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# Heavy metals concentrations in hake *Merluccius merluccius* (Linnaeus, 1758) from the Bay of Oran (Mediterranean Sea): Potential human health risk estimation

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**Abstract:** To evaluate the potential human health risk to hake consumers, the bioaccumulation of heavy metals (Cd, Pb, and Zn) was measured in the edible tissues of hake *Merluccius merluccius* (Linnaeus, 1758) caught from Oran Bay on the western Algerian Mediterranean coast. Results revealed that the average metal concentrations varied significantly among sex and in target organs. The concentrations of metal accumulation take place preferentially in the liver compared to the muscle of hake species, with the highest levels found for Zn, followed by Pb and Cd. The Estimated daily intake (EDI) of each trace metal did not exceed the tolerable daily intake (TDI). Human health risk assessment from heavy metal exposure through fish consumption from Oran Bay for adults showed no significant non-carcinogenic adverse health risk since all calculated values for Target Hazard Quotient (THQ) and Total Target Hazard Quotient (TTHQ) were <1.
***Keywords*:** *Merluccius merluccius*; heavy metals; health risk; Target Hazard Quotient; western Algerian Mediterranean coast

**Sažetak:** KONCENTRACIJA TEŠKIH METALA U TKIVU OSLIĆA *MERLUCCIUS MERLUCCIUS* (LINNAEUS, 1758) IZ ORANSKOG ZALJEVA (SREDOZEMNO MORE): PROCJENA POTENCIJALNOG RIZIKA ZA LJUDSKO ZDRAVLJE. Da bi se odredio potencijalni rizik za ljudsko zdravlje pri konzumaciji oslića, određena je bioakumulacija teških metala (Cd, Pb i Zn) u jestivom tkivu oslića *Merluccius merluccius* (Linnaeus, 1758) ulovljenog u zapadnom Sredozemlju u alžirskom zaljevu Oran. Rezultati pokazuju da prosječne koncentracije metala značajno variraju ovisno o spolu i testiranom organu oslića. Metali se više akumuliraju u jetri oslića nego u mišićima, a najviše razine utvrđene su za cink, nakon čega slijede olovo i kadmij. Procijenjeni dnevni unos (eng. *Estimated daily intake, EDI*) svakog metala nije premašio prihvatljivi dnevni unos (eng. *Tolerable daily intake, TDI*). Procjena rizika za ljudsko zdravlje zbog izloženosti teškim metalima pri konzumaciji ribe iz zaljeva Oran pokazala je da za odrasle osobe nema značajnog nekancerogenog ali štetnog zdravstvenog rizika jer su sve izračunate vrijednosti za kvocijent ciljane opasnosti (eng. *Target hazard quotient, THQ*) i ukupni kvocijent ciljane opasnosti (eng. *Total target hazard quotient, TTHQ*) bile <1.
***Ključne riječi*:** *Merluccius merluccius*; teški metali; zdravstveni rizik; kvocijent ciljane opasnosti; zapadna alžirska Sredozemna obala

## INTRODUCTION

Heavy metals discharged into aquatic environments by anthropogenic activities are one of the most commonly accumulated pollutants in fish and are nondegradable, bioaccumulative, and toxic (Varol and Sünbül, 2017; Trevizani *et al*., 2019). Fish exposure to toxicants has had some harmful effects on the quality, diversity, and health of humans who depend on them for their protein needs (Varol *et al*., 2022). Lead (Pb) and cadmium (Cd) are toxic even in trace concentrations and have no essential role in biological systems. On the other hand, zinc (Zn) is an essential element with significant importance in physiological processes, but it can be toxic when present at concentrations higher than optimal levels (Rejomon *et al*., 2010; El-Moselhy *et al*., 2014; Liu *et al*., 2015).

The consumption of fish is recommended mainly due to their superior nutritional content of high protein, vitamins, essential minerals, and Omega-3 fatty acids (Arulkumar *et al*., 2017; Chen *et al*., 2022). The nutritional profile of fish has been associated with several health benefits, such as anti-oxidation, anti-inflammation, wound healing, neuroprotection, cardioprotection, and hepatoprotection properties (Chen *et al*., 2022; Durazzo *et al*., 2022). However, the content of heavy metals discovered in some fish may affect the role of fish consumption on a healthy diet (Castro-González and Méndez-Armenta, 2008). Heavy metal marine pollution poses a risk not only to fish health but also to human health as these toxins can bioaccumulate to hazardous levels through the food chain (e.g. Gu *et al*., 2017; Varol *et al*., 2022). Effectively, the consumption of prolonged contaminated fishes by the trace metals

