Journal of Applied Biosciences 202: 21422 – 21433
ISSN 1997-5902

Contamination of *Merluccius merluccius* (L., 1758) by Metallic Trace Elements along the Eastern Algerian Sector

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Submission 3rd June 2024. Published online at https://www.m.elewa.org/Journals/ on 30th November 2024.
https://doi.org/10.35759/JABs.202.3

ABSTRACT
Objective: This study aims to assess the level of contamination of the species *Merluccius merluccius(Hake) considered* as a bioindicator of pollution by Metallic Trace Elements(MTEs).
Methodology and |Results: Six metallic trace elements (Zn, Fe, Cu, Ni, Pb, and Cd) were analysed in *Merluccius merluccius* specimens caught in the Gulf of Béjaïa, the Bay of Jijel, and the Gulf of Annaba during the year 2016. Inductively Coupled Plasma Mass Spectrometry (ICP/AES) was used to assess the level of contamination by these heavy metals. The study's results reveal significant variation in values both among the target organs and across the study areas. The most notable contamination pertains to essential Metal Trace Elements, specifically zinc and iron. Following these, essential Metallic Trace Elements are present in lower concentrations, including copper and nickel, and finally, there are the purely toxic MTE, namely lead and cadmium. Based on the average concentration limit values in fish muscles, the contamination hierarchy of metallic elements in the muscle tissue of *Merluccius merluccius* is as follows: zinc > iron > copper > nickel > lead > cadmium. Hake sourced from the Gulf of Béjaïa accumulates more essential Trace Metal Elements (zinc, iron) compared to those from Jijel Bay and the Gulf of Annaba. Conversely, essential Metal Trace Elements (copper, nickel) at low doses are more concentrated in hake from the Gulf of Annaba. However, cadmium and lead are predominant in the muscle tissue of hake from the Gulf of Béjaïa and Jijel Bay, respectively. Depending on the target organs, zinc and iron exhibit higher accumulation in liver tissue, while copper, nickel, lead, and cadmium are more prominent in muscle tissue than in the liver. Statistical data analysis for each metal considered indicates highly significant differences among the sampling areas.
Conclusion and Application of results: The presence of alarming levels of Fe and Ca in Hake flesh suggests that the entire trophic network could be contaminated, which presents a real danger to public health, thus requiring periodic biomonitoring of the coastline.
Keywords*:* Metallic trace elements; Contamination; *Merluccius merluccius*; Gulf of Bejaia; Inductively coupled plasma spectroscopy

INTRODUCTION

Fishery resources are primarily used as food, making them a crucial component of national food security policies. However, for more than half a century, pollution has emerged as a substantial threat to both human well-being and the integrity of diverse ecosystems on our planet (Abdennour *et al.*, 2000; Gochfeld, 2003, Eggleton & Thomas, 2004;). Among the various chemical substances that pose serious environmental and health risks, heavy metals emerge as notable culprits, particularly in aquatic environments (Wu *et al.*, 2013a, 2013b). These highly toxic elements are increasingly utilized in industrial and agricultural processes (Bisone, 2012; Chang *et al.*, 2014; Pradhan & Kumar, 2014). Even certain non-toxic metals can present significant dangers due to their potential for bioaccumulation and their persistence in the environment, particularly within sedimentary deposits of aquatic systems, where degradation does not occur (Saher & Siddiqui, 2016). This study not only complements previous research (Belhoucine *et al.*, 2014; Benguedda & Amara, 2011; Boucetta *et al.*, 2016; Ouali *et al.*, 2018) but also offers new insights, aiming to enhance our understanding of the impact of pollution caused by various metallic trace elements on aquatic ecosystems. *Merluccius merluccius,* a benthic fish recommended by FAO-UNEP as a bioindicator of marine contamination, serves as the subject of this study. This species choice enables the detection of specific micropollutants in two critical organs: the muscle (edible portion) and the liver (a detoxification and storage organ). This approach additionally aids in assessing the overall health of marine ecosystems along the eastern Algerian coast, illuminating the significance of anthropogenic contributions of heavy metals. The results obtained facilitate a comparison of contamination levels in the muscle tissue of *Merluccius merluccius* within the study area against international recommendations and standards that define acceptable limits for public health. To achieve this, we selected the coastal regions of the Gulf of Béjaïa, the Bay of Jijel, and the Gulf of Annaba as our study sites. The choice of these aquatic ecosystems is guided by the distinct characteristics inherent to each respective region.

