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Long-term monitoring and bioaccumulation of heavy metals in European anchovy (*Engraulis encrasicolus*, Linnaeus, 1758) from the Romanian Black Sea area: A risk assessment perspective

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Abstract. This study examines the ecological and consumer safety implications of heavy contamination in anchovy (*Engraulis encrasicolus*, Linnaeus 1758), one of the most widely consumed small pelagic fish in the Romanian Black Sea. Long-term data (1994–2019) were analyzed to assess the accumulation of copper (Cu), cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr) in dorsal muscle tissues, focusing on contamination trends and bioaccumulation patterns. While concentrations generally align with established European Commission's safety thresholds, occasional exceedances, particularly for Cd and Pb, highlight episodic contamination events, especially during the 1990s and early 2000s. To evaluate potential health concerns, key risk assessment metrics: estimated daily intake (EDI), target hazard quotient (THQ), total target hazard quotient (TTHQ), and carcinogenic risk index (CRI) were applied. Cadmium consistently emerged as the most critical contaminant, with THQ and TTHQ values exceeding 1 in specific years, particularly among children due to higher intake per body weight. Although CRI values for Pb remained within the acceptable range, the cumulative non-carcinogenic risk during peak contamination periods warrants concern. The declining contamination trend observed in recent years reflects the effectiveness of environmental regulations. However, prolonged exposure to these metals may impact marine ecosystem stability and trophic interactions. Given the potential for cumulative pollutant effects and the possibility of re-emergence due to episodic contamination events, continuous surveillance and periodic risk assessments are recommended. This study highlights the significance of sustainable environmental monitoring and targeted mitigation efforts to protect marine ecosystems, sustain fisheries, and ensure seafood safety in the Black Sea region.

***Keywords*:** anchovy; bioaccumulation; Black Sea; heavy metals; risk assessment.

1. Introduction

The Black Sea is a vital marine ecosystem that supports biodiversity and provides essential resources for coastal communities. Among its commercially significant fish species, European anchovy (*Engraulis encrasicolus*), a small pelagic fish, plays a crucial role in the trophic chain and serves as a primary target for fisheries, including the Romanian sector [1, 2]. This species exhibits distinct seasonal migration patterns: migrate toward coastal waters from April to September and move to deeper waters for overwintering. Spawning occurs at night between June and September, while their diet mainly consists of zooplankton and small larvae [3]. Historically, Romanian anchovy fisheries played a major role in the Black Sea fishing sector. However, since the early 2000s, catch levels have fluctuated due to overfishing, short fishing seasons, and changing environmental conditions [2]. Since the 1990s, Turkiye has led Black Sea anchovy catches [4], while in Romania, high fishing costs and administrative challenges have limited anchovy exploitation. In addition, broader environmental pressures, including the input of various pollutants into marine ecosystems, may have contributed to this decline, posing significant risks to both fish stock sustainability and human health [35]. Heavy metal (HM) accumulation in marine environments is largely influenced by human activities, including industry, agriculture, wastewater discharge, and shipping operations [5]. These contaminants are persistent, toxic, and bioaccumulative, posing risks to marine ecosystems and public health [6, 7].

As these metals accumulate and biomagnify through the food web, they can disrupt fish reproduction, growth, and overall population dynamics. Ultimately, this contamination affects higher trophic levels, including human consumers [8], increasing the potential for toxic exposure [9].

Small pelagic fish, such as anchovy, are sensitive to anthropogenic inputs and serve as bioindicators of marine pollution. Because of their zooplankton-based diet and rapid metabolism, anchovies could accumulate more heavy metals than benthic species, reflecting the environmental burden of contaminants in the water column [10]. Factors like metal properties, environmental conditions, and species traits influence

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their uptake and retention, impacting both ecosystems and consumers [11]. Most research on HM in marine organisms from the Romanian coast has focused on bioaccumulation [12-14], with limited investigation into consumer health risks, despite the dietary importance of these species.