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such as cadmium (Cd), lead (Pb), and zinc (Zn) results in several adverse effects such as liver damage, food poisoning, cardiovascular diseases, and even death (Ullah *et al*., 2017; Marengo *et al*., 2018). Fish bioaccumulate metals from surrounding water, sediment, and their diet (Jayaprakash *et al*., 2015; Liu *et al*., 2015). According to the literature survey, the level of heavy metal bioaccumulation in fish tissues depends upon several factors such as age and size of the fish, detoxifying mechanisms, metabolic processes of fish, feeding behavior, physicochemical parameters of the environment, etc. (Traina *et al*., 2019; Delahaut *et al*., 2020; Nyantakyi *et al*., 2021).

Biomonitoring, a widely recognized practice for assessing the presence and concentrations of pollutants in living organisms, offers valuable insights into the overall ecological well-being. The foundation of biomonitoring lies in the understanding that chemicals present in organisms leave detectable markers, providing evidence of their exposure. These markers include the chemicals themselves, their byproducts, and the biological changes that occur within organisms as a result of the chemical's influence (Zhou *et al*., 2008). Fish are regarded as noteworthy models in assessing potential environmental stressors due to their capability to metabolize, accumulate, and retain pollutants present in sediments and water (Ali *et al*., 2008). They offer early indicators of toxicant-induced environmental changes and degradation, making them valuable in monitoring environmental health (Boettcher *et al*., 2010; Bücker *et al*., 2012; Deutschmann *et al*., 2016). Additionally, since fish exhibit similar responses to toxicants as mammals (Kligerman, 1982), they serve as effective screening tools to identify potentially harmful chemicals to humans. Furthermore, they serve as indicators of human exposure risk through the aquatic food chain (Hallare *et al*., 2016; Prabhakaran *et al*., 2017; Igbo *et al*., 2018).

Indeed, suggested as a sentinel species in many biomonitoring programs (UNEP/RAMOGE, 1999), European hake (*Merluccius merluccius*) represents a good sampling candidate due to its bottom-feeding niche, availability in Algerian waters all year round and its consumption by a majority of people living along the coast. The fishing ports of Oran produced, for example, about 66.5 tonnes of hake in 2010 (Belhoucine *et al*., 2022). On the other hand, since hake is placed at higher trophic levels, the uncontrolled and anarchic consumption of seafood can carry a serious risk to the population health of the region (Alik *et al*., 2021). Fish is the top consumer in the aquatic food web and are considered ideal organisms for toxicology and toxicogenomics studies (Aliko *et al*., 2019; Burgos-Aceves *et al*., 2019).

The bay of Oran located on the west coast of Algeria is characterized by an important fish market participating in the economic consolidation, job creation, and food security of the city. Althrought many investigations on the status of the fish resources in different parts of the Algerian west coast have been carried out in the recent past (Bouhadiba *et al*., 2017; Tabeche *et al*., 2021; Kaddour *et al*., 2021; Belhoucine *et al*., 2022; Kaddour *et al*., 2022), very few data are still available for evaluating potential effects of contamination by anthropogenic activities on commercial fish resources caught in Oran Bay which represents one of the most important fish markets of the basin.

Therefore, the purpose of this work was to investigate the levels of potentially toxic elements (Cd, Pb, and Zn) concentrations content in samples tissues of *M. merluccius* from Oran Bay to evaluate the dynamics of these three contaminants according to biological parameters, to assess potential human health risks (Estimated daily intake, EDI; Target Hazard Quotient, THQ and Total Target Hazard Quotient, TTHQ) associated to the intake of contaminants are the main focus of this work.

## MATERIALS AND METHODS

### Fish collection and sample preparation

The sampling took place in the Bay of Oran (N 35°43', W 00° 38') (Fig.1), located on the west coast of Algeria and in the southwest of the Mediterranean, an area that belongs to the coastal chain of Tel Septentrional (Djebel Murdjadjo and Djebel Khar) (Leclaire, 1972). The Oran coast is severely affected by the nuisances of the civilized world including: industrial activities, intensive tourism, and massive urbanization with an ever-increasing level of domestic pollution (Remili and Kerfouf, 2010). *M. merluccius* specimens were sampled in Oran Bay by professional fishermen who operated trawlers. The sampling was conducted within the coastal zone with a maximum depth of 500 m, throughout the study period spanning from October 2019 to July 2020.