MATERIAL AND METHODS

Study Zones: To conduct a comprehensive study on the biology of *Merluccius merluccius*, three primary zones along the Algerian east coast were selected: the Gulf of Béjaïa, the Bay of Jijel, and the Gulf of Annaba. The Béjaïa region (36°45'0" N, 5°19'60" E) is located on the southern Mediterranean shore, in the eastern sector of the Algerian coast. It is bounded to the east by Cape Cavale and to the west by Cape Carbon, with the Gulf of Béjaïa opening northward onto the Mediterranean (*Figure 01*).

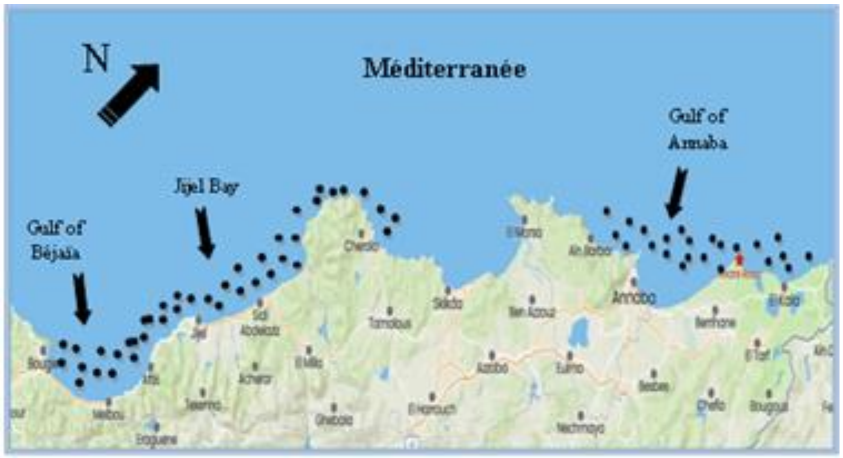

Figure 01: Satellite image depicting the study areas of *Merluccius merluccius* in the eastern Algerian sector.

The Béjaïa region is intersected by several wadis that channel runoff water into the Gulf of Béjaïa. Among these, the most significant are Djemaa wadi (46 km), Agarioun wadi (80 km), Zitoun wadi (30 km), and Sommam wadi. These wadis collectively form an extensive hydrographic network, with a total watershed area of 9,200 km2, distributed as follows: (a) Sahel wadi basin from Sour El-Ghozlane (Bouira region) to Akbou: 3,750 km2; (b) Bou Sellam wadi basin from Aïn-Oulmane (Sétif region) to Akbou: 4,500 km2; (c) Soummam basin, specifically from Akbou to the sea: 950 km^2. Jijel Bay stretches between northern latitudes 36°:10' and 36°:50', and eastern longitudes 5°:25' and 6°:30'. The western part of Jijel town features rocky coves interspersed with a few small sandy beaches, while the eastern coast boasts a straight and fairly wide sandy beach. To the north, the imposing Babor Mountains, part of the Tell Atlas of Algeria, dominate the landscape. This region includes the El Kebir wadi basin, covering an area of around 1,110 km2. The fishing area (within national jurisdiction) spans 10,660 km2, with a coastline of 120 km (*Figure 01*). The Gulf of Annaba lies at the far eastern end of the Algerian coast, between Chetaïbi in the west and El Kala in the east (*Figure 01*).This wide-opening bay extends approximately 40 km and opens to the Mediterranean Sea to the north. It encompasses the continental waters of the Seybouse River, Algeria's second-longest river with a watershed of about 6,470 km2, along with the Mafrag River connected to the Bounamoussa and ElKebir rivers, which drain a watershed of 2,252 km2(Belabed *et al.*, 2011).