Heavy metal exposure can cause neurological damage, developmental delays, and carcinogenic effects [15]. Moreover, the presence of other hazardous substances, such as persistent organic pollutants and polycyclic aromatic hydrocarbons, often detected in biota [16], adds complexity to such assessments, emphasizing the importance of an integrated evaluation approach.

This study investigates the concentrations of Cu, Cd, Pb, Ni, and Cr in anchovy from the Romanian Black Sea coast, analyzing data collected between 1994 and 2019. It assesses long-term contamination trends, bioaccumulation patterns, and associated health risks. Given the ecological [1] and economic [17, 18] importance of anchovy, it was selected as an indicator species for the study, serving as an indicator of environmental burden.

While our dataset has been partially published in previous studies focusing solely on bioaccumulation levels, this study represents our first attempt to apply risk assessment indices to evaluate the potential human health impacts of anchovy consumption in Romania. By integrating bioaccumulation data with comprehensive risk metrics, this work provides a more complete assessment of seafood safety and public health implications over a 25-year period.

This study addresses a significant research gap by exploring the wider effects of HM contamination on both marine ecosystems and public health. It underscores the need for long-term environmental monitoring and cumulative impact assessments to support sustainable seafood safety and marine resource conservation in the Black Sea region.

2. Experimental

2.1. Study area

The 244 km Romanian coast experiences significant pressures from both natural and human activities. The northern sector, heavily influenced by the Danube River, receives substantial pollutant loads, while the southern sector is affected by industrial activity, urban wastewater, maritime transport, and tourism [19, 20]. These anthropogenic inputs not only elevate HM concentrations in the environment but also facilitate their bioaccumulation in marine organisms [16, 21].

Anchovy samples were collected from fixed fishing locations (Figure 1) along the entire Romanian coast, using pound nets [13]. Composite samples of anchovy (10-15 adult individuals) were selected from the catches for contaminant analysis.

Over the entire study period, a total of 32 composite anchovy samples were analyzed for HM content (Table S1). Sample distribution by year was as follows: 1 sample/year in 2001, 2002, 2005, 2011 and 2016; 2 samples/year in 2006 and 2019; 3 samples/year in 2003; 4 samples/year across 1994-1999 period and 2010; 5 samples/year in 2014 and 7 samples/year in 2013.

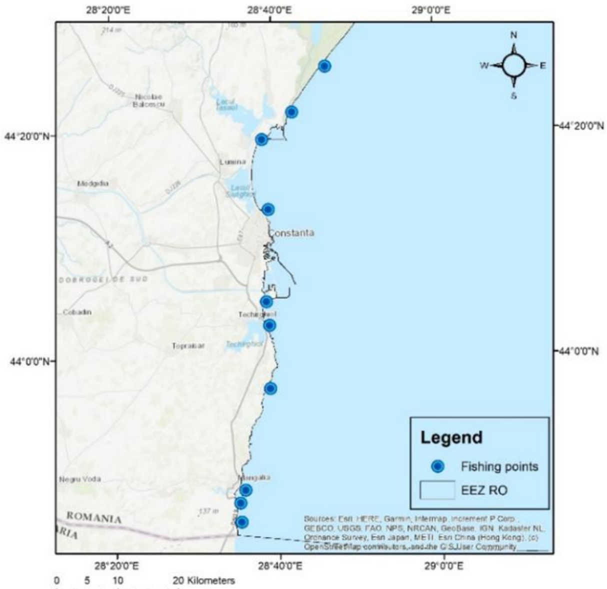

Figure 1. Map with the fishing locations (@Dragoș Niculescu, NIMRD)

2.2. Samples analysis

Once collected, the fish specimens were immediately stored in freezer containers to preserve their integrity and then transported to the laboratory for analysis. Dorsal muscle tissues were carefully dissected using sterilized instruments, freeze-dried, homogenized with an electric grinder, and analyzed [22].

