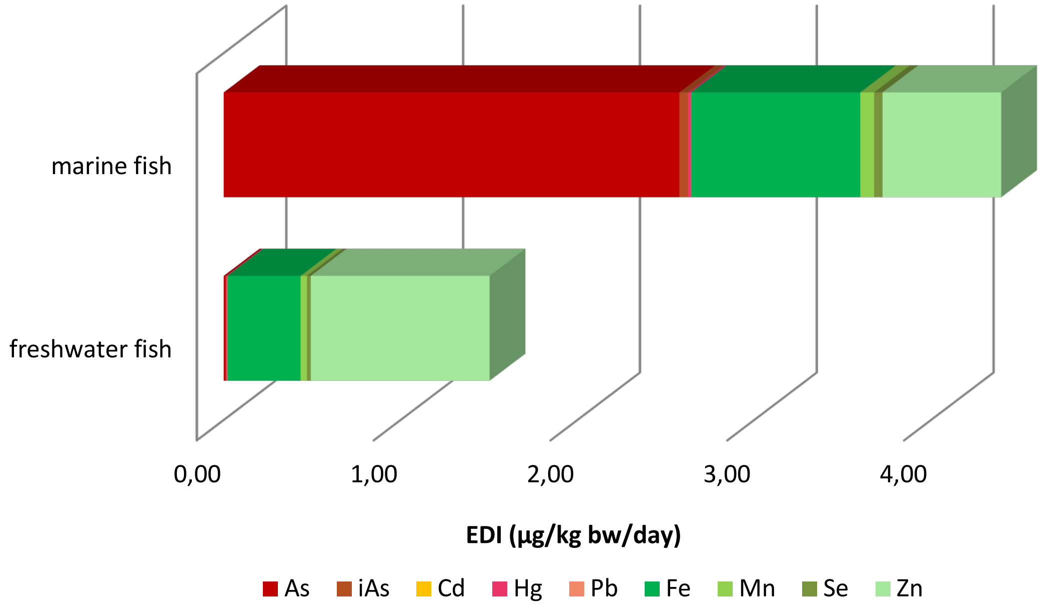

Figure 1. Estimated daily intakes (EDIs) for all the elements analyzed in marine fish and freshwater fish samples. Toxic elements (TEs) are represented in red shades and essential elements (EEs) in green shades. TEs = arsenic (As), inorganic arsenic (iAs), mercury (Hg), lead (Pb), cadmium (Cd). EEs = iron (Fe), manganese (Mn), selenium (Se), zinc (Zn).

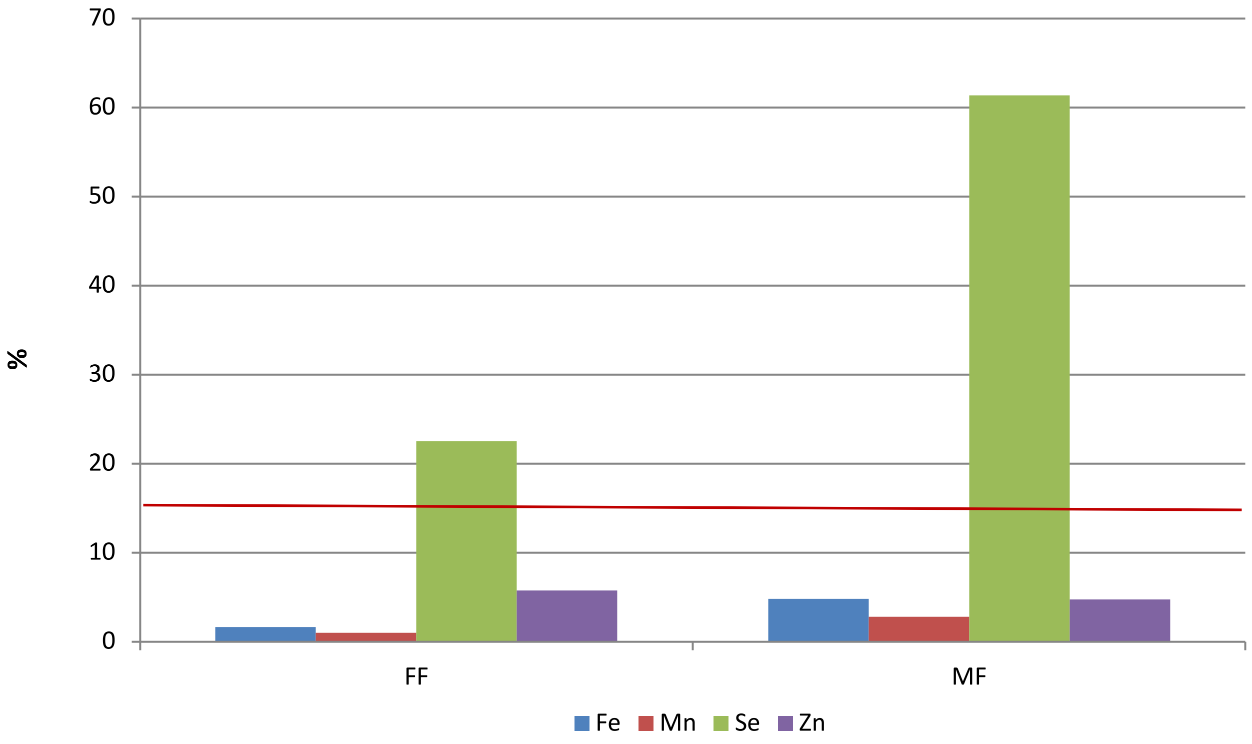

Figure 2. Contribution (%) to Nutrient Reference Values (NRVs) of the essential elements (EEs) in freshwater (FF) and in marine fish species (MF). Red line: 15% threshold reported in EC Reg. 1169/2011.