Sampling: *Merluccius merluccius* specimens were sampled along the eastern Algerian sector by professional fishermen. This fish species is abundant in the Mediterranean, particularly in the western region compared to the eastern part (Arneri & Morales-Nin, 2005; Morales-Nin & Moranta, 2004; Recasens *et al.*, 1998), as well as in the North-East Atlantic (from England to Senegal) (Domínguez-Petit *et al.*, 2008; Khoufi *et al.*, 2014; Lannin, 2006; Lucio *et al.*, 2000, 1996–1997; Murua *et al.*, 2006; Piñeiro, 2003).It inhabits variable depths on the continental shelf, near the coast for juveniles and up to 1000 m in depth for adults (Bouaziz *et al.*, 1998). Throughout the study year of 2016, over 1000 individuals ranging in length from 9.8 cm to 81.9 cm were collected for biological material. The collected samples were immediately sent to the laboratory for biological analysis. Each individual underwent a series of measurements, enabling their classification into size and sex categories. Muscle tissue and liver samples were taken from each individual, dried in an oven for 72 hours at 90°C, crushed, and sieved. Sub-samples were weighed, labelled, and stored in pill containers before chemical analysis. In a subsequent step, mineralization took place at the laboratory of the National Agricultural Research Institute of Oued-Ghir (I.N.R.A). The samples underwent a mineralization process using a Gildhard device equipped with a series of six tubes. Each flask contained 1 g of tissue in dry weight (muscle, liver) along with 1 ml of nitric acid (HNO3). The flasks were heated at a constant temperature of 95°C for one hour. After filtration, the solution was ready for analysis using an inductively coupled plasma spectrophotometer (ICP/AES).

Statistical Analysis: Statistical analysis was conducted to compare mean concentrations (mean ± standard deviation) of heavy metals. The ANOVA test was applied to assess variations in metal pollutant concentrations based on the target organ, gender, and study area. A significance level of 5% (<0.05) was considered to indicate a significant difference.

RESULTS AND DISCUSSION

Variations in Heavy Metal Contents in *Merluccius merluccius* Based on Study Stations and Target Organs: Graphical representations of metal element content percentages based on study stations are provided in Figure 02, shedding light on the variations in metal element distribution across different study sites. Broadly speaking, the findings uncover consistent trends in micropollutant percentages within hake specimens from the eastern Algerian sector. Notably, these variations occur in pairs (Zn and Fe; Cu and Ni; Pb and Cd) and are influenced by specific study sites. Among the metals, essential elements (Zn and Fe) prominently emerge, exerting dominance across the study areas, particularly within the liver tissue in comparison to the muscle tissue. Specifically, in hake muscle, zinc takes precedence over iron, comprising 84.59% to 10.25% in the Gulf of Béjaïa, 82.85% to 10.57% in the Bay of Jijel, and 79.04% to 13.34% in the Gulf of Annaba (Fig. 02a). This pattern is even more pronounced in hake liver, with percentages ranging from 86.19% to 11.07% in the Gulf of Béjaïa, 84.55% to 11.34% in the Bay of Jijel, and 81.04% to 15.36% in the Gulf of Annaba (Fig. 02b).