The fish were carefully stored in clean labeled polythene bags and transported in ice bags to the laboratory for subsequent analysis of the metal content in their tissues. Each individual underwent a series of measurements, allowing them to be categorized into size and sex classes. The sex of each fish was determined visually, and the total length was measured from the tip of the upper jawbone, adjusted to the lower 1 cm. A total of 160 *M. merluccius* individuals were collected (80 males and 80 females), with all individuals falling within the


**Fig. 1.** Map showing sampling location: Oran Bay (Algerian west coast).

same length range (33.5 to 43.7 cm). Biometric measurements were taken, and samples of muscle and liver tissues were extracted for each individual.

The preparation of subsamples and trace metal analysis were made according to FAO/WHO (2012). Fish was dissected using stainless steel instruments. Muscles were dried in an oven for 24 hours at 90 °C then ground and passed through a sieve. The obtained subsamples were weighed and placed in labeled pillboxes, and prepared for chemical analysis. For subsequent analysis, composite samples weighing 2 g of dry weight from each liver and muscle sample were utilized. The composite samples underwent digestion by utilizing ultra-pure nitric acid ($HNO_3$) through the hot plate method at a temperature of 100 °C for 20 minutes. The resulting solution was then adjusted to a known volume using deionized distilled water and stored in polyethylene vessels.

All concentrations of Cd, Pb, and Zn are expressed as mg/g wet weight and were determined by Atomic Absorption Spectrophotometer (AAS model GPC A932 ver. 1.1) (Amiard *et al*., 1987). All reagents were of analytical grade; glassware was soaked in 10% nitric acid and later rinsed with distilled water before use to avoid metal contamination. Accuracy and precision were verified by using reference materials (MA-A-2/TM) provided by the International Atomic Agency (IAEA). Analytical results of the quality control samples indicated a satisfactory performance of heavy metal determination within the range of certified values of 95-111% recovery for the metals studied.

### Data analysis

The obtained results are presented as mean ± standard error. The verification of the normality and the homogeneity of covariance matrices were conducted using the Levene test. The statistical treatment of results was performed using the computer program SPSS9 (SPSS, Chicago, IL) for PC. To compare concentrations of heavy metals between two independent groups of the same population, the ANOVA test is applied to estimate the importance of differences between the concentrations of metallic pollutants either by target organ or sex. This difference is considered significant with a probability (p) of less than 5% ($p<0.05$).

Risk assessment is a tool to estimate the probability of health effects due to exposure to a hazard, which in this study is the exposure through the consumption of fish. Human health risks due to the determined metals in the muscles of fish species via their consumption were characterized using estimated dietary intakes (EDI), target hazard quotient (THQ), and Total Target Hazard Quotient (TTHQ)

EDI is directly linked to metal concentration, food consumption, and body weight. To assess the potential health risks associated with metal intake, EDI values are compared to tolerable daily intake (TDI) values recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1982, 2011), and European Food Safety Authority (EFSA, 2014). The daily intake of metals for adults was calculated using the following equation (Copat *et al*., 2013; Griboff *et al*., 2017):

$$\mathrm{EDI} = \frac{\mathrm{MC} \times \mathrm{IRd}}{\mathrm{BW}} \quad (1)$$

Where MC is the metal concentration in fish muscle (µg/g wet weight), IRd is the daily average fish ingestion rate (12 g/person/day), calculated by MADRP (2018) for an Algerian population, BW is the average body weight for an adult (75 kg) (Abbes, 2017). EDI was expressed as µg/kg BW/day.

The THQ was developed by the United States Environmental Protection Agency for the estimation of potential health risks associated with long-term exposure to chemical pollutants. THQ is the ratio of an exposure level of a single substance over a specified period (e.g. subchronic) to a reference dose (RfD) for that substance derived from a similar exposure period (USEPA, 2000).

If the value of THQ is <1, it means that there are no adverse effects for the exposed population; when THQ >1, there is a potential risk related to the metal studied in the exposed population (Chien *et al*., 2002). It is calculated using the following equation (USEPA, 2000):

$$\mathrm{THQ} = \frac{\mathrm{EFr} \times \mathrm{EDtot} \times \mathrm{Wfood} \times \mathrm{Ci}}{\mathrm{RfDo} \times \mathrm{BW} \times \mathrm{ATn}} \times 10^{-3} \quad (2)$$

EFr is the frequency of exposure (set at 365 days/year), EDtot is the duration of exposure (76 years) equivalent to life expectancy at birth, Wfood is the fish intake rate in Algeria (12 g/person/day) (MADRP, 2018), Ci is the concentration of the metal element in the sample (µg/g ww), RfDo (mg/person/day) the oral reference dose, BW is the average body weight (75 kg for adults) (Abbes, 2017) and ATn the period of average exposure for non-carcinogens (365 days/year× several exposure years, 70 years).