Tissue samples were digested with 10 mL concentrated HNO_3 in sealed Teflon vessels on an electric hot plate at 120 °C. Trace metal concentrations (Cu, Cd, Pb, Ni, Cr) were assessed using graphite furnace atomic absorption spectrometry (GF-AAS). Throughout the study period, different GF-AAS instruments (ThermoElectron, Analytic Jena) were used. All analytical procedures followed consistent and comparable digestion protocols and AAS operating principles, including electrothermal program, matrix correction and calibration, thus ensuring continuity and reliability of the data [21-23].

Each sample was measured in three replicates, and the average value was reported. The detection limits for HMs ranged from 0.001 to 0.01 µg/L, depending on the element. Tissue concentrations are expressed as µg/g tissue wet weight (ww). To ensure the accuracy of the analytical procedures, reference methods were used [23]. High-purity reagents and certified glassware were used throughout the procedures to minimize external contamination.

2.3. Risk assessment

Health risks from consuming HMs contaminated fish were assessed using dietary risk indices [24]. The evaluation integrated fish consumption rates, HMs intake levels, and toxicological reference values to comprehensively assess potential adverse health effects from dietary exposure.

The **Estimated Daily Intake** (EDI) of HM (mg/kg/day) was calculated based on metal concentrations in fish and average consumption rates, offering an estimate of dietary exposure [25, 26]:

$$EDI\ (mg/kg/day) = \frac{C_{metal}\ \times FIR}{BW}$$

where C_{metal} = concentration of HM in anchovy (mg/kg, wet wt.), FIR (Food Ingestion Rate) (kg/day) = daily fish consumption rate, based on FAOSTAT data (https://www.fao.org/faostat/), and BW = Body Weight (30 kg for children, 70 kg for adults) [21].

The **Target Hazard Quotient (THQ)** was employed to evaluate non-carcinogenic health risks arising from the dietary intake of toxic metals via fish consumption. THQ is calculated as the ratio between the Estimated Daily Intake (EDI, mg/kg/day) and the Chronic Oral Reference Dose (RfD, mg/kg/day) [27]:

$$THQ = \frac{EDI}{RfD}$$

where *RfD* represents the maximum acceptable oral exposure level for each metal without significant risk of adverse health effects. The reference doses applied were 0.0001 mg/kg/day for Cd, 0.04 mg/kg/day for Cu, 0.02 mg/kg/day for Ni, and 0.003 mg/kg/day for Cr, based on values provided by the U.S. EPA Regional Screening Levels (RSLs) (https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables) and the Risk Assessment Information System (RAIS) (https://rais.ornl.gov/cgi-bin/tools/TOX_search). A THQ value exceeding 1.0 indicates that the EDI surpasses the corresponding RfD, implying potential health concerns. The magnitude of risk is influenced by both the concentration of the metal in fish tissue and the rate of consumption [21, 28].

The **Total Target Hazard Quotient (TTHQ)** metric was used to estimate the overall non-carcinogenic health risk posed by combined exposure to multiple toxic metals through fish consumption:

$$TTHQ = \sum_{i=1}^{n} THQ_i$$

TTHQ reflects the cumulative non-carcinogenic risk associated with simultaneous exposure to multiple heavy metals and is calculated as the sum of individual THQ values [21]. A TTHQ exceeding 1 indicates a potential risk of chronic health effects, whereas values below 1 suggest no significant concern [29]. Evaluating this index is important for capturing the combined impact and potential interactions of multiple metal exposures on human health [30].

The **Carcinogenic Risk Index (CRI)** is used to estimate the potential lifetime cancer risk resulting from exposure to carcinogenic contaminants [31]. It is calculated using the following equation:

$$CRI = EDI\ \times CSF$$

where CSF is Cancer Slope Factor $(mg/kg/day)^{-1}$, a parameter that quantifies the probability of developing cancer over a lifetime due to chronic exposure to a specific substance [32]. CSF value for Pb is 0.0085 $(mg/kg/day)^{-1}$, based on data from USEPA RSLs [28] and the RAIS.