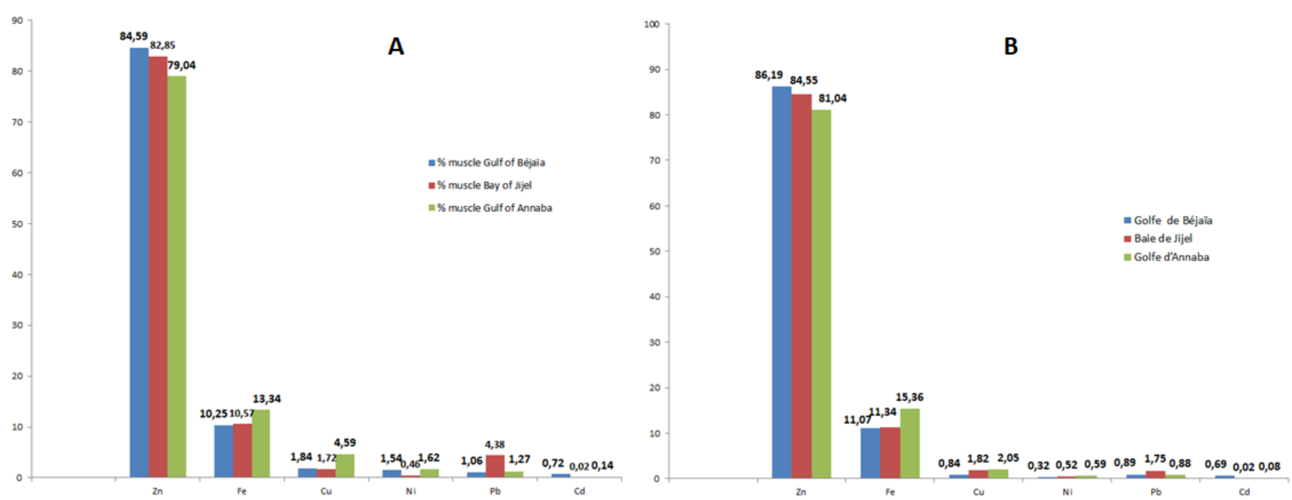

Figure 02: Overall content percentage of Essential Trace Metals (ETMs) analysed in muscle (a) and liver (b) tissues of *Merluccius merluccius* from various areas along the eastern Algerian coast.

Zinc alone constitutes approximately three-quarters of the aggregate content of the other recorded elements. Essential elements such as copper and nickel also exhibit fluctuations across study areas and target organs. For hake muscle, the concentrations of copper and nickel are elevated in the Gulf of Annaba (4.59% to 1.62%), surpassing those in the Gulf of Béjaïa (1.84% to 1.54%) and Jijel Bay (1.72% to 0.46%) (Fig. 02a). Similarly, hake liver displays higher levels of copper and nickel compared to muscle tissue, being more pronounced in the Gulf of Annaba (2.05% to 0.59%) relative to the Bay of Jijel (1.82% to 0.52%) and the Gulf of Béjaïa (0.84% to 0.32%) (Fig. 02b). Conversely, lead and cadmium exhibit the lowest concentrations. Lead content demonstrates variability in muscle tissue (1.06%, 4.38%, and 1.27%) (Fig. 02a), while displaying distinct proportions in liver tissue (0.89%, 1.75%, 0.88%) in the Gulf of Béjaïa (Fig. 02b), Bay of Jijel, and Gulf of Annaba, respectively. It's worth noting that lead predominance is observed in the Bay of Jijel compared to the other two study areas. In Figure 02a, the graphical representation and analysis portray the distribution of metal elements within the muscle tissue of *Merluccius merluccius* samples from the three study stations. For instance, in the Gulf of Béjaïa, zinc (Zn) constitutes 84.59% of the metal content in muscle tissue, followed by iron (Fe) at 10.25%, copper (Cu) at 1.84%, nickel (Ni) at 1.54%, lead (Pb) at 1.06%, and cadmium (Cd) at 0.72%. Similar distributions are observed in the Bay of Jijel and the Gulf of Annaba. Regarding Liver Tissue: Figure 02b illustrates the percentage distribution of metal elements in the liver tissue of the same *Merluccius merluccius* samples. For example, in the Gulf of Béjaïa, zinc (Zn) accounts for 86.19% of the metal content in liver tissue, followed by iron (Fe) at 11.07%, copper (Cu) at 0.84%, nickel (Ni) at 0.32%, lead (Pb) at 0.89%, and cadmium (Cd) at 0.69%. Similar distributions are observed for the Bay of Jijel and the Gulf of Annaba.