In this study, the total target hazard quotient (TTHQ) was also performed, since humans are often exposed to more than one contaminant with associated combined or interactive effects (Tao *et al*., 2012; Li *et al*., 2013; Alamdar *et al*., 2017). TTHQ is expressed as the sum of the hazard quotients, using the following equation (Núñez *et al*., 2018):

$$\mathrm{TTHQ} = \Sigma\, \mathrm{THQi} \quad (3)$$

As with the hazard quotient, overall exposures below 1 calculated using hazard quotients are unlikely to result in any non-carcinogenic adverse health effects during a lifetime of exposure and would normally be considered acceptable (Sparling, 2016).

## RESULTS

### Determination of heavy metals

The Fig. 2 illustrates the evaluation of variations in concentrations of metallic trace elements in *M. merluccius* tissues based on their sex within Oran Bay. Among the three metals analyzed (Cd, Pb, and Zn), there are noteworthy differences in concentrations between female and male individuals, demonstrating statistical

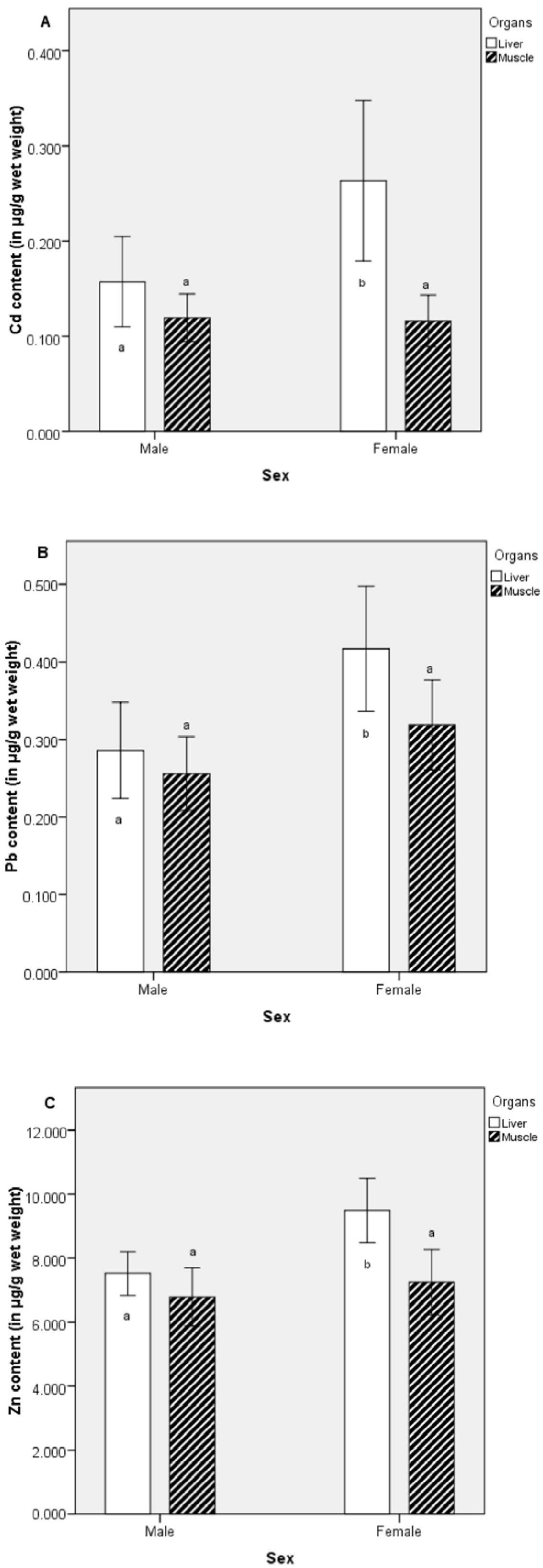


**Fig. 2.** Variations in heavy metal concentrations according to tissue and sex and tissue of *Merluccius merluccius* from Oran Bay. Cadmium (**A**), lead (**B**), and zinc (**C**). (The letters a and b indicate significant differences for the same matrix).

significance ($p<0.05$). Specifically, females exhibit elevated levels of Cd and Zn in the liver, with recorded values of 0.263 ± 0.042 µg/g wet weight and 9.469 ± 0.498 µg/g wet weight, respectively. Conversely, males display a higher concentration of Pb in the liver, measuring 0.417 ± 0.040 µg/g wet weight (Fig. 2).