According to risk classification thresholds [31], a CRI value below 10^{-6}, is considered negligible, values between 10^{-6} and 10^{-4} are regarded as acceptable or tolerable, while values above10^{-4} indicate a significant carcinogenic risk. [21].

Data analysis was carried out using Microsoft Excel 365 and Statistica software [33].

3. Results and discussion

Copper (Cu) concentrations exhibited a highly skewed distribution, with most values clustering at low levels (1–2 µg/g wet weight) but occasional spikes reaching 30.20 µg/g wet weight. Cd and Pb levels were predominantly below 1 µg/g wet weight, yet episodic exceedances up to 5.002 µg/g for Cd and 4.350 µg/g for Pb were recorded, indicating potential contamination events. Ni and Cr displayed lower overall concentrations, but sporadic peaks suggested exposure to transient contamination sources. The high variability in metal concentrations, particularly for Cu, Cd, and Pb, underscores the influence of episodic pollution inputs from industrial and agricultural activities, stormwater runoff, and maritime operations (Table 1; Figure S1).

Table 1. Concentrations of Cu, Cd, Pb, Ni, and Cr (µg/g ww) in anchovy samples during 1994-2019

	Valid N	Mean	Median	Min	Max	25th Percentile	75th Percentile	Coef. Var.	Skewness	Kurtosis
Cu	32	3.525	1.998	0.150	30.200	0.883	4.044	151.864	4.257	20.921
Cd	32	0.496	0.080	0.003	5.002	0.019	0.331	218.684	3.183	10.480
Pb	32	0.563	0.094	0.007	4.350	0.008	0.981	165.969	2.516	7.822
Ni	25*	1.154	0.184	0.002	7.747	0.065	0.380	197.909	2.215	3.736
Cr	17*	0.269	0.135	0.001	1.622	0.058	0.277	145.840	2.890	9.408

** Ni and Cr were analyzed starting with 2003 and 2006, respectively*

Over the study period, most HM exhibited either a declining or stabilizing trend, likely reflecting the effectiveness of pollution mitigation measures. Cu displayed episodic peaks, particularly in the late 1990s and early 2000s, while Cd and Pb showed more pronounced fluctuations (Figure 2), with occasional spikes persisting into the 2010s. These variations were likely influenced by point-source pollution, heavy rainfall events mobilizing contaminants, or changes in water circulation patterns that resuspended contaminated sediments. In contrast, Ni and Cr exhibited sporadic peaks without a clear upward or downward trend, suggesting exposure to transient pollution sources rather than sustained contamination (Figure S2).

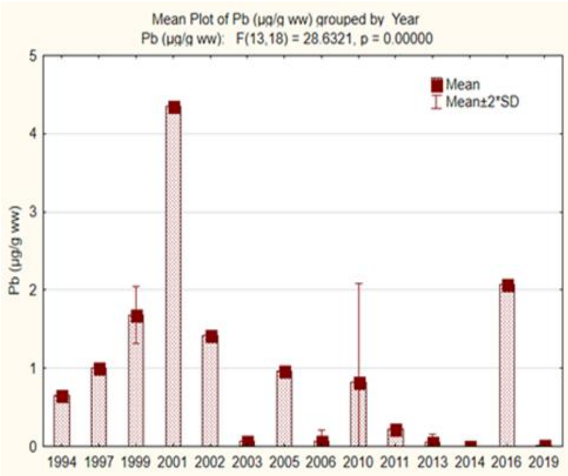

Figure 2. Temporal trends of mean Pb concentrations in anchovy during 1994-2019

The elevated HMs concentrations in anchovy during the earlier period resulted from a combination of historical pollution and environmental factors shaping contamination dynamics in the Black Sea basin [34]. In addition to environmental factors, some variation may also stem from analytical uncertainties, particularly in earlier data (1994–1999) sourced from the NIMRD database. However, all measurements followed the same analytical procedures, ensuring general methodological consistency across the dataset.