Global Content of Analysed Heavy Metals: Pollutant concentrations in *Merluccius merluccius* are compared to the Maximum Allowable Dose Levels (MADL) defined by various organizations.

Essential Metallic Trace Elements (Zinc, Iron): The presented Figure 3 provides a comprehensive visualization of metal element content percentages, specifically highlighting the concentrations of zinc (Zn) and iron (Fe) across three key study areas: the Gulf of Béjaïa, Jijel Bay, and the Gulf of Annaba. Notably, zinc concentrations exhibit a significant elevation, measuring 71.85 mg/kg F.W in the Gulf of Béjaïa, 58.78 mg/kg F.W in the Bay of Jijel, and 65.99 mg/kg F.W in the Gulf of Annaba (Fig. 03a). This observation holds particular importance as it exceeds the thresholds established by international standards, such as WHO-IPCS (1998) and IAEA-407 (2003), which recommend a range of 30 - 48.5 mg/kg F.W. Conversely, iron concentrations demonstrate variations, with levels recorded at 40.13 mg/kg F.W in the Gulf of Béjaïa, 37.74 mg/kg F.W in Jijel Bay, and 33.04 mg/kg F.W in the Gulf of Annaba (Fig. 03b). It is noteworthy that significant differences in iron concentrations exist among hake specimens from the three study areas ($F = 11.55$; $P \leq 0.001$). Intriguingly, these recorded iron concentrations align with the standard outlined by JORADP No. 25 (2011), which recommends a threshold of 50 mg/kg F.W. The distinctive content percentages of these essential metal elements underscore the spatial heterogeneity of their distribution and emphasize their integral roles within the composition and ecological dynamics of the marine ecosystem.

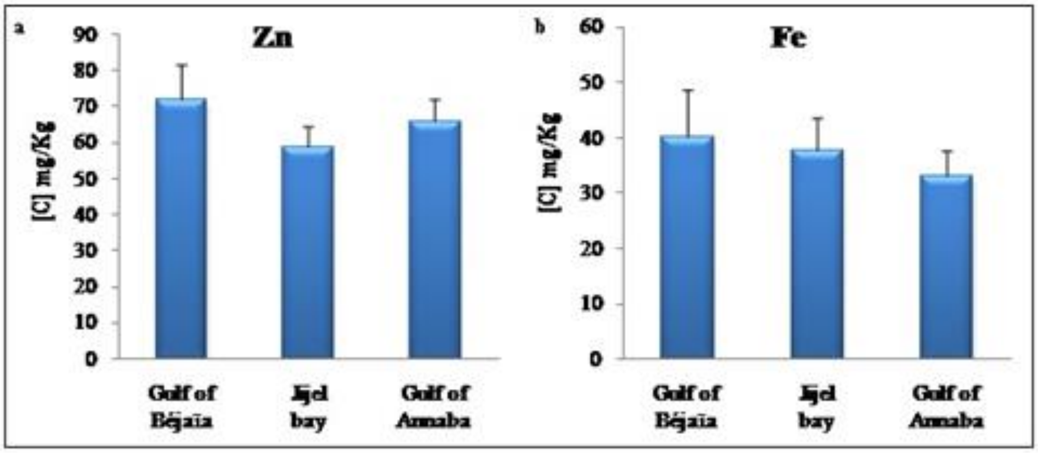

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Figure 03: Average concentrations of essential metallic trace elements (zinc, iron) in *Merluccius merluccius* samples from diverse fishing regions along the eastern Algerian coast.