The assessment of variations of toxic element concentrations in tissues of *M. merluccius* (Table 1) followed a clear pattern. The highest concentrations of the three metals studied (Zn, Pb, and Cd) were recorded in the liver, regardless of sex. In addition, the mean concentration of elements follows the order: Zn > Pb > Cd in the two organs studied.

The EDI values for three metals (Cd, Pb, and Zn), along with their corresponding THQ and TTHQ values, are presented in Table 2. In this study, the EDI values of Cd, Pb, and Zn resulting from fish consumption by adult individuals in Algeria were found to be well below the TDI limits. Furthermore, the THQ and TTHQ values for each heavy metal due to fish consumption never surpassed the hazard quotient threshold of 1.

## DISCUSSION

Monitoring the bioavailability of metals is crucial in addressing the global issue of metal pollution, primarily because they tend to accumulate throughout the food chain. The uptake and accumulation of metals in marine organisms are well-known to be influenced by a complex interplay of both non-biological and biological factors. Physicochemical parameters such as pH, salinity, temperature, and dissolved oxygen, as well as the physiological characteristics, metabolic processes, and ecological role of the organisms (size, age, sex, diet, metabolism, and position in the trophic web) (Jakimska *et al*., 2011; Bawuro *et al*., 2018; Alik *et al*., 2021), all play crucial roles in shaping the dynamics of metal uptake and accumulation in these organisms.

Sex is one of the predominant biological factors. In 2011 Lacoue-Labarthe *et al*., showed that from a certain stage of embryonic development of individuals, sex plays an important role in the accumulation of different Metallic Trace Elements (Cd, Cu, Zn, etc.), while Webb (1997) announced that the sex undoubtedly, plays an important role in the distribution of MTEs. The obtained results demonstrate significant variations in average concentrations of non-essential heavy metals (Pb, Cd), with notably higher levels of Cd predominantly observed in females, while Pb predominates in males. Additionally, Fig. 2 illustrates that the essential trace element (Zn) exhibited elevated concentrations in the liver of female fish compared to males. A similar accumulation of these metallic trace elements was reported by Belhoucine *et al*. (2014) and Alik *et al*. (2021) in *M. merluccius* from the Bay of Oran and the Gulf of Bejaïa in Algeria, respectively. The nature of hormones, such as estrogen and testosterone, have distinct roles and levels in females and males, respectively. These hormones can influence the expression and activity of cytochrome P-450 enzymes, which play a crucial role

**Table 1.** Levels of heavy metals (µg/g wet weight) in the liver and muscle of *Merluccius merluccius* (male and female) collected from the Bay of Oran.

| **Tissues** | **Sex** | **Heavy metals** | | |
|---|---|---|---|---|
| | | Cd* | Pb* | Zn* |
| Liver | Male<br>Female | 0.157 ± 0.023<br>0.263 ± 0.042 | 0.417 ± 0.040<br>0.286 ± 0.031 | 7.469 ± 0.326<br>9.469 ± 0.498 |
| Muscle | Male<br>Female | 0.116 ± 0.013<br>0.117 ± 0.014 | 0.315 ± 0.028<br>0.258 ± 0.025 | 6.437 ± 0.413<br>7.286 ± 0.487 |

* indicates a statistically significant difference in the accumulation of heavy metals between male and female hake.

**Table 2**. Estimated daily intakes (EDI) from *Merluccius merluccius* consumption by adults (TDI: tolerable daily intake, RfDo: oral reference dose, THQ: ratio of an exposure level of a single substance over a specified period).

| Metals | Mean (µg/g wet weight) | EDI (µg/kg bw/day) | TDI (µg/kg/bw/day) | RfDo (µg/kg/bw/day) | THQ |
|---|---|---|---|---|---|
| Cd | 0.118 | 0.019 | 0.8 (JECFA, 2011) | 1 (USEPA, 2000) | $1.888\times 10^{-5}$ |
| Pb | 0.286 | 0.046 | 1.50 (EFSA, 2014) | 1.50 (EFSA, 2010) | $3.051 \times 10^{-4}$ |
| Zn | 6.920 | 1.107 | 300 (JECFA, 1982) | 300 (USEPA, 2000) | $3.691 \times 10^{-6}$ |