Decades of heavy industrialization and intensive agriculture under communist regimes contributed to long-term marine degradation, with contaminants persisting in sediments and ecosystems even after their collapse in the late 1980s [35]. The Danube River, as a major freshwater input to the Black Sea, transported pollutants from its extensive and industrialized basin, while events like the Yugoslav Wars and NATO bombings of industrial facilities in the 1990s further introduced contaminants into the Danube and Black Sea [34, 35]. Despite Romania’s economic decline in the 1990s, legacy pollution from sediments and untreated waste delayed ecosystem recovery, exacerbated by the Black Sea’s limited connection to open oceans. A turning point came with Romania’s EU accession in 2007, which led to the adoption of EU environmental directives and major investments in wastewater treatment, sustainable agriculture, and industrial modernization [36]. These efforts contributed to a gradual decline in pollutant level in marine ecosystems [37]. However, episodic events such as extreme weather or upstream incidents occasionally reintroduced contaminants, causing temporary spikes in hazardous substances levels, including in biota.

Despite these improvements, the presence of heavy metals in marine organisms remains a concern, as periodic contamination events continue to impact seafood safety. In particular, cadmium (Cd) and lead (Pb) concentrations in anchovy have exceeded regulatory limits in a significant portion of the analyzed samples.

During the study period, Cd levels exceeded regulatory limits in 33% of the total samples, while Pb exceeded limits in 35% of the samples (Figure S3).

These levels surpassed the European Commission's maximum allowable concentrations (MAC) for seafood consumption (0.25 mg/kg w.w. for Cd and 0.30 mg/kg w.w. for Pb) (Commission Regulation (EU) 2023/915; https://eur-lex.europa.eu/eli/reg/2023/915/oj/eng).
These exceedances were particularly prevalent during earlier years (1990s and 2000s), aligning with historical pollution events and industrial discharges. Although the declining trend in recent years reflects progress in environmental policies and pollution control efforts [38], the continued detection of elevated levels in some samples highlights the ongoing need for sustained monitoring and targeted mitigation measures.

Given these persistent contamination risks, assessing their potential impact on human health is crucial. Therefore, this study applied key health risk indices: EDI, THQ, TTHQ, and CRI, to evaluate the safety of anchovy consumption (Table S1). Among the analyzed metals, cadmium (Cd) consistently posed the greatest concern, with EDI values frequently exceeding its EFSA health-based guidance value (HBGV) of 0.00036 mg/kg/day [39] particularly in children. Peaks in Cd exposure (THQ>1) were observed during the same high-risk periods (1994–1999 and 2001), likely reflecting specific events, including industrial discharges, agricultural runoff, or sediment disturbances. Lead (Pb) occasionally exceeded its HBGV (0.00063 mg/kg/day) [40], particularly during isolated years such as 2001. In contrast, nickel (Ni) remained consistently below its HBGV (0.01300 mg/kg/day) [41]. Copper (Cu), despite being present at the highest concentrations had a minimal influence on risk indices due to its significantly lower toxicity.

TTHQ values showed a strong correlation with Cd levels ($R^2 = 0.986$) rather than total heavy metal content (Figure 3), highlighting the dominant role of Cd’s high toxicity in driving cumulative health risks. Even when metals such as Cu or Ni exhibited higher concentrations, their lower toxicological impact minimized their contribution to cumulative risk indices. The health risks associated with anchovy consumption may be influenced by a combination of heavy metal toxicity and food ingestion rate (FIR) (Figure 4). In children, the higher FIR relative to lower body weight amplified EDI, THQ, and TTHQ values, leading to significantly higher risk levels than in adults. During peak contamination years, children consuming anchovy frequently exceeded the acceptable cumulative risk threshold (TTHQ > 1). In contrast, adults generally exhibited lower risks but occasionally approached the threshold, particularly during periods of elevated Cd levels.