Essential Metallic Trace Elements at Low Dose (Copper, Nickel): The concentrations of two essential metal elements, copper (Cu) and nickel (Ni), are illustrated in Figure 4, showcasing their distribution across three distinct study areas: the Gulf of Béjaïa, Jijel Bay, and the Gulf of Annaba. Within the Gulf of Béjaïa, copper (Cu) concentrations measure 0.35 mg/kg P.F, while nickel (Ni) exhibits an average concentration of 0.71 mg/kg P.F (Fig. 04a). In comparison, Jijel Bay displays a slightly higher copper (Cu) concentration of 0.37 mg/kg P.F, with nickel (Ni) exhibiting a lower concentration of 0.29 mg/kg P.F. Shifting to the Gulf of Annaba, copper (Cu) maintains a concentration of 0.36 mg/kg P.F, while nickel (Ni) demonstrates a higher concentration of 0.58 mg/kg P.F. Interestingly, the average copper concentrations do not exhibit significant differences among the study areas ($F = 1.23$; $P \geq 0.05$); however, all recorded results surpass the national standard of 0.15 mg/kg P.F outlined in JORADP No. 25 (2011). Conversely, average nickel concentrations vary significantly, measuring 0.71 mg/kg P.F in the Gulf of Béjaïa, 0.29 mg/kg P.F in Jijel Bay, and 0.58 mg/kg P.F in the Gulf of Annaba (Fig. 04b). Notably, statistical analysis reveals highly significant differences in mean nickel concentrations ($F = 1873$; $P \leq 0.001$). Nickel concentrations exceed the standards proposed by WHO-IPCS (1998) and JORADP No. 25 (2011) in both the Gulf of Béjaïa and Gulf of Annaba, while adhering to standards in the Bay of Jijel. These findings underscore the varying concentrations of copper (Cu) and nickel (Ni) among the studied areas, shedding light on their spatial distribution within the marine environment. These differences provide valuable insights into potential variations in environmental conditions and sources of contamination across the studied regions, further emphasizing the dynamic nature of metal element distribution in marine ecosystems.

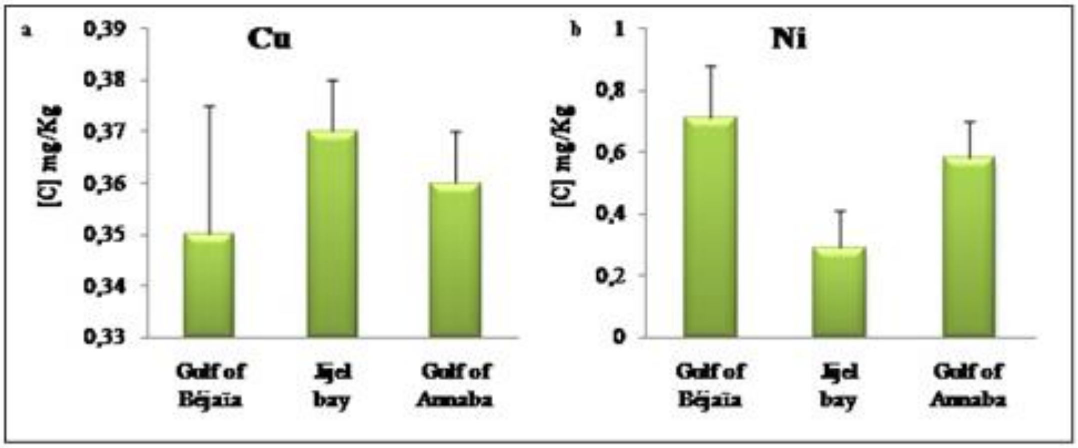

Figure 04: Average concentrations of essential metallic trace elements (copper, nickel) in *Merluccius merluccius* samples from different fishing areas along the eastern Algerian coast.