**Table 3.** Comparison of heavy metal content in the muscle of *Merluccius merluccius* (µg/g wet weight) from the maximum permissible limit (MPL) international standards.

| **Heavy metals** | **Present study** | **Standards** | | | |
|---|---|---|---|---|---|
| | | UNEP (1985) | WHO-IPCS (1992, 1977, 2001) | USEPA (2000) | FAO/WHO (2001) |
| Cd | 0.103 | 0.3 | 0.1 | 2 | 0.1 |
| Pb | 0.221 | 0.3 | 0.5 | 4 | 0.3 |
| Zn | 6.83 | - | 30 | 120 | - |

in the metabolism of various compounds, including Zn (Jørgensen and Pedersen, 1994). Also, Lacoue-Labarthe *et al*. (2011) showed that from a certain stage of embryonic development of individuals, sex plays an important role in the accumulation of different metallic trace elements (Cd, Pb, Zn, etc.).

Belhoucine *et al*. (2014) reported that the decrease in contaminant concentrations in female fish can be attributed to the reproductive process. Specifically, the act of spawning induces a significant decontamination effect in females, as evidenced by a clear decrease in metal pollutant concentrations observed from their initial reproductive cycle.

The results presented here also showed that *M. merluccius* sampled from the Bay of Oran, showed the lowest concentration of trace metals in muscle than in the liver. Similar results were obtained by Aissioui *et al*. (2021), Tabeche *et al*. (2021), and Belhoucine *et al*. (2022), for *Mullus barbatus, Solea solea,* and *M. merluccius*, respectively, collected from Algerian Coast. This organ tropism is probably due to the capacity of the liver for accumulating higher metal concentrations than the other organs (Yilmaz *et al*., 2007). It is well known that the accumulation of essential metals in the liver is likely linked to its role in metabolism (Zhao *et al*., 2012). So, the liver is a good indicator of chronic metal exposure and plays an important role in their storage and inactivation (Chahid, 2016).

Görür *et al*. (2012) affirmed that high levels of Zn in hepatic tissues are usually related to natural binding proteins such as metallothioneins (MT) which act as an essential metal storage (i.e., Zn) to fulfill enzymatic and other metabolic demands (Amiard *et al*., 2006; Atli and Canli, 2008). On the other hand, the liver also showed high levels of non-essential metals such as Cd and Pb. This finding could be explained by the ability of Cd to displace the normally MT-associated essential metals in hepatic tissues (Amiard *et al*., 2006). Results presented in Table 3 indicate that the concentrations of Zn and the purely toxic elements (Pb and Cd) in the tissues of *M. merluccius* are below the limits recommended by WHO-IPCS (1977, 1992, 2001), UNEP (1985), USEPA (2000) and FAO/WHO (2001). It may be noted that these fish are consumed throughout the year by the Algerian population and may lead to subsequent bioaccumulation in humans as well. FAOCd and Pb are heavy metals identified among the 10 most toxic substances as reported by the "Priority List of Hazardous Substances" (ATSDR, 2020).

In this study, EDI values of Cd, Pb, and Zn from fish consumption by adult people in Algeria (Table 2) were far below the TDI limits, indicating that health risks associated with the intake of studied heavy metals through the consumption of examined fish samples were currently not present.

In addition, THQ and TTHQ values of individual heavy metals due to fish consumption presented in Table 2 never exceeded the hazard quotient threshold of 1. This implies that the consumption of *M. merluccius* currently does not pose a toxic risk to the Algerian population. Thus, we suggest no possible health risk in the *M. merluccius* with these levels of human exposure to the metals examined. In this regard, Traina *et al*. (2019) and Kontas *et al*. (2022) revealed that THQ of toxic metals (Cd, Pb, and Zn) was indeed lower than 1, indicating no danger to human health from consumption of contaminated *M. merluccius* from Sicilian coasts and Edremit Bay of the Mediterranean Sea, respectively.

## CONCLUSION

The data obtained demonstrated, in terms of the distribution of heavy metals, a good state of the fish resources studied. Indeed, the pollutants studied (Cd, Pb, and Zn) present a low health risk for the population. Nevertheless, heavy metal contamination of coastal and marine ecosystems is becoming an increasingly serious risk to aquatic organisms and humans. Thus, it is recommended that regular monitoring of trace metal contamination of fish species be carried out. The toxicological risk characterization must be communicated to the public, to ensure that nature and human health are in perfect balance.

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