The CRI for Pb remained well below the acceptable threshold (10^{-6}–10^{-4}) in anchovy, indicating minimal long-term cancer risk from their consumption (Figure 4). These findings suggest that while Pb occasionally contributed to non-carcinogenic risks through elevated EDI values, it did not pose a significant carcinogenic threat. Temporal spikes in Cd exposure coincide with periods of increased anthropogenic pressures, emphasizing the necessity for stricter regulatory controls on industrial and agricultural discharges. Given the observed risk profiles, dietary recommendations should limit consumption for high-risk groups, particularly children, during contamination peaks. Furthermore, public health campaigns should raise awareness of pollutant exposure risks while promoting the safe and sustainable consumption of seafood.

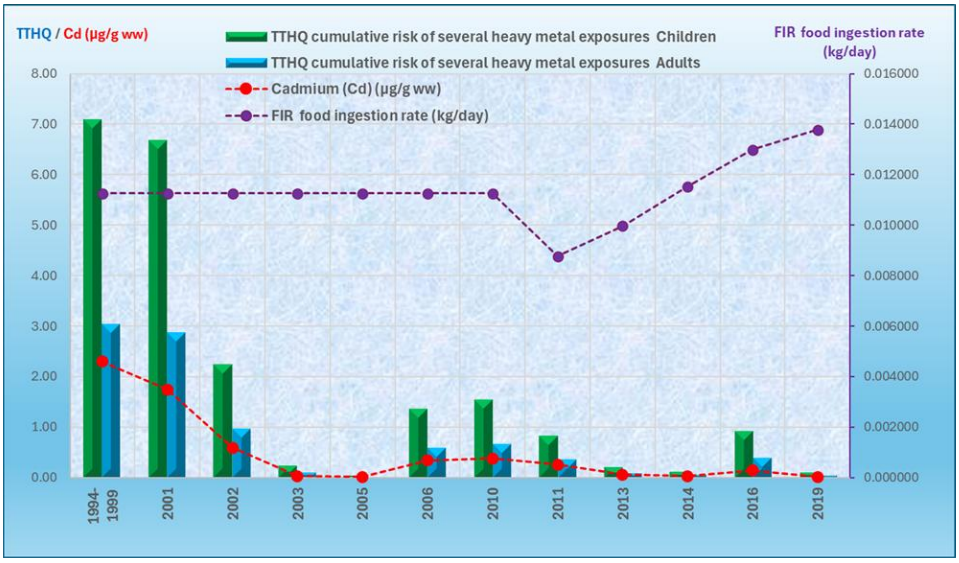

Figure 3. Temporal trends in the cumulative risk of heavy metal exposure (TTHQ) from anchovy consumption

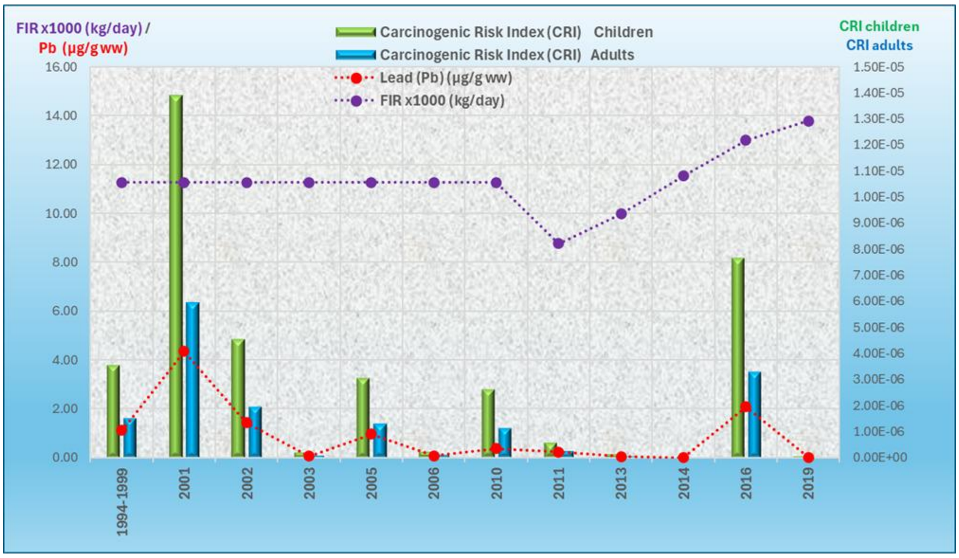

Figure 4. Temporal trends in the carcinogenic risk index (CRI) from anchovy consumption