Toxic Metallic Trace Elements: The concentrations of two specific heavy metal elements, lead (Pb) and cadmium (Cd), are graphically depicted in Figure 5, illustrating their distribution across three distinct study areas: the Gulf of Béjaïa, Jijel Bay, and the Gulf of Annaba. Lead concentrations exhibit a range from 0.31 mg/kg P.F in the Gulf of Béjaïa to 1.45 mg/kg P.F in the Bay of Jijel, with the Gulf of Annaba recording a concentration of 0.49 mg/kg P.F (Fig. 05a). Statistical analysis reveals highly significant differences in mean lead concentrations ($F = 557.56$; $P \leq 0.001$). Notably, concentrations in the Gulf of Béjaïa and Gulf of Annaba remain within recommended thresholds, while the elevated concentration in Jijel Bay raises concern. In parallel, average cadmium concentrations are approximately 0.02 mg/kg P.F in the Gulf of Béjaïa, 0.01 mg/kg P.F in the Bay of Jijel, and also 0.01 mg/kg P.F in the Gulf of Annaba (Fig. 05b). Further statistical analysis indicates significant differences in average cadmium concentrations ($F = 5.17$; $P \leq 0.05$) among hake specimens from the eastern sector of Algeria. It's essential to emphasize that the recorded cadmium concentrations remain below the Maximum Allowable Daily Load (MADL) values recommended by various regulatory bodies, including EEC (1982), WHO-IPCS (1998), CSHPF (1990), and IAEA-407 (2003). These findings, as depicted in Figure 5, offer valuable insights into the distribution of lead (Pb) and cadmium (Cd) concentrations across the studied marine regions. The notable variation in lead concentrations, particularly the elevated level in Jijel Bay, underscores potential differences in contamination sources and their ecological implications. Similarly, the consistently low cadmium concentrations align with regulatory standards, highlighting the overall health of the marine ecosystem within the investigated areas.

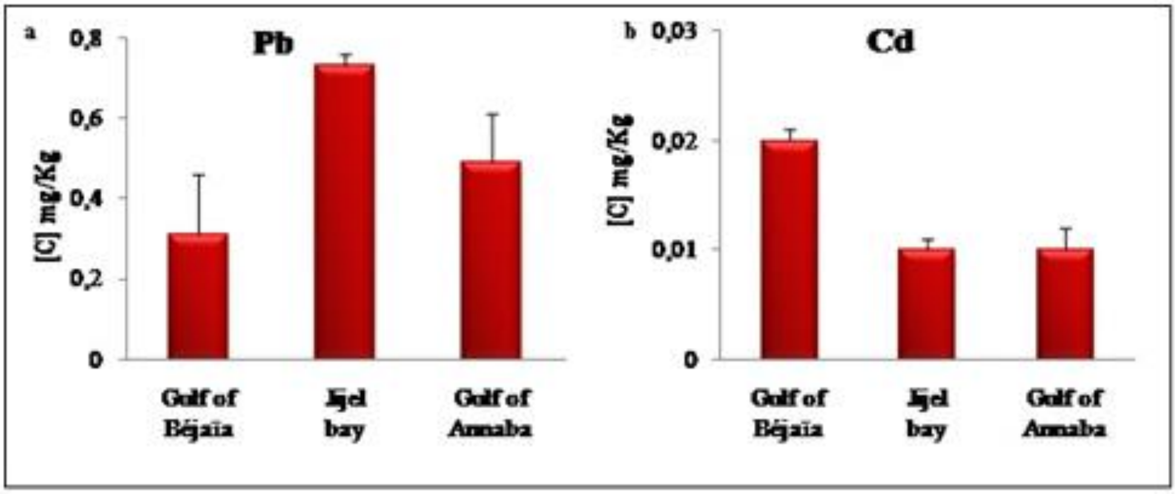

Figure 05: Average concentrations of toxic ETMs (lead, cadmium) in *Merluccius merluccius* samples from distinct fishing areas along the eastern Algerian coast.

Metallic Trace Element Impact on Ecosystems and Human Health: Metallic trace elements find their way into aquatic ecosystems and oceans through diverse pathways, encompassing industrial tributaries, rivers, atmospheric deposition, and soil leaching (Bourhane-Eddine *et al.*, 2013; Diop *et al.*, 2014; El Zrelli *et al.*, 2015). Coastal regions, often marked by urbanization and industrialization, remain particularly vulnerable to anthropogenic discharges (Pekey, 2006; Waltham *et al.*, 2011; Wu *et al.*, 2022). These elements permeate the aquatic environment, becoming concentrated within sediments(Sin *et al.*, 2001; Buggy & Tobin, 2008; Yi *et al.*, 2011; Medeiros *et al.*, 2013; Genç & Yilmaz, 2017), acting as vectors for aquatic organisms(Diop *et al.*, 2016; Türkmen *et al.*, 2008; Yildirim *et al.*, 2008). The presence of metallic trace elements can lead to physiological imbalances in aquatic organisms, affecting factors such as growth, reproduction, and mortality rates (Bird *et al.*, 2008; Canli & Atli, 2003; Farkas *et al.*, 2000). Additionally, these elements disrupt the ecological equilibrium of aquatic ecosystems, influencing the diversity and structure of biological communities (Leer *et al.*, 2005; Papagiannis *et al.*, 2004; Yakimov *et al.*, 2007), thus posing threats to human well-being. Our measurements unveil elevated levels of essential metals (zinc, iron) in hake sourced from the Gulf of Béjaïa compared to the Bay of Jijel and the Gulf of Annaba. Meanwhile, copper and nickel accumulate at lower levels in the Gulf of Béjaïa and Annaba. Cadmium exhibits a higher presence in the Gulf of Béjaïa, whereas lead dominates in the Bay of Jijel. The Gulf of Béjaïa faces contamination from multiple sources, including fuel reserves, maritime traffic, and substantial human activity. Furthermore, various emissions, including those from the Eghzar-Amokrane Agro-food complex and Tala-hamza Zinc mill, contribute to the contamination of metal elements. The Gulf's hydrological network exacerbates the issue by transporting pollutants from agricultural and industrial activities. The rapid expansion of the industrial and tourism sectors, coupled with population concentration, presents marine environmental challenges in the Jijel region. The Gulf of Annaba confronts pollution from diverse origins, including thirteen sewers, and heavy metal influx from the Oued Seybouse watershed, which receives untreated wastewater. These sources of pollution collectively contribute to the contamination of both water and sediment by heavy metals. Importantly, it's observed that zinc and iron tend to accumulate more in liver tissue, while copper, nickel, lead, and cadmium show higher concentrations in muscle tissue. This pattern aligns with earlier findings in other fish species.

CONCLUSION AND APPLICATION OF RESULTS

This study highlights the significant importance of the recorded heavy metal contents in hake sourced from the eastern sector of Algeria, providing a clear reflection of pollution levels along these coastlines. The collected measurements reveal elevated levels of essential metals (zinc, iron) in hake from the Gulf of Béjaïa, surpassing those found in hake from Jijel Bay and the Gulf of Annaba. In contrast, hake from the Gulf of Annaba accumulates lower doses of copper and nickel. Notably, cadmium shows a higher prevalence in hake from the Gulf of Béjaïa, while lead predominates in hake from Jijel Bay. These findings underscore hake's significance as an indicator species for pollution. The measured levels exhibit considerable values when compared to documented levels in the Mediterranean by various researchers. This dataset contributes to a comprehensive and insightful understanding of environmental quality in terms of metallic trace element contamination. As an initial step, it is advisable to maintain continuous, annual, and systematic monitoring to formulate a strategy addressing the issue of industrial and urban discharges. Furthermore, it is imperative to explore additional perspectives for more comprehensive studies concerning the evolution of anthropogenic contamination in these three study sites along the eastern Algerian sector, specifically through heavy metal analysis. This approach should encompass the sampling of additional sites and the analysis of heavy metal concentrations in various marine organisms, plants, and animals within the food chain.

Conflicts of Interest

The authors declare that no conflicts of interest have been declared regarding the publication of this paper.

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