HM bioaccumulation in small pelagic fish not only creates ecosystem instability through significant ecological risks, such as its impacts on fish physiology, reproduction, and biodiversity [42], while also raising public health concerns associated with the consumption of contaminated seafood. These contaminants persist in aquatic environments even after pollution sources decline, emphasizing the need for continuous monitoring [43]. Long-term fish consumption can lead to cumulative health risks, particularly in regions with ongoing pollutant discharge [44]. Our results are consistent with previous regional research and underscore the need for continued monitoring to safeguard food safety.

Studies in the Black Sea and Mediterranean show species- and region-specific accumulation patterns in commercially important fish. Seasonal variations in heavy metal concentrations have been observed, with levels fluctuating but largely remaining within regulatory limits [45-47]. In some cases, higher metal concentrations were detected in non-muscle tissues, while improved environmental regulations, particularly after 2006, have contributed to a reduction in dietary exposure [48]. Long-term assessments from the southern and eastern Black Sea (2009–2019) indicate that heavy metal levels have remained stable, reflecting the effectiveness of pollution control measures [49]. Additional studies on marine fish confirm compliance with international safety standards, with both carcinogenic and non-carcinogenic risks remaining within acceptable limits [27, 50]. Similar research on anchovy from Turkey, Georgia, and Abkhazia [9, 50, 51], as well as multiple species from the Turkish Black Sea [24, 27], supports these findings, though vulnerable groups such as children and frequent fish consumers may face slightly higher exposure levels [9, 51].

Considering these findings, our results emphasize the necessity of ongoing monitoring of HM levels in marine fish, particularly in species susceptible to metal accumulation, to ensure food safety. While no consistent upward trend was observed, periodic exceedances of Cd and Pb in the 1990s and 2000s point to industrial and agricultural pollution, posing risks for vulnerable populations, like anchovy. Stricter environmental regulations since Romania's EU accession in 2007 have contributed to declining metal concentrations, aligning with regional trends of reduced ecological risks due to improved pollution control [17, 24, 27]. Current health risk indices (THQ, TTHQ, CRI) indicate low consumer risks, though past exceedances stress the importance of continued surveillance. Our findings confirm that Cd remains a key contributor to cumulative risk, reinforcing the need for targeted mitigation efforts.

Future research should incorporate spatial analyses, examine a broader range of contaminants, and consider specific dietary patterns to enhance seafood safety evaluations. Addressing the cumulative effects of various pollutants is a key knowledge gap, as these compounds may have additive or synergistic toxic effects [52]. This highlights the need for integrated risk assessments to better evaluate seafood safety in future studies.

4. Conclusions

This research provides a long-term assessment of HM contamination in *Engraulis encrasicolus* from the Romanian waters, showing that recent levels generally comply with international safety limits, indicating low health risks. However, historical pollution resulted in exceedances of Cd and Pb thresholds, posing potential risks in earlier periods. The recent decline in contamination levels [16, 21] reflects the impact of stricter environmental regulations following Romania's EU accession. Health risk indices (THQ, TTHQ, CRI) confirm Cd as a key contributor to cumulative risk, particularly for vulnerable populations. The study highlights the need for continued monitoring in regions affected by industrial, agricultural, and riverine pollution sources. Future research should focus on assessing the cumulative impacts of heavy metals and hazardous substances on marine ecosystems and human health, thus improving our understanding of their combined effects. While current risks from anchovy consumption appear minimal, ongoing surveillance and pollution control remain essential for protecting both marine ecosystems and public health.

Conflict of interest

The authors declare no conflicts of interest